TRANSCRIPTIONAL RESPONSE OF O^6- METHYLGUANINE METHYLTRANSFERASE DEFICIENT YEAST TO METHYL-N-NITRO-N-NITROSOGUANIDINE (MNNG)

by

Anoop Rao

Submitted to the Biological Engineering Division,

School of Engineering, Massachusetts Institute of Technology

in partial fulfillment of the requirements for the degree of

Master of Science in Biological Engineering

at the

February 2004

Signature of the Author _____

Biological Engineering Division

February 5, 2004

Approved by _____

Leona Samson

Professor

Thesis supervisor

Certified by _____

Alan J Grodzinsky

Professor

Chairman of Biological Engineering Graduate Committee

TRANSCRIPTIONAL RESPONSE OF O^6- METHYLGUANINE METHYLTRANSFERASE DEFICIENT YEAST TO METHYL-N-NITRO-N-NITROSOGUANIDINE (MNNG)

by

Anoop Rao

Submitted to the Biological Engineering Division on February 5, 2004, in partial fulfillment of the requirements for the Degree of Master of Science in Biological Engineering.

Abstract

Damage to DNA can occur by means of endogenous biochemical processes or exogenous chemicals such as alkylating agents. If left unrepaired, alkylated bases, most notably, O^6 Methylguanine (O^6MeG) can be mutagenic and cytotoxic to the cell. Luckily, DNA methyltransferase (encoded by the gene *MGT1* in yeast), repairs this damage. By using transcriptional profiling as a tool, an attempt to elucidate the role of *MGT1* has been made. First, the basal expression profile of the *mgt1* was established. Then, the response of wild-type (WT) yeast and yeast lacking *MGT1* (*mgt1*) to the alkylating agent, MNNG was studied using exponentially growing WT and *mgt1* cultures which were exposed to 30μg/ml of MNNG for 10 to 60 minutes.

Basal expression profile of yeast lacking *MGT1* showed up-regulation of *REV7*, a gene implicated in spontaneous mutagenesis. Response to MNNG was invoked immediately and was dramatic and widespread involving 30% of the genome in both WT and *mgt1*. Cell-cycle checkpoints, damage signal amplifiers, DNA repair genes (nucleotide excision repair, photoreactive repair, mismatch repair) and chromatin remodeling genes were induced. Genes involved in maintaining mitochondrial structure and mitochondrial genome were also induced. Intriguingly, *RPN4*, a key regulator of proteasomal system was found to be repressed. Environmental stress response genes were culled out to examine the effects of MNNG on WT and *mgt1*, more carefully.

Temporal gene expression profiles in WT and *mgt1* were informative in delineating differences in the distinct responses mounted by WT and *mgt1*. The magnitude of response in *mgt1* is more profound than in WT. The differences in the dynamic trends between the two suggest that *mgt1* initiates a coordinated response involving repression of transcription factors and subsequently, induction of RNA processing (35% of genes incrementally induced) and kinases involved in protein phosphorylation. In the WT, the response was restricted to a transient repression of fundamental biochemical processes. Interestingly, a gene whose repression is known to mimic apoptosis was found to be repressed in the WT. The overwhelming induction of ribosomal protein synthesis genes in both WT and *mgt1* in response to MNNG is an unexpected result that could signify a successful recovery following wide-spread cellular damage.

Thesis supervisor: Leona D Samson
Title: Professor

Acknowledgements

I thank Prof. Leona Samson for her guidance, advice and suggestions during the course of the experiments and data analysis. I also thank her for providing unflinching financial support. I extend a special word of thanks to Dr Rebecca Fry for offering excellent advice with the experiments, data analysis and her words of encouragement. Brad Hogan was superb with his technical assistance in running the first set of arrays. Mervy, from the BioPolymer center, provided timely help in scanning the arrays. Sean and Sanchita provided excellent support via the BioMicrocenter. Other members of the Samson Lab added to the hustle.

The experiments were possible because of funding from the NIH and the excellent infrastructure that was provided by the Biological Engineering Division, BioMicro Center, BioPolymer Center and Massachusetts Institute of Technology.

Table of Contents

List of Figures

List of Tables

Chapter 1: Introduction

BACKGROUND

Genetic information in any cell is chemically stored in the form of deoxyribonucleic acid (DNA) and it essentially comprises a sequence of repeating nucleotides. Nucleotides in turn consist of a pentose sugar, a nitrogenous base and a variable number of phosphate groups stacked and aligned in an orderly fashion. Maintaining the physical and chemical integrity of the DNA structure is vital for its function. Unfortunately, however, errors can be introduced during replication, recombination and even repair. They can also be introduced via damage due to physical and chemical agents. Eventually, if errors remain uncorrected, it may lead to instability of the chemical structure and modification of the molecular structure. Such an alteration classifies as DNA damage and this may sometimes preclude the semi-conservative replication of DNA, lead to cell cycle arrest and often, cell death. Another ominous outcome of unchecked DNA damage is the accumulation of mutations that can lead to cancer. (Figure 1)

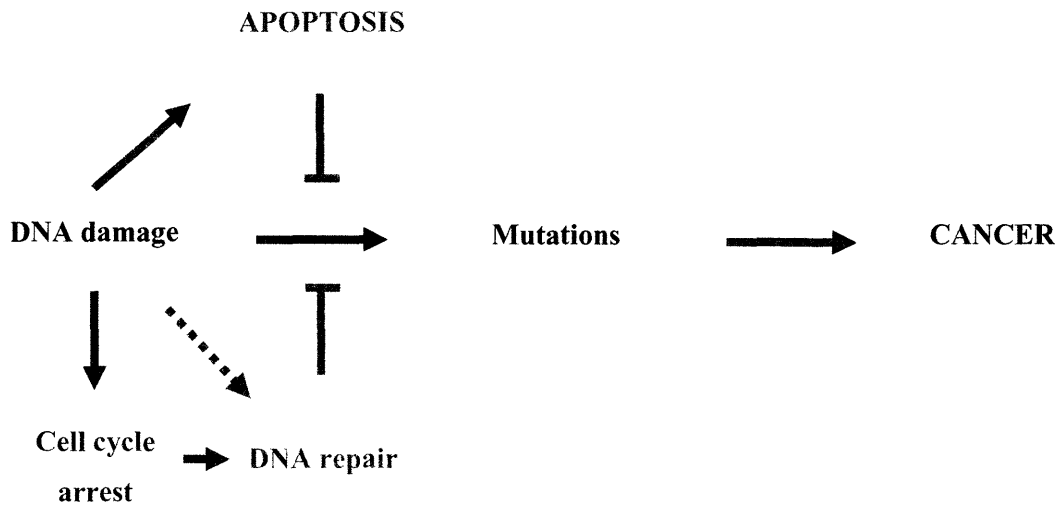


Figure 1: Unchecked DNA damage may lead to cancer.

The mechanism of damage to DNA

Spontaneous alterations

The nitrogenous bases, purines and pyrimidines, occasionally undergo a spontaneous alteration in their chemistry. The bases can lose their exocyclic amino group and undergo deamination. This modification increases the propensity for anomalous pairing of bases and an inappropriately incorporated base can introduce a transition or transversion mutation. In addition, instability of base pairing may also lead to replication arrest. Purines and pyrimidines can also be spontaneously hydrolyzed and lost. (Lindahl, 1993). Abasic sites that are produced can also lead to mutations during replication. (Loeb, 1986)

Unlike the fleeting deamination and hydrolysis reactions, oxidative damage to DNA is more elaborate and once initiated, results in a chain reaction. The cause of damage can be exogenous or endogenous. Exogenous sources of oxidative DNA damage include radiation, near UV light at 320 to 380nm and several drugs (Friedberg, 1995). Endogenously, redox reactions which ubiquitously occur in cells, are the major source of reactive oxygen species. Notable among them are the by-products of aerobic mitochondrial respiration. Singlet oxygen and hydrogen peroxide inflict damage via formation of hydroxyl radicals through metal catalyzed reactions. The intermediates and by-products of such reaction are independently capable of inflicting more damage on intracellular macromolecules. Fortunately, there are several cellular defense mechanisms help to mitigate the effect of these reactive oxygen species (ROS). These include antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and other scavengers, Vitamin C and α tocopherol. Potentially unfavorable cellular responses triggered by the presence of ROS may include the inhibition of cell cycle progression, the initiation of apoptosis and the activation of a degradative process to replace macromolecules. Oxygen radicals are also known to induce chromosome breaks. In general, irrespective of the source and mechanism of alteration or damage, the most ominous outcome is typically a mismatch of base pairs during DNA synthesis. The most important oxidative base adduct, 8-oxoguanine can mispair and be mutagenic (Lindahl., 1993, Friedberg, 2002).

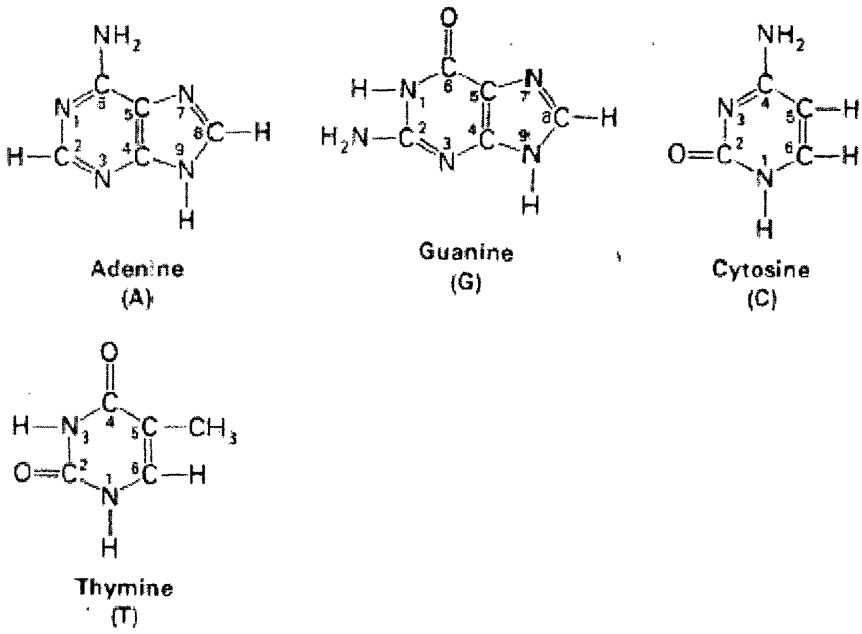


Figure 2: The nitrogenous bases; Purines (A,G) and Pyrimidines (C,T,U)

Environmental damage

Physical agents - Ionizing Radiation: Apart from spontaneous alterations and damage to DNA, physical and chemical agents in the environment inflict a substantial amount of damage. Exposure to ionizing radiation as a therapeutic, diagnostic or occupational hazard induces a variety of lesions by direct damage. Radiolysis of water generates reactive oxygen species that damage cellular macromolecules. Glutathione, a radioprotector, can counteract the damage at several tiers of radical production. Damage to bases, sugar moieties and direct induction of strand breaks can occur. Strand breaks are a special problem since mere DNA ligation may not be sufficient to repair the lesion. (Burrows, 1998). Ultraviolet (UV) radiation induces covalent linkage of adjacent pyrimidines producing cyclobutane pyrimidine dimers (CPD). These lesions distort the helix and lead to extensive bending of DNA, albeit variably. A less frequent lesion is the pyrimidine-pyrimidone (6-4) photoproduct that, like CPD's, distorts the helix (Ravanat, 2001). These physical distortions may result in an obligatory arrest of replication.

Chemical agents: Environmental exposure to chemicals included as food agents (Sugimura., 2002), inhalation of polluted air and ingestion of contaminated water are by far

the most common modes of encountering DNA damaging agents. Occupational hazards and therapeutic intervention, most notably by anti-cancer agents are responsible for most of the DNA damage in humans. The diverse class of chemicals known to cause DNA damage includes psoralens, benzo[a]pyrene, aflatoxins and nitroquinolones and alkylating agents. Historically, there has been an interest in examining the potential for food additives to be potent carcinogens. Most of the evidence has relied on demonstrating that electrophilic metabolites of the parent compound can forms DNA or protein adducts. Their metabolism is dependent on an inducible system of membrane proteins called the cytochrome P-450 system. Apart from this P-450 system, there are other enzymes that conjugate compounds to make them more water soluble and permit easy elimination from the system. They include acetyltransferases, glucoronyl transferases, adenosylating enzymes and methylating enzymes. In principle, the cell has several mechanisms of dealing with DNA damage but it is unfortunate, however, that metabolites can themselves be more harmful than the parent compounds.

Means of response to DNA damage

In essence, physical and chemical damage to DNA is a universal phenomenon across living systems. Damage by physical and chemical agents can lead to arrest of replication and transcription. Fortunately, there are sub-cellular systems that operate in a coordinated way to sense and respond to damage. Broadly, they can be classified as DNA repair mechanisms (specific set of events to eliminate the primary lesion) and DNA damage checkpoint mechanisms - accessory events that stall the cell cycle and permit the specific DNA repair mechanisms to act.

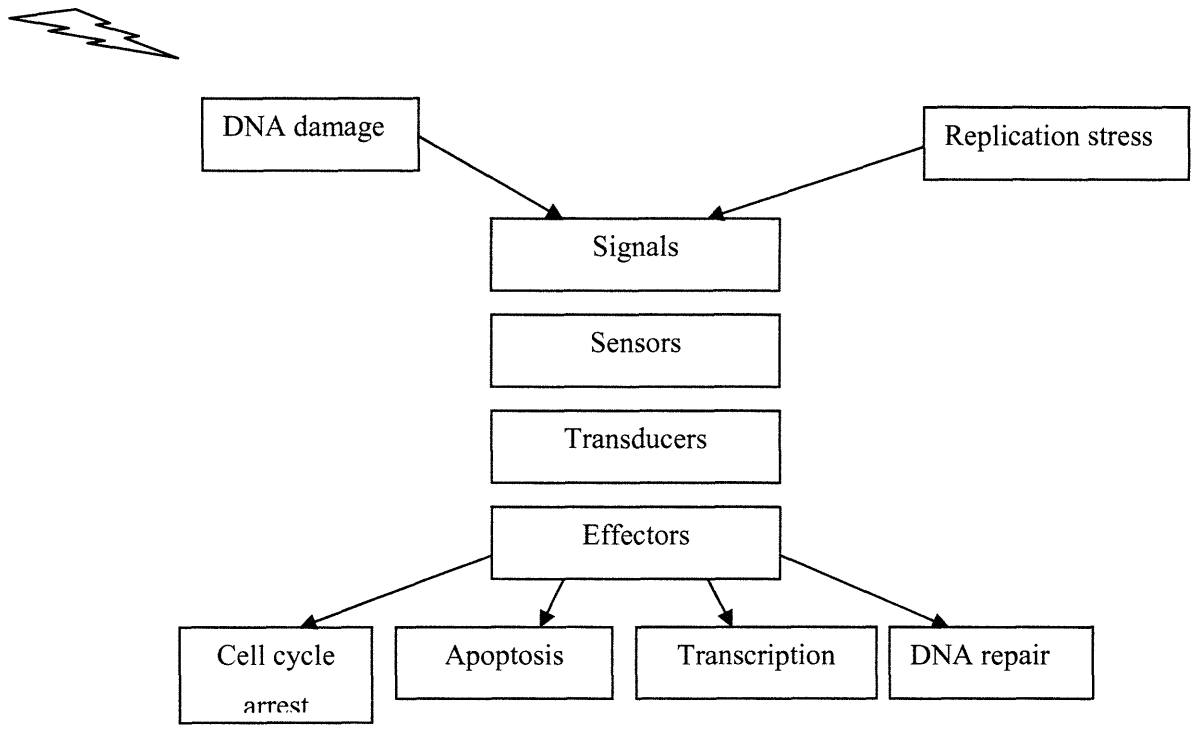


Figure 3: Causes and consequences of DNA damage.

An elaborate, overlapping set of enzymes, proteins and mechanisms deal with these deleterious lesions. Cells respond to DNA damage by delaying cell cycle progression and by increasing the expression of a few genes involved in the repair and tolerance of DNA damage (Friedberg et al,1995). Surveillance mechanisms in eukaryotic cells monitor and regulate the cell cycle and its progress. The cell-cycle checkpoints are activated by one or more signals and ultimately results in the inhibition of cell cycle progression. The checkpoint mechanism first detects damaged DNA and then generates a signal that arrests cells in the G_1/S or G_2/M phase of the cell cycle. It also slows down S phase (DNA synthesis). This mechanism is thought to prevent the replication of damaged templates and the segregation of broken chromosomes.

Consequences of unrepaired lesions

Despite the orchestrated response, however, the damage may not be mitigated. If lesions are not repaired, they would pose a problem by being mutagenic or lethal. In response to DNA damage the cell has four major routes of responses. Cell-cycle arrest provides the crucial time for repairing the damage. If the damage load is too profound for the cell to handle, it may undergo apoptosis (programmed cell death) to avoid the propagation of highly defective cells. The lesions may be fixed by DNA repair pathways or alternatively, the

unrepaired lesions may generate sequence changes in the genome to be passed on as a mutation.

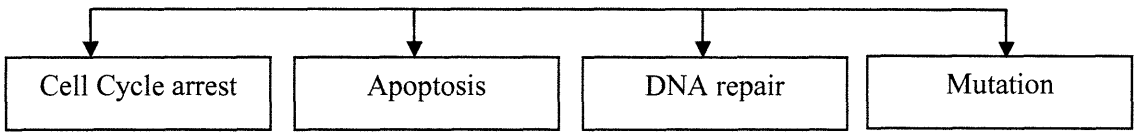


Figure 4: Outcomes of DNA damage

Overview of repair mechanisms

Photoreactivation by photolyases

UV light exposure generates 2 major classes of stable DNA lesions - cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts (6-4 PD). Unless repaired, these lesions may lead to blockage of transcription, mutations, cell death and cancer. CPD's and 6-4 PD's are removed by two pathways: (i) nucleotide excision repair (NER); and (ii) photoreactivation. Some plants, bacteria, and yeast possess a photolyase that preferentially reverses the CPD's in the non-transcribed strand of active genes. DNA photolyases catalyze the light-dependent repair of pyrimidine dimers in DNA. (Carell, 2001). Photolyases bind tightly to CPDs and, on excitation by 340–400-nm light, catalyse the cleavage of the cyclobutane linkage between the adjacent pyrimidines and restore the monomeric bases without cutting the phosphodiester backbone of DNA.

In yeast, the Phr1 gene that codes for photolyase has been shown to be upregulated by several DNA-damaging agents such as UV radiation, 4NQO, MMS and MNNG (Sebastian et al, 1990). If Phr1 is unable to resolve cyclobutane linkages then it will also try to enhance the NER of CPDs. The photolyase enzyme functions as a model for proteins that interact with sites of DNA damage and have the potential to facilitate DNA-damage recognition by repair pathways. (Sebastian and Sancar, 1991)

Methylguanine methyltransferase

The methylguanine methyltransferase reverts O^6-methylguanine to guanine by transferring the methyl group from DNA to a reactive cysteine group of the protein in an irreversible reaction. This covalent attachment of the alkyl group to the cysteine residue

inactivates the enzyme. Alkylguanine methyl transferase is a suicide enzyme. The mechanism for this reaction is indicated in Figure 7.

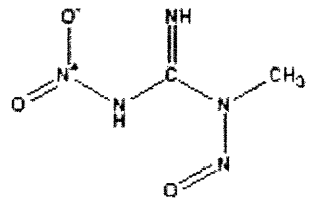


Figure 5: Alkylating agent, MNNG

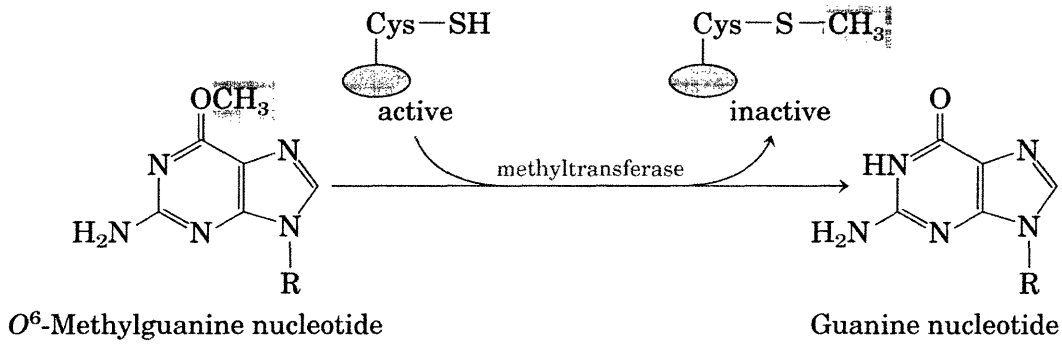


Figure 6: O⁶Mehyltguanine methyltransferase accepts the methyl group at its cysteine residue

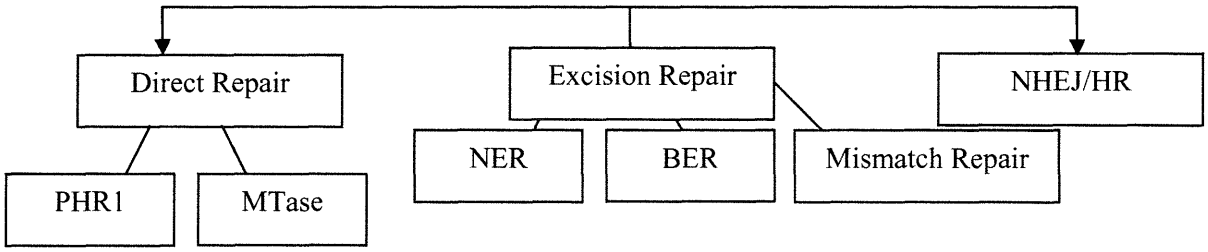


Figure 7: Overview of DNA repair mechanisms

Excision Repair

In contrast to direct repair there is cleavage of the sugar phosphate backbone in excision repair.

Base Excision Repair

Damage to DNA from deamination, oxidation and alkylation is mainly repaired by BER. In base excision repair, the DNA bases that are altered by small chemical modifications are replaced through the excision of only the damaged nucleotide (short patch BER) or through the removal of 2–13 nucleotides containing the damaged nucleotide (long-patch BER). DNA glycosylases initiate BER by excising damaged bases from DNA and generating abasic sites.

Nucleotide Excision Repair (NER)

In cases where the alteration involves the addition of large chemical additions or cross-links, the DNA bases are excised using the nucleotide excision repair where a short, single-stranded segment containing the damage is removed. NER helps in repair of bulky base adducts formed by UV radiation, various environmental mutagens, and certain chemotherapeutic agents. In NER. (Wood, 1997)

Mismatch Repair (MMR).

An important replication-associated correction function is provided by the post-replicative mismatch repair system. Base-base mismatches or loops of extra bases if left unrepaired, will generate point or frameshift mutations respectively. Misincorporation of non-complementary bases by DNA polymerases is a major source of the occurrence of promutagenic base-pairing errors during DNA replication or repair. MMR is conserved from bacteria to humans. It identifies and corrects mispaired bases and 1–3-nucleotide loops that result from DNA polymerase errors during replication.

Double strand break (DSB) repair

Double strand breaks are rare and two independent pathways handle them; homologous recombination (HR) and non-homologous end joining (NHEJ). Homologous recombination uses extensive homology to code DNA and maintain accuracy. Non-homologous end joining involves a coordinated rejoining of the broken ends and uses no or extremely limited regions of homology as a template for repair. Consequently, this process is inaccurate and the deletions of a few nucleotides are introduced at the site of the DSB. HR

and NHEJ are important in all eukaryotes and HR is more important in rapidly dividing cells and NHEJ is more important in quiescent or terminally differentiated cells. HR is important for meiosis or the repair of inter-strand cross-links, while NHEJ is required for joining of DNA fragments while generating the diversity of the immune system.

Mechanism of damage by alkylation

Alkylating agents are electrophilic compounds with affinity for nucleophilic centers in organic macromolecules. They are probably the broadest class of chemicals that have the potential to cause profound damage to DNA. Alkylating agents are classified as monofunctional or bifunctional depending on the number of reactive groups, and therefore, the ability to react with multiple sites within DNA. Alkylating agents attack nitrogen and oxygen at various sites within nitrogenous bases with different reactivity's. Apart from these veritable hot spots within nitrogenous bases, alkylating agents can react with oxygen in the phosphodiester linkage to form a phosphotriester.

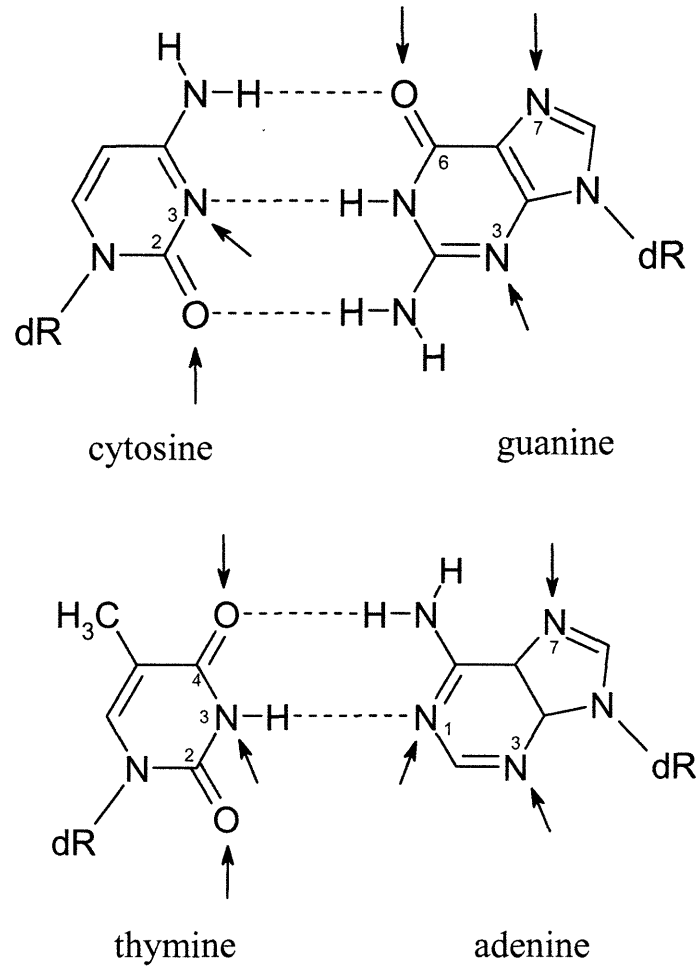


Figure 8: Sites within the nitrogenous bases susceptible to alkylation damage.

By virtue of its ability to reach several nucleophilic sites within the nitrogenous bases, inter and intra-strand cross links can occur as a consequence of exposure to bifunctional alkylating agents. The covalent link sustains this anomaly and prevents strand separation (if there is an inter-strand crosslink) leading to a complete block of replication and transcription.

Damage to DNA bases

Alkylating agents are structurally diverse group of chemicals that cause a wide range of biological effects including cell death, mutation and cancer. DNA damaged by these agents contains widely different amounts of 12 alkylated purines/pyrimidines and two

phosphotriester isomers. They are used in anticancer therapy and are also found in cigarette smoke.

Lesions caused by alkylation

Attack in O^6 position of guanine leads to the formation of the adduct, O^6 Methylguanine (O^6 MeG). This is a relatively minor lesion compared to O^4Methylthymine (O^4MeT), but potentially the most deleterious lesion if left unrepaired in the system. Other potentially harmful lesions include 3 Methyl Adenine (3MeA). If the O^6 MeG lesion remains unrepaired, then it permits G→A transition mutation following 2 rounds of replication. This happens in both eukaryotes and prokaryotes. Recombination and cell death that may ensue but both need a functional MMR system.

In *E.coli*, it has been shown that the miscoding alkylation adducts on the template strand would lead to anomalous base pairs upon replication. Provocation of mismatch repair by such lesions would result in a futile turnover of the newly synthesized strand because the offending adduct is not removed from the template DNA, a process that could lead to cell death. Luckily, the O^6 methylguanine MTase protein is able to counter this effect by irreversibly and covalently binding to the methyl group and plucking it off the base. Since the cysteine which is methylated is not regenerated at all, the capacity for repair of O^6-methylguanine is limited by the number of molecules of the MTase available within the cell.

Alkyltransferases across systems

Methyltransferase belongs to a class of proteins, the alkyltransferases. There are close to a 100 alkyltransferases but the structure of only 3 three family members: the Ada-C protein from *Escherichia coli* (Moore et al 1994), the human alkyltransferase (hAGT) (Daniels, 2000), and *Pyrococcus kodakaraensis* (Hashimoto 1999) are known. The protein is non-enzymatic in nature and therefore the protection by alkyltransferase depends on the regulation of its synthesis and degradation.

Structure and function of Yeast methyltransferase (MGT1)

Repair of O^6-MeG in yeast extracts was shown to be performed by a 25-kilodalton protein Methyl transfer was accompanied by the formation of S-methylcysteine. The *S. cerevisiae MGT1* codes for a 188 amino acid protein. About half of the *MGT1* protein has

homology with four bacterial MTases and also the human DNA MTase. (Xiaoand Samson 1992)

Exponentially growing yeast cultures have about 150 molecules of MTase in each cell. The yeast MTase has a half-life of about 4 min at 37°C. Synthesis of the yeast DNA MTase is not inducible by sublethal exposures to alkylating agent. The substrates for yeast MTase include O^6 MeG and O^4MeT. Unlike this, the human MTase is very specific for O^6MeG.

Spontaneous mutations

Mutations are defined as spontaneous when they arise in cells that are not actively exposed to exogenous, xenobiotic mutagens. Spontaneous mutations occur due to either uncorrected DNA replication errors, or endogenous metabolites that cause lesions on DNA. Oxidative damage and alkylation damage are the 2 major sources of endogenous DNA damage. It results as a consequence of cellular metabolism and failure to correct this damage due to genetic defects results in significantly increased spontaneous mutation rates. Spontaneous mutations have been studied earlier using several systems including *MGT1* deletions in yeast. *MGT1* deleted mutants were shown to have an increased spontaneous mutation rate suggesting an endogenous source of DNA methylation damage. (Xiao and Samson, 1993).

Alkylation due to endogenous processes

S-adenosyl methionine (SAM) a cellular methylase co-factor has a reactive methyl group and is responsible for enzymatic methylation of DNA, RNA and proteins. Under physiological conditions, SAM has been shown to non-enzymatically methylate DNA to form 3-Methyl adenine and O^6MeG (Rydberg and Lindahl 1982). Endogenous processes might include the 'aberrant' methylation of guanine by *S*-adenosylmethionine and the endogenous nitrosation of compounds containing primary amino groups and their subsequent breakdown to methylating species (Sedgwick, 1997). *n*-nitrosoglycocholic acid has been shown to be able to methylate DNA *in vitro* and *in vivo* (Shuker and Margison, 1997).

***Saccharomyces cerevisiae* as a model system to study genome-wide expression.**

The baker's yeast *S. cerevisiae* is an informative model organism in traditional genetic studies. It also presents an ideal model genome for large-scale functional analysis. Relative to other eukaryotes, *S. cerevisiae* has a compact genome. Approximately, 70% of its total (non-ribosomal DNA) genetic complement is protein-coding sequence. Encompassing 16 chromosomes, the 12-megabase (Mb) yeast genome is predicted to encode about 6,200 genes, with 1 gene per 2 kb of genomic sequence. (Goffeau, 1996).*S. cerevisiae* is an informative predictor of human gene function; nearly 50% of human genes implicated in heritable diseases have yeast homologues. (Bassett, 1996, 1997. Venter. 2001).

Since its development in the mid-1990s (Schena, 1995, Chee,. et al 1996), the DNA microarray has emerged as the pre-eminent tool for functional genomics. The ability to analyse thousands of DNA samples simultaneously by hybridization-based assay has provided a popular method for analysing the relative levels of mRNA transcripts on a genome-wide scale. Typically, DNA microarrays have been used to identify genes, the expression of which is either induced or repressed during specific cellular responses. For example, DeRisi (DeRisi *et al.* 1997) used DNA microarrays to monitor relative changes in mRNA levels during the shift from anaerobic fermentation to aerobic respiration in yeast. Microarrays have also been used to identify genes differentially expressed during sporulation (Chu et al 1998), as well as genes periodically expressed during the cell cycle (Cho. et al 1998, Spellman, 1998). Jelinsky and Samson (1999) used oligonucleotide arrays to identify over 400 genes that are either induced or repressed in response to the DNA-damaging, alkylating agent methyl methanesulphonate (MMS). These and other microarray-based studies have identified genes that putatively function in common regulatory pathways; such pathways are also being delineated by transcriptional profiling of strains mutated for key regulatory components.

Affymetrix has used the genomic sequence of the budding yeast Saccharomyces cerevisiae to design and synthesize high-density oligonucleotide arrays for monitoring the expression levels of nearly all yeast genes. This direct and highly parallel approach involves the hybridization of total mRNA populations to a set of four arrays that contain a total of more than 260,000 specifically chosen oligonucleotides synthesized in situ using light-directed combinatorial chemistry.

Chapter 2: Expression Profiling

Transcriptional response of *Saccharomyces cerevisiae* wild type and DNA methyltransferase mutants.

Materials and Methods

Yeast strains and growth conditions

To study the genome-wide transcriptional response of *Saccharomyces cerevisiae* upon exposure to MNNG two strains were obtained from Research Genetics, Carlsbad, CA. The wild-type (WT) BY4741 (*MAT* a *his3*Δ1 *leu2*Δ0 *met15*Δ0 *ura3*Δ0) and the methylguanine methyltransferase lacking strain, BY4741 *mgt1* Δ (*mgt1*). The *mgt1* Δ strain was originally created using a PCR based gene deletion strategy (Baudin et al., 1993 and Wach et al., 1994). This gene deletion is viable since *MGT1* is a non-essential gene. The cells were grown and maintained on YPD (10 g yeast extract, 20 g peptone, 20 g dextrose, 20 g agar/liter) containing 200μg/ml of G418 (Geneticin, Sigma Chemicals).

N-Methyl-N'-Nitro-N-Nitrosoguanidine

A 1% stock solution of DNA damaging agent, N-Methyl-N'-Nitro-N-Nitrosoguanidine (MNNG) was prepared and stored in amber tubes away from light. This stock was used for all the experiments. First, the phenotypes of the strains were established using a gradient plate assay. Briefly, equal number of BY4741 and BY4741 *mgt1* Δ cells were plated in YPD+G418 plates which had MNNG in concentrations of 0 μg/ml, 5 μg/ml, 10 μg/ml, 15 μg/ml, 20 μg/ml and 25 μg/ml. The agar was allowed to settle at an angle in the plates. This permitted variable exposure of the cells to a fixed concentration of MNNG. Experiments were performed in duplicate. Colony formation was observed after 3 days of growth at 30°C.

Growth Curve

Single colonies of yeast were picked from YPD+G418 plates to inoculate 5 ml of YPD culture in a test-tube that was rotated overnight at 250 rpm at 30°C. 100μl of each strain was inoculated in 150 ml of YPD+G418 rotating at 300 rpm, at 30°C. OD and cell counts were taken over time to follow growth in cell number. Growth curves were plotted for BY4741 and BY4741 *mgt1* Δ.

MNNG-induced cell killing

The difference in the MNNG-induced cell killing between BY4741 and BY4741 *mgt1* was studied. One colony of wild-type (WT) and MTase lacking mutant (*mgt1*), was picked and grown in 2 separate test-tubes with 5ml YPD (with G418), overnight. Then, 100μl was transferred into 150 ml of media (with G418) in a 250 ml flask. This was grown for 12 hours at 30 °C after which 10 ml of culture was transferred into four 15ml tubes. To this 150μl of 1% MNNG stock was added to establish the final concentration of MNNG at 30μg/ml. After incubating them for 0, 20, 40 and 60 minutes, the test-tubes were centrifuged. The supernatant was discarded and the cells were re-suspended in 10 ml of distilled water. Over a series of dilutions, the re-suspended cells were plated onto YPD agar plates with G418. Colonies were counted after 3 days of incubation at 30 °C.

Cell preparation for Microarray Analysis

Single colonies of WT and *mgt1* were picked from YPD plates to inoculate 5 ml of YPD culture. They were incubated at 250 rpm at 30°C, overnight. 100μl of each strain was inoculated in 150 ml of YPD+G418 media in a flask rotating at 300 rpm maintained at 30°C. The cells were grown to mid-log phase (OD = 0.8). The cultures were split into 3 volumes of

50 ml before being exposed to MNNG (30 μg/ml) for a variable length of time as shown in Table 1. The control samples were mock-treated with same amount of double distilled water. (DDW). After exposure for the appropriate length of time, the cells were spun down in 50 ml tubes centrifuged at 8000 rpm. The cells were snap-frozen and stored at -80°C.

Table 1 : Experimental design of oligonucleotide expression study.

The number of BY4741 and BY4741 *mgt1* Δ samples that were treated with double distilled water (DDW) or MNNG.

	Control (DDW)	**Treated with 30 μg/ml MNNG**					
		10 min	20 min	30 min	40 min	50 min	60 min
WT	3	3	3	3	3	3	3
mgt1	3	3	3	3	3	3	3

Total RNA preparation

Total RNA was extracted from the frozen cells using the enzymatic lysis protocol (Qiagen RNEasy Mini Protocol – Standard Version) as detailed in the Appendix of Protocols. Briefly, the cells were incubated for 20-30 minutes (with gentle shaking, every 5 minutes) with 2 ml of buffer made from Sorbitol (1M), EDTA (0.1M) β mercaptoethanol (0.1%) and 50 U of lyticase (Sigma) per $1x10^7$ cells. The cells were then centrifuged to pellet the spheroplasts. In a series of steps the cell wall was lysed and the lysate was made to pass across a silica-gel membrane to trap the RNA. Finally, RNAse free water was used to elute the RNA out before estimating the concentration using a spectrophotometer. 260/280 absorbance readings were measured for total RNA. A ratio of 260/280 ratios between 1.8 to 2.1 was considered acceptable. If the ratio was below 1.8 (indicates possible contamination) or above 2.1

(indicates presence of degraded RNA truncated cRNA transcripts, and/or excess free nucleotides), the total-RNA process was repeated.

Pre-hybridization quality control

Forty-two samples of total RNA from the different experimental groups (Table 1) was isolated and stored at -20°C. The samples were also tested using the Agilent 2100 Bioanalyser system. This permits rapid visualization of RNA sample quality and quantity. A rRNA ratio of 28S/18S close to 2 implies minimal degradation of RNA, a prerequisite for efficient reverse transcription, cDNA synthesis and in-vitro transcription and to ensure the highest quality RNA hybridization to the gene expression microarrays. The steps for cRNA synthesis from total RNA is illustrated in Figure 9 and details are included in the Appendix.

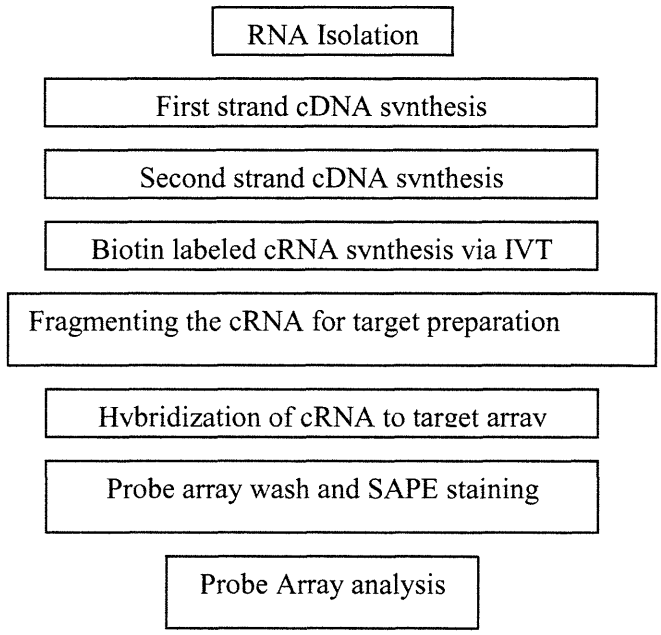


Figure 9: Steps in cRNA preparation and hybridization to GeneChip.

GeneChip® hybridizations and Image analysis.

Fragmented cRNA samples were hybridized to GeneChip® arrays containing the complete yeast genome for a total of 42 arrays (YG-S98 arrays, Affymetrix, CA). The GeneChip® Yeast Genome S98 Array contains probe sets for approximately 6,400 *S. cerevisiae* (S288C strain) genes identified in the Saccharomyces Genome Database (December 1998). This array also contains approximately 600 additional probe sets representing putative open reading frames (ORFs) identified by SAGE analysis, mitochondrial proteins, TY proteins, plasmids, and a small number of ORFs for strains other than S288C.

Scanning was carried out at the MIT Biopolymers Laboratory after hybridizing fragmented cRNA at a concentration of 0.05 ug/μl to GeneChip®s in 200μl of Affy buffer (100 mM MES, 1 M NaCl, 20 mM EDTA, 0.01% Tween 20) with GeneChip® eukaryotic hybridization controls (GeneChip® Eukaryotic Hybridization Controls Kit, Affymetrix, CA) in the presence of 0.1 mg/ml herring sperm DNA and 0.5 mg/ml acetylated BSA at 40 °C for 16 h with constant rotation. Arrays were rinsed after hybridization with 200 μl of stringent wash buffer (100 mM MES, 0.1 M NaCl, 0.01% Tween 20) followed by a non-stringent wash (6XSSPE, 0.01% Tween 20). 20XSSPE had the following composition (3 M NaCl, 0.2 M NaH_2PO_4, 0.02 M EDTA). Staining was done with 2 ug/ml streptavidin–phycoerytherin and 1 mg/ml acetylated BSA in 6×SSPE-T. Arrays were scanned by a HP G2500A GeneArray scanner.

Data analysis

A total of 42 hybridizations were performed and the scanned images from Micorarray Suite 5.0 were stored for computational analysis that was performed using Spotfire, MS Excel and S-plus Array analyzer.

Data Normalization

The .cel files generated after scanning using the Affymetrix suite were used for this analysis. All the 42 .cel files from the control and the experimental groups were analyzed simultaneously.

The variation between high-density oligonucleotide arrays was reduced by normalizing the data. The Robust Multichip Average (RMA) algorithm was used to adjust the background and perform quantile normalization. The expression results for each ORF/gene were represented as logarithm (to base 2) of the expression value. The software package RMAExpress 0.2 alpha 1 version for Windows was used for this purpose. RMAExpress combines the 16-20 probe pair intensities for a given gene to define a measure of expression that represents the amount of the corresponding mRNA species. The normalization takes only perfect matches into account and the mismatch probe cells are not used for calculation the signal intensity/measure of expression.

Analysis of RMA output files

The output of the RMAExpress, in the form of $\log_2$ values of expression, was exported to MS Excel. For further analysis, average expression value from 3 biological replicates (Table 1), for each time point was computed. Average expression values derived from all the arrays were compared with the average expression of untreated WT to get an expression ratio (ER). This

blanket comparison to compute ER ensures that all the data are compared to an unambiguous baseline. The comparisons were represented as $\log_2$ of expression ratio's ($\log_2$ER).

Post-normalization cut-off

A $\log_2$ ER for a gene/ORF > 1 indicates average fold change induction factor of 2 for that particular gene/ORF. Analogously, a $\log_2$ ER < -1 indicates an average fold change repression factor of 2. If $\log_2$ ER's were between 1 and -1, they were classified as not significant (NS). For the purpose of visualization, the $\log_2$ ER's across all treatment time-points for both WT and *mgt1* were exported and visualized in Spotfire's functional genomics module.

RESULTS

MNNG gradient plate

The phenotype of the WT and *mgt1* strains, in response to MNNG, was ascertained using gradient plates (Figure 10). Upon exposure to increasing concentrations of MNNG, fewer colonies of *mgt1* survived when compared to WT.

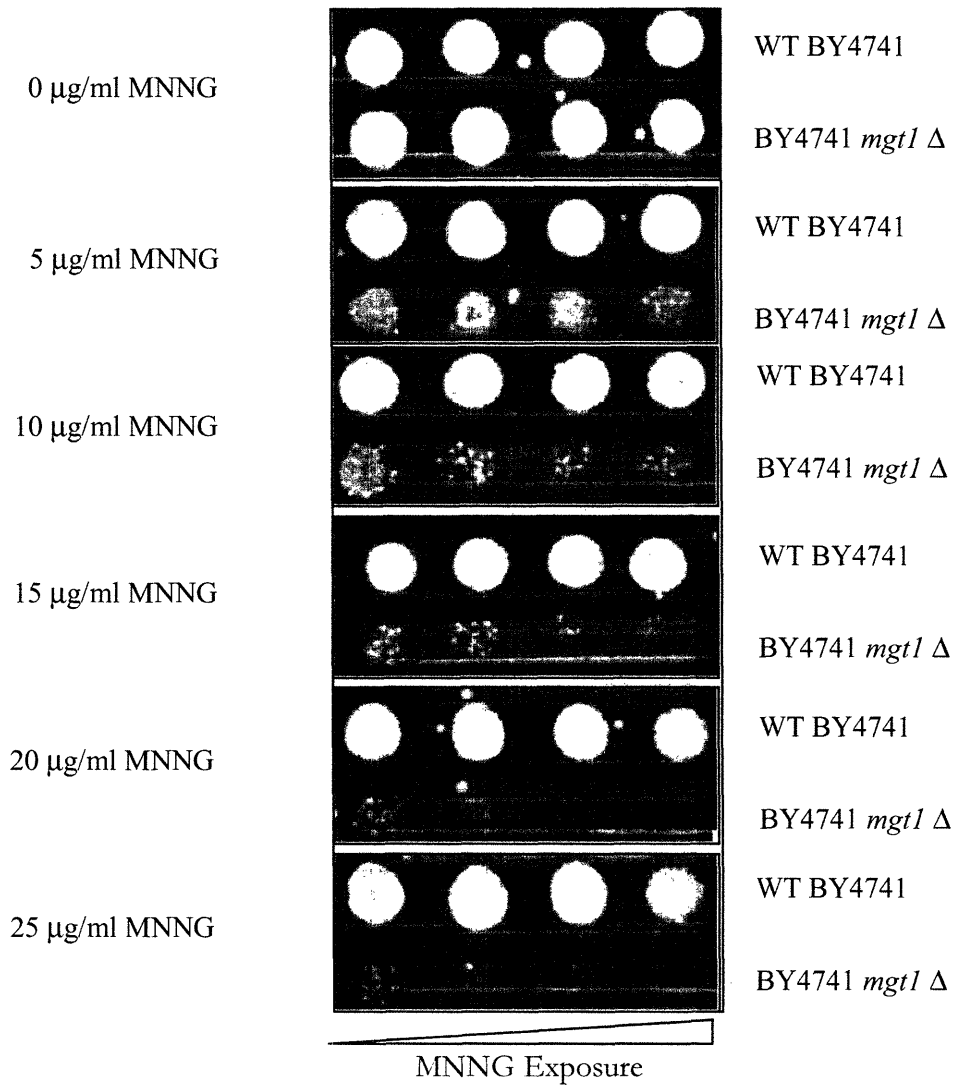


Figure 10: MNNG gradient plate assay for Wild-type BY4741 (WT) and BY4741 *mgt1* Δ.

The agar was allowed to settle at an angle in the plates. This permitted variable exposure of the cells to a fixed concentration of MNNG. The same numbers of cells were inoculated on the surface of agar plates with increasing concentrations of MNNG (5-

25μg/ml). The cells were grown at 30°C for 3 days before being observed and photographed. The increased sensitivity of *mgt1* to killing by MNNG can be attributed to the lack of MTase.

MNNG-induced cell killing

MNNG-induced cell killing in cultures was compared between WT and *mgt1*. Exposure to MNNG at 30 μg/ml cause significantly more killing in *mgt1* than in the WT. The lack of MTase and the inability to repair alkylation induced repair in the *mgt1* imparts this difference.

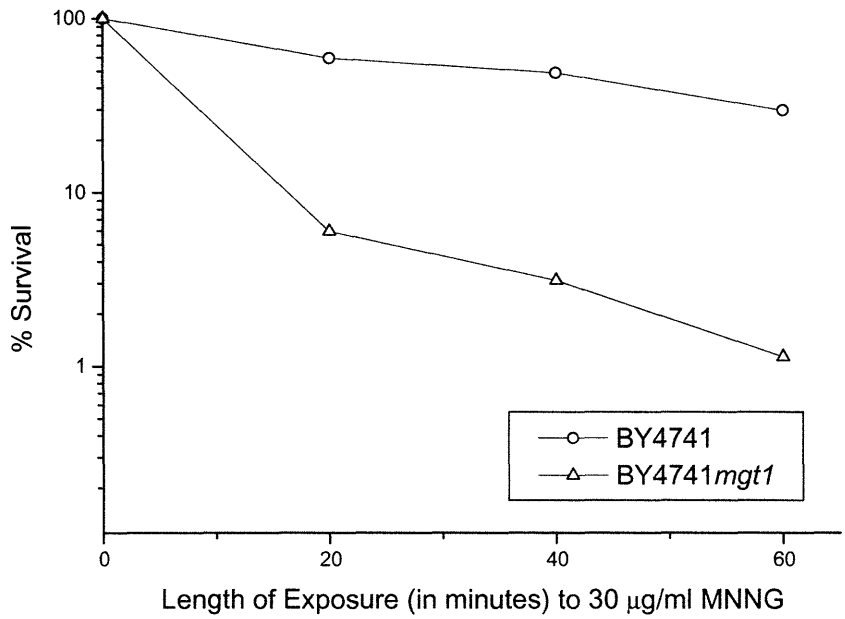


Figure 11: MNNG-induced killing in wild-type (WT) and MTase deficient yeast (*mgt1*).

WT and *mgt1* cells were picked and grown separately in 5ml YPD (with G418), overnight. Then, 100μl was transferred into 150 ml of media (with G418) in a 250 ml flask. After growth

for 12 hours at 30 °C, 10 ml of culture was transferred to four 15ml tubes. To each, 150μl of 1% MNNG stock was added to establish the final concentration of MNNG at 30μg/ml. After incubating them for 0, 20, 40 and 60 minutes, the test-tubes were centrifuged. The supernatant was discarded and the cells were re-suspended in 10 ml of distilled water. Over a series of dilutions, the re-suspended cells were plated onto YPD agar plates with G418. Colonies were counted after 3 days of incubation at 30 °C. The colony count, for each duration of exposure (0, 20, 40 or 60 minutes), was compared to the colony count at time point 0. This was expressed as the percent survival (% survival) for that duration. The % survival plot for increasing length of exposure to MNNG, for WT and *mgt1*, is shown in Figure 11. *mgt1* was more sensitive to MNNG than WT. In addition, increasing the length of exposure to MNNG killed more *mgt1* cells than WT.

Total RNA extraction from exponentially growing cells.

WT or *mgt1* cells were grown to mid-log phase (OD = 0.8) as described earlier. The cultures were split into 3 volumes of 50 ml before being exposed to MNNG (30 μg/ml) for a variable length of time as shown in Table 1. The control samples were mock-treated with same amount of double distilled water. After exposure for the appropriate length of time, the cells were spun down in 50 ml tubes centrifuged at 8000 rpm. The cells were snap-frozen and stored at -80°C before total RNA extraction using the Qiagen RNEasy protocol.

Pre hybridization quality control of total RNA samples

A few of the total RNA samples were tested for their quality using the Agilent Bioanalyzer system. Lane 1 in figure 12 shows the ladder. Lane 2 in Figure 12 depicts a sample that had a good 28s:18s ratio (1.84).

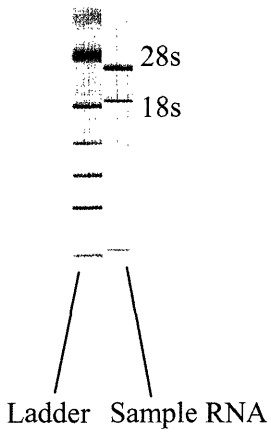


Figure 12: Pre-hybridization quality control gel of total RNA sample. This sample was from exponentially growing wild-type (WT) cells treated with 30μg/ml of MNNG for 40 minutes.

The total RNA samples from the different experimental groups were tested using the Agilent 2100 Bioanalyzer system. This system permits rapid visualization and quality control of the RNA sample. The rRNA ratio (28S/18S) was between 1.61-1.87 in the tested samples. Quality of RNA is a prerequisite for efficient in vitro-transcription reaction.

Post-hybridization quality control.

After hybridization to the target Affymetrix YGS98 array, the quality of the arrays was judged by the following factors; percent present calls, presence of spiked control cRNA, background values and noise. All these quality control standards were met satisfactorily (summarized in Table 2). The hybridization efficiency was judged by the percentage of absent and present calls. On an average, the present calls were > 74% before normalization. Hybridization controls, *BioB, bioC,* and *bioD* represent genes in the biotin synthesis pathway of *E. coli. Cre* is the recombinase gene from P1 bacteriophage. The GeneChip Eukaryotic Hybridization Control Kit contains 20x Eukaryotic Hybridization controls composed of a mixture of biotin-labeled cRNA transcripts of *bioB*, *bioC*, *bioD,* and *cre*, prepared in staggered concentrations (1.5 pM, 5 pM, 25 pM, and 100 pM for *bioB*, *bioC*, *bioD,* and *cre*, respectively).

The 20x Eukaryotic hybridization controls are spiked into the hybridization cocktail, independent of RNA sample preparation, and are thus used to evaluate sample hybridization efficiency to gene expression arrays. *BioB* is at the level of assay sensitivity and should be called "Present" at least 50% of the time. *BioC, bioD,* and *cre* should always be called "Present" with increasing Signal values, reflecting their relative concentrations. The 20x Eukaryotic Hybridization Controls can be used to indirectly assess RNA sample quality among replicates. The overall intensity for a degraded RNA sample, or a sample that has not been properly amplified and labeled, will be lower when compared to a normal replicate sample.

Controls that were spiked-in were detected as expected (BioB-90%, BioC, D, Cre -100%). Their relative intensities were also in accordance with expectations (BioB < BioC < BioD < Cre). Ideally, the BioB control cRNA is spiked in at the detection threshold (1.5 pM) and should receive 'present' detection call in approximately 50 percent of all samples. BioB was present in 90% of the samples. Average background values ideally range from 20 to 100 for arrays scanned with GeneArray® Arrays. In our data, the average background value was about 60. Noise is a measure of the pixel-to-pixel variation of probe cells on a GeneChip array. The two main factors that contribute to noise - electrical noise of the GeneArray Scanner and sample quality. The datasets had an average noise of 1.4.

Parameters	Outcome
Percent present calls	> 74%
Spiked controls	As expected, BioB < BioC < BioD < Cre
Background value	60 (Normal range 20-100)
Noise (Q)	1.4

Table 2: Parameters of post-hybridization quality control.

Four parameters; percent present calls, presence of spike-in controls in the appropriate order, low background value and the presence of noise were assessed and found to be favorable.

RMA normalization

The data from 42 arrays were normalized using RMA method of normalization (Bolstad, 2003). The benefit of RMA normalization is depicted in Figure 13. Probe-set intensities from six arrays (three replicates each, from 2 experimental groups) are shown before (Figure 13A) and after (Figure 13B) normalization. After normalization, the variation between the arrays was minimized dramatically. S-plus's Array analyzer module was used to create and compare these plots directly from the Affymetrix .cel files obtained upon scanning.

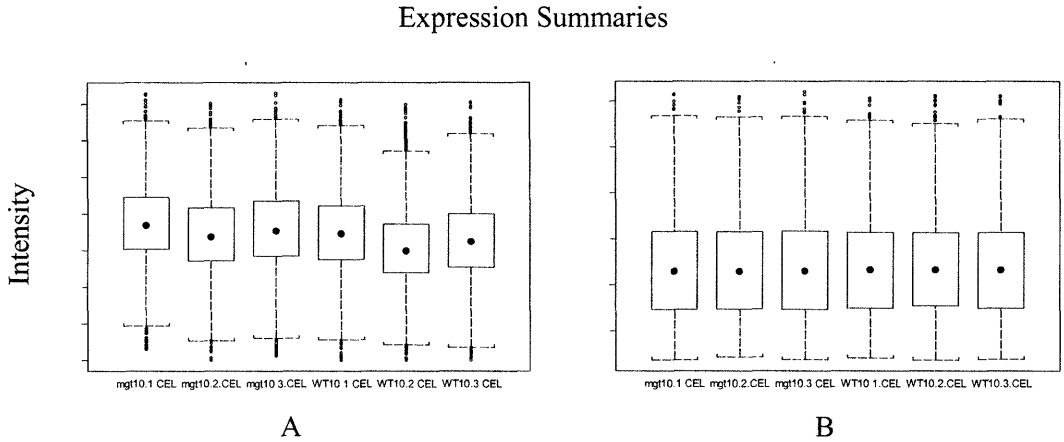


Figure 13: Box plots of intensities before (A) and after (B) RMA quantile normalization.

The intensities for six sample Affymetrix .cel files (3 WT and 3 *mgt*1) are plotted on $\log_2$ scale before (A) and after RMA quantile normalization. The Affymetrix .cel file intensities were imported to S-plus Arrayanalyzer module and RMA quantile normalization was performed. The normalization of the intensities reduces the variation between the samples and thus helps in making comparisons between disparate sets of oligonucleotide arrays.

Variability between the replicates

The variability between the replicates was assessed by computing the R^2 value between the $\log_2$ of RMA-normalized intensities (Table 3).

Table 3A

	WT1	WT2	WT3
WT1	1	0.94	0.96
WT2	0.94	1	0.95
WT3	0.96	0.95	1

Table 3B

	mgt1	*mgt2*	*mgt3*
mgt1	1	0.97	0.94
mgt2	0.97	1	0.93
mgt3	0.94	0.93	1

Table 3: R^2 values of WT (3A) and *mgt1* (3B) replicates

R^2 value for the intensity plots for comparing the replicate arrays from the wild-type (WT) **(3A)** and the methylguanine methyltransferase mutant (*mgt1*) **(3B)**. The samples used here included triplicate untreated WT and untreated *mgt1* arrays.

Computation of $\log_2$ Expression Ratio

Mean gene expression profiles from all experimental groups were compared to mean expression from untreated WT. This uniform denominator allows comparisons to be made between the experimental groups. The numerator was either mean expression from triplicate arrays at a given time point or mean expression across all the arrays in WT or *mgt1* (18 each, representing 3 for each of the 6 time-points). To delineate genes of importance, the $\log_2$ expression ratio ($\log_2$ER) was used to classify genes. A $\log_2$ ER > 1 indicates average fold change induction factor of 2 for that particular gene. Analogously, a $\log_2$ ER < -1 indicates an average fold change repression factor of 2. If $\log_2$ ER's were between 1 and -1, they were classified as not significant (NS). The $\log_2$ ER's across all treatment time-points for both WT and *mgt1* were exported and visualized in Spotfire's functional genomics module. The expression ratios were the basis of making comparisons between the experimental groups in this study.

Comparison with the phenotypic database

We also studied the expression profile in the context of the phenotypic database (http://genomicphenotyping.mit.edu) which includes information on sensitivity of 4800 yeast gene deletion strains to MMS. These deletions strains are of those genes that are nonessential. It was shown earlier that several of these genes are important for cellular recovery after mutagen exposure. In addition, it was observed that transcriptional responsiveness to these mutagens was not predictive of contribution of a gene to the recovery from the damage.

Basal gene expression profile in methylguanine methyltransferase deficient mutant (*mgt1*).

The basal gene expression profile of a gene in the O^6MeG methylguanine methyltransferase deficient mutant (*mgt1*) was assessed by computing a ratio of mean expression in *mgt1* (for that gene

from triplicate arrays) to mean expression in WT for the same gene (from triplicate arrays). If the expression ratio (ER) for any gene/ORF was > 2, then that gene was classified as being up-regulated and if the ratio was < 0.5, then it was classified as down-regulated. A plot of the gene expression ratio's for *mgt1* versus WT for the entire gene population is shown in Fig 14. In the examination of *mgt1* expression profile, 148/9275 (1.6%) genes were found to be up-regulated (maximum fold change was 14.8x) and 92 genes (<1%) were found to be down-regulated (maximum fold change was 95x).

Genes up-regulated in basal *mgt1*

A subset of these genes that were up-regulated in basal *mgt1* expression, listed by function, is shown in Table 4A (Appendix of Tables). Genes that were up-regulated but can otherwise be a part of the environmental stress response are indicated in table 4B. Notable among the genes up-regulated in the basal *mgt1* are those involved in detoxification and drug transport (*YER185W, YOR378W, YHL047C, YEL065W*), amino-acid biosynthesis (*BAT1, LEU1*) and transport (*BAP3, ALP1*). Other important genes included metabolism (*CHA1*) genes, genes involved in cell cycle control (*BAT1, BUB1, CKA1*), and transcription (*CKA1, TFG1, GAL80, GCN5, CBP2, RNA15*). Cell cytoskeleton and mitochondrial biogenesis (*SMP2, DCG1, GIC1, Q0183*) and *REV7*, a subunit of DNA polymerase-zeta (Pol-zeta) were also induced.

Genes down-regulated in basal *mgt1*

A subset of these genes that were down-regulated in basal *mgt1* has been ordered by function and is shown in Table 5A. Sixty-five of them had no known function and have not been shown. Genes that were down-regulated but is otherwise a part of the environmental stress response are indicated in table 5B. Among the genes that are repressed were those involved in amino acid

(*MET14, CYS3*, and *BAS1*) and carbohydrate metabolism (*SUC2, MIG2, HXT1, HXT3 and HXT4*) and transcription (*NFS1, CPR6 and MIG2*).

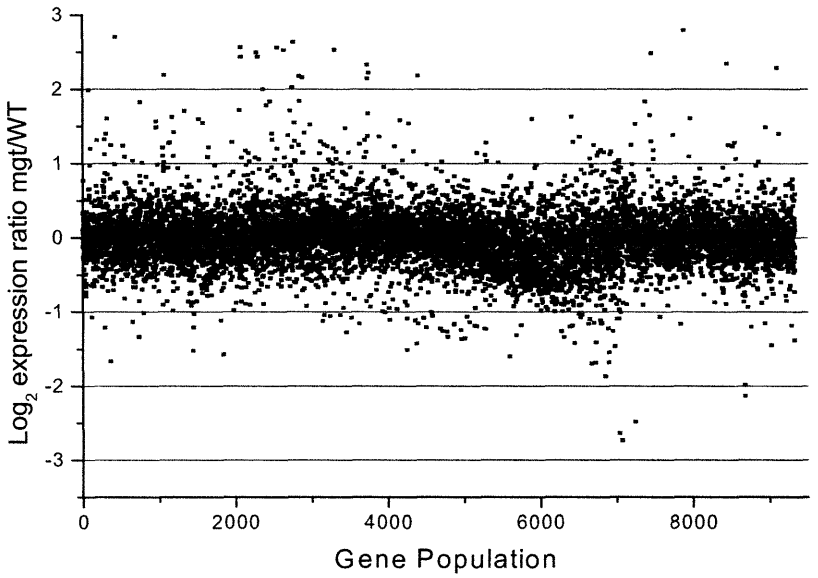


Figure 14: Expression ratio plot for yeast methylguanine methyltransferase mutant (*mgt1*).

Expression values (triplicate) of *mgt1* were compared to wild-type (WT) yeast expression and $\log_2$ transformed. Points above the y-axis grid line 1 indicate genes that are up-regulated in *mgt1*. Points below the y-axis grid line of -1 indicate genes that are down-regulated in *mgt1*.

Principal Component Analysis of experimental groups

The data from different experimental groups were also analyzed using Principal component analysis (PCA) in Matlab v6.0. Three untreated WT arrays 3 untreated *mgt1* datasets were compared with 6 datasets each from WT and *mgt1* treated with MNNG. Each WT and *mgt1* dataset included the mean expression from triplicate arrays. The PCA plot (Figure 15) shows 4 distinct clusters of data representing the untreated WT, untreated *mgt1*, treated WT and treated *mgt1*.

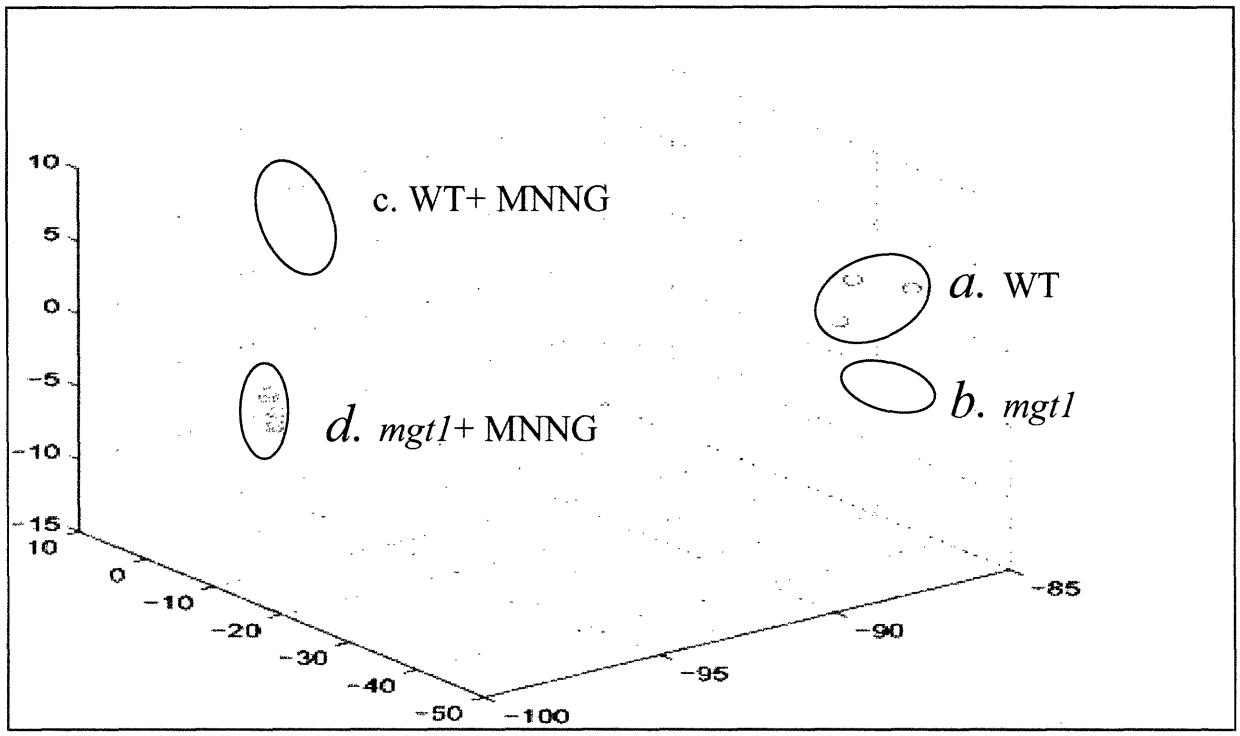


Figure 15: Principal component analysis of the experimental groups.

Three wild-type (WT) arrays (**a**) and 3 methylguanine methyltransferase mutants (*mgt1*) (**b**) were compared with 6 datasets (each representing the mean from triplicate arrays) from WT treated with alkylating agent, MNNG (**c**) and 6 datasets (each representing the mean from triplicate arrays) from *mgt1* treated with alkylating agent, MNNG.

Effect of MNNG on transcriptional profile - Cluster Analysis

The log_2 expression ratio's (ER's) were calculated for different experimental groups and exported to Spotfire. Hierarchical clustering (using Wards Method) of log_2ER's was used to generate a qualitative picture of the effect of MNNG on yeast. This heat map was generated using the data from 39 arrays (3 arrays were used as baseline for comparison).

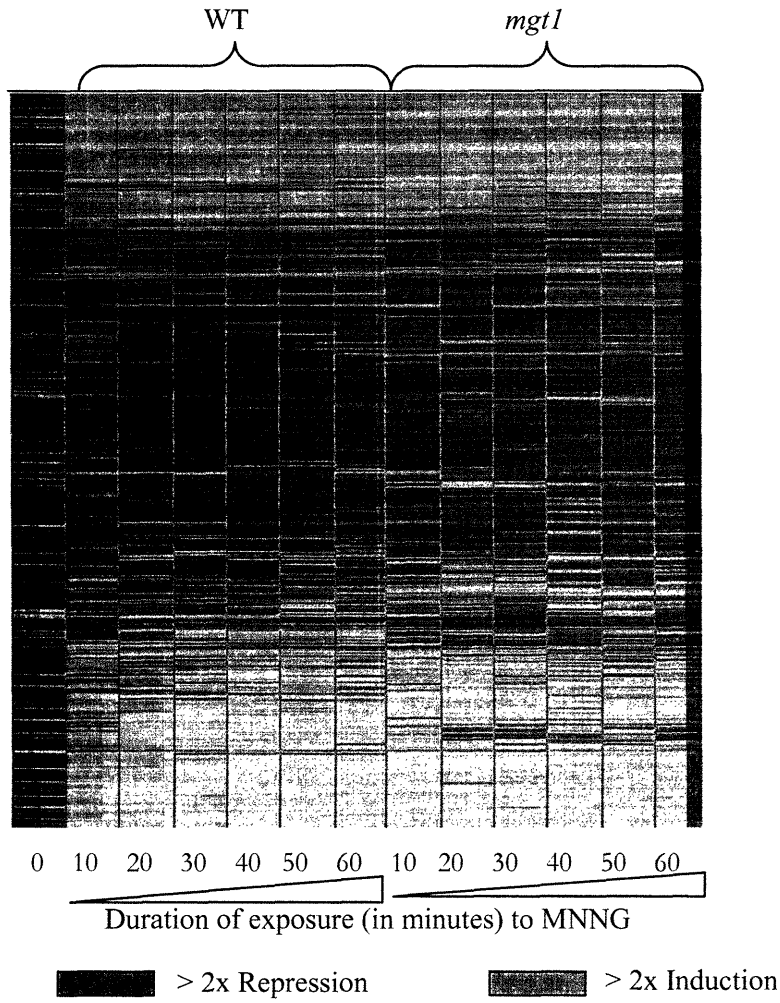


Figure 16: Heat map of the expression ratio from wild-type (WT) and Mtase mutant (*mgt1*). Log_2 expression ratio (log_2ER) was calculated for 9335 probe-sets by dividing the mean expression (from triplicates) for each probe-set by the mean expression for the same probe-set in the untreated WT (WT0). The first column of data represents the basal expression in *mgt1*. The other columns of data represent the mean expression value of triplicate arrays where yeast strains, either WT or *mgt1*, that was exposed to MNNG for variable length of time (10-60 minutes). This indicates that the genome is very responsive to the treatment with MNNG.

Temporal effects of gene categories

The temporal effects of MNNG on the yeast strains on a genome-wide scale were examined using the average expression profile for the treated WT (Figure 17A) and *mgt1* (Figure 17B). About 1200-1400 genes are induced or repressed upon treatment with MNNG in both WT and *mgt1*.

Figure 17A

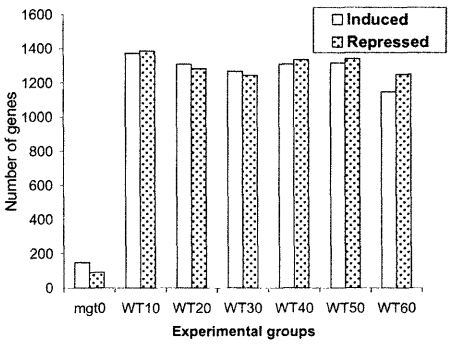


Figure 17B

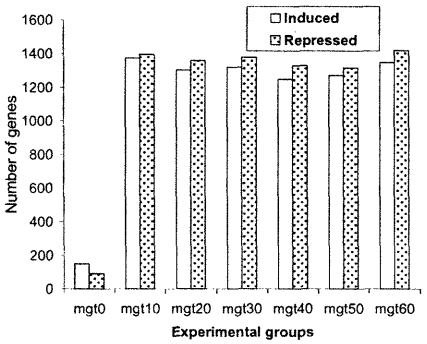


Figure 17A: Genes responsive to treatment with MNNG in the wild-type (WT) yeast.

The number of genes that are either induced or repressed in the WT yeast upon exposure to MNNG for varying lengths of time (10-60 minutes, WT10 through WT60) is shown. The untreated *mgt1* (*mgt*0) serves as a comparison.

Figure 17B: Genes responsive to treatment with MNNG in the methylguanine methyltransferase (*mgt1*) yeast.

The number of genes that are either induced or repressed in the methylguanine methyltransferase mutant (*mgt1*) yeast, upon exposure to MNNG for varying lengths of time (10-60 minutes, mgt10 through mgt60) is shown. The untreated *mgt1* (*mgt*0) category serves as a comparison.

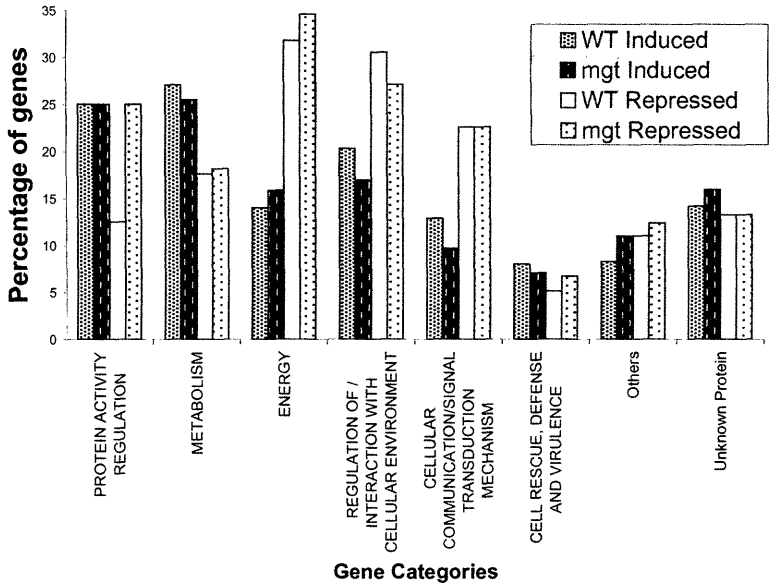


Figure 18: Gene expression responsiveness for some functional categories.

Average gene expression ratios for each gene within a particular functional category were compared across the experimental groups. The percentages of genes within a particular category, that are induced or repressed upon treatment with MNNG are indicated.

The expression profiles from entire categories of genes were examined to study the effect of MNNG on them and in particular if the gene expression was different between WT and *mgt1*. Mean fold induction and fold repression for each ORF/gene, across all the WT and *mgt1* arrays was calculated. In each category, the percentage of genes that were responsive upon MNNG treatment is shown in Figure 18. To calculate this, data from 18 WT and 18 *mgt1* arrays was used.

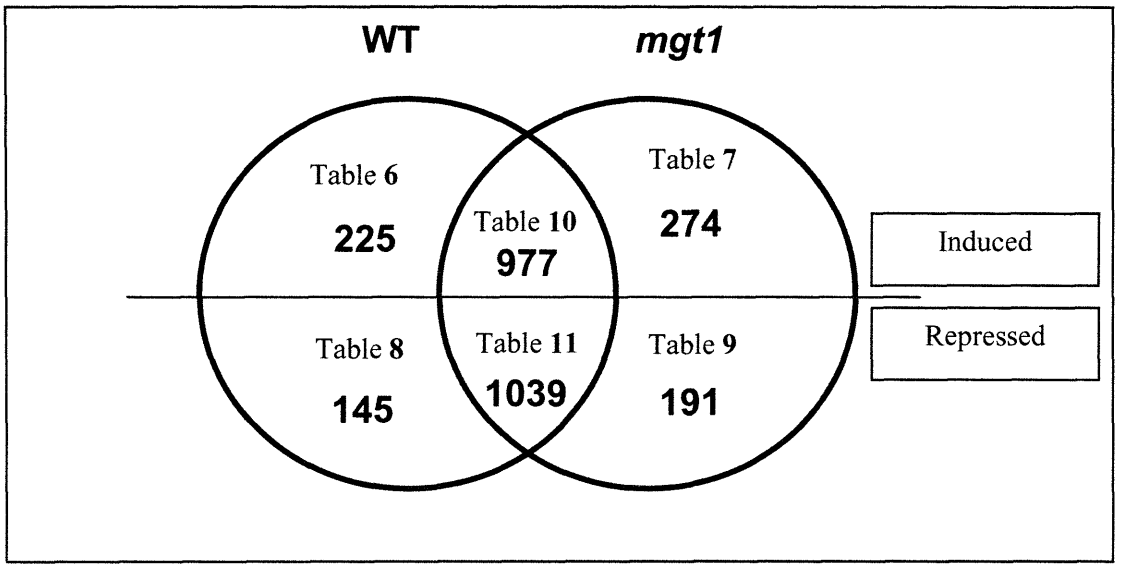


Figure 19: Venn-diagram of gene expression responsiveness in wild-type (WT) and methylguanine methyltransferase deficient yeast (*mgt1*) upon treatment with MNNG.

Genes that had a fold change (FC) > 2 that were either induced or repressed in WT and/or *mgt1* are represented using the Venn-diagram. This representation allows examination of effects that are unique to WT or *mgt1* or common both, upon exposure to MNNG. Gene expression response, induction or repression, that is specific to WT and *mgt1* can therefore be distinguished from a response that is found in both WT and *mgt1*. The upper-middle panel in the Venn diagram represents genes that are induced in both WT and *mgt1* (977 genes) as a common response to MNNG exposure. Alternatively, genes that are induced only in MNNG treated WT (225), or only in MNNG treated *mgt1* (274) are represented by the non-overlapping segments of the 2 circles. The lower-middle panel in the Venn diagram represents genes that are repressed in both WT and *mgt1* (1039 genes) as a common response to MNNG exposure. Alternatively, genes that are repressed only in MNNG treated WT (145), or only in MNNG treated *mgt1* (191) are represented by the non-overlapping segments of the 2 circles in the lower panel.

Genes induced specifically in WT upon treatment with MNNG

The upper-left panel in the Venn diagram (Figure 19) illustrates the response that can be attributed to WT strain upon exposure to MNNG. Upon MNNG-treatment, a total of 225 ORF's were specifically induced only in the WT. Since the only difference between WT and *mgt1* is the lack of MTase because of the deletion, it could be postulated that these genes are induced in the WT because of MNNG's effect in the presence of MTase. A total of 127 genes had a known function (listed in Tables 6A and 6B). Table 6B includes genes that are a part of the ESR. Table 6A includes

17 (17%) genes that are essential (highlighted in red). Interestingly 12 (12%) of the induced genes were involved in protein biosynthesis. Three DNA repair genes *UNG1*, *SIR2* and *RAD52* were also induced. Two mitochondrial genes, *RIM1* and *ERV1*, were found to be induced. Table 7B includes 30 genes that are a part of the ESR. This included 12 genes (40%) that are otherwise essential in yeast (highlighted in red).

Genes that are induced specifically in *mgt1* upon treatment with MNNG

There were 274 genes in this category. 103 of them had a known function and are listed in Table 7A and 7B. Table 7A includes 87 known genes that are induced in the *mgt1* upon MNNG treatment. Of them, 20 (22%) were essential genes. Notable among them were DNA repair genes *PRI2* and *CCE1* and several mitochondrial associated genes (*MRS11 TIM8, CCE1, COQ3, IDH2* and *DIC1*). In contrast to WT, only 3 protein synthesis genes were found to be specifically induced in *mgt1*. Interestingly, *SWI6*, a substrate of Rad53 in the G(1)/S DNA damage checkpoint was activated. The homothallic switching (HO) endonuclease, which creates a site-specific double-strand break (DSB) in the genome at the mating-type (MAT) locus, was also induced. Table 7B includes 16 genes with known function that are induced in *mgt1* and are a part of the ESR. Nine (56%) were found to be essential.

Genes repressed in WT upon MNNG treatment

A total of 145 genes were repressed exclusively in WT. Table 8A lists 70 genes with known function that are repressed exclusively in WT upon MNNG treatment. This included 7 essential genes (10%). Six genes (*SUM1, DOT6, ESC1, ISW2, NGG1 and SET3*) involved in chromatin silencing and histone modification were repressed. Two DNA repair genes (RAD16 and RAD28)

were repressed. Table 8B lists 10 ESR genes that are repressed in WT. Only 1 gene was found to be essential.

Genes repressed in *mgt1* upon MNNG treatment

A total of 191 genes were repressed exclusively in *mgt1*. Table 9A lists 70 genes with known function that are repressed exclusively in *mgt1* upon MNNG treatment. This includes 14 essential genes (20%). Three genes (*SNF11, SPT10, TBF1*) involved in chromatin remodeling were repressed. *CKS1*, a cyclin-dependent kinase regulatory subunit, was also repressed. Table 9B lists 3 ESR genes that are repressed in *mgt1*.

Genes induced upon treatment with MNNG in both WT and mgt1

In contrast to a damage induced response exclusive to WT or *mgt1*, there were several genes that were induced upon treatment with MNNG in both WT and *mgt1*. This overlapping response indicated in the upper-middle panel of Figure 19 included 977 genes that were induced in both WT and *mgt1*. Of them, 127 genes were included as ESR genes. The function of 282 genes was not known. The remaining 568 genes, whose function was known and those that were not a part of the ESR are listed by function in Table 10A. 121 (21%) of these are essential genes. Briefly, the genes that were induced upon treatment with MNNG, and were not a part of ESR, included those involved in maintaining cellular structure and function. Primarily, these included genes involved in cell wall organization, ergosterol biosynthesis, amino acid metabolism, mitochondrial organization and biogenesis. A few other genes of interest included chromatin silencing genes (*APC5, ISW1, ORC3, MRC1, ORC5, NNT1, SWD1, SWD3*) genes involved in DNA damage response (*HUG1, DUN1, PCL2*), DNA recombination (CDC9), DNA repair (*HAM1, MSH1, RAD18, RHC18, POL1*), DNA replication (*POL5, RNR3, RNR4, RNR1*), DNA topological change (TOF1) and

DNA unwinding (*CDC46, HFM1, MCM2*). Specific DNA repair genes included those involved in nucleotide excision repair (*RFA 1, RFA2, RFA3, CDC2, POL30* and *DPB2*). Interestingly, 68 genes (11%) of the genes induced in both WT and *mgt1* were involved in protein synthesis. Table 10B lists 127 ESR genes that are induced upon treatment with MNNG in both WT and *mgt1*. Interestingly, 57 (44%) of these are essential genes. Eleven genes are involved in ubiquitin-dependent protein catabolism.

Genes repressed upon treatment with MNNG in both WT and *mgt1*

A total of 1039 genes were repressed in both WT and *mgt1*. Of them, 545 had a known function and are shown in Tables 11A and 11B. Table 11A includes genes that were repressed in both WT and *mgt1* and are not a part of the ESR. These included 75 genes (13%) that were essential. Notable among them were genes involved in cell wall organization, fatty acid metabolism, G1/S cell cycle transition genes, mRNA splicing, methionine biosynthesis and mitochondrial organization. The largest category of genes that were affected was involved in transcription and its regulation. About 43 genes (7%) of the genes belonged to this category. Interestingly several DNA repair genes were repressed. These included genes involved in DNA recombination and repair (*HEX3, SLX8, IXR31, NSE)* DNA replication (*RRM3, TAH11,RIM4)* DSB repair *(YKU80, SIR4, LRP1, FYV6)* nucleotide excision repair *(TFB3, DPB11, RAD4).* Table 11B includes 60 ESR genes with known function that are repressed in both WT and *mgt1* upon treatment with MNNG.

DNA damage response and repair genes

The DNA damage response and repair genes were of additional interest and were therefore examined as a separate class. The mean expression profile of the DNA damage response and repair genes is indicative of DNA repair pathways are likely to be activated in WT and *mgt1* in response to

MNNG. A total of 133 genes were pooled into this category based on Affymetrix annotations that are derived from the SGD annotations. Twenty five genes were induced (Table 12A) and 20 genes were repressed (Table 12B). More than half the induced genes are essential or are sensitive upon deletion (Table 12A). 80 genes did not have an appreciable fold change to be classified as induction or repression. A total of 8 of the DNA repair and replication genes were classified as ESR genes (Table 12C).

Temporal effects of individual genes

To further elucidate the differences between WT and *mgt,1* the temporal profiles of individual genes were examined. Mean expression profiles at 6 time points for WT (3 arrays per time point) and 6 time points for *mgt1* (3 arrays per time point) were used. The temporal profile of a gene indicates a change in its mean expression upon increasing length of exposure to MNNG. The change was judged by 2 methods; a) the slope of the expression and b) the net fold change. The net fold-change can be defined as the ratio of expression at the 60^{th} minute (Exp_{60}) to expression at the 10^{th} minute (Exp_{10}) after exposure to MNNG. All genes that had an Exp_{60}/Exp_{10} ratio >2 were classified as induced and Exp_{60}/Exp_{10} ratio < 0.5 were classified as repressed. The Venn diagram in Figure 20 summarizes the differences and similarities in the responses between WT and *mgt1*. The upper panel represents genes that are incrementally induced starting at the first time of exposure (10^{th} minute). The lower panel represents genes that are repressed over time, starting at the first time point. There are unique and shared responses by the WT and *mgt1* to MNNG.

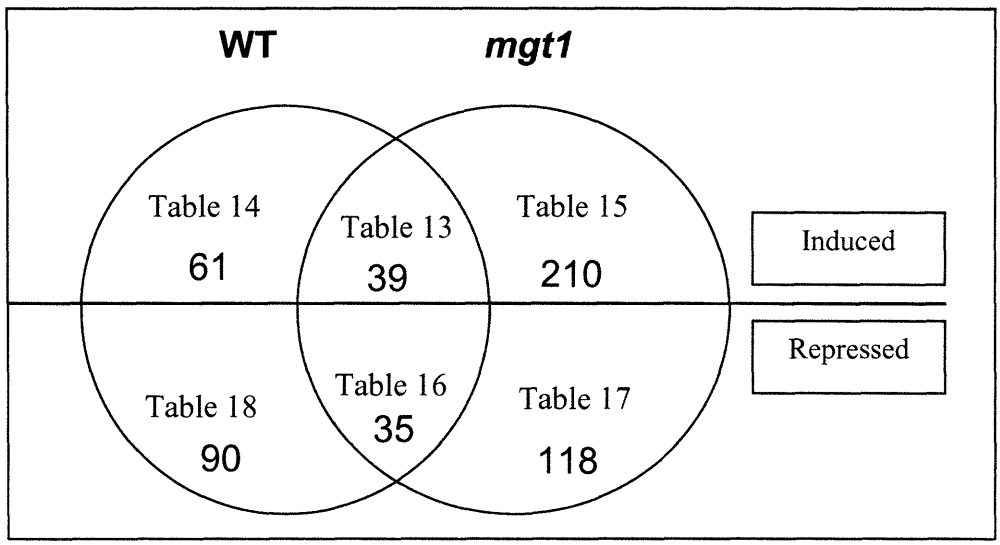


Figure 20: Venn-diagram of genes that are incrementally induced or repressed upon increasing length of exposure to MNNG.

The net fold-change (FC) over time, was calculated from ratio of mean expression at the 60th minute to mean expression at the 10th minute. A cut-off of FC>2 (for induction) and FC<0.5 (for repression) was used to select genes that have been included in this representation.

Genes induced over time

When all such profiles were examined, the expression of 60 genes was found to have an incremental induction over time in the WT. Similarly, 210 genes in *mgt1* were found to increase upon increasing the length of exposure to MNNG. The incremental response of 39 genes was common to WT and *mgt1*. Induced genes that were known to have a function are represented in Table 13. In the WT, only 2 (out of 24 genes with known function, 8%) were essential. In contrast, 34 (out of 79 genes with known function, 43%) were found to be essential genes.In the WT, DNA damage effectors *HUG1* and *RNR3* were incrementally induced with increasing length of exposure to MNNG. In contrast to WT (61), there were more genes in *mgt1* (210) that were responsive. These

genes are involved in ribosomal RNA processing, mRNA processing and transcription were found to be incrementally induced. Several other ESR genes were also found to be a part of pre-rRNA processing and ribosomal protein synthesis. Overall, there were about 28 genes (35%) involved in mRNA, rRNA, tRNA and ribosomal function, that were incrementally induced in *mgt1* upon exposure to MNNG over time. 20 (out of 52 genes) fitted a profile of a dramatic initial repression (after 10 minutes of exposure to MNNG) followed by a steady increment towards the basal levels. In contrast, 7 genes were consistently induced. The reflex response by 20 genes is likely to represent a response that follows the perturbation. The 7 genes that are incrementally induced over and above basal levels are likely to represent processes that are integrated with damage response and recovery.

Genes repressed over time

Ninety genes were repressed exclusively in WT. In contrast 118 genes were repressed in *mgt1*. Only 35 genes were seemed to be repressed in both WT and *mgt1*. Repressed genes that were known to have a function are represented in Table 14. The corresponding sets of genes that belong to ESR are also listed.

In the WT, only 1 gene (out of 18) was found to be essential. Notable among them was the homothallic switching (HO) endonuclease, which creates a site-specific double-strand break (DSB) in the genome at the mating-type (MAT) locus. This gene was listed earlier as a gene that was induced in *mgt1* but not in WT. Closer examination of the profile revealed that there is an instantaneous induction of HO after the first exposure to MNNG in both WT and *mgt1*. While this induction is sustained in the *mgt1* (hence the mean $\log_2$ ER>2), there is a decline in the induction over time in the WT (therefore, explaining its classification as "repression").

Genes that were repressed in *mgt1* includes 6 genes (out of 47) that are essential. Notable among them are genes involved in protein folding, methionine metabolism and translation. 18 (out of 47

genes) fitted a profile of a dramatic initial induction (after 10 minutes of exposure to MNNG) followed by a decline. In contrast, 13 genes were consistently repressed beyond the basal levels. The reflex response by 18 genes is likely to represent a response that follows the perturbation. The 13 genes that are incrementally repressed below basal levels are likely to represent processes that are integrated with damage response and recovery. The differential response between WT and *mgt1* can provide valuable insight into the differences in the mechanism of response to MNNG in the presence and absence of MTase.

DISCUSSION

Several authors have studied the genome-wide transcriptional effects of chemical and physical agents on yeast using microarrays. The yeast transcriptome response to MMS (Jelinsky and Samson 1999) and MNNG (Jelinsky et al 2000) have yielded valuable data on how the yeast adapts to these alkylating agents. Specifically, these studies explored the transcriptional response of *S. cerevisiae* to a wide range of chemical and physical damaging agents in an attempt to delineate the response of each ORF to these agents. Agarwal et al 2003, studied the genome-wide effects of antifungal agents on yeast in an attempt to characterize their mechanism of action. Several other microarray-based studies have examined the effect of single gene deletions on yeast transcriptome. For example, Ohkuni et al 2003, studied the genome-wide expression in the Deltanap1 cells in order to study the transcriptional control of *NAP1*, a nucleosomal assembly protein. Fry et al 2003, studied the effect of *SGS1* deletion on transcriptional profile in yeast because of its homology with human genes involved in Werner and Blooms syndrome. Gasch et al 2001, studied basal expression profile in *MEC1, DUN1* and *CRT1* deficient yeast and their transcriptional changes in response to MMS. The study uncovered the role of *MEC1*, the human ATR homolog in yeast. It was also concluded that *MEC1* was an integral part of controlling the environmental stress response. A literature survey yielded no genome-wide expression profile studies where deletion strains of DNA repair genes were used. The current study is the first report of the transcriptional responsiveness where a known DNA repair deficient (O^6 methylguanine methyltransferase deficient strain, *mgt1*) has been studied. While undertaking this study, the goals were two-fold. The first was to study the effects of deletion of *mgt1* on the yeast transcriptome. The second was to study the effect of alkylation induced transcriptional changes over time in the WT and *mgt1* and to examine the differences between them.

MNNG specific response genes enriched by culling out genes involved in environmental stress response (ESR).

In response to environmental perturbations, *S. cerevisiae* cells elicit rapid transcriptional reprogramming involving both activation and repression of gene expression. Some of these transcriptional changes represent responses that are common to chemical and physical stresses. Removing these ESR genes from the observed response, will help to enrich the set of genes that are specific to MNNG treatment and/or presence or absence of MTase. This was achieved by comparing the expression profile in the current study with ESR dataset from Gasch et al 2000 to identify ESR genes that might confound interpretation of the data. In the second tier of comparison, phenotypic sensitivity information of yeast deletion strains from (http://genomicphenotyping.mit.edu) was incorporated along with expression.

Deletion of *MGT1* induces dramatic basal transcription changes, activates cell cycle checkpoints, transcription factors and a gene involved in spontaneous mutagenesis.

MGT1 is involved in direct repair of O^6 Methylguanine and O^4Methylthymine lesions. Genes that are induced upon deletion of *MGT1* are likely to be involved in a) a direct interaction with *MGT1* or b) a downstream effect of a lack of *MGT1*. A direct interaction can follow from the argument that *MGT1* normally, represses these genes and removing *MGT1* induces them. Alternatively, lack of *MGT1* can lead to increased spontaneous mutations and DNA damage and genes induced might be a part of the downstream cellular processes that are involved in handling this damage. It was interesting to note the up-regulation of 148 genes upon the deletion of one single gene (*MGT1*). The deletion appears to up-regulate a gene involved in spontaneous mutagenesis and several genes that are transcription factors and 2 others that are involved in cell cycle control. The 7-fold up-regulation of *REV7*, a subunit of DNA polymerase-zeta (Pol-zeta, Pol

ζ) is important in the context of spontaneous mutations. The other subunit of pol-zeta, is *REV3* (which was not up-regulated). *REV7* is the processivity factor for *REV3* and complex together, to get involved in translesion (TLS) synthesis, a mechanism that probably helps cells cope with DNA lesions that have escaped the efficient DNA repair systems. TLS is invoked when there is a replication blocking lesion that the normal polymerases are not able to copy past. O^6Methylguanine (O^6MeG) is a mutagenic lesion and is not considered as a replication blocking lesion. It is likely, therefore, that the endogenous lesion leading to the up-regulation of *REV7*, is probably not because of O^6 MeG. At the same time that TLS helps to copy past the lesion, it has potentially mutagenic consequences making it responsible for the majority of spontaneous mutations (Friedberg 1995). Increase in spontaneous mutations in *mgt1* has been observed earlier (Xiao and Samson 1992). The same study found that wild-type mutation rate was restored when the *mgt1* mutant was transformed with a functional *MGT1*. The seven-fold induction of *REV7* in this context may suggest a downstream effect of the deletion of *MGT1* rather than via O^6 MeG. Cell cycle control genes, *BUB1* and *CKA1* were up-regulated. *BUB1* is a protein kinase and serves as a mitotic spindle checkpoint. *CKA1* is the alpha unit of protein kinase CK2 and is known to be involved in DNA damage response and cell-cycle control. Among the transcription factors that were up-regulated *GCN5* has two tiers of significance. First, Gcn5p plays a role in controlling the expression of 5 % of the yeast genome (Holstege et al, 1998). Secondly, *GCN5*, a histone acetyl transferase allows efficient access of the repair machinery to chromosomal DNA damage either indirectly via influencing transcription or directly via modifying chromatin structure. Gcn5 functions before or during the DNA repair process. An earlier report suggested that Gcn5 is recruited upstream of the damaged area by a hitherto unknown DNA damage sensor (Teng et al, 2002). Overall, it appears as though the deletion of *MGT1* is leads to increased DNA damage good reason why cell cycle checkpoints are up-regulated. Simultaneous up-regulation of a key component of the translesion synthesis suggests the

role of error-prone damage tolerance mechanisms in response to possible replication blocking lesions. Up-regulation of *GCN5*, a gene that aids DNA repair and controls expression of 5% of the yeast genome is indeed remarkable.

Cellular response to MNNG in WT and *mgt1*

About 30% of the yeast genome (as represented on the Affymetrix GeneChip Array YGS98) was responsive to treatment with MNNG (Figure 16). As the heat map in Figure 16 indicates, response to most of the damage that was inflicted by MNNG was initiated in the first 10 minutes of exposure. Thereafter, the total number of responsive genes did not change dramatically. Evaluation of the genome-wide response across the length of time might lead us to miss changes in a subset of genes that might be instrumental in understanding the response to MNNG. Therefore, in order to dissect the transcriptional response further, genes belonging to several functional sub-categories were examined. This, however, did not yield any substantial change in the responsiveness in gene expression over time, to MNNG (Figure 17A and 17B).

MNNG induced damage activates cell-cycle checkpoint cascade, DNA damage signal amplifiers and downstream effectors

As the cells respond to an adverse condition such as exposure to MNNG, several cellular responses are mounted by WT and *mgt1*. The Venn diagram (Figure 19) indicates that a majority (977 genes, 66%) of this response was common to both WT and *mgt1*. The response that is shared by WT and the *mgt1* is indicative of cellular processes that are common to both strains in response to MNNG. Among the shared response are genes that serve as a part of S-phase checkpoint. The checkpoint regulatory mechanism has an important role in maintaining the integrity of the genome and results in a temporary cessation of DNA replication. Eukaryotic cells activate checkpoint

pathways that arrest cell cycle progression and induce the expression of genes that are required for DNA repair. This checkpoint machinery consists of proteins that recognize DNA damage and initiate the signaling response. The identification of the damage also needs to be amplified in order to recruit other mediators of DNA damage response. *MRC1* and *TOF1* are DNA damage signal amplifiers. *TOF1* and *MRC1* were induced in both WT and *mgt1*. Upon damage to DNA, *TOF1* gets activated and forms a part of a replication-pausing complex. *TOF1*, located at the arrested forks activates checkpoint cascades, leading to repair of the damaged DNA. Recently, it was demonstrated that Tof1 and Mrc1 interact directly with the damaged DNA (Katou et al 2003). It has also been postulated that Tof1p links Mec1p with Rad53p (Foss, 2001). This is an interesting finding in the context that *MEC1* and *RAD53* is an indispensable component of DNA damage response. Rad53 and Mec1 are protein kinases required for DNA replication and recovery from DNA damage in *S. cerevisiae*. DNA damage during S phase slows down the rates of replication fork elongation (Tercero and Diffley, 2001) and triggers a Rad53/Mec1-dependent block. As a result, DNA damage leads to an abrupt decrease in DNA synthesis (Paulovich and Hartwell, 1995). In addition, Mec1 and Rad53 are required to prevent DNA damage-induced collapse of replication forks (Tercero and Diffley 2001), via their ability to phosphorylate replication and repair proteins at stalled replication forks. The essential function of Mec1 and Rad53 in *S. cerevisiae* is to promote deoxyribonucleotide triphosphate (dNTP) production during S phase to coincide with DNA replication. This is achieved via phosphorylation and subsequent degradation of Sml1 (Zhao et al., 2001), an inhibitor of ribonucleotide reductase (*RNR*). Ribonucleotide reductase (*RNR*) catalyzes the rate limiting step in the production of deoxyribonucleotides needed for DNA synthesis. Its synthesis is tightly regulated at the level of transcription. It is cell-cycle regulated and provides a metabolic state that facilitates DNA replicational repair processes. Dun1, a protein kinase, controls inducibility of *RNR1*, 2 and 3

in response to DNA damage and replication blocks. RNR genes in yeast form a regulon that is coordinately regulated by protein phosphorylation in response to DNA damage.

In our dataset, the $\log_2$ER for *MEC1* and *RAD53* was not induced more than 2 fold. It is likely that this is because they are kinases and hence present transiently. *DUN1* and *HUG1* are DNA damage response genes down-stream of *MEC1* and *RAD53* and were induced. *HUG1* (**h**ydroxyurea and **U**V and **g**amma radiation induced) is a component of the *MEC1*-mediated checkpoint response to DNA damage and leads to replication arrest. The *HUG1* gene was identified as a component of the DNA-damage checkpoint response using deletion and overexpression mutants of *S. cerevisiae* (Kaplun 2000). DNA damage-specific induction of *HUG1* is independent of the cell cycle stage. *HUG1* induction also increased with increasing exposure to MNNG in both WT and *mgt1*. Its induction response to MNNG is therefore consistent with its role in DNA damage response.

MNNG induced damage activates chromatin silencing.

Mec1 is the central transducer of these stress-response signals (Zhou and Elledge 2000). Both Rad53 and Mec1 are key proteins involved in the response to replication blocks and they act together with a novel regulator of Rad53, Mrc1. The DNA damage response pathway has been linked to the control of chromatin organization. In response to DNA damage, certain proteins that are normally relocalize to silence telomeric chromatin (Martin et al. 1999, Mills et al. 1999). This relocation is dependent on Mec1 (Craven and Petes 2000). In the current data, 6 genes involved in chromatin silencing are induced and 3 of them are also known to be essential. This chromatin-mediated maintenance of transcriptional inactivation is in accordance with expectations.

MNNG induced damage activates genes involved in DNA replication and repair.

DNA repair mechanisms are by far the most important components of the cellular response that gets induced upon damage to DNA by MNNG. DNA polymerase alpha (*POL1)* is an essential gene required for initiation of replication and lagging-strand synthesis. It was also found to be a part of the ESR. The *MCM2 is* a part of the Mcm2-Mcm7 protein complex that forms a DNA helicase that unwinds the DNA ahead of the replication fork (Labib and Diffley, 2001). Additionally, all the essential subunits of replication protein A (*RPA*) were induced. *RPA* is a single-stranded DNA binding protein (SSB) involved in DNA replication, recombination and repair (Kim C et al 1992). It has been recently shown that RPA facilitates telomerase action (Schramke, 2004). Proliferating cell nuclear antigen (PCNA), encoded by the *POL30* gene, is essential for DNA replication (in association with *RFC*) and DNA repair. PCNA is a ring-shaped DNA polymerase accessory protein that can encircle duplex DNA. PCNA interacts with Pol eta to permit efficient lesion bypass. Another notable gene that was induced in response to MNNG was *HAM1*. It is known that overexpression of the yeast HAM1 gene prevents 6-N-hydroxylaminopurine mutagenesis in *E. coli* (Kozmin et al 1998) suggesting that it might play a protective role in MNNG induced damage. *HAM1* controls 6-N-hydroxylaminopurine (HAP) sensitivity and mutagenesis in *S. cerevisiae.* The *HAM1* protein protects the cell from HAP, either on the level of deoxynucleoside triphosphate or the DNA level by a yet unidentified set of reactions (Noskov, 1996). It was intriguing to note that *HAM1* deletion phenotype was not sensitive to MMS

Repair of MNNG induced mitochondrial DNA damage

CDC9 gene encodes a DNA ligase protein that is targeted to both the nucleus and the mitochondria and this yeast rely upon a single DNA ligase, Cdc9p, to carry out mitochondrial DNA replication and recovery from both spontaneous and induced mitochondrial DNA damage

(Donahue 2001). *MSH1* is a DNA-binding protein in yeast mitochondria that recognizes nucleotide mismatches in DNA and plays a role in mitochondrial mutation avoidance. *MSH1* protein is targeted to the mitochondria where its mitochondrial-targeting sequence is removed (Chi and Kolodner, 1994). Taken together, the induction of *CDC9* and MSH1 appears to be a part of a program to repair damage to mitochondrial DNA.

MNNG induced damage activates ubiquitin mediated protein catabolism

Twenty genes involved in protein ubiquitination and ubiquitin mediated protein catabolism were induced (*RPT1, DOA1, RPN2, RPN1, PRE9, SCL1, RPN9, UFD2, RPN7, RPT5, PRE8*). Among them, *DOA1* is thought to encode a regulatory component of the proteasome pathway, which involves ubiquitin (Ub)-dependent protein degradation (Ghislain, 1996).

MNNG induced damage activates protein synthesis genes

The yeast ribosomal proteins (RPs) of are encoded by more than 100 genes. These are among the most transcriptionally active genes in the yeast genome. It consumes a prodigious amount of the cell's resources and, consequently, is tightly regulated. Interestingly, 68 genes (11%) of the genes induced in both WT and *mgt1* were involved in ribosomal protein synthesis. This is in contrast to earlier observations where protein synthesis genes were found to be repressed upon exposure to MMS (Jelinsky et al 1999) and osmotic stress (Rep, 2000). Notable among these include *RAP1*, a multifunctional transcription factor that has a BRCT domain. The BRCT domain is found predominantly in proteins involved in cell cycle checkpoint functions responsive to DNA damage. *RAP1* is essential for cell viability and can function as either an activator or a repressor of transcription, depending upon the context of its binding site. *RAP1* was incidentally found to be

repressed and it is likely that repression of *RAP1* is responsible for induction of the 68 ribosomal protein synthesis genes.

In an earlier study *MAG1*, 3-methyladenine DNA glycosylase, an integral component of the base excision repair pathway, was shown to be induced upon damage to DNA by MMS. It was also shown that *MAG1* and *MGT1* have a common upstream regulatory sequence. In this dataset, *MAG1* was not found to be induced more than 2-fold.

Ubiquitin-proteasome regulator, *RPN4*, is repressed upon MNNG treatment.

Intracellular proteolysis in yeast occurs mainly via the ubiquitin-proteasome system. Expression of this system is under the control of the transcription factor, Rpn4p (Mannhaupt G et al, 1999). It has been shown earlier that alkylating agent MMS resulted in activation of genes that are involved in ubiquitin and 26S proteasome-dependent protein degradation. Rpn4p is a major transcription regulator that acts by binding to proteasome-associated control element (PACE) with a unique upstream activating sequence (5'-GGTGGCAAA-3'). This binding either stimulated or inhibited transcription. In this dataset, however, *RPN4* was found to be repressed 4.5 times.

Genes involved in non-homologous end joining (NHEJ), recombination and some elements of nucleotide excision repair are repressed in MNNG exposed yeast cells.

Among the genes repressed in both WT and *mgt1* are those involved in DSB repair via NHEJ. (*YKU80, SIR4, LRP1, FYV6*). The components of the non-homologous end joining (NHEJ) repair pathway were repressed 2-3.5 fold. Additionally, few other genes involved in recombination and replication were also repressed. *HEX3* and *SLX8* have DNA binding activity and are implicated in recombination repair. *SLX8* is required to resolve recombination intermediates that arise in response to DNA damage. The *RAD4* gene of yeast is required DNA binding and for

the incision of damaged DNA during nucleotide excision repair (NER) (Owsianik 2002). *TFB2* is a transcription/repair factor (TFIIH) subunit that is a transcription initiation factor required for NER.

Finally, two discrete set of genes, the "WT-specific MNNG-damage signature" and "*mgt1*-specific MNNG-damage signature" was created. These include uniquely up-regulated in the WT or *mgt1* upon exposure to MNNG. This signature may help define new candidates for involvement in cellular responses to MNNG in a wild-type and upon O^6 methylguanine methyltransferase deletion.

MNNG induces genes involved in mRNA turnover and protein synthesis in WT

Since the only difference between WT and *mgt1* is the lack of MTase, it could be postulated that the set of transcripts are specifically induced in WT are because of MNNG's effect in the WT MTase background. Expression of 7 genes involved in mRNA splicing (MSL1), cleavage (*PTI1*) and catabolism (*NMD4, PUB1*), specifically in the WT is indicative of more mRNA turnover. There were 3 others that were classified as a part of ESR. Accordingly, there were 18 protein synthesis genes (6 were ESR genes) that were induced. It is likely that the overwhelming induction of protein synthesis genes is representative of a recovery response after the initial insult. More protein synthesis genes are induced in the WT as opposed to *mgt*, probably because it is able to recover faster (than the *mgt1*). Mitochondrial DNA damage response was exemplified by induction of *RIM1*. This has single stranded binding (SSB) activity and is involved in mitochondrial genome maintenance. *RIM1* forms an essential component of the yeast mtDNA replication apparatus (Van Dyck, 1992). *ERV1* gene is essential for cell viability and for the biogenesis of functional mitochondria.

Repression of chromatin remodeling genes following MNNG induced damage

While the genes that are specifically induced in the WT upon MNNG damage are involved in fundamental metabolic processes, the genes that are repressed include 5 chromatin remodeling

and histone modification genes (of 18). In addition, *RPI1*, a repressor of the ras-cAMP pathway and *UBP10,* a deubiquitinating enzyme are repressed. Loss of *UBP10* function is known to lead to partial impairment of silencing at telomeres. A study of ubp10 deletion revealed that it mimicked oxidative damage by intracellular accumulation of reactive oxygen species and eventually leading to DNA fragmentation and phosphatidylserine externalization, which happen to be the 2 markers of apoptosis (Orlandi, 2003).

MNNG induces mitochondrial damage in *mgt1*

It appears as though the lack of MTase in *mgt1* results in increased damage to mitochondria. This was reflected in the responsiveness of several mitochondrial proteins that were induced specifically in *mgt1*. These genes are involved in fundamental biochemical processes in the mitochondria. *MRS11* and *TIM8* are protein transporters in the mitochondria. *CCE1* is involved in DNA recombination and is also present in the inner-mitochondrial membrane. *COQ3* is involved in ubiquinone biosynthesis, and is a component of the inner mitochondrial membrane. *IDH2* is involved in the TCA cycle and also localizes to the inner mitochondrial membrane. *DIC1* is involved in dicarboxylic transport across the mitochondrial membrane. There were only 3 protein synthesis genes

MNNG induced damage activates multifunctional transcription factor *SWI6*, in *mgt1*

SWI6 is a transcription factor involved in controlling genes involved in cell wall biogenesis and architecture. It is also a key component of G1/S checkpoint. When a cell detects damaged DNA, Rad53 checkpoint kinase activity is dramatically increased, which ultimately leads to changes in DNA replication, repair, and cell division. *SWI6*, a substrate of Rad53 in the G(1)/S DNA damage checkpoint was activated in *mgt1* indicating that there is more DNA damage in *mgt1*. *SWI6*

enhances the expression level of the recombination genes in meiosis in a dosage-dependent manner, which results in an effect on the frequency of meiotic recombination (Leem 1998). Another gene involved in recombination is *CCE1*.

Ho endonuclease introduces a site-specific double strand break (DSB) in the mating type (*MAT*) gene of yeast and its expression is tightly regulated. This endonuclease is known to be induced for a short duration and quickly degraded via the ubiquitin-26 S proteasome system (Kaplun, L et al 2000). Taken together, it appears as though *mgt1* has a propensity to undergo more mitochondrial damage and DNA recombination repair.

An exaggerated response of *mgt1*-specific MNNG-damage was limited to genes that seem to be involved in remodeling of the cell cytoskeleton, translation and signal transduction activity.

Role of environmental stress response genes

The environmental stress response, ESR is a stereotypical pattern of changes in the expression of approximately 900 genes evoked by a large variety of environmental stresses, including heat shock, osmotic shock, DTT, nitrogen starvation, and peroxide. Many of the genes in this program are induced in response to stressful environments and therefore may play a critical role of maintaining internal homeostasis. As expected, the ESR was rapidly initiated in wild-type and *mgt1* cells responding to MNNG, and it was sustained through the entire course of the experiment. From Gasch et al 2000, a total of 95 microarray hybridization experiments were used to deduce the environmental stress response genes that were found to be responsive in this study. Approximately 48% of the genes found in the ESR were also induced/repressed upon treatment with MNNG. Approximately 78% of these ESR genes were common to the response by WT and *mgt1*. This overlap is very suggestive of cellular responses that are common to processes that help the cell survive and achieve internal homeostasis.

In an earlier study Gasch et al 2000 reported the presence of a DNA damage signature cluster comprising nine genes including the ribonucleotide reductase subunits *RNR2* and *RNR4*, the DNA-damage repair genes *RAD51* and *RAD54*, the DNA-damage activated kinase *DUN1*, the DNA-damage-inducible mitochondrial nuclease *DIN7*, *PLM2*, which has homology to the fork-head associated-domain found in several transcription factors and kinases, and two uncharacterized ORFs (*YER004W* and *YBR070C*). A total of 4 genes from the 9 from their cluster (*DUN1, PLM2 RNR4*, and the ORF *YBR070C*) were found to be induced in the current study.

Temporal response to MNNG.

A comparison of temporal expression profiles of genes incrementally induced or repressed in WT and *mgt1* revealed interesting trends. Overall, there were more genes that showed induction or repression in the *mgt1* (210 induced, 118 repressed) than WT (61 induced, 90 repressed). There were a few genes that showed trends (39 induced, 35 repressed) that were shared between WT and *mgt1*. This result indicates that the perturbation in the *mgt1* is more profound than in the WT.

In the WT there is a reflex repression of several fundamental biochemical processes when the cells are first exposed to MNNG. These processes include glucose metabolism, lipid signaling pathways, fatty acid metabolism, electron transport system and the glyoxylate cycle. The repression is transient and is eased as the cell tries to recover from the perturbation. Almost simultaneously, there is a reflex induction of processes involved in maintaining the cell wall structure and function. Their induction wanes over time. Other genes that follow this pattern are involved in phosphatidylethanolamine and serine biosynthesis and threonine catabolism. Other interesting genes in this category included, *TLC1* which encodes the RNA subunit of telomerase (Singer, 1994) and HO endonuclease. The components of the DNA damage response pathway are known to degrade HO endonuclease via the ubiquitin 26s proteasome system (Kaplun L). This could explain the

waning of "induction" over time. These responses are likely to be related to processes that are involved in damage recovery. A few genes were consistently induced after the initial exposure to MNNG. These responses need to be distinguished from the ones that are observed above. These responses are sustained as long as the cells are exposed to MNNG. Therefore, these are likely to be critical and related to processes that are directly related to the damage caused by MNNG or its downstream processes. To exemplify, *GTT2* codes for a glutathione transferase and its deletion strain is also found to be very sensitive to MMS. In addition to its role as an antioxidant, glutathione has several physiological functions, such as detoxification of various cytotoxic compounds, acting as a co-factor for enzymes, protection of proteins' SH groups. Upon invasion by xenobiotics, glutathione-S-conjugates are formed by glutathione S-transferase and the conjugates or their degraded compounds are exported from the cytoplasm by some transporters. In *S. cerevisiae*, two glutathione S-transferase genes (*GTT1* and *GTT2*) have been identified (Choi 1998). and glutathione-S-conjugates are transported into the vacuole by the *YCF1* gene product, which is an ATP-binding-cassette transporter on the vacuolar membrane (Li, 1996). Induction of GSH synthesis in yeast has been shown to protect the mitochondrial DNA (mtDNA) from oxidative damage (Kei-ichi, 2001).

The damage by MNNG is more profound in *mgt1* than WT. Unlike the WT, the initial exposure to MNNG represses genes that are more likely to be transcriptional factors. After the initial repression these genes were incrementally induced and tended to approach WT levels. By virtue of affecting transcription factors, their influence on the expression profile is more pronounced than what is apparent. Other genes in this category included ones involved in RNA processing and kinases involved in protein phosphorylation. It was very intriguing to find *RAD28* in this category of genes. It is a homolog of the Cockayne syndrome A (CSA) gene. CSA patients exhibit severe developmental and neurological abnormalities. In contrast genes that were induced as

a reflex response and waned over time included those involved in copper and lipid transport, glucan, sulfur and methionine metabolism. An interesting class of genes here were 5 genes involved in translation initiation, elongation and regulation. The temporal responsiveness in *mgt1* was striking on several other counts. About 35% of the induced genes were involved in ribosomal function and 43% of all the genes induced were found to be essential.

Overall, the propensity for damage to macromolecules and cellular processes is much more in the *mgt1* than the WT. Given that *mgt1* is not able to repair O^6Methylguanine and O^4Methylthymine, it can be postulated that the profound damage in *mgt1* could be because of these lesions itself or due to downstream processes. The repression of transcription factors followed by the induction of ribosomal and RNA components is an interesting finding that suggests that the recovery after damage is coordinated at the transcriptional level. This is unlike the WT that can repair the O^6MeG lesion to a greater extent than the *mgt1*. In the WT, the reprogramming involves a transient repression of genes restricted to fundamental metabolic processes indicating that the damage is limited compared to *mgt1*.

Summary

In conclusion, the transcriptional changes precipitated by deleting MTase in yeast are indicative of DNA damage induction, cell cycle checkpoint activation and eventually, damage tolerance via *REV7*. This finding of error-prone translesion bypass polymerase activity correlates well with an earlier result where increased spontaneous mutagenesis was demonstrated in MTase deficient yeast.

The effect of alkylating agent MNNG on yeast is dramatic and about 30% of the genome is instantly responsive. The initial insult with MNNG is rapidly followed by a repression of major metabolic processes but an induction of genes involved in maintaining cell wall structure and function. Other processes that try to maintain homeostasis after the initial insult with MNNG set in early and are sustained. A reflex response orchestrated by cell-cycle checkpoints serve to stall the cell-cycle and provide sufficient time to repair the DNA. This was evidenced by induction of DNA damage sensors, signal amplifiers and effectors. Nucleotide excision repair genes were the predominant class of repair proteins induced.

While most of the response to DNA damage is shared by the wild-type and *mgt1*, a fraction of the genes respond differentially and they include individual components of DNA repair systems. It appears as though the lack of MTase in *mgt1* leads to increased damage in mitochondria and a program that increases the transcription of genes pre-mRNA processing, mRNA splicing and ribosomal biogenesis. Damage due to alkylation does not limit itself to genetic material in the nucleus. It affects organelles such as mitochondria which appear to be very sensitive.

In the WT treated with MNNG, double-strand break repair was induced along with uracil DNA glycosylase (*UNG1*). There was more protein synthesis and transport across the subcellular organelles. In contrast, in *mgt1*, there was more mismatch repair (*MSH2*), and mitochondrial repair genes. The induction, over increasing length of exposure to MNNG, of 30 genes involved in pre-

RNA processing, mRNA splicing and ribosome maintenance could be attributed to the induction of *SWI6*, a transcriptional co-activator.

Culling out environmental stress responsive genes (ESR genes) from the current study permitted a study of gene expression attributes specific to WT or *mgt1*. Five out of the 9 genes identified as the DNA damage cluster by Gasch et al 2000 were found to be induced upon MNNG exposure. These genes included *DUN1*, *PLM2, RNR4* and the ORF of yet unknown function, *YBR070C.*

It appears that the MNNG induced damage is not limited to the DNA alone. Other macromolecular processes are affected considerably. An equal dose of MNNG imparts more damage in *mgt1* than the WT. While the O^6Methylguanine lesion is successfully repaired by the MTase in WT, lack of MTase in *mgt1* is not able to do so. With more O^6Methylguanine in the genome, the *mgt1* cells are killed faster than WT. The transcriptional changes that accompany imply that the cellular processes in *mgt1* sustain more damage. Hence the transcriptional responsiveness is more elaborate than in the WT. As a rule, the fundamental metabolic processes (glucose metabolism, amino acid metabolism and fatty acid metabolism) are transiently repressed in order to cope up with this stress and cell wall synthesis genes are induced in both WT and *mgt1*.

Bibliography

Agarwal AK, Rogers PD, Baerson SR, Jacob MR, Barker KS, Cleary JD, Walker LA, Nagle DG, Clark AM. Genome-wide expression profiling of the response to polyene, pyrimidine, azole, and echinocandin antifungal agents in Saccharomyces cerevisiae. *J Biol Chem.* 2003 Sep 12;278(37):34998-5015.

Bassett, D. E. et al. Genome cross-referencing and XREFdb: implications for the identification and analysis of genes mutated in human disease. *Nature Genet.* 15, 339-344 (1997).

Bassett, D. E., Boguski, M. S. & Hieter, P. Yeast genes and human disease. *Nature* 379, 589-590 (1996).

Baudin, A., Ozier-Kalogeropoulos, O., Denouel, A., Lacroute, F. & Cullin, C. A simple and efficient method for direct gene deletion in Saccharomyces cerevisiae. *Nucleic Acids Res.* 21, 3329-3330 (1993).

Begley TJ, Rosenbach AS, Ideker T, Samson LD. Damage recovery pathways in Saccharomyces cerevisiae revealed by genomic phenotyping and interactome mapping. *Mol Cancer Res.* 2002 Dec;1(2):103-12.

Bhatia PK, Verhage RA, Brouwer J, Friedberg EC. Molecular cloning and characterization of Saccharomyces cerevisiae RAD28, the yeast homolog of the human Cockayne syndrome A (CSA) gene. *J Bacteriol.* 1996 Oct;178(20):5977-88.

Bolstad, B.M., Irizarry R. A., Astrand, M., and Speed, T.P. (2003), A Comparison of Normalization Methods for High Density Oligonucleotide Array Data Based on Bias and Variance. *Bioinformatics* 19(2):185-193.

Gasch AP, Spellman PT, Kao CM, Carmel-Harel O, Eisen MB, Storz G, Botstein D, Brown PO. Genomic expression programs in the response of yeast cells to environmental changes. *Mol Biol Cell.* 2000 Dec;11(12):4241-57.

Burrows C. J., J. G. Muller, Oxidative Nucleobase Modifications Leading to Strand Scission. *Chem Rev.* 1998 May 7;98(3):1109-1152.

Carell T, Burgdorf LT, Kundu LM, Cichon M. The mechanism of action of DNA photolyases. *Curr Opin Chem Biol.* 2001 Oct;5(5):491-8

Chee, M. et al. Accessing genetic information with high-density DNA arrays. *Science* 274, 610-614 (1996).

Chi NW, Kolodner RD. The effect of DNA mismatches on the ATPase activity of MSH1, a protein in yeast mitochondria that recognizes DNA mismatches. *J Biol Chem.* 1994 Nov 25;269(47):29993-7.

Cho, R. J. et al. A genome-wide transcriptional analysis of the mitotic cell cycle. *Mol. Cell* 2, 65-73 (1998).

Choi, J. H., Lou, W. and Vancura, A. (1998) A novel membrane-bound glutathione S-transferase functions in the stationary phase of the yeast Saccharomyces cerevisiae. *J. Biol. Chem.* 273, 29915–29922

Chu, S. et al. The transcriptional program of sporulation in budding yeast. *Science* 282, 699-705 (1998).

Craven RJ, Petes TD. Involvement of the checkpoint protein Mec1p in silencing of gene expression at telomeres in Saccharomyces cerevisiae. *Mol Cell Biol.* 2000 Apr;20(7):2378-84.

Daniels DS, Mol CD, Arvai AS, Kanugula S, Pegg AE, Tainer JA. Active and alkylated human AGT structures: a novel zinc site, inhibitor and extrahelical base binding. *EMBO J.* 2000 Apr 3;19(7):1719-30.

Daniels DS, Tainer JA. Conserved structural motifs governing the stoichiometric repair of alkylated DNA by O(6)-alkylguanine-DNA alkyltransferase. *Mutat Res.* 2000 Aug 30;460(3-4):151-63.

DeRisi, J. L., Iyer, V. R. & Brown, P. O. Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* 278, 680-686 (1997).

Donahue SL, Corner BE, Bordone L, Campbell C. Mitochondrial DNA ligase function in Saccharomyces cerevisiae. *Nucleic Acids Res.* 2001 Apr 1;29(7):1582-9.

Estruch F. Stress-controlled transcription factors, stress-induced genes and stress tolerance in budding yeast. *FEMS Microbiol Rev.* 2000 Oct;24(4):469-86.

Foss EJ. Tof1p regulates DNA damage responses during S phase in Saccharomyces cerevisiae. *Genetics.* 2001 Feb;157(2):567-77.

Friedberg, E. C., Wagner, R., and Radman, M. Specialized DNA Polymerases, Cellular Survival and the Genesis of Mutations (2002) *Science* 296: 1627-1630.

Friedberg, E.C., Walker, G.C., and Siede, W. (1995) *DNA Repair and Mutagenesis*, American Society of Microbiology, Washington, DC.

Fry RC, Sambandan TG, Rha C. DNA damage and stress transcripts in Saccharomyces cerevisiae mutant sgs1. *Mech Ageing Dev.* 2003 Jul;124(7):839-46.

Gasch, A. P., Huang, M., Metzner, S., Botstein, D., Elledge, S. J., & Brown, P. O. (2001). *Mol. Biol. Cell* 12, 2987

Gasch, A.P., Spellman, P.T., Kao, C.M., Carmel-Harel, O., Eisen, M.B., Storz, G., Botstein, D., Brown, P.O. (2000). Genomic expression programs in the response of yeast cells to environmental changes. *Mol. Biol. Cell* 11(12):4241-4257.,

Ghislain M, Dohmen RJ, Levy F, Varshavsky A. Cdc48p interacts with Ufd3p, a WD repeat protein required for ubiquitin-mediated proteolysis in Saccharomyces cerevisiae.
EMBO J. 1996 Sep 16;15(18):4884-99.

Goffeau, A. et al. Life with 6000 genes. *Science* 274, 546, 563-567 (1996).

Hashimoto, H., Inoue, T., Nishioka, M., Fujiwara, S., Takagi, M., Imanaka, T., and Kai, Y. (1999) J. Mol. Biol. 292, 707-716. *J Biol Chem. 1997 Sep 19;272(38):23465-8*

Jacob S, Praz F. DNA mismatch repair defects: role in colorectal carcinogenesis. *Biochimie.* 2002 Jan;84(1):27-47

Jamieson ER, Lippard SJ. Structure, Recognition, and Processing of Cisplatin-DNA Adducts. *Chem Rev.* 1999 Sep 8;99(9):2467-98.

Jelinsky, S. A. & Samson, L. D. Global response of Saccharomyces cerevisiae to an alkylating agent. *Proc. Natl Acad. Sci.* USA 96, 1486-1491 (1999).

Kaplun, L., Ivantsiv, Y., Kornitzer, D., and Raveh, D. (2000) Functions of the DNA damage response pathway target Ho endonuclease of yeast for degradation via the ubiquitin-26S proteasome system *Proc. Natl. Acad. Sci.* U. S. A. 97, 10077–10082

Karran, P., and Marinus, M. G. (1982). Mismatch correction at O6-methylguanine residues in E. coli DNA. *Nature.* 1982 Apr 29;296(5860):868-9.

Katou Y, Kanoh Y, Bando M, Noguchi H, Tanaka H, Ashikari T, Sugimoto K, Shirahige K. S-phase checkpoint proteins Tof1 and Mrc1 form a stable replication-pausing complex. *Nature.* 2003 Aug 28;424(6952):1078-83.

Kei-ichi S, Atsuki K, Shingo I and Yoshiharu I Role of glutathione in heat-shock-induced cell death of *Saccharomyces cerevisiae Biochem. J.* (2000) 352 (71–78)

Kim C, Snyder RO, Wold MS. Binding properties of replication protein A from human and yeast cells. *Mol Cell Biol.* 1992 Jul;12(7):3050-9.

Kozmin SG, Leroy P, Pavlov YI. Overexpression of the yeast HAM1 gene prevents 6-N-hydroxylaminopurine mutagenesis in Escherichia coli. *Acta Biochim Pol.* 1998;45(3):645-52.

Labib K, Diffley JF. *Curr Opin Genet Dev.* 2001 Feb; 11(1): 64-70. Is the MCM2-7 complex the eukaryotic DNA replication fork helicase?

Lawley PD, Phillips DH. Related Articles, Links DNA adducts from chemotherapeutic agents. *Mutat Res.* 1996 Aug 17;355(1-2):13-40.

Leem SH, Chung CN, Sunwoo Y, Araki H. Meiotic role of SWI6 in Saccharomyces cerevisiae. *Nucleic Acids Res.* 1998 Jul 1;26(13):3154-8.

Li, Z. S., Szczypka, M., Lu, Y. P., Thiele, D. J. and Rea, P. A. (1996) The yeast cadmium factor protein (YCF1) is a vacuolar glutathione S-conjugate pump. *J. Biol. Chem.* 271, 6509–6517

Lindahl T and R. D. Wood Quality Control by DNA Repair. *Science*, Dec 3, 1999; 286(5446): 1897 - 1905.

Lindahl T, Sedgwick B, Sekiguchi M, Nakabeppu Y Regulation and expression of the adaptive response to alkylating agents. *Annu Rev Biochem.* 1988;57:133-57.

Lindahl T. Instability and decay of the primary structure of DNA. *Nature.* 1993 Apr 22;362(6422):709-15.

Loeb L. A, B. D. Preston, Mutagenesis by apurinic/apyrimidinic sites. *Annu. Rev. Genet.* 1986, 20, 201

Mannhaupt G, Schnall R, Karpov V, Vetter I, Feldmann H. Rpn4p acts as a transcription factor by binding to PACE, a nonamer box found upstream of 26S proteasomal and other genes in yeast. *FEBS Lett.* 1999 Apr 30;450(1-2):27-34.

Martin SG, Laroche T, Suka N, Grunstein M, Gasser SM. Relocalization of telomeric Ku and SIR proteins in response to DNA strand breaks in yeast. *Cell.* 1999 May 28;97(5):621-33.

Mills KD, Ferguson DO, Alt FW. The role of DNA breaks in genomic instability and tumorigenesis. *Immunol Rev.* 2003 Aug; 194:77-95.

Moore,M.H., Gulbus,J.M., Dodson,E.J., Demple,B. and Moody,P.C.E. (1994) Crystal structure of a suicidal DNA repair protein: the Ada O6-methylguanine-DNA methyltransferase from E.coli. *EMBO J.*, 13, 1495–1501

Modrich P Mechanisms and biological effects of mismatch repair. *Annu Rev Genet.* 1991;25:229-53.

Noskov VN, Staak K, Shcherbakova PV, Kozmin SG, Negishi K, Ono BC, Hayatsu H, HAM1, the gene controlling 6-N-hydroxylaminopurine sensitivity and mutagenesis in the yeast Saccharomyces cerevisiae. *Yeast.* 1996 Jan;12(1):17-29.

Ohkuni K, Shirahige K, Kikuchi A. Genome-wide expression analysis of NAP1 in Saccharomyces cerevisiae. *Biochem Biophys Res Commun.* 2003 Jun 20;306(1):5-9.

Orlandi I, Bettiga M, Alberghina L, Vai M Transcriptional profiling of ubp10 null mutant reveals subtelomeric gene expression and insurgence of oxidative stress response. *J Biol Chem.* 2003 Nov 17

Paulovich AG, Hartwell LH. A checkpoint regulates the rate of progression through S phase in S. cerevisiae in response to DNA damage. *Cell.* 1995 Sep 8;82(5):841-7.

Pavlov YI. HAM1, the gene controlling 6-N-hydroxylaminopurine sensitivity and mutagenesis in the yeast Saccharomyces cerevisiae. *Yeast* 1996;12:17-29.

Pegg AE. Repair of O(6)-alkylguanine by alkyltransferases. *Mutat Res.* 2000 Apr;462(2-3):83-100.

Ravanat JL, Douki T, Cadet J. Direct and indirect effects of UV radiation on DNA and its components. *J Photochem Photobiol* B. 2001 Oct;63(1-3):88-102.

Rep, M., Reiser, V., Gartner, U., Thevelein, J.M., Hohmann, S., Ammerer, G., and Ruis, H. (1999). Osmotic stress-induced gene expression in Saccharomyces cerevisiae requires Msn1p and the novel nuclear factor Hot1p. *Mol. Cell. Biol.* 19, 5474-5485

Rydberg B, Lindahl T. Nonenzymatic methylation of DNA by the intracellular methyl group donor S-adenosyl-L-methionine is a potentially mutagenic reaction. *EMBO J.* 1982;1(2):211-6.

Schena, M., Shalon, D., Davis, R. W. & Brown, P. O. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* 270, 467-470 (1995).

Schramke V, Luciano P, Brevet V, Guillot S, Corda Y, Longhese MP, Gilson E, Geli V. RPA regulates telomerase action by providing Est1p access to chromosome ends.
Nat Genet. 2004 Jan;36(1):46-54.

Sebastian J, Kraus B, Sancar GB. Expression of the yeast PHR1 gene is induced by DNA-damaging agents. Mol Cell Biol. 1990 Sep;10(9):4630-7.

Sebastian J, Sancar GB. A damage-responsive DNA binding protein regulates transcription of the yeast DNA repair gene PHR1. Proc Natl Acad Sci U S A. 1991 Dec 15;88(24):11251-5.

Sedgwick, 1997. *Carcinogenesis.* 1997 Aug;18(8):1561-7. Nitrosated peptides and polyamines as endogenous mutagens in O6-alkylguanine-DNA alkyltransferase deficient cells.

Seeberg E, Eide L, Bjoras M. The base excision repair pathway. Trends Biochem Sci. 1995 Oct;20(10):391-7.

Shuker, D. E. G. & Margison, G. P. (1997) Nitrosated glycine derivatives as a potential source of O6-methylguanine in DNA. Cancer Res 57:366-369

Singer, M. S., and D. E. Gottschling. 1994. TLC1: template RNA component of Saccharomyces cerevisiae telomerase. *Science* 266:404-409

Spellman, P. T. et al. Comprehensive identification of cell cycle-regulated genes of the yeast Saccharomyces cerevisiae by microarray hybridization. *Mol. Biol. Cell* 9, 3273-3297 (1998).

Sugimura T, Food and cancer, *Toxicology*, Volumes 181-182, 27 December 2002, 17-21

Swanson, Rebecca L., Natalie J. Morey, Paul W. Doetsch, and Sue Jinks-Robertson. 1999. Overlapping specificities of base excision repair, nucleotide excision repair, recombination, and translesion synthesis pathways for DNA base damage in *Saccharomyces cerevisiae. Mol. Cell. Biol.* 19:2929-2935

Teng Y, Yu Y, Waters R. The Saccharomyces cerevisiae histone acetyltransferase Gcn5 has a role in the photoreactivation and nucleotide excision repair of UV-induced cyclobutane pyrimidine dimers in the MFA2 gene. *J Mol Biol.* 2002 Feb 22;316(3):489-99.

Tercero JA, Diffley JF. Regulation of DNA replication fork progression through damaged DNA by the Mec1/Rad53 checkpoint. *Nature.* 2001 Aug 2;412(6846):553-7.

Torres-Ramos, Carlos A., Robert E. Johnson, Louise Prakash, and Satya Prakash. 2000. Evidence for the involvement of nucleotide excision repair in the removal of abasic sites in yeast. *Mol. Cell. Biol.* 20:3522-3528

Van Dyck E, Foury F, Stillman B, Brill SJ. A single-stranded DNA binding protein required for mitochondrial DNA replication in *S. cerevisiae* is homologous to E. coli SSB. *EMBO J.* 1992 Sep;11(9):3421-30.

Venter, J. C. et al. The sequence of the human genome. *Science* 291, 1304-1351 (2001).

Wach A, Brachat A, Pohlmann R, Philippsen P. New heterologous modules for classical or PCR-based gene disruptions in Saccharomyces cerevisiae. Yeast. 1994 Dec;10(13):1793-808.

Ward, J. H. Hierarchical grouping to optimize an objective function. *J. Am. Stat. Assoc.* 58, 236-244 (1963).

Wibley, J. E. , Pegg, A. E, P. C. Moody, Crystal structure of the human O6-alkylguanine-DNA alkyltransferase *Nucleic Acids Res.* 2000, 28, 39

Winzeler, E. A. et al. Functional characterization of the S. cerevisiae genome by gene deletion and parallel analysis. *Science* 285, 901-906 (1999).

Xiao W, L Samson. In vivo Evidence for Endogeneous DNA Alkylation Damage as a Source of Spontaneous Mutation in Eukaryotic Cells *PNAS*, Vol 90, 2117-2121, 1993

Xiao W, Derfler B, Chen J, Samson L. Primary sequence and biological functions of a Saccharomyces cerevisiae O6-methylguanine/O4-methylthymine DNA repair methyltransferase gene. *EMBO J.* 1991 Aug;10(8)

Xiao W, Treena Fontanie, Sonya Bawa and Lester Kohalmi REV3 is required for spontaneous but not methylation damage-induced mutagenesis of Saccharomyces cerevisiae cells lacking O6-methylguanine DNA methyltransferase Mutation Research Vol 431, 16 1999, Pages 155-165

Xiao W, Samson L, The Saccharomyces cerevisiae MGT1 DNA repair methyltransferase gene: its promoter and entire coding sequence, regulation and in vivo biological functions. Nucleic Acids Res. 20 (1992), pp. 3599–3606

Yang W, Rogozin IB, Koonin EV. Yeast POL5 is an evolutionarily conserved regulator of rDNA transcription unrelated to any known DNA polymerases. Cell Cycle. 2003 Mar-Apr;2(2):120-2.

Zhou BB, Elledge SJ. The DNA damage response: putting checkpoints in perspective. Nature. 2000 Nov 23;408(6811):433-9.

Zhao X, Chabes A, Domkin V, Thelander L, Rothstein R. The ribonucleotide reductase inhibitor Sml1 is a new target of the Mec1/Rad53 kinase cascade during growth and in response to DNA damage.

Appendix of Tables

Table 4: A subset of the genes that are up-regulated in basal *mgt1*.

A total of 148 genes upregulated ($log_2ER>2$) in basal *mgt1*.The function for 57 of them was known and is shown in Table 4A. Table 4B shows genes that upregulated in basal *mgt1* but are a part of the environmental stress response (ESR). The corresponding fold changes upon treatment in WT and *mgt1* are shown for reference. The information on phenotypic sensitivity of deletion strains of yeast to MMS was obtained from http://genomicphenotyping.mit.edu. Sensitivity score can range for 0-30. A score of > 2 implies sensitivity to MMS. Essential genes are highlighted in red.

ORF	Gene	Function	Basal *mgt1*	Sensitivity
YMR165C	SMP2	aerobic respiration	2.35	E
YIR031C	DAL7	allantoin catabolism	3.55	0
YHR061C	GIC1	axial budding	2.15	0
YGR286C	BIO2	biotin biosynthesis	3.04	4
YHR208W	BAT1	branched chain family AA biosynthesis	2.18	E
YFR013W	IOC3	chromatin modeling	3.03	0
YER058W	PET117	cytochrome c oxidase biogenesis	2.26	0
YIL004C_ex2	BET1	ER to Golgi transport	2.64	0
YGR087C	PDC6	ethanol metabolism	2.46	0
YOR388C	FDH1	formate catabolism	2.32	E
YML051W	GAL80	galactose metabolism	2.43	0
YGR252W	GCN5	histone acetylation	3.08	7
YHL047C	ARN2	iron-siderochrome transport	2.48	0
YEL065W	SIT1	iron-siderochrome transport	2.38	4
YIL138C	TPM2	isotropic bud growth	2.80	0
YGL009C	LEU1	leucine biosynthesis	2.13	E
YGL144C	ROG1	lipid metabolism	3.05	0
YGL044C	RNA15	mRNA cleavage	2.13	E
YIL139C	REV7	mutagenesis	6.93	18
YIR030C	DCG1	nitrogen metabolism	2.01	2
YPR194C	OPT2	oligopeptide transport	3.57	0
YHR123W_ex1	EPT1	phosphatidylethanolamine biosynthesis	2.80	0
YHR123W_ex2	EPT1	phosphatidylethanolamine biosynthesis	2.96	0
YIL035C	CKA1	protein amino acid phosphorylation	2.13	12
YGR188C	BUB1	protein amino acid phosphorylation	2.38	15
YMR225C_ex2	MRPL44	protein biosynthesis	2.44	0
YER153C	PET122	protein biosynthesis	2.08	0
YHR189W	PTH1	protein biosynthesis	2.61	4
YHR060W	VMA22	protein complex assembly	2.63	13
YIL071C	PCI8	protein deneddylation	3.14	0
YGR218W	CRM1	protein-nucleus export	2.05	E
YHL038C	CBP2	RNA splicing	6.50	6
YOR382W	FIT2	siderochrome transport	4.56	0
YOR383C	FIT3	siderochrome transport	2.02	0
YNL334C	SNO2	thiamin biosynthesis	2.42	0
YNL333W	SNZ2	thiamin biosynthesis	2.08	0
YCL064C	CHA1	threonine catabolism	2.36	0
YHR124W	NDT80	transcription	2.89	0
YGR186W	TFG1	transcription initiation from Pol II promoter	2.03	E
YNL270C	ALP1	transport	2.30	0
YDR046C	BAP3	transport	2.50	0
YEL003W	GIM4	tubulin folding	2.68	6
YPL276W	FDH2	NA	2.15	E
YGR090W	UTP22	NA	2.11	E

ORF	Gene	Function	Basal *mgt1*	Sensitivity
YHR093W	AHT1	NA	3.97	0
CEN8	CEN8	NA	12.70	0
YGL110C	CUE3	NA	2.30	0
YHR059W	FYV4	NA	2.42	0
YMR195W	ICY1	NA	2.21	0
YHR156C	LIN1	NA	2.32	0
YEL035C	UTR5	NA	4.89	0
YGR089W	NNF2	NA	10.02	4

Table 4B: ESR subset of genes that are up-regulated in *mgt1*

ORF	Gene	Function	Basal *mgt1*	Sensitivity
YLR267W	BOP2	NA	2.27	0
YGR187C	HGH1	NA	3.26	4
YPL263C	KEL3	NA	2.92	0
YLR134W	PDC5	ethanol fermentation	5.60	5
YIL104C	SHQ1	snoRNA metabolism	5.08	E

Table 5: A subset of the genes that are down-regulated in basal *mgt1*.

A total of 89 genes down regulated ($log_2ER<0.5$) in basal *mgt1*.The function for 24 of them was known and is shown in Table 5A. Table 5B shows genes that down-regulated in basal *mgt1* but are a part of the environmental stress response (ESR). The corresponding fold changes upon treatment in WT and *mgt1* are shown for reference. The information on phenotypic sensitivity of deletion strains of yeast to MMS was obtained from http://genomicphenotyping.mit.edu. Sensitivity score can range for 0-30. A score of > 2 implies sensitivity to MMS. Essential genes are highlighted in red.

Table 5A

ORF	Gene	Function	Basal *mgt1*	Sensitivity
YDL200C	MGT1	DNA dealkylation	-26.49	12
YGL209W	MIG2	glucose metabolism	-2.97	0
YHR094C	HXT1	hexose transport	-2.88	0
YDR345C	HXT3	hexose transport	-2.31	0
YKR099W	BAS1	histidine biosynthesis	-2.32	0
YGR142W	BTN2	intracellular protein transport	-3.17	0
YCL017C	NFS1	iron-sulfur cluster assembly	-5.60	E
YCL018W	LEU2	leucine biosynthesis	-126.12	E
YCL050C	APA1	nucleotide metabolism	-2.10	6
YGL158W	RCK1	protein AA phosphorylation	-2.24	4
YLR216C	CPR6	protein folding	-2.19	4
YMR238W	DFG5	pseudohyphal growth	-2.02	0
YGR211W	ZPR1	regulation of cell cycle	-2.60	E
YLR262C	YPT6	retrograde (endosome to Golgi) transport	-2.27	0
YIL162W	SUC2	sucrose catabolism	-2.30	0
YKL001C	MET14	sulfate assimilation	-2.16	6
YAL012W	CYS3	sulfur amino acid metabolism	-2.52	0
YOL154W	ZPS1	NA	-6.66	E
YHR049W	FSH1	NA	-2.39	0
YPL250C	ICY2	NA	-2.02	0
YOR104W	PIN2	NA	-2.09	0
YKR098C	UBP11	NA	-2.73	2

Table 5B

ORF	Gene	Function	Basal *mgt1*	Sensitivity
YER103W	SSA4	response to stress	-4.39	0
YLR183C	TOS4	NA	-2.22	0

Table 6: Subset of genes that are specifically induced in WT upon MNNG treatment

A total of 225 genes were induced ($log_2ER>2$) specifically in WT. The function for 127 of them was known and is shown in Table 6A. Table 6B shows genes that induced in WT but are a part of the environmental stress response (ESR). The corresponding fold changes upon treatment in other categories are shown for reference. The information on phenotypic sensitivity of deletion strains of yeast to MMS was obtained from http://genomicphenotyping.mit.edu. Sensitivity score can range for 0-30. A score of > 2 implies sensitivity to MMS. Essential genes are highlighted in red.

ORF	Gene	Function	WT FC	Sens
YLR429W	CRN1	actin filament organization	2.18	0
YCR105W	ADH7	alcohol metabolism	2.06	0
YFL057C	NA	aldehyde metabolism	3.13	E
YDL243C	AAD4	aldehyde metabolism	2.59	10
YIR027C	DAL1	allantoin catabolism	2.57	2
YBR085W	AAC3	ATP ADP exchange	3.12	0
YPR185W	APG13	autophagy	2.02	0
YNR058W	BIO3	biotin biosynthesis	2.34	0
YDR226W	ADK1	cell proliferation	2.13	2
YDR141C	DOP1	cellular morphogenesis	2.09	E
YJL072C	NA	DNA dependent DNA replication	2.24	E
YML021C	UNG1	DNA repair	2.02	0
YDL042C	SIR2	DSB repair via NHEJ	2.07	4
YML032C	RAD52	DSB repair via NHEJ	2.32	23
YHR007C	ERG11	electron transport	2.02	E
YNR075W_1	COS10	endocytosis	2.46	0
YHR098C	SFB3	ER to Golgi transport	2.04	E
YGR284C	ERV29	ER to Golgi transport	2.29	0
YGR166W	KRE11	ER to Golgi transport	2.25	0
YAR002C-A	ERP1	ER to Golgi transport	2.14	4
YLR208W	SEC13	ER-associated protein catabolism	2.04	E
YMR015C	ERG5	ergosterol biosynthesis	2.18	6
YHR044C	DOG1	glucose metabolism	2.17	0
YMR006C	PLB2	glycerophospholipid metabolism	2.71	0
YMR205C	PFK2	glycolysis	2.11	0
YJL099W	CHS6	Golgi to plasma membrane transport	2.25	0
YMR079W_ex2	SEC14	Golgi to plasma membrane transport	2.41	0

ORF	Gene	Function	WT FC	Sens
YDR044W	HEM13	heme biosynthesis	2.67	E
YER145C	FTR1	high affinity iron ion transport	2.15	0
YLR214W	FRE1	iron ion transport	2.03	0
YDL066W	IDP1	isocitrate metabolism	2.11	7
YCR086W	CSM1	meiotic chromosome segregation	2.02	0
YKL104C	GFA1	metabolism	2.04	E
YLR180W	SAM1	methionine metabolism	2.98	0
YPL124W	SPC29	microtubule nucleation	2.11	E
YDR488C	PAC11	microtubule-based process	2.40	0
YCR028C-A_ex2	RIM1	mitochondrial genome maintenance	2.34	0
YGR029W_ex2_alt	ERV1	mitochondrion organization and biogenesis	2.53	0
YCL055W	KAR4	mitosis	2.28	0
YDL008W	APC11	mitotic metaphase anaphase transition	2.16	E
YDR180W	SCC2	mitotic sister chromatid cohesion	2.24	E
YLR363C	NMD4	mRNA catabolism, nonsense-mediated	2.35	0
YNL016W	PUB1	mRNA catabolism, nonsense-mediated	2.21	5
YGR156W	PTI1	mRNA cleavage	2.39	E
YIR009W	MSL1	mRNA splicing	2.95	0
YOL103W	ITR2	myo-inositol transport	2.18	0
YBR218C	PYC2	NADPH regeneration	2.03	5
YCL026C-A	FRM2	negative regulation of fatty acid metabolism	4.33	0
YDR451C	YHP1	negative regulation of transcription from Pol II promoter	2.80	2
YDL232W	OST4	N-linked glycosylation	2.48	0
YMR246W	FAA4	N-terminal protein myristoylation	2.15	0
YIL016W	SNL1	nuclear pore organization and biogenesis	2.02	2
YCR073C	SSK22	osmosensory signaling pathway	2.06	0
YOL028C	YAP7	positive regulation of transcription from Pol II promoter	2.25	0
YGR109C	CLB6	premeiotic DNA synthesis	2.03	0

ORF	Gene	Function	WT FC	Sens
YMR142C_ex2	RPL13B	protein biosynthesis	2.50	0
YJL177W_ex1	RPL17B	protein biosynthesis	2.03	0
YBL027W_ex2	RPL19B	protein biosynthesis	2.11	0
YBR191W_ex2	RPL21A	protein biosynthesis	2.71	0
YOL127W_ex2	RPL25	protein biosynthesis	2.76	0
YMR194W_ex2	RPL36A	protein biosynthesis	2.21	0
YPR043W_ex2	RPL43A	protein biosynthesis	2.03	0
YPR043W_ex2	RPL43A	protein biosynthesis	2.02	0
YMR143W_ex2	RPS16A	protein biosynthesis	2.15	0
YOR096W_ex1	RPS7A	protein biosynthesis	2.28	0
YNL096C_ex1	RPS7B	protein biosynthesis	2.62	0
YNL096C_ex2	RPS7B	protein biosynthesis	2.75	0
YML019W	OST6	protein complex assembly	2.47	0
YDL141W	BPL1	protein modification	2.18	E
YMR300C	ADE4	purine base metabolism	2.09	0
YGR061C	ADE6	purine nucleotide biosynthesis	2.04	0
YCR059C	YIH1	regulation of amino acid metabolism	2.72	0
YML028W	TSA1	regulation of redox homeostasis	2.00	13
YPR052C	NHP6A	regulation of transcription from Pol II promoter	2.14	0
YMR179W	SPT21	regulation of transcription from Pol II promoter	2.01	19
YOR018W	ROD1	response to drug	2.22	10
YBR008C	FLR1	response to toxin	3.32	2
YCR106W	RDS1	response to xenobiotic stimulus	2.88	0
YNL287W	SEC21	retrograde (Golgi to ER) transport	2.20	E
YHR114W	BZZ1	salinity response	2.03	0
YML049C	RSE1	spliceosome assembly	2.38	E
YPL145C	KES1	steroid biosynthesis	2.42	0
YPR167C	MET16	sulfate assimilation	2.36	0
YMR260C	TIF11	translational initiation	2.00	E
YBR170C	NPL4	tRNA-nucleus export	2.20	12
YOL038W	PRE6	ubiquitin-dependent protein catabolism	2.48	E
YER031C	YPT31	vesicle-mediated transport	2.03	2
YER143W	DDI1	vesicle-mediated transport	2.01	11
YGL263W	COS12	NA	3.40	0
YKL219W	COS9	NA	2.35	0
YDL178W	DLD2	NA	2.01	0
YGL083W	SCY1	NA	2.04	0
YGL259W	YPS5	NA	2.51	0
YLR466W_0	YRF1-4	NA	2.51	0
YPL171C	OYE3	NA	2.43	4

ORF	Gene	Function	WT FC	Sens
YGR136W	LSB1	NA	2.18	11
YGR136W	LSB1	NA	2.06	11

Table 6B: ESR subset of genes induced only in WT

ORF	Gene	Function	WT FC	Sensitivity
YLR062C	BUD28	bud site selection	2.01	0
YLR299W	ECM38	cell wall organization and biogenesis	2.28	0
YBR283C	SSH1	cotranslational membrane targeting	2.31	0
YJL014W	CCT3	cytoskeleton organization and biogenesis	2.14	E
YJR064W	CCT5	cytoskeleton organization and biogenesis	2.24	E
YNL256W	FOL1	folic acid and derivative biosynthesis	2.30	0
YLR017W	MEU1	glutamate biosynthesis	2.04	5
YMR217W	GUA1	GMP metabolism	2.08	0
YGR195W	SKI6	mRNA catabolism	2.17	E
YHR065C	RRP3	mRNA splicing	2.10	E
YIL079C	AIR1	mRNA-nucleus export	2.01	0
YPL037C	EGD1	nascent polypeptide association	2.19	0
YBR106W	PHO88	phosphate transport	2.06	0
YOR253W	NAT5	protein amino acid acetylation	2.01	0
YPR102C	RPL11A	protein biosynthesis	2.30	E
YLR029C	RPL15A	protein biosynthesis	2.54	E
YNL067W	RPL9B	protein biosynthesis	2.04	0
YOR369C	RPS12	protein biosynthesis	2.15	0
YOR167C	RPS28A	protein biosynthesis	2.21	0
YLR264W	RPS28B	protein biosynthesis	2.20	0
YML106W	URA5	pyrimidine base biosynthesis	2.16	0
YLR397C	AFG2	response to drug	2.38	E
YHR148W	IMP3	rRNA modification	2.33	E
YMR235C	RNA1	rRNA-nucleus export	2.01	E
YPR110C	RPC40	transcription from Pol III promoter	2.20	E
YDR382W	RPP2B	translational elongation	2.04	0
YOR260W	GCD1	translational initiation	2.18	E
YMR309C	NIP1	translational initiation	2.54	E
YDL167C	NRP1	NA	2.05	0
YLR221C	RSA3	NA	2.26	0

Table 7: A subset of the genes that are induced specifically in *mgt1*

A total of 274 genes were induced ($log_2ER>2$) specifically in *mgt1*. The function for 87 of them was known and is shown in Table 7A. Table 7B shows genes that induced in *mgt1* but are a part of the environmental stress response (ESR). The corresponding fold changes upon treatment in other categories are shown for reference. The information on phenotypic sensitivity of deletion strains of yeast to MMS was obtained from http://genomicphenotyping.mit.edu. Sensitivity score can range for 0-30. A score of > 2 implies sensitivity to MMS. Essential genes are highlighted in red. There were 29 essential genes induced in *mgt1* 9 of which were induced in the ESR subset (Table 7B)

ORF	Gene	Function	*mgt1* FC	Sensitivity
YBR058c-a	TSC3	3-keto-sphinganine metabolism	2.13	0
YDL029W_ex2	ARP2	actin filament organization	2.08	0
YLR139C	SLS1	aerobic respiration	2.09	0
YHR011W	DIA4	aerobic respiration	2.12	15
YNR044W	AGA1	agglutination during conjugation with cellular fusion	2.05	E
YKL008C	LAC1	aging	2.31	0
YLL048C	YBT1	bile acid transport	2.08	6
YIL003W	DRE3	cell growth and	2.02	0
YHR142W	CHS7	cell wall chitin biosynthesis	2.03	0
YCR089W	FIG2	cellular morphogenesis during conjugation with cellular fusion	2.12	2
YBR093C	PHO5	cellular response to phosphate starvation	3.21	0
YLR381W	CTF3	chromosome segregation	2.46	0
YGL028C	SCW11	cytokinesis, completion of separation	2.31	0
YLR348C	DIC1	dicarboxylic acid transport	3.43	0
YKL011C	CCE1	DNA recombination	2.14	10
YKL045W	PRI2	DNA repair synthesis	2.00	E
YER073W	ALD5	electron transport	3.16	0
YNL272C	SEC2	exocytosis	2.28	E
YJL167W	ERG20	farnesyl diphosphate biosynthesis	2.06	E
YDL132W	CDC53	G1 S transition of mitotic cell cycle	2.21	E
YLR182W	SWI6	G1 S-specific transcription in mitotic cell cycle	2.12	20
YDL227C	HO	gene conversion at MAT locus	2.24	0
YPR160W	GPH1	glycogen catabolism	2.40	23
YHR092C	HXT4	hexose transport	2.62	0
YMR319C	FET4	intracellular copper ion transport	2.92	4
YNL188W	KAR1	karyogamy during conjugation with cellular fusion	2.24	E

ORF	Gene	Function	*mgt1* FC	Sensitivity
YLR382C	NAM2	leucyl-tRNA aminoacylation	2.40	0
YDL131W	LYS21	lysine biosynthesis, aminoadipic pathway	2.59	0
YJR135w-a	TIM8	mitochondrial translocation	2.52	0
YHR005C-A	MRS11	mitochondrial translocation	2.11	E
YHR152W	SPO12	mitotic cell cycle	2.24	9
YGL116W	CDC20	mitotic metaphase anaphase transition	2.01	E
YDR016C	DAD1	mitotic spindle assembly	2.34	E
YGL061C	DUO1	mitotic spindle assembly	2.31	E
YMR178W	NA	Mo-molybdopterin cofactor biosynthesis	2.03	0
YLR115W	CFT2	mRNA cleavage	2.06	E
YER105C	NUP157	mRNA-nucleus export	2.13	E
YJR131W	MNS1	N-linked glycosylation	2.05	0
YKR061W	KTR2	N-linked glycosylation	2.45	4
YLR148W	PEP3	nonselective vesicle docking	2.32	17
YPR018W	RLF2	nucleosome assembly	2.04	15
YGL119W	ABC1	oxidative phosphorylation, ubiquinone to cytochrome c	2.15	E
YKL209C	STE6	peptide pheromone export	2.81	E
YNR013C	PHO91	phosphate transport	2.21	0
YLR305C	STT4	phosphatidylethanolamine biosynthesis	2.11	E
YLR386W	VAC14	phospholipid metabolism	2.01	7
YOR181W	LAS17	polar budding	2.01	E
YAL021C	CCR4	poly(A) tail shortening	2.05	17
YOR270C	VPH1	polyphosphate metabolism	2.04	4
YBR015C	MNN2	protein amino acid glycosylation	2.16	0
YGR092W	DBF2	protein amino acid phosphorylation	2.28	18
YGL143C	MRF1	protein biosynthesis	2.29	0
YGR084C	MRP13	protein biosynthesis	2.18	0

ORF	Gene	Function	*mgt1* FC	Sensitivity
YDR237W	MRPL7	protein biosynthesis	2.09	0
YIL098C	FMC1	protein complex assembly	2.05	4
YJR099W	YUH1	protein deubiquitination	2.19	0
YDR390C	UBA2	protein sumoylation	2.50	E
YIL005W	EPS1	protein-ER retention	2.08	2
YGL238W	CSE1	protein-nucleus export	2.18	E
YML097C	VPS9	protein-vacuolar targeting	2.52	7
YGL151W	NUT1	regulation of transcription from Pol II promoter	2.47	13
YMR267W	PPA2	respiratory gaseous exchange	2.19	0
YNL239W	LAP3	response to antibiotic	2.01	0
YMR092C	AIP1	response to osmotic stress	2.25	0
YHR136C	SPL2	response to temperature	2.01	0
YGL223C	COG1	retrograde (vesicle recycling within Golgi) transport	2.07	0
YGR116W	SPT6	RNA elongation from Pol II promoter	2.27	E
YHR110W	ERP5	secretory pathway	2.02	5
YDR461W	MFA1	signal transduction during conjugation with cellular fusion	2.03	0
YNL145W	MFA2	signal transduction during conjugation with cellular fusion	2.35	0

ORF	Gene	Function	*mgt1* FC	Sensitivity
YKL092C	BUD2	small GTPase mediated signal transduction	2.17	0
YKL120W	OAC1	sulfate transport	3.61	0
YJR130C	STR2	sulfur metabolism	2.05	0
YOR337W	TEA1	transcription	2.27	0
YGL049C	TIF4632	translational initiation	2.47	0
YNR055C	HOL1	transport	2.04	0
YOR136W	IDH2	tricarboxylic acid cycle	2.13	4
YGR119C	NUP57	tRNA-nucleus export	2.09	E
YOL096C	COQ3	ubiquinone biosynthesis	2.04	5
YFL004W	VTC2	vacuole fusion (non-autophagic)	2.18	0
YNL085W	MKT1	viral life cycle	2.01	0
YML132W_1	COS3	NA	2.16	0
YIL171W	HXT12	NA	2.04	0
YGL221C	NIF3	NA	2.00	0
YBR162C	TOS1	NA	2.03	0
YGL161C	YIP5	NA	2.05	0
YBR111C	YSA1	NA	2.24	5

Table 7B: ESR subset of genes specifically induced in *mgt1* upon MNNG treatment

ORF	Gene	Function	*mgt1* FC	Sensitivity
YLR129W	DIP2	processing of 20S pre-rRNA	2.14	E
YLR222C	UTP13	processing of 20S pre-rRNA	3.39	E
YML093W	UTP14	processing of 20S pre-rRNA	2.30	E
YKL144C	RPC25	transcription from Pol III promoter	2.27	E
YGR083C	GCD2	translational initiation	2.13	E
YBR079C	RPG1	translational initiation	2.09	E
YKL035W	UGP1	UDP-glucose metabolism	2.31	E
YNL182C	IPI3	NA	2.36	E
YLR409C	UTP21	NA	2.29	E

ORF	Gene	Function	*mgt1* FC	Sensitivity
YMR105C	PGM2	glucose 6-phosphate utilization	2.32	0
YIL113W	SDP1	MAPKKK cascade (cell wall biogenesis)	2.81	0
YDR097C	MSH6	mismatch repair	2.21	0
YJL198W	PHO90	phosphate transport	2.16	0
YDR440W	DOT1	regulation of meiosis	2.05	0
YDR496C	PUF6	NA	2.58	0
YAL035W	FUN12	translational initiation	2.29	14

Table 8: **A subset of the genes that are repressed specifically in WT**

A total of 145 genes were induced ($log_2ER<0.5$) specifically in WT. The function for 70 of them was known and is shown in Table 8A. Table 8B shows genes that induced in WT but are a part of the environmental stress response (ESR). The information on phenotypic sensitivity of deletion strains of yeast to MMS was obtained from http://genomicphenotyping.mit.edu. Sensitivity score can range for 0-30. A score of > 2 implies sensitivity to MMS. Essential genes are highlighted in red. There were 8 essential genes repressed in WT, 1 of which were induced in the ESR subset (Table 8B)

ORF	Gene	Function	WT FC	Sensitivity
YML129C	COX14	aerobic respiration	-2.01	0
YPR100W	MRPL51	aerobic respiration	-2.52	0
YPL132W	COX11	aerobic respiration	-2.33	2
YBR128C	APG14	autophagy	-2.07	0
YKR063C	LAS1	bud growth	-2.00	E
YNL192W	CHS1	budding	-2.24	0
YDL179W	PCL9	cell cycle	-2.10	0
YOR304W	ISW2	chromatin modeling	-2.21	0
YDR310C	SUM1	chromatin silencing at HML and HMR (sensu Saccharomyces)	-2.24	5
YER088C	DOT6	chromatin silencing at ribosomal DNA (rDNA)	-2.35	7
YMR219W	ESC1	chromatin silencing at telomere	-2.14	0
YBR095C	RXT2	conjugation with cellular fusion	-2.03	6
YDR030C	RAD28	DNA repair	-2.80	4
YMR020W	FMS1	electron transport	-2.03	0
YOR375C	GDH1	glutamate biosynthesis, using glutamate dehydrogenase	-2.17	0
YDR176W	NGG1	histone acetylation	-2.04	4
YKR029C	SET3	histone deacetylation	-2.12	8
YER075C	PTP3	inactivation of MAPK (osmolarity sensing)	-2.27	0
YBR066C	NRG2	invasive growth	-2.02	0
YOR354C	MSC6	meiotic recombination	-2.20	0
YIL144W	TID3	microtubule nucleation	-2.20	E
YPR141C	KAR3	mitosis	-2.02	18
LSR1_1	LSR1	mRNA splicing	-2.65	0
YCR033W	SNT1	negative regulation of meiosis	-2.09	10
YDR397C_ex2	NCB2	negative regulation of transcription from Pol II promoter	-2.11	0
YDR028C	REG1	negative regulation of transcription from Pol II promoter	-2.14	5
YLR231C	BNA5	nicotinamide adenine dinucleotide biosynthesis	-2.32	0
YDR075W	PPH3	nitrogen metabolism	-2.12	19
YBR114W	RAD16	nucleotide-excision repair, DNA damage recognition	-2.58	0
YKR093W	PTR2	peptide transport	-2.24	6
YGR138C	TPO2	polyamine transport	-6.30	0

ORF	Gene	Function	WT FC	Sensitivity
YOR078W	BUD21	processing of 20S pre-rRNA	-2.43	0
YJL186W	MNN5	protein amino acid glycosylation	-2.25	0
YOL016C	CMK2	protein amino acid phosphorylation	-2.41	0
YNR037C	RSM19	protein biosynthesis	-2.01	0
YPL013C	MRPS16	protein biosynthesis	-2.06	2
YGR174C	CBP4	protein complex assembly	-2.27	2
YBR083W	TEC1	pseudohyphal growth	-3.45	0
YKL048C	ELM1	pseudohyphal growth	-2.05	11
YER040W	GLN3	regulation of nitrogen utilization	-2.12	0
YKL139W	CTK1	regulation of transcription from Pol II promoter	-2.26	18
YJL006C	CTK2	regulation of transcription from Pol II promoter	-2.06	21
YCR097W_ex2	HMRA1	regulation of transcription, mating-type specific	-2.54	0
YDR423C	CAD1	response to cadmium ion	-2.12	0
YOR153W	PDR5	response to drug	-2.33	0
YNL074C	MLF3	response to drug	-2.08	4
YOR075W	UFE1	retrograde (Golgi to ER) transport	-2.41	E
YLR223C	IFH1	rRNA processing	-2.03	E
YBR257W	POP4	rRNA processing	-2.11	E
YOL010W	RCL1	rRNA processing	2.33	E
YNL091W	NST1	salinity response	-2.64	0
YDL194W	SNF3	signal transduction	-2.13	0
YDR104C	SPO71	spore wall assembly (sensu Saccharomyces)	-2.04	0
YAR044W	OSH1	steroid biosynthesis	-2.01	0
YIL119C	RPI1	thiamin biosynthesis	-2.61	0
YOR194C	TOA1	transcription initiation from Pol II promoter	-2.20	E
YAL001C_ex2	TFC3	transcription initiation from Pol III promoter	-2.46	0
YOR245C	DGA1	triacylglycerol biosynthesis	-2.07	0
YLR304C	ACO1	tricarboxylic acid cycle	-2.02	0
YHL016C	DUR3	urea transport	-2.11	10
YDR202C	RAV2	vacuolar acidification	-2.19	0

ORF	Gene	Function	WT FC	Sensitivity
YGR196C	FYV8	NA	-2.60	0
YDL001W	RMD1	NA	-2.18	0
YMR156C	TPP1	NA	-2.04	0
YJR127C	ZMS1	NA	-2.11	2
YBR033W	EDS1	NA	-2.01	3
YJR122W	CAF17	NA	-2.03	6
YNL215W	IES2	NA	-2.29	7
YDL115C	IWR1	NA	-2.12	15
YAL011W	SWC1	NA	-2.13	25

Table 8B: ESR Subset of genes that are repressed only in WT upon MNNG treatment

ORF	Gene	Function	Basal *mgt1*	WT FC	*mgt1* FC	Sensitivity
YOR173W	DCS2	NA	1.01	-3.23	-1.89	0
YLR080W	EMP46	ER to Golgi transport	-1.17	-2.26	-1.50	0
YML128C	MSC1	meiotic recombination	-1.08	-2.35	-1.52	0
YMR304W	UBP15	protein deubiquitination	-1.22	-2.12	-1.44	0
YNL186W	UBP10	protein deubiquitination	-1.08	-2.08	-1.96	E
YPR149W	NCE102	protein secretion	-1.18	-2.10	-1.76	0
YMR271C	URA10	pyrimidine base biosynthesis	-1.12	-2.17	-1.84	2
YFL014W	HSP12	response to dessication	-1.00	-2.32	-1.73	4
YBR072W	HSP26	response to stress	1.01	-2.66	-1.77	0
YNL015W	PBI2	vacuole fusion (non-autophagic)	-1.08	-2.29	-1.74	0

Table 9: A subset of the genes that are repressed specifically in *mgt1*

A total of 191 genes were induced ($log_2ER<0.5$) specifically in *mgt1*. The function for 70 of them was known and is shown in Table 9A. Table 9B shows genes that induced in *mgt1* but are a part of the environmental stress response (ESR). The corresponding fold changes upon treatment in other categories are shown for reference. The information on phenotypic sensitivity of deletion strains of yeast to MMS was obtained from http://genomicphenotyping.mit.edu. Sensitivity score can range for 0-30. A score of > 2 implies sensitivity to MMS. Essential genes are highlighted in red. There were 14 essential genes repressed in *mgt1*.

ORF	Gene	Function	*mgt1* FC	Sensitivity
YLR395C	COX8	aerobic respiration	-2.11	0
YDL067C	COX9	aerobic respiration	-2.54	0
YJR055W	HIT1	aerobic respiration	-2.18	0
YKL055C	OAR1	aerobic respiration	-2.20	28
YDR538W	PAD1	aromatic compound catabolism	-2.26	0
YKL106W	AAT1	asparagine biosynthesis from oxaloacetate	-2.29	4
YLR295C	ATP14	ATP synthesis coupled proton transport	-2.05	0
YBR039W	ATP3	ATP synthesis coupled proton transport	-2.05	E
YCR068W	CVT17	autophagy	-2.19	0
YCR068W	CVT17	autophagy	-2.45	0
YIL155C	GUT2	carbohydrate metabolism	-2.10	0
YBR297W	MAL33	carbohydrate metabolism	-2.27	0
YBL101C	ECM21	cell wall organization and biogenesis	-2.61	0
YDR073W	SNF11	chromatin modeling	-2.54	0
YJL127C	SPT10	chromatin modeling	-2.01	26
YJR060W	CBF1	DNA replication and chromosome cycle	-2.26	9
YNL044W	YIP3	ER to Golgi transport	-2.01	0
YDR143C	SAN1	establishment and or maintenance of chromatin architecture	-2.25	11
YNL161W	CBK1	exit from mitosis	-2.04	E
YDL168W	SFA1	formaldehyde assimilation	-2.05	0
YIL135C	NA	G1 S transition of mitotic cell cycle	-2.14	0
YJL219W	HXT9	hexose transport	-2.71	E
YFL041W	FET5	iron ion transport	-2.49	0
YER013W	PRP22	ianat formation, 5'-splice site cleavage	-2.11	E
YPL128C	TBF1	loss of chromatin silencing	-2.35	E

ORF	Gene	Function	*mgt1* FC	Sensitivity
YJL146W	IDS2	meiosis	-2.06	0
YLR329W	REC102	meiotic recombination	-2.63	2
YDR502C	SAM2	methionine metabolism	-2.22	0
YOL060C	MAM3	mitochondrion organization and biogenesis	-2.35	0
YJR091C	JSN1	mRNA catabolism, deadenylation-dependent	-2.09	0
YPR042C	PUF2	mRNA catabolism, deadenylation-dependent	-2.34	0
YDL160C	DHH1	mRNA catabolism, nonsense-mediated	-2.80	15
YEL036C	ANP1	N-linked glycosylation	-2.08	0
YDR441C	APT2	nucleoside metabolism	-3.10	0
YLR005W	SSL1	nucleotide-excision repair	-2.27	E
YDR019C	GCV1	one-carbon compound metabolism	-2.08	0
YOR298C-A	MBF1	positive regulation of transcription from Pol II promoter	-2.52	0
YBR101C	NA	protein biosynthesis	-3.32	16
YOR027W	STI1	protein folding	-2.13	9
YGL058W	RAD6	protein monoubiquitination	-2.18	7
YEL059C-A	SOM1	proteolysis and peptidolysis	-2.05	0
YOR128C	ADE2	purine base metabolism	-2.30	0
YBR135W	CKS1	regulation of cell cycle	-2.47	E
YML010W	SPT5	regulation of transcription, DNA-dependent	-2.21	E
YML010W	SPT5	regulation of transcription, DNA-dependent	-2.09	E
YPL179W	PPQ1	regulation of translation	-2.51	0
YBL005W	PDR3	response to drug	-2.26	0
YLR006C	SSK1	response to hydrogen peroxide	-2.19	18
YLR037C	DAN2	response to stress	-2.12	7
YOR025W	HST3	short-chain fatty acid metabolism	-2.52	11

ORF	Gene	Function	*mgt1* FC	Sensitivity
YBR213W	MET8	siroheme biosynthesis	-2.28	0
YDR403W	DIT1	spore wall assembly (sensu Saccharomyces)	-2.34	0
YGR055W	MUP1	sulfur amino acid transport	-2.96	0
YMR095C	SNO1	thiamin biosynthesis	-2.07	0
YBR240C	THI2	thiamin biosynthesis	-2.03	5
YCR020C	PET18	thiamin metabolism	-2.22	0
YLR237W	THI7	thiamin transport	-2.14	0
YDR156W	RPA14	transcription from Pol I promoter	-2.13	0
YBL014C	RRN6	transcription from Pol I promoter	-2.03	E
YPR168W	NUT2	transcription from Pol II promoter	-2.02	E
YIL125W	KGD1	tricarboxylic acid cycle	-2.33	0
YNR034W	SOL1	tRNA processing	-2.41	0

ORF	Gene	Function	*mgt1* FC	Sensitivity
YDR151C	CTH1	NA	-2.33	0
YNL245C	CWC25	NA	-2.80	E
YDR373W	FRQ1	NA	-2.15	E
YGR002C	GOD1	NA	-2.10	E
YMR161W	HLJ1	NA	-2.40	0
RDN37-1_2	RDN37-1	NA	-2.96	0
YMR266W	RSN1	NA	-2.11	0
YPL239W	YAR1	NA	-2.75	0

Table 9B ESR subset of genes that are repressed specifically in *mgt1* upon MNNG treatment

ORF	Gene	Function	Basal *mgt1*	WT FC	*mgt1* FC	Sensitivity
YJR059W	PTK2	polyamine transport	-1.06	-1.98	-2.16	2
YBL064C	NA	regulation of redox homeostasis	-1.08	-1.73	-2.16	4
YEL060C	PRB1	vacuolar protein catabolism	-1.09	-1.79	-2.11	0

Table 10: Genes that are induced in WT and *mgt1* upon MNNG treatment

This overlapping response indicated in the upper-middle panel of Figure 19 included 977 genes that were induced in both WT and *mgt1*. Of them, 127 genes were included as ESR genes. The function of 282 genes was not known and is not indicated in these tables. The remaining 568 genes, whose function was known and those that were not a part of the ESR are listed by function in Table 10A. 121 (21%) of these are essential genes.

Table 10A ORF	Gene	Function	WT FC	*mgt1* FC	Sens
YLR153C	ACB1	acetyl-CoA biosynthesis	2.61	2.68	E
YKL013C	ARC19	actin cortical patch assembly	2.63	2.67	E
YCR088W	ABP1	actin cortical patch assembly	3.27	3.44	0
YLR144C	ACF2	actin cytoskeleton organization and biogenesis	2.28	2.17	3
YBR234C	ARC40	actin filament organization	2.37	2.43	E
YIL034C	CAP2	actin filament organization	2.87	3.67	2
YDL135C	RDI1	actin filament organization	2.14	2.09	0
YGR080W	TWF1	actin polymerization and or depolymerization	2.85	2.90	0
YLR452C	SST2	adaptation to pheromone during conjugation with cellular fusion	2.36	3.08	0
YNL220W	ADE12	adenosine biosynthesis	2.92	2.50	7
YDR487C	RIB3	aerobic respiration	2.61	2.22	E
YIL070C	MAM33	aerobic respiration	2.02	2.39	4
YGL032C	AGA2	agglutination during conjugation with cellular fusion	2.21	3.79	0
YBR145W	ADH5	alcohol metabolism	5.25	5.86	4
YMR318C	ADH6	alcohol metabolism	16.76	16.00	0
YFL056C	AAD6	aldehyde metabolism	5.82	2.08	3
YIR028W	DAL4	allantoin transport	3.14	2.87	2
YGL105W	ARC1	amino acid activation	2.74	2.49	0
YFR055W	NA	amino acid metabolism	14.64	13.62	0
YLL058W	NA	amino acid metabolism	3.58	3.05	0
YIL111W_ex2	COX5B	anaerobic respiration	2.06	2.70	0
YGL106W	MLC1	apical bud growth	2.53	2.20	E

Table 10A ORF	Gene	Function	WT FC	*mgt1* FC	Sens
YNL298W	CLA4	apical bud growth	2.59	3.38	23
YJL071W	ARG2	arginine biosynthesis	2.33	2.87	0
YHR018C	ARG4	arginine biosynthesis	7.10	8.30	0
YER069W	ARG5,6	arginine biosynthesis	2.41	2.19	0
YDR127W	ARO1	aromatic amino acid family biosynthesis	4.66	5.32	0
YDR035W	ARO3	aromatic amino acid family biosynthesis	2.86	2.95	0
YGL202W	ARO8	aromatic amino acid family metabolism	5.73	5.36	0
YLR088W	GAA1	attachment of GPI anchor to protein	2.86	2.97	E
YHR188C	GPI16	attachment of GPI anchor to protein	2.31	2.13	E
YIL109C	SEC24	autophagy	2.26	2.16	E
YDR309C	GIC2	axial budding	2.14	2.02	2
YDR135C	YCF1	bilirubin transport	2.87	2.85	0
YGL065C	ALG2	biosynthesis	2.29	2.16	E
YER090W	TRP2	biosynthesis	4.58	4.33	0
YJR016C	ILV3	branched chain family amino acid biosynthesis	2.29	3.00	E
YER086W	ILV1	branched chain family amino acid biosynthesis	4.21	4.46	21
YCL009C	ILV6	branched chain family amino acid biosynthesis	2.65	2.28	0
YNR027W	BUD17	bud site selection	3.39	2.33	4
YOR301W	RAX1	bud site selection	3.37	3.02	4
YIL140W	AXL2	bud site selection	3.49	3.52	0
YGR041W	BUD9	bud site selection	2.02	2.27	0
YLR084C	RAX2	bud site selection	2.93	2.31	0
YEL031W	SPF1	calcium ion homeostasis	3.40	3.11	0
YOR087W	YVC1	cation homeostasis	4.21	3.56	5
YOR087W	YVC1	cation homeostasis	4.30	3.28	5
YHL003C	LAG1	cell aging (sensu Saccharomyces)	3.18	3.54	4
YDL127W	PCL2	cell cycle	2.19	2.24	0
YML058w-a	HUG1	cell cycle arrest	11.42	9.32	0
YDL101C	DUN1	cell cycle checkpoint	6.55	7.72	18
YDL226C	GCS1	cell cycle dependent actin filament reorganization	4.44	3.92	5
YKL101W	HSL1	cell morphogenesis checkpoint	2.44	2.45	7
YMR109W	MYO5	cell wall organization and biogenesis	3.05	2.62	6
YHL030W	ECM29	cell wall organization and biogenesis	5.92	5.64	4
YJL158C	CIS3	cell wall organization and biogenesis	4.78	4.50	0
YGL027C	CWH41	cell wall organization and biogenesis	3.27	3.92	0

Table 10A					
ORF	Gene	Function	WT FC	*mgt1* FC	Sens
YHR143W	DSE2	cell wall organization and biogenesis	3.70	3.78	0
YBR078W_ex1	ECM33	cell wall organization and biogenesis	3.00	2.78	0
YBR078W_ex2	ECM33	cell wall organization and biogenesis	2.46	2.80	0
YMR062C	ECM40	cell wall organization and biogenesis	5.76	5.70	0
YLR342W	FKS1	cell wall organization and biogenesis	3.41	3.78	0
YOR109W	INP53	cell wall organization and biogenesis	2.38	2.16	0
YGL178W_ex2	MPT5	cell wall organization and biogenesis	2.07	2.40	0
YNL283C	WSC2	cell wall organization and biogenesis	6.04	7.23	0
YOR249C	APC5	chromatin assembly disassembly	2.15	2.62	E
YBR245C	ISW1	chromatin modeling	2.10	2.24	12
YLL004W	ORC3	chromatin silencing at HML and HMR (sensu Saccharomyces)	2.10	2.12	E
YNL261W	ORC5	chromatin silencing at HML and HMR (sensu Saccharomyces)	2.80	2.53	E
YCL061C	MRC1	chromatin silencing at HML and HMR (sensu Saccharomyces)	2.61	2.76	9
YLR285W	NNT1	chromatin silencing at ribosomal DNA (rDNA)	3.18	2.91	0
YAR003W	SWD1	chromatin silencing at telomere	2.80	2.20	E
YBR175W	SWD3	chromatin silencing at telomere	5.88	4.46	7
YAL034W-A	MTW1	chromosome segregation	2.53	2.74	E
YPR046W	MCM16	chromosome segregation	2.68	2.01	2
YOL058W	ARG1	citrulline metabolism	9.98	11.09	5
YAR031W	PRM9	conjugation with cellular fusion	4.23	3.14	0
YMR305C	SCW10	conjugation with cellular fusion	3.53	3.65	0
YNL259C	ATX1	copper ion transport	3.30	3.44	5
YCR075C	ERS1	cystine transport	2.04	2.39	0
YHR107C	CDC12	cytokinesis	2.47	2.04	E
YLR314C	CDC3	cytokinesis	7.12	7.76	E
YNL327W	EGT2	cytokinesis	2.18	2.63	12
YNL233W	BNI4	cytokinesis	2.28	2.22	0
YDL117W	CYK3	cytokinesis	2.19	2.55	0
YIL142W	CCT2	cytoskeleton organization and biogenesis	2.36	2.42	E
YJL008C	CCT8	cytoskeleton organization and biogenesis	2.20	2.13	E
YDR212W	TCP1	cytoskeleton organization and biogenesis	2.74	2.30	E
YNL138W	SRV2	cytoskeleton organization and biogenesis	2.12	2.29	0
YDL219W_ex1	DTD1	D-amino acid catabolism	11.36	9.61	0
YDL219W_ex2	DTD1	D-amino acid catabolism	7.55	7.00	0
YKL212W	SAC1	dephosphorylation	5.03	5.07	4
YDR489W	NA	DNA dependent DNA replication	3.01	2.67	E

Table 10A					
ORF	Gene	Function	WT FC	*mgt1* FC	Sens
YEL055C	POL5	DNA dependent DNA replication	3.18	3.41	E
YDL164C	CDC9	DNA recombination	3.70	3.95	E
YLR383W	RHC18	DNA repair	2.29	3.54	E
YCR066W	RAD18	DNA repair	2.55	2.61	30
YJR069C	HAM1	DNA repair	9.17	8.81	0
YHR120W	MSH1	DNA repair	2.18	2.20	0
YGR180C	RNR4	DNA replication	3.14	3.06	9
YIL066C	RNR3	DNA replication	4.18	6.16	0
YNL273W	TOF1	DNA topological change	4.95	6.19	15
YLR274W	CDC46	DNA unwinding	4.83	5.30	E
YBL023C	MCM2	DNA unwinding	2.88	2.77	E
YGL251C	HFM1	DNA unwinding	2.58	2.27	0
YOR067C	ALG8	dolichol-linked oligosaccharide biosynthesis	2.65	2.57	0
YNR030W	ECM39	dolichol-linked oligosaccharide biosynthesis	4.31	4.29	0
YBL082C	RHK1	dolichol-linked oligosaccharide biosynthesis	3.81	3.13	0
YOR074C_ex1	CDC21	dTMP biosynthesis	2.50	2.45	0
YOR074C_ex2	CDC21	dTMP biosynthesis	6.45	6.30	0
YNL111C	CYB5	electron transport	2.69	2.33	4
YJL204C	RCY1	endocytosis	2.16	2.18	15
YNR075W_0	COS10	endocytosis	3.33	2.53	0
YNR075W_1	COS10	endocytosis	5.25	3.79	0
YBR080C	SEC18	ER to Golgi transport	2.55	2.58	E
YDL195W	SEC31	ER to Golgi transport	2.12	2.08	E
YDR407C	TRS120	ER to Golgi transport	2.31	2.27	E
YBR254C	TRS20	ER to Golgi transport	2.46	2.59	E
YNL263C	YIP1	ER to Golgi transport	2.40	2.00	E
YCL001W	RER1	ER to Golgi transport	2.18	2.02	4
YAL007C	ERP2	ER to Golgi transport	6.14	7.08	0
YOR216C	RUD3	ER to Golgi transport	3.45	3.89	0
YCR067C	SED4	ER to Golgi transport	5.04	4.16	0
YOR115C	TRS33	ER to Golgi transport	2.93	3.08	0
YNR026C	SEC12	ER-associated protein catabolism	3.75	3.37	0
YGR175C	ERG1	ergosterol biosynthesis	2.17	2.04	E
YPL028W	ERG10	ergosterol biosynthesis	2.59	2.00	E
YML126C	ERG13	ergosterol biosynthesis	5.72	3.82	E
YGR060W	ERG25	ergosterol biosynthesis	5.44	3.76	E
YGL001C	ERG26	ergosterol biosynthesis	3.94	3.30	E
YLR100W	ERG27	ergosterol biosynthesis	3.10	3.07	E
YHR190W	ERG9	ergosterol biosynthesis	2.47	2.57	E

Table 10A ORF	Gene	Function	WT FC	*mgt1* FC	Sens
YER044C	ERG5	ergosterol biosynthesis	3.10	3.13	0
YLR056W	ERG3	ergosterol biosynthesis	3.19	2.51	29
YML008C	ERG6	ergosterol biosynthesis	6.15	6.18	25
YMR202W	ERG2	ergosterol biosynthesis	5.24	3.97	23
YNL280C	ERG24	ergosterol biosynthesis	2.31	2.86	9
YMR208W	ERG12	ergosterol biosynthesis	2.55	2.26	0
YGL012W	ERG4	ergosterol biosynthesis	13.68	13.96	0
YGL019W	CKB1	establishment of cell polarity (sensu Saccharomyces)	2.20	2.26	10
YDL225W	SHS1	establishment of cell polarity (sensu Saccharomyces)	2.63	2.86	0
YLR313C	SPH1	establishment of cell polarity (sensu Saccharomyces)	3.26	2.65	0
YJL196C	ELO1	fatty acid elongation, unsaturated fatty acid	5.69	6.36	0
YGR037C	ACB1	fatty acid metabolism	4.77	4.26	4
YOL158C	ENB1	ferric-enterobactin transport	4.11	2.59	2
YBR160W	CDC28	G1 S transition of mitotic cell cycle	2.92	2.72	E
YGL229C	SAP4	G1 S transition of mitotic cell cycle	2.60	2.28	0
YPR119W	CLB2	G2 M transition of mitotic cell cycle	2.14	2.30	13
YER003C_ex2	PMI40	GDP-mannose biosynthesis	2.71	2.32	0
YLR300W	EXG1	glucan metabolism	2.66	2.57	0
YKL127W	PGM1	glucose 6-phosphate utilization	8.29	7.42	0
YHR183W	GND1	glucose metabolism	10.63	8.44	8
YOL049W	GSH2	glutathione biosynthesis	4.96	5.98	0
YLL060C	GTT2	glutathione metabolism	4.01	3.64	7
YGL084C	GUP1	glycerol transport	2.24	2.28	4
YHR174W	ENO2	glycolysis	4.60	4.67	E
YOL056W	GPM3	glycolysis	4.59	4.18	0
YGR240C	PFK1	glycolysis	2.52	2.49	0
YIL068C	SEC6	Golgi to plasma membrane transport	2.38	2.72	E
YGR261C	APL6	Golgi to vacuole transport	2.70	3.21	0
YNL038W	GPI15	GPI anchor biosynthesis	3.06	4.26	E
YGR216C	GPI1	GPI anchor biosynthesis	3.15	3.17	0
YKL165C	MCD4	GPI anchor biosynthesis	2.47	2.49	0
YDR047W	HEM12	heme biosynthesis	2.74	3.25	E
YOR278W	HEM4	heme biosynthesis	2.47	2.50	0
YMR058W	FET3	high affinity iron ion transport	2.20	2.30	0
YBR248C	HIS7	histidine biosynthesis	3.85	2.99	20
YCL030C	HIS4	histidine biosynthesis	3.16	3.07	4

Table 10A ORF	Gene	Function	WT FC	*mgt1* FC	Sens
YER055C	HIS1	histidine biosynthesis	9.06	7.68	0
YFR025C	HIS2	histidine biosynthesis	4.40	4.52	0
YOR202W	HIS3	histidine biosynthesis	7.82	6.80	0
YIL116W	HIS5	histidine biosynthesis	3.73	3.14	0
YGR191W	HIP1	histidine transport	2.34	2.48	E
YDL236W	PHO13	histone dephosphorylation	5.93	5.83	0
YFL037W	TUB2	homologous chromosome segregation	3.71	3.39	E
YML085C_ex2	TUB1	homologous chromosome segregation	2.88	3.14	0
YML124C_ex2	TUB3	homologous chromosome segregation	4.46	3.88	0
YLR113W	HOG1	hyperosmotic response	2.80	2.72	11
YBR288C	APM3	intracellular protein transport	2.49	2.81	0
YDR093W	DNF2	intracellular protein transport	3.13	3.23	0
YDR093W	DNF2	intracellular protein transport	2.62	2.85	0
YHR108W	GGA2	intracellular protein transport	4.25	4.01	0
YBR210W	NA	intracellular signaling cascade	3.00	2.64	4
YNL123W	NA	intracellular signaling cascade	3.04	2.73	0
YEL022W	GEA2	intra-Golgi transport	2.17	2.28	E
YDL192W	ARF1	intra-Golgi transport	2.54	2.62	0
YOR080W	DIA2	invasive growth	2.78	2.79	24
YIL114C	POR2	ion transport	4.28	3.04	0
YMR177W	MMT1	iron ion homeostasis	4.24	2.85	0
YIR008C	PRI1	lagging strand elongation	2.76	2.87	E
YBR087W	RFC5	leading strand elongation	4.13	3.81	E
YOR108W	NA	leucine biosynthesis	4.40	7.10	0
YBR041W	FAT1	lipid transport	4.15	3.96	5
YIR034C	LYS1	lysine biosynthesis, aminoadipic pathway	3.37	2.99	4
YBR115C	LYS2	lysine biosynthesis, aminoadipic pathway	2.24	2.52	0
YOR079C	ATX2	manganese ion homeostasis	6.39	5.30	0
YKL116C	PRR1	MAPKKK cascade	2.59	2.72	0
YBR073W	RDH54	meiotic recombination	2.74	2.94	12
YHR039C	MSC7	meiotic recombination	2.33	2.37	9
YPR028W_ex1	YOP1	membrane organization and biogenesis	3.02	3.24	0
YPR028W_ex2	YOP1	membrane organization and biogenesis	2.17	2.59	0
YIL123W	SIM1	microtubule cytoskeleton organization and biogenesis	4.87	6.01	0
YHR172W	SPC97	microtubule nucleation	2.38	2.42	E
YPL241C_ex2	CIN2	microtubule-based process	4.77	5.26	0
YIL051C	MMF1	mitochondrial genome maintenance	3.26	3.14	E
YDL198C	YHM1	mitochondrial genome maintenance	13.44	13.70	2

Table 10A ORF	Gene	Function	WT FC	*mgt1* FC	Sens
YCR028C-A_ex1	RIM1	mitochondrial genome maintenance	2.51	2.12	0
YHR024C	MAS2	mitochondrial processing	5.14	5.84	E
YNL070W	TOM7	mitochondrial translocation	2.60	2.09	4
YIL134W	FLX1	mitochondrial transport	2.54	2.44	17
YOR222W	ODC2	mitochondrial transport	2.29	2.33	0
YMR012W	CLU1	mitochondrion organization and biogenesis	6.55	6.11	0
YGR029W	ERV1	mitochondrion organization and biogenesis	2.14	2.02	0
YGL020C	MDM39	mitochondrion organization and biogenesis	3.88	4.44	0
YGL021W	ALK1	mitosis	2.37	2.03	0
YHR129C	ARP1	mitotic anaphase B	2.23	2.08	7
YDL003W	MCD1	mitotic chromosome condensation	3.52	2.89	E
YER031C	SMC2	mitotic chromosome condensation	3.22	4.58	E
YFL008W	SMC1	mitotic chromosome segregation	2.67	2.94	E
YIL026C	IRR1	mitotic sister chromatid cohesion	2.72	3.55	E
YJL074C	SMC3	mitotic sister chromatid cohesion	2.08	2.58	E
YLR212C	TUB4	mitotic spindle assembly (sensu Saccharomyces)	4.09	3.98	E
YOR250C	CLP1	mRNA cleavage	2.34	2.45	E
YBR130C	SHE3	mRNA localization, intracellular	2.85	2.73	0
YFR005C	SAD1	mRNA splicing	3.48	3.74	E
YLR147C	SMD3	mRNA splicing	2.54	2.54	E
YMR125W	STO1	mRNA splicing	2.63	2.61	8
YIR021W	MRS1	mRNA splicing	4.82	6.19	0
YOR046C	DBP5	mRNA-nucleus export	2.22	2.19	E
YML103C	NUP188	mRNA-nucleus export	2.13	2.85	5
YNL253W	NA	mRNA-nucleus export	2.17	2.21	0
YDR395W	SXM1	mRNA-nucleus export	2.55	2.62	0
YHR046C	INM1	myo-inositol metabolism	2.29	2.42	0
YGL067W	NPY1	NADH metabolism	2.69	2.66	0
YDR252W	BTT1	nascent polypeptide association	2.36	2.11	2
YLL002W	RTT109	negative regulation of DNA transposition	2.82	3.24	30
YJR001W	AVT1	neutral amino acid transport	5.63	5.70	0
YER001W	MNN1	N-glycan processing	2.56	3.44	0
YBR243C	ALG7	N-linked glycosylation	4.85	5.48	E
YPR183W	DPM1	N-linked glycosylation	2.96	2.69	E
YOR103C	OST2	N-linked glycosylation	2.26	2.32	E
YGL022W	STT3	N-linked glycosylation	3.06	2.90	E
YGR036C	CAX4	N-linked glycosylation	3.37	3.23	3

Table 10A ORF	Gene	Function	WT FC	*mgt1* FC	Sens
YPL227C	ALG5	N-linked glycosylation	3.72	3.90	0
YGR227W	DIE2	N-linked glycosylation	2.40	2.27	0
YBR205W	KTR3	N-linked glycosylation	2.45	2.37	0
YBR205W	KTR3	N-linked glycosylation	2.22	2.14	0
YGL226C-A_ex2	OST5	N-linked glycosylation	3.19	3.47	0
YIL076W	SEC28	nonselective vesicle coating	4.22	4.25	2
YDR164C	SEC1	nonselective vesicle fusion	2.31	2.21	E
YLR195C	NMT1	N-terminal peptidyl-glycine N-myristoylation	2.45	2.88	0
YOR130C	ORT1	nuclear migration (sensu Saccharomyces)	5.02	5.96	0
YKR091W	SRL3	nucleobase, nucleoside, nucleotide and nucleic acid metabolism	3.14	2.47	2
YOR247W	SRL1	nucleobase, nucleoside, nucleotide and nucleic acid metabolism	3.71	3.95	0
YOR247W	SRL1	nucleobase, nucleoside, nucleotide and nucleic acid metabolism	3.54	4.02	0
YAL022C	FUN26	nucleoside transport	2.76	2.75	0
YDL102W	CDC2	nucleotide-excision repair	2.32	2.44	E
YPR175W	DPB2	nucleotide-excision repair	2.11	2.28	E
YBR088C	POL30	nucleotide-excision repair	4.05	3.42	E
YAR007C	RFA1	nucleotide-excision repair	3.96	4.13	E
YJL173C	RFA3	nucleotide-excision repair	2.12	2.31	E
YNL312W_ex2	RFA2	nucleotide-excision repair	4.69	4.27	0
YLR188W	MDL1	oligopeptide transport	2.29	2.13	3
YDL095W	PMT1	O-linked glycosylation	2.80	3.00	0
YAL023C	PMT2	O-linked glycosylation	3.14	3.29	0
YOR321W	PMT3	O-linked glycosylation	2.32	2.29	0
YDL093W	PMT5	O-linked glycosylation	2.87	2.79	0
YOR241W	MET7	one-carbon compound metabolism	2.12	2.01	2
YIL145C	PAN6	pantothenate biosynthesis	11.57	11.26	2
YBR176W	ECM31	pantothenate biosynthesis	3.92	4.24	0
YLR354C	TAL1	pentose-phosphate shunt	4.17	4.24	0
YDR410C	STE14	peptide pheromone maturation	4.18	3.31	0
YGL115W	SNF4	peroxisome organization and biogenesis	2.77	3.37	0
YNL316C	PHA2	phenylalanine biosynthesis, prephenate pathway	2.75	2.49	0
YAR071W	PHO11	phosphate metabolism	4.80	3.50	E
YML123C	PHO84	phosphate transport	2.27	2.13	0
YLR133W	CKI1	phosphatidylcholine biosynthesis	2.24	2.01	8

Table 10A					
ORF	Gene	Function	WT FC	*mgt1* FC	Sens
YPR113W	PI[illegible]	phosphatidylinositol biosynthesis	3.10	3.03	0
YDR284C	DPP1	phospholipid metabolism	3.64	3.72	7
YOR386W	PHR1	photoreactive repair	2.25	2.53	2
YHR201C	PPX1	polyphosphate metabolism	3.78	4.23	E
YOR281C	PLP2	positive regulation of transcription from Pol II promoter by pheromones	4.59	4.65	E
YDR183W	PLP1	positive regulation of transcription from Pol II promoter by pheromones	2.34	2.32	0
YOR265W	RBL2	post-chaperonin tubulin folding pathway	2.43	2.33	2
YGL246C	RAI1	processing of 27S pre-rRNA	3.67	4.10	0
YER023W	PRO3	proline biosynthesis	4.27	4.09	E
YOR323C	PRO2	proline biosynthesis	3.18	3.15	4
YDL040C	NAT1	protein amino acid acetylation	4.39	4.69	0
YPR073C	LTP1	protein amino acid dephosphorylation	4.06	3.47	0
YJL031C	BET4	protein amino acid geranylgeranylation	3.99	4.99	E
YOL113W	SKM1	protein amino acid phosphorylation	2.76	2.83	5
YNL154C	YCK2	protein amino acid phosphorylation	2.36	2.24	2
YDR507C	GIN4	protein amino acid phosphorylation	3.35	3.48	0
YLL018C	DPS1	protein biosynthesis	2.35	2.32	E
YBL076C	ILS1	protein biosynthesis	3.36	3.71	E
YHL015W	RPS20	protein biosynthesis	3.49	2.82	E
YKR084C	HBS1	protein biosynthesis	2.24	2.08	0
YLR192C	HCR1	protein biosynthesis	2.78	2.65	0
YGR076C	MRPL25	protein biosynthesis	2.37	2.56	0
YCR003W	MRPL32	protein biosynthesis	2.75	3.27	0
YPR047W	MSF1	protein biosynthesis	2.99	4.18	0
YDL082W_ex2	RPL13A	protein biosynthesis	4.10	3.01	0
YKL006W_ex1	RPL14A	protein biosynthesis	3.91	3.49	0
YKL006W_ex1	RPL14A	protein biosynthesis	3.76	3.44	0
YIL133C_ex2	RPL16A	protein biosynthesis	2.75	2.44	0
YNL069C_ex2	RPL16B	protein biosynthesis	3.97	2.88	0
YKL180W_ex1	RPL17A	protein biosynthesis	2.22	2.08	0
YKL180W_ex2	RPL17A	protein biosynthesis	2.14	2.33	0
YJL177W_ex1	RPL17B	protein biosynthesis	3.20	2.34	0
YJL177W_ex2	RPL17B	protein biosynthesis	2.85	2.70	0
YJL177W_ex2	RPL17B	protein biosynthesis	2.43	2.13	0
YOL120C_ex1	RPL18A	protein biosynthesis	3.68	3.34	0
YNL301C_ex1	RPL18B	protein biosynthesis	5.76	4.85	0
YNL301C_ex2	RPL18B	protein biosynthesis	3.84	3.38	0

Table 10A					
ORF	Gene	Function	WT FC	*mgt1* FC	Sens
YPL079W_ex2	RPL21B	protein biosynthesis	3.54	2.51	0
YLR061W_ex2	RPL22A	protein biosynthesis	5.03	4.64	0
YFL034C-A_ex2	RPL22B	protein biosynthesis	2.54	3.49	0
YBL087C_ex2	RPL23A	protein biosynthesis	2.48	2.25	0
YHR010W_ex2	RPL27A	protein biosynthesis	3.07	2.71	0
YDR471W_ex2	RPL27B	protein biosynthesis	2.64	2.18	0
YFR032C-A	RPL29	protein biosynthesis	3.63	3.64	0
YFR031C-A_ex2	RPL2A	protein biosynthesis	3.09	2.69	0
YDL075W_ex1	RPL31A	protein biosynthesis	2.90	2.54	0
YDL075W_ex2	RPL31A	protein biosynthesis	2.17	2.04	0
YLR406C_ex1	RPL31B	protein biosynthesis	2.43	2.23	0
YLR406C_ex2	RPL31B	protein biosynthesis	2.43	2.21	0
YPL143W_ex2	RPL33A	protein biosynthesis	3.13	2.86	0
YER056C-A_ex2	RPL34A	protein biosynthesis	3.38	3.02	0
YLR185W_ex2	RPL37A	protein biosynthesis	2.43	2.38	0
YIL148W_ex2	RPL40A	protein biosynthesis	2.25	2.45	0
YKR094C_ex2	RPL40B	protein biosynthesis	3.16	2.49	0
YKR094C_ex2	RPL40B	protein biosynthesis	2.23	2.56	0
YNL162W_ex2	RPL42A	protein biosynthesis	2.44	2.06	0
YJR094W-A_ex2	RPL43B	protein biosynthesis	2.44	2.32	0
YLR448W_ex2	RPL6B	protein biosynthesis	4.59	3.20	0
YGL076C_ex2	RPL7A	protein biosynthesis	4.64	4.44	0
YGL076C_ex2	RPL7A	protein biosynthesis	4.11	3.18	0
YPL198W_ex2	RPL7B	protein biosynthesis	3.47	3.17	0
YPL198W_ex2	RPL7B	protein biosynthesis	4.38	4.43	0
YPL198W_ex3	RPL7B	protein biosynthesis	2.32	2.11	0
YGR214W_ex1	RPS0A	protein biosynthesis	3.89	2.96	0
YGR214W_ex2	RPS0A	protein biosynthesis	3.27	2.99	0
YLR048W_ex1	RPS0B	protein biosynthesis	4.19	3.07	0
YLR048W_ex2	RPS0B	protein biosynthesis	3.86	3.41	0
YLR048W_ex2	RPS0B	protein biosynthesis	2.97	2.51	0
YOR293W_ex1	RPS10A	protein biosynthesis	2.57	2.03	0
YDR064W_ex2	RPS13	protein biosynthesis	2.90	2.44	0
YCR031C_ex2	RPS14A	protein biosynthesis	2.28	2.19	0
YMR143W_ex2	RPS16A	protein biosynthesis	2.61	3.11	0

Table 10A ORF	Gene	Function	WT FC	*mgt1* FC	Sens
YDL083C_ex2	RPS16B	protein biosynthesis	2.92	2.34	0
YML024W_ex2	RPS17A	protein biosynthesis	2.31	2.15	0
YML026C_ex2	RPS18B	protein biosynthesis	3.15	2.76	0
YNL302C_ex2	RPS19B	protein biosynthesis	3.00	2.45	0
YJL136C_ex2	RPS21B	protein biosynthesis	2.55	2.30	0
YJL136C_ex2	RPS21B	protein biosynthesis	2.03	2.02	0
YLR367W_ex2	RPS22B	protein biosynthesis	6.10	6.72	0
YER074W_ex2	RPS24A	protein biosynthesis	3.76	3.12	0
YHR021C_ex2	RPS27B	protein biosynthesis	2.81	2.46	0
YPL090C_ex2	RPS6A	protein biosynthesis	3.09	2.21	0
YOR096W_ex2	RPS7A	protein biosynthesis	2.73	2.18	0
YBR173C	UMP1	protein catabolism	2.87	2.40	0
YGR105W	VMA21	protein complex assembly	2.30	2.42	11
YOR085W	OST3	protein complex assembly	3.02	2.56	0
YKL119C	VPH2	protein complex assembly	2.39	2.43	0
YFR010W	UBP6	protein deubiquitination	2.39	2.64	2
YDR304C	CPR5	protein folding	2.77	2.92	4
YDR518W	EUG1	protein folding	2.61	2.57	0
YOL088C	MPD2	protein folding	2.53	2.36	0
YKL117W	SBA1	protein folding	3.07	2.79	0
YMR214W	SCJ1	protein folding	2.83	2.46	0
YHR109W	CTM1	protein modification	2.10	2.55	0
YMR275C	BUL1	protein monoubiquitination	3.80	3.24	7
YLR024C	UBR2	protein monoubiquitination	2.22	2.15	0
YGR082W	TOM20	protein targeting	3.00	3.63	E
YDR414C	ERD1	protein-ER retention	2.89	2.47	9
YBL040C_ex2	ERD2	protein-ER retention	2.11	2.10	0
YDR292C	SRP101	protein-ER targeting	2.37	2.52	E
YPL243W	SRP68	protein-ER targeting	3.31	3.37	E
YPL210C	SRP72	protein-ER targeting	2.15	2.38	E
YBL069W	AST1	protein-membrane targeting	2.28	2.04	0
YBL069W_alt	AST1	protein-membrane targeting	2.04	2.54	0
YGL016W	KAP122	protein-nucleus import	5.27	4.29	10
YGL241W	KAP114	protein-nucleus import	2.58	3.11	0
YNL246W_ex2	VPS75	protein-vacuolar targeting	4.29	4.12	0
YDR144C	MKC7	proteolysis and peptidolysis	4.47	3.13	10
YBL022C	PIM1	proteolysis and peptidolysis	2.15	2.10	0
YCL057W	PRD1	proteolysis and peptidolysis	2.65	2.31	0

Table 10A ORF	Gene	Function	WT FC	*mgt1* FC	Sens
YIL049W	DFG10	pseudohyphal growth	3.04	3.28	0
YJR147W	HMS2	pseudohyphal growth	3.13	3.43	0
YIR019C	MUC1	pseudohyphal growth	2.44	2.72	0
YJR105W	ADO1	purine base metabolism	2.50	2.20	2
YLR209C	PNP1	purine nucleoside catabolism	3.71	3.57	0
YGL224C	SDT1	pyrimidine base metabolism	5.47	5.52	0
YPL256C	CLN2	regulation of CDK activity	2.86	3.11	8
YGR108W	CLB1	regulation of CDK activity	3.76	7.01	0
YLR103C	CDC45	regulation of cell cycle	4.31	4.14	E
YDR353W	TRR1	regulation of redox homeostasis	4.62	4.17	E
YIL010W	DOT5	regulation of redox homeostasis	3.81	3.39	6
YPR052C	NHP6A	regulation of transcription from Pol II promoter	2.40	2.19	0
YHL009C	YAP3	regulation of transcription from Pol II promoter	2.43	2.49	0
YFL028C	CAF16	regulation of transcription, DNA-dependent	4.60	5.31	2
YGL030W_ex2	RPL30	regulation of translation	2.19	2.23	0
YGL195W	GCN1	regulation of translational elongation	3.72	3.72	6
YFR009W	GCN20	regulation of translational elongation	2.25	2.88	4
YBR048W_ex2	RPS11B	regulation of translational fidelity	2.91	2.54	0
YGR118W_ex1	RPS23A	regulation of translational fidelity	3.96	3.96	0
YPL081W_ex2	RPS9A	regulation of translational fidelity	3.06	2.34	0
YPL081W_ex2	RPS9A	regulation of translational fidelity	2.78	2.38	0
YBR189W_ex2	RPS9B	regulation of translational fidelity	4.52	4.58	0
YBR189W_ex2	RPS9B	regulation of translational fidelity	3.48	3.32	0
YOL090W	MSH2	removal of nonhomologous ends	6.66	8.13	5
YPL163C	SVS1	response to chemical substance	3.19	3.68	7
YDL166C	FAP7	response to oxidative stress	3.90	3.77	E
YBR244W	GPX2	response to oxidative stress	4.18	4.50	15
YFL026W	STE2	response to pheromone during conjugation with cellular fusion	3.05	4.44	4
YHR043C	DOG2	response to stress	3.22	2.98	0
YER011W	TIR1	response to stress	5.61	5.06	0
YGR234W	YHB1	response to stress	7.20	8.04	0
YCR044C	PER1	response to unfolded protein	5.00	3.72	0
YER089C	PTC2	response to unfolded protein	2.71	2.57	0
YDL145C	COP1	retrograde (Golgi to ER) transport	3.94	3.62	E
YER051C	RET2	retrograde (Golgi to ER) transport	3.30	2.89	E
YPL010W	RET3	retrograde (Golgi to ER) transport	3.65	3.61	E

Table 10A					
ORF	Gene	Function	WT FC	*mgt1* FC	Sens
YGL137W_ex2	SEC21	retrograde (Golgi-to-ER) transport	2.12	2.09	E
YHL034C	SBP1	RNA metabolism	2.41	2.02	0
YDL014W	NOP1	RNA methylation	2.74	2.65	E
YFL001W	DEG1	RNA processing	3.04	2.85	12
YLR059C	REX2	RNA processing	2.51	2.15	5
YGR276C	RNH70	RNA processing	2.41	2.67	0
YJL133W	MRS3	RNA splicing	2.30	2.97	0
YML031W	NDC1	RNA-nucleus export	2.21	2.11	E
YHR062C	RPP1	rRNA processing	2.41	2.02	E
YOL080C	REX4	rRNA processing	2.49	2.69	4
YMR285C	NGL2	rRNA processing	2.34	2.39	2
YLR306W_ex2	UBC12	RUB1-protein conjugation	2.22	2.72	0
YER019W	ISC1	salinity response	3.11	2.76	10
YDL018C	ERP3	secretory pathway	3.62	4.07	0
YIL074C	SER33	serine family amino acid biosynthesis	3.96	2.84	2
YGR208W	SER2	serine family amino acid biosynthesis	3.91	2.43	0
YIR022W	SEC11	signal peptide processing	3.06	3.22	E
YLR066W	SPC3	signal peptide processing	2.62	2.51	E
YML055W	SPC2	signal peptide processing	2.30	2.30	6
YFR027W	ECO1	sister chromatid cohesion	2.85	3.06	E
YPR069C	SPE3	spermidine biosynthesis	3.08	2.79	0
YDL052C	SLC1	sphingolipid biosynthesis	2.70	2.45	4
YOR198C	BFR1	spindle assembly	4.18	3.93	0
YDL043C	PRP11	spliceosome assembly	3.21	2.70	E
YDR260C	SWM1	spore wall assembly (sensu Saccharomyces)	2.59	2.12	9
YCR002C	CDC10	spore wall assembly (sensu Saccharomyces)	3.82	4.35	0
YER012W	PRE1	sporulation (sensu Saccharomyces)	2.85	2.52	E
YGR253C	PUP2	sporulation (sensu Saccharomyces)	2.46	2.46	E
YJL001W_ex2	PRE3	sporulation (sensu Saccharomyces)	2.67	2.86	0
YML052W	SUR7	sporulation (sensu Saccharomyces)	2.46	2.75	0
YML105C	SEC65	SRP-dependent cotranslational membrane targeting, signal sequence recognition	2.07	2.22	E
YOR254C	SEC63	SRP-dependent cotranslational membrane targeting, translocation	2.73	3.21	E
YBR171W	SEC66	SRP-dependent cotranslational membrane targeting, translocation	3.02	2.79	0
YGR177C	ATF2	steroid metabolism	3.06	2.20	0
YOL064C	MET22	sulfate assimilation	3.21	2.58	6
YJR137C	ECM17	sulfate assimilation	3.64	2.14	0

Table 10A					
ORF	Gene	Function	WT FC	*mgt1* FC	Sens
YJL212C	OPT1	sulfur metabolism	2.75	3.57	0
YBR275C	RIF1	telomerase-dependent telomere maintenance	2.13	2.28	8
YIL009C-A_ex1	EST3	telomerase-dependent telomere maintenance	2.69	2.92	0
YIL009C-A_ex2	EST3	telomerase-dependent telomere maintenance	2.86	3.77	0
TLC1_0	TLC1	telomerase-dependent telomere maintenance	2.87	4.91	0
TLC1_1	TLC1	telomerase-dependent telomere maintenance	3.28	6.08	0
YOR143C	THI80	thiamin biosynthesis	2.10	2.28	E
YBR092C	PHO3	thiamin transport	5.53	10.27	5
YER052C	HOM3	threonine metabolism	9.09	8.58	12
YDR158W	HOM2	threonine metabolism	8.15	7.21	0
YKL194C	MST1	threonyl-tRNA aminoacylation	2.49	2.60	0
YOR340C	RPA43	transcription from Pol I promoter	2.98	3.08	E
YDR308C	SRB7	transcription from Pol II promoter	2.75	2.67	E
YIL128W	MET18	transcription from Pol II promoter	2.20	2.28	18
YNL236W	SIN4	transcription from Pol II promoter	3.54	3.20	8
YHR041C_ex2	SRB2	transcription from Pol II promoter	4.39	3.64	0
YDR045C	RPC11	transcription from Pol III promoter	2.87	2.03	E
YCR065W	HCM1	transcription initiation from Pol II promoter	3.10	2.20	0
YPL007C	TFC8	transcription initiation from Pol III promoter	2.04	2.61	E
YDL130W_ex1	RPP1B	translational elongation	2.66	2.49	0
YDL130W_ex2	RPP1B	translational elongation	2.33	2.03	0
YKL081W_ex1	TEF4	translational elongation	5.17	3.23	0
YKL081W_ex2	TEF4	translational elongation	4.53	3.40	0
YJR047C	ANB1	translational initiation	2.82	2.07	0
YOR306C	MCH5	transport	2.41	2.15	7
YPR058W	YMC1	transport	7.15	6.17	7
YPR021C	NA	transport	2.38	2.22	5
YOL119C	MCH4	transport	2.85	2.76	4
YBR104W	YMC2	transport	7.03	9.14	4
YBL089W	AVT5	transport	2.13	2.11	0
YMR261C	TPS3	trehalose biosynthesis	2.57	2.66	0
YDL112W	TRM3	tRNA methylation	2.01	2.30	0
YHR163W	SOL3	tRNA processing	4.82	3.30	0
YER107C	GLE2	tRNA-nucleus export	2.68	2.45	E

Table 10A					
ORF	Gene	Function	WT FC	*mgt1* FC	Sens
YGL092W	NAM8	tRNA-nucleus export	4.18	4.17	E
YDR354W	TRP4	tryptophan biosynthesis	5.05	5.65	12
YKL211C	TRP3	tryptophan biosynthesis	5.00	5.19	2
YGL026C	TRP5	tryptophan biosynthesis	4.26	4.33	0
YDR268W	MSW1	tryptophanyl-tRNA aminoacylation	3.10	3.89	0
YML094W_ex2	GIM5	tubulin folding	2.91	2.68	0
YBR166C	TYR1	tyrosine metabolism	4.44	5.32	3
YBR003W	COQ1	ubiquinone metabolism	2.09	2.34	4
YNR041C	COQ2	ubiquinone metabolism	4.13	4.29	0
YKL210W	UBA1	ubiquitin cycle	2.18	2.24	E
YML092C	PRE8	ubiquitin-dependent protein catabolism	3.37	2.68	E
YHR027C	RPN1	ubiquitin-dependent protein catabolism	2.41	2.20	E
YIL075C	RPN2	ubiquitin-dependent protein catabolism	2.04	2.28	E
YPR108W	RPN7	ubiquitin-dependent protein catabolism	2.33	2.06	E
YDR427W	RPN9	ubiquitin-dependent protein catabolism	2.72	2.88	E
YKL145W	RPT1	ubiquitin-dependent protein catabolism	2.49	2.52	E
YOR117W	RPT5	ubiquitin-dependent protein catabolism	2.53	2.54	E
YGL011C	SCL1	ubiquitin-dependent protein catabolism	2.31	2.20	E
YKL213C	DOA1	ubiquitin-dependent protein catabolism	3.46	4.27	30
YGR135W	PRE9	ubiquitin-dependent protein catabolism	3.38	3.27	0
YDL190C	UFD2	ubiquitin-dependent protein catabolism	2.05	2.20	0
YPL244C	HUT1	UDP-galactose transport	3.80	3.86	0
YDL103C	QRI1	UDP-N-acetylglucosamine biosynthesis	10.89	10.81	E
YEL051W	VMA8	vacuolar acidification	2.52	2.82	18
YGR020C	VMA7	vacuolar acidification	3.53	3.82	7
YLR043C	TRX1	vacuole fusion (non-autophagic)	2.47	2.71	8
YPL019C	VTC3	vacuole fusion (non-autophagic)	2.59	2.69	0
YJL012C	VTC4	vacuole fusion (non-autophagic)	2.51	2.16	0
YBR164C	ARL1	vesicle-mediated transport	2.40	2.27	7
YEL053C	MAK10	virus-host interaction	2.35	2.42	4
YBR256C	RIB5	vitamin B2 biosynthesis	2.11	2.20	E
YHR063C	PAN5	NA	4.40	5.59	E
YPR048W	TAH18	NA	2.16	2.14	E
YHR047C	AAP1'	NA	3.74	3.90	10
YIL040W	APQ12	NA	2.11	2.30	9
YOL002C	PHO36	NA	4.98	4.25	7
YHR117W	TOM71	NA	2.17	2.45	7
YER170W	ADK2	NA	2.29	2.18	6
YIL027C	KRE27	NA	2.26	2.44	5

Table 10A					
ORF	Gene	Function	WT FC	*mgt1* FC	Sens
YDR261C	EXG2	NA	4.49	4.45	4
YOL030W	GAS5	NA	2.40	2.63	4
YJR118C	ILM1	NA	2.48	2.47	4
YLR315W	NKP2	NA	2.92	2.29	4
YPL246C	QUT1	NA	2.60	2.58	4
YOL093W	TRM10	NA	2.63	2.83	4
YKR013W	PRY2	NA	4.34	4.89	2
YDR257C	RMS1	NA	3.18	2.64	2
YKL218C	SRY1	NA	2.51	2.16	2
YDL100C	ARR4	NA	2.78	2.50	0
YMR116C_ex1	ASC1	NA	4.12	2.77	0
YMR116C_ex2	ASC1	NA	3.93	3.13	0
YGR295C_0	COS6	NA	2.01	2.05	0
YER060W	FCY21	NA	2.75	2.66	0
YOR280C	FSH3	NA	2.73	2.56	0
YER057C	HMF1	NA	2.34	2.50	0
YML056C_ex1	IMD4	NA	6.74	5.32	0
YML056C_ex2	IMD4	NA	7.17	7.75	0
YFR024C-A_ex2	LSB3	NA	3.21	2.42	0
YDR033W	MRH1	NA	3.38	4.38	0
YIL164C	NIT1	NA	3.13	2.40	0
YLR351C	NIT3	NA	2.71	2.44	0
YGR038W	ORM1	NA	2.70	2.24	0
YHR179W	OYE2	NA	3.91	4.09	0
YBR233W	PBP2	NA	3.15	2.78	0
YMR123W	PKR1	NA	2.68	2.53	0
YDR501W	PLM2	NA	4.25	4.42	0
YKL128C	PMU1	NA	7.18	6.15	0
YDL189W_ex2	RBS1	NA	2.45	2.62	0
YIL011W	TIR3	NA	3.42	3.37	0
YER175C	TMT1	NA	3.28	4.62	0
YGR221C	TOS2	NA	2.20	2.35	0
YLR467W_2	YRF1-5	NA	2.88	2.37	0
YHR017W	YSC83	NA	2.59	2.76	0
YNR065C	YSN1	NA	3.01	2.30	0

Table 10B: ESR subset of genes that are induced in both WT and *mgt1*. 127 ESR genes that are induced upon treatment with MNNG in both WT and *mgt1*. Interestingly, 57 (44%) of these are essential genes.

Table 10B					
ORF	Gene	Function	WT FC	*mgt1* FC	Sensitivity
YGR264C	MES1	amino acid activation	2.82	3.48	E
YDR023W	SES1	amino acid activation	3.11	3.23	E
YGR185C	TYS1	amino acid activation	9.65	9.35	E
YDR321W	ASP1	amino acid metabolism	3.06	2.74	2
YML022W	APT1	AMP biosynthesis	4.92	4.10	15
YLR150W	STM1	anti-apoptosis	2.93	2.76	11
YGL148W	ARO2	aromatic amino acid family biosynthesis	6.87	6.59	2
YBR249C	ARO4	aromatic amino acid family biosynthesis	11.26	9.64	4
YGR124W	ASN2	asparagine biosynthesis	3.65	3.21	5
YHR019C	DED81	asparaginyl-tRNA aminoacylation	5.99	5.86	E
YML060W	OGG1	base-excision repair, AP site formation	4.85	4.95	0
YEL040W	UTR2	cell wall organization and biogenesis	11.35	8.29	0
YMR307W	GAS1	cell wall organization and biogenesis	5.71	6.11	4
YMR212C	EFR3	cellular morphogenesis	3.52	3.89	E
YDL143W	CCT4	cytoskeleton organization and biogenesis	3.74	3.30	E
YNL102W	POL1	DNA repair synthesis	2.75	3.26	E
YER070W	RNR1	DNA replication	23.03	22.30	5
YHR042W	NCP1	ergosterol biosynthesis	3.01	2.49	0
YLR372W	SUR4	fatty acid biosynthesis	12.68	11.59	0
YDR454C	GUK1	GMP metabolism	3.15	3.19	0
YHR216W	IMD2	GTP biosynthesis	3.20	2.91	E
YBL068W	PRS4	histidine biosynthesis	2.69	2.77	0
YPR033C	HTS1	histidyl-tRNA aminoacylation	4.99	4.96	E
YHR068W	DYS1	hypusine biosynthesis from peptidyl-lysine	5.46	4.39	E
YNR043W	MVD1	isoprenoid biosynthesis	5.10	3.46	E
YGL120C	PRP43	lariat formation, 5'-splice site cleavage	2.23	2.30	E
YPL160W	CDC60	leucyl-tRNA aminoacylation	3.17	3.34	E
YDR037W	KRS1	lysyl-tRNA aminoacylation	4.74	5.21	E
YPR145W	ASN1	metabolism	8.31	7.62	0
YER043C	SAH1	methionine metabolism	2.76	2.23	E
YNL066W	SUN4	mitochondrion organization and biogenesis	4.04	3.29	0
YGL213C	SKI8	mRNA catabolism	2.14	2.68	0
YJL080C	SCP160	mRNA localization, intracellular	8.14	7.26	10
YMR308C	PSE1	mRNA-nucleus export	2.26	2.59	E
YJR025C	BNA1	nicotinamide adenine dinucleotide biosynthesis	3.79	4.79	2
YGR019W	UGA1	nitrogen utilization	2.49	2.86	0
YGL225W	VRG4	N-linked glycosylation	3.10	2.72	E
YBR084W	MIS1	nucleobase, nucleoside, nucleotide and nucleic acid metabolism	6.28	6.61	7
YJR143C	PMT4	O-linked glycosylation	5.56	5.32	E
YPR074C	TKL1	pentose-phosphate shunt	5.55	4.13	7
YLR060W	FRS1	phenylalanyl-tRNA aminoacylation	7.34	6.62	E
YFL022C	FRS2	phenylalanyl-tRNA aminoacylation	2.87	3.16	E
YDR324C	UTP4	processing of 20S pre-rRNA	2.16	2.41	E
YGR128C	UTP8	processing of 20S pre-rRNA	2.22	2.25	E
YDR300C	PRO1	proline biosynthesis	2.27	2.22	0
YHR013C	ARD1	protein amino acid acetylation	4.93	5.44	22
YEL042W	GDA1	protein amino acid glycosylation	2.37	2.22	0
YGR123C	PPT1	protein amino acid phosphorylation	3.39	2.78	9
YPR102C	RPL11A	protein biosynthesis	2.20	2.03	E
YLR029C	RPL15A	protein biosynthesis	2.49	2.02	E
YBL092W	RPL32	protein biosynthesis	2.59	2.30	E
YOL040C	RPS15	protein biosynthesis	2.86	2.26	E
YGL189C	RPS26A	protein biosynthesis	2.23	2.09	E
YJR123W	RPS5	protein biosynthesis	3.06	2.33	E
YIL078W	THS1	protein biosynthesis	6.15	6.39	E
YGR085C	RPL11B	protein biosynthesis	2.16	2.49	0
YEL054C	RPL12A	protein biosynthesis	2.99	2.02	0
YMR242C	RPL20A	protein biosynthesis	2.71	2.32	0
YGL031C	RPL24A	protein biosynthesis	2.34	2.25	0
YHL033C	RPL8A	protein biosynthesis	3.17	2.64	0
YLL045C	RPL8B	protein biosynthesis	2.37	2.14	0
YLL045C	RPL8B	protein biosynthesis	3.62	3.37	0

Table 10B					
YGL147C	RPL9A	protein biosynthesis	2.90	3.37	0
YLR441C	RPS1A	protein biosynthesis	3.79	3.06	0
YLR441C	RPS1A	protein biosynthesis	2.73	2.54	0
YML063W	RPS1B	protein biosynthesis	3.23	3.08	0
YER131W	RPS26B	protein biosynthesis	3.48	3.43	0
YOR167C	RPS28A	protein biosynthesis	2.88	2.67	0
YLR388W	RPS29A	protein biosynthesis	2.23	2.35	0
YDR418W	RPL12B	protein biosynthesis	4.77	3.87	5
YDR418W	RPL12B	protein biosynthesis	4.35	3.84	5
YJL190C	RPS22A	protein biosynthesis	5.95	5.15	7
YGR148C	RPL24B	protein biosynthesis	2.67	2.33	8
YHR052W	CIC1	protein catabolism	2.45	2.41	E
YGR285C	ZUO1	protein folding	4.50	4.47	2
YKL154W	SRP102	protein-ER targeting	2.50	2.60	E
YER009W	NTF2	protein-nucleus import	2.65	2.31	E
YER118C	SHO1	pseudohyphal growth	2.39	2.68	0
YOR243C	PUS7	pseudouridine synthesis	3.95	3.85	0
YBR252W	DUT1	pyrimidine deoxyribonucleoside triphosphate catabolism	3.33	3.34	E
YHR144C	DCD1	pyrimidine nucleotide metabolism	4.93	4.62	E
YHR128W	FUR1	pyrimidine salvage	3.32	2.55	E
YGL008C	PMA1	regulation of pH	2.53	2.15	E
YDR453C	TSA2	regulation of redox homeostasis	5.84	9.75	0
YGL123W	RPS2	regulation of translational fidelity	2.14	2.05	E
YLR276C	DBP9	ribosomal large subunit assembly and maintenance	2.48	2.38	E
YIR012W	SQT1	ribosomal large subunit assembly and maintenance	2.36	2.09	E
YGL111W	NSA1	ribosomal large subunit biogenesis	2.09	2.24	E
YLR186W	EMG1	ribosome biogenesis	3.12	3.14	E
YMR131C	RRB1	ribosome biogenesis	2.50	2.61	E
YHR089C	GAR1	rRNA modification	4.92	4.30	E
YOR310C	NOP58	rRNA modification	3.01	2.53	E
YLR197W	SIK1	rRNA modification	3.40	4.00	E
YPL211W	NIP7	rRNA processing	2.35	2.05	E

Table 10B					
YDR087C	RRP1	rRNA processing	2.80	2.78	E
YGL097W	SRM1	rRNA-nucleus export	3.74	3.48	E
YGR152C	RSR1	small GTPase mediated signal transduction	2.78	2.54	0
YLR146C	SPE4	spermine biosynthesis	3.78	3.88	0
YCR034W	FEN1	sphingolipid biosynthesis	7.58	7.23	0
YIR026C	YVH1	sporulation (sensu Saccharomyces)	4.16	3.78	0
YPL273W	SAM4	sulfur amino acid metabolism	23.63	20.90	0
YHR025W	THR1	threonine metabolism	8.31	8.29	12
YCR053W	THR4	threonine metabolism	3.72	3.54	13
YPR010C	RPA135	transcription from Pol I promoter	2.50	2.56	E
YNL248C	RPA49	transcription from Pol I promoter	4.70	4.37	0
YIL021W	RPB3	transcription from Pol II promoter	2.10	2.24	E
YGL070C	RPB9	transcription from Pol II promoter	2.59	2.31	19
YBR121C	GRS1	transcription termination	3.17	3.05	6
YLR340W	RPP0	translational elongation	2.26	2.14	E
YOL039W	RPP2A	translational elongation	2.81	2.35	4
YDR429C	TIF35	translational initiation	2.37	2.05	E
YKR026C	GCN3	translational initiation	2.88	2.99	6
YBR143C	SUP45	translational termination	2.39	2.36	E
YLR083C	EMP70	transport	3.42	3.10	0
YNL292W	PUS4	tRNA modification	3.53	3.02	0
YOL097C	WRS1	tryptophanyl-tRNA aminoacylation	4.82	4.35	E
YNL153C	GIM3	tubulin folding	3.77	3.54	6
YHR026W	PPA1	vacuolar acidification	2.48	2.47	4
YKL080W	VMA5	vacuolar acidification	2.78	2.54	15
YKL103C	LAP4	vacuolar protein catabolism	2.55	2.74	0
YGR094W	VAS1	valyl-tRNA aminoacylation	4.57	4.47	E
YLR196W	SWP1	NA	2.13	2.15	E
YDR091C	RLI1	NA	3.23	2.49	E
YDR516C	EMI2	NA	2.40	2.37	0
YLR449W	FPR4	NA	3.52	3.56	0
YMR215W	GAS3	NA	5.52	7.08	0
YNL175C	NOP13	NA	2.69	2.72	2

Table 11: Genes repressed in both WT and *mgt1* upon MNNG treatment.

A total of 1039 genes were repressed in both WT and *mgt1*. Of them, 545 had a known function and are shown here. Table 11A includes genes that were repressed in both WT and *mgt1* and are not a part of the ESR. These included 75 genes (13%) that were essential. The largest category of genes that were affected was involved in transcription and its regulation. About 43 genes (7%) of the genes belonged to this category.
Table 11B includes the ESR subset of genes that are repressed in both WT and *mgt1*

Table 11A ORF	Gene	Function	WT FC	*mgtFC*	Sensitivity
YAL054C	ACS1	acetyl-CoA biosynthesis	-18.63	-25.51	4
YBL015W	ACH1	acetyl-CoA metabolism	-6.48	-7.72	0
YDR208W	MSS4	actin cable assembly	-2.76	-2.69	E
YNL020C	ARK1	actin filament organization	-3.04	-3.05	0
YJL100W	LSB6	actin filament organization	-2.63	-2.42	0
YGL191W	COX13	aerobic respiration	-3.85	-3.98	E
YGL187C	COX4	aerobic respiration	-2.14	-2.17	E
YEL024W	RIP1	aerobic respiration	-2.88	-4.04	4
YMR256C	COX7	aerobic respiration	-2.62	-2.41	4
YNL052W	COX5A	aerobic respiration	-3.30	-5.35	0
YMR188C	MRPS17	aerobic respiration	-5.42	-3.81	0
YDR529C	QCR7	aerobic respiration	-2.20	-2.26	0
YFR033C	QCR6	aerobic respiration	-4.14	-4.16	0
YBL045C	COR1	aerobic respiration	-2.90	-2.56	0
YJL166W	QCR8	aerobic respiration	-2.59	-2.39	0
YDL174C	DLD1	aerobic respiration	-3.41	-3.06	0
YOL025W	LAG2	aging	-2.61	-2.43	0
YER024W	YAT2	alcohol metabolism	-8.95	-7.88	0
YAR035W	YAT1	alcohol metabolism	-27.04	-30.28	0
YJR155W	AAD10	aldehyde metabolism	-4.41	-5.53	0

Table 11A ORF	Gene	Function	WT FC	*mgt FC*	sitivity
YHR033W	NA	amino acid biosynthesis	-56.64	-43.69	0
YHR139C	SPS100	amino acid metabolism	-76.93	-53.29	0
YHL036W	MUP3	amino acid transport	-3.91	-7.01	4
YGR121C	MEP1	ammonium transport	-10.74	-11.93	0
YOR303W	CPA1	arginine biosynthesis	-2.75	-2.64	0
YLR438W	CAR2	arginine catabolism	-8.23	-8.52	0
YPL111W	CAR1	arginine catabolism to ornithine	-10.28	-10.03	0
YMR042W	ARG80	arginine metabolism	-2.22	-2.72	0
YMR056C	AAC1	ATP ADP exchange	-5.35	-6.24	0
YML081C-A	ATP18	ATP synthesis coupled proton transport	-2.83	-2.73	2
YDL181W	INH1	ATP synthesis coupled proton transport	-14.10	-10.92	0
YDL149W	APG9	autophagic vacuole formation	-3.16	-3.37	0
YPL149W	APG5	autophagy	-2.03	-2.03	4
YDL049C	KNH1	beta-1,6 glucan biosynthesis	-4.10	-6.54	0
YCR047C	BUD23	bud site selection	-2.18	-2.25	8
YOR360C	PDE2	cAMP-mediated signaling	-5.92	-5.59	9
YDR006C	SOK1	cAMP-mediated signaling	-6.08	-3.82	0
YER062C	HOR2	carbohydrate metabolism	-2.69	-2.92	0
YOR040W	GLO4	carbohydrate metabolism	-5.67	-5.64	0
YCR008W	SAT4	cation homeostasis	-4.12	-3.81	0
YIL046W	MET30	cell cycle	-6.31	7.33	E
YGR003W	NA	cell cycle	-2.08	-2.51	0
YMR052W	FAR3	cell cycle arrest in response to pheromone	-3.29	-3.66	0
YBR050C	REG2	cell growth and or maintenance	-3.81	-3.35	0
YNL322C	KRE1	cell wall organization and biogenesis	-2.55	-2.56	4
YIL146C	ECM37	cell wall organization and biogenesis	-2.40	-2.67	4
YBR065C	ECM2	cell wall organization and biogenesis	-7.74	-8.16	0
YJR106W	ECM27	cell wall organization and biogenesis	-2.72	-2.42	0
YBL043W	ECM13	cell wall organization and biogenesis	-29.31	-27.13	0
YDL047W	SIT4	cell wall organization and biogenesis	-2.84	-3.43	0
YKL163W	PIR3	cell wall organization and biogenesis	-6.57	-7.73	0
YDR528W	HLR1	cell wall organization and biogenesis	-2.55	-3.55	0
YPR030W	CSR2	cell wall organization and biogenesis	-13.47	-14.70	0
YGR032W	GSC2	cell wall organization and biogenesis	-7.23	-3.56	0

Table 11A ORF	Gene	Function	WT FC	*mgtFC*	Sensitivity
YOR092W	ECM3	cell wall organization and biogenesis	-7.65	-5.87	0
YHR102W	KIC1	cellular morphogenesis	-2.21	-2.27	E
YDL223C	HBT1	cellular morphogenesis during conjugation with cellular fusion	-11.21	-12.92	0
YMR140W	SIP5	cellular response to glucose starvation	-2.26	-2.91	6
YFR034C	PHO4	cellular response to phosphate starvation	-4.31	-5.60	0
YJL156C	SSY5	chemosensory perception	-2.23	-2.13	E
YFR029W	PTR3	chemosensory perception	-2.79	-2.97	0
YER096W	SHC1	chitin biosynthesis	-2.75	-3.74	0
YBR010W	HHT1	chromatin assembly disassembly	-2.08	-2.33	0
YOR191W	RIS1	chromatin assembly disassembly	-2.21	-2.07	0
YHL025W	SNF6	chromatin modeling	-2.04	-2.07	7
YMR044W	IOC4	chromatin modeling	-2.55	-2.50	0
YHR090C	YNG2	chromatin modification	-2.38	-2.51	E
YOR064C	YNG1	chromatin modification	-7.96	-7.73	0
YKL112W	ABF1	chromatin silencing at HML and HMR (sensu Saccharomyces)	-6.06	-5.64	E
YKR101W	SIR1	chromatin silencing at HML and HMR (sensu Saccharomyces)	-3.96	-3.90	4
YMR127C	SAS2	chromatin silencing at telomere	-2.43	-2.42	0
YOR156C	NFI1	chromosome condensation	-3.31	-3.48	0
YJR089W	BIR1	chromosome segregation	-2.46	-2.39	E
YHR002W	LEU5	coenzyme A transport	-2.79	-2.68	0
YER068W	MOT2	conjugation with cellular fusion	-2.28	-2.36	19
YJL108C	PRM10	conjugation with cellular fusion	-10.11	-13.28	0
YPL156C	PRM4	conjugation with cellular fusion	-5.08	-4.68	0
YBR295W	PCA1	copper ion homeostasis	-2.63	-3.63	0
YLR038C	COX12	cytochrome c oxidase biogenesis	-2.33	-2.37	0
YAL039C	CYC3	cytochrome c-heme linkage	-3.15	-2.67	4
YFR023W	PES4	DNA dependent DNA replication	-2.23	-2.58	0
YCR010C	ADY2	DNA metabolism	-36.50	-44.54	0
YER116C	SLX8	DNA recombination	-4.19	-3.13	11
YDL013W	HEX3	DNA recombination	-3.80	-3.34	8
YKL032C	IXR1	DNA repair	-4.21	-4.35	5
YLR007W	NSE1	DNA repair	-2.52	-2.51	0

Table 11A ORF	Gene	Function	WT FC	*mgt FC*	sitivity
YHR031C	RRM3	DNA replication	-3.32	-2.88	17
YJR046W	TAH11	DNA replication licensing	-4.95	-7.41	E
YMR106C	YKU80	double-strand break repair via nonhomologous end-joining	-3.44	-3.19	5
YNL133C	FYV6	double-strand break repair via nonhomologous end-joining	-2.97	-3.40	3
YDR227W	SIR4	double-strand break repair via nonhomologous end-joining	-2.28	-2.29	2
YHR081W	LRP1	double-strand break repair via nonhomologous end-joining	-2.46	-2.55	0
YMR272C	SCS7	electron transport	-2.52	-2.15	19
YML054C	CYB2	electron transport	-14.86	-14.16	0
YOR089C	VPS21	endocytosis	-2.52	-2.41	8
YNL093W	YPT53	endocytosis	-14.58	-13.05	0
YDR207C	UME6	entry into meiosis	-2.83	-3.21	19
YKR068C	BET3	ER to Golgi transport	-2.33	-2.92	E
YOL013C	HRD1	ER-associated protein catabolism	-2.39	-2.19	4
YER114C	BOI2	establishment of cell polarity (sensu Saccharomyces)	-2.14	-2.22	0
YDL085W	NDE2	ethanol fermentation	-23.45	-17.83	0
YMR303C	ADH2	ethanol metabolism	-68.41	-81.67	0
YGL205W	POX1	fatty acid beta-oxidation	-27.80	-26.42	E
YOR180C	DCI1	fatty acid beta-oxidation	-3.03	-3.34	3
YLR284C	ECI1	fatty acid beta-oxidation	-3.32	-2.94	0
YIL160C	POT1	fatty acid beta-oxidation	-35.44	-32.98	0
YNL202W	SPS19	fatty acid catabolism	-9.18	-8.05	0
YBR132C	AGP2	fatty acid metabolism	-2.96	-2.55	9
YOR100C	CRC1	fatty acid metabolism	-10.92	-12.64	0
YJR019C	TES1	fatty acid oxidation	-3.62	-3.62	0
YPL147W	PXA1	fatty acid transport	-17.45	-13.70	4
YOR049C	RSB1	fatty acid transport	-5.10	-2.86	3
YAL063C	FLO9	flocculation	-2.37	-2.42	E
YDR054C	CDC34	G1 S transition of mitotic cell cycle	-2.10	-2.40	E
YOR054C	NA	G1 S transition of mitotic cell cycle	-5.41	-4.33	4
YER167W	BCK2	G1 S transition of mitotic cell cycle	-3.71	-3.54	0
YKR072C	SIS2	G1S transition of mitotic cell cycle	-2.12	-2.73	3
YLR081W	GAL2	galactose metabolism	-2.71	-3.23	0

Table 11A ORF	Gene	Function	WT FC	*mgtFC*	Sensitivity
YPL248C	GAL4	galactose metabolism	-2.21	-2.58	0
YBR020W	GAL1	galactose metabolism	-2.10	-2.32	0
YOL126C	MDH2	gluconeogenesis	-12.75	-11.23	2
YKR097W	PCK1	gluconeogenesis	-15.85	-23.63	2
YLR377C	FBP1	gluconeogenesis	-20.73	-24.54	0
YGL035C	MIG1	glucose metabolism	-4.84	-6.04	7
YKL038W	RGT1	glucose metabolism	-2.63	-2.79	6
YCR005C	CIT2	glutamate biosynthesis	-8.28	-3.97	0
YAL062W	GDH3	glutamate biosynthesis	-27.15	-36.05	0
YHL032C	GUT1	glycerol metabolism	-3.73	-4.83	4
YMR311C	GLC8	glycogen biosynthesis	-2.61	-2.45	6
YJL137C	GLG2	glycogen biosynthesis	-3.85	-3.29	2
YNL117W	MLS1	glyoxylate cycle	-46.40	-52.59	2
YFL030W	NA	glyoxylate cycle	-4.51	-4.37	2
YER065C	ICL1	glyoxylate cycle	-14.23	-19.04	0
YDR153C	ENT5	Golgi to endosome transport	-2.27	-2.20	0
YOR327C	SNC2	Golgi to plasma membrane transport	-3.14	-3.43	0
YOR036W	PEP12	Golgi to vacuole transport	-4.21	-3.60	12
YOR107W	RGS2	G-protein signaling, coupled to cAMP nucleotide second messenger	-33.60	-34.47	0
YDR232W	HEM1	heme biosynthesis	-2.49	-2.67	0
YER061C	CEM1	hexadecanal biosynthesis	-2.61	-3.02	8
YJR158W	HXT16	hexose transport	-2.34	-3.02	E
YFL011W	HXT10	hexose transport	-2.89	-3.25	2
YGL255W	ZRT1	high-affinity zinc ion transport	-3.03	-3.30	0
YLR055C	SPT8	histone acetylation	-2.24	-2.19	5
YPL055C	LGE1	histone methylation	-7.67	-8.64	23
YMR172W	HOT1	hyperosmotic response	-2.55	-2.56	0
YLR094C	GIS3	intracellular signaling cascade	-5.38	-4.48	8
YOR030W	DFG16	invasive growth	-2.41	-2.60	24
YDR043C	NRG1	invasive growth	-13.28	-12.99	0
YLL027W	ISA1	iron ion transport	-2.60	-3.59	0
YOR381W	FRE3	iron-siderochrome transport	-4.31	-4.92	0
YNR060W	FRE4	iron-siderochrome transport	-4.55	-5.51	0
YPL135W	ISU1	iron-sulfur cluster assembly	-5.63	-6.19	7
YLR174W	IDP2	isocitrate metabolism	-10.90	-12.28	0

Table 11A ORF	Gene	Function	WT FC	*mgt FC*	sitivity
YKL217W	JEN1	lactate transport	-23.93	-26.18	2
YMR313C	NA	lipid metabolism	-2.10	-2.03	2
YDR072C	IPT1	mannosyl diphosphorylinositol ceramide metabolism	-3.63	-2.78	0
YDL214C	PRR2	MAPKKK cascade	-38.22	-40.91	0
YJR094C	IME1	meiosis	-2.97	-2.93	0
YPL200W	CSM4	meiotic chromosome segregation	-11.14	-11.33	0
YJR021C_ex1	REC107	meiotic recombination	-3.24	-3.13	0
YJR021C_ex2	REC107	meiotic recombination	-2.32	-2.07	0
YLR219W	MSC3	meiotic recombination	-2.78	-2.82	0
YLL018C-A	COX19	metal ion transport	-2.56	-2.50	2
YBR290W	BSD2	metal ion transport	-3.90	-3.49	0
YGL184C	STR3	methionine biosynthesis	-2.51	-4.22	E
YNL277W	MET2	methionine biosynthesis	-11.46	-24.01	0
YOR257W	CDC31	microtubule nucleation	-2.61	-2.51	E
YPL060W	LPE10	mitochondrial magnesium ion transport	-2.66	-2.39	0
YPL134C	ODC1	mitochondrial transport	-2.92	-3.84	0
YML091C	RPM2	mitochondrion organization and biogenesis	-2.33	-2.61	E
YGL219C	MDM34	mitochondrion organization and biogenesis	-2.71	-2.27	0
YOR147W	MDM32	mitochondrion organization and biogenesis	-3.41	-2.68	0
YOL027C	MDM38	mitochondrion organization and biogenesis	-2.18	-2.07	0
YPR083W	MDM36	mitochondrion organization and biogenesis	-2.50	-2.24	0
YBR179C	FZO1	mitochondrion organization and biogenesis	-3.46	-2.50	0
YJL116C	NCA3	mitochondrion organization and biogenesis	-27.10	-19.36	0
YOR058C	ASE1	mitotic anaphase B	-3.60	-4.07	4
YBL084C	CDC27	mitotic metaphase	-2.95	-3.70	E
YKL022C	CDC16	mitotic metaphase anaphase transition	-2.64	-2.99	E
YFR036W	CDC26	mitotic metaphase anaphase	-2.19	-2.38	14

Table 11A ORF	Gene	Function	WT FC	*mgtFC*	Sensitivity
		transition			
YGL003C	CDH1	mitotic metaphase anaphase transition	-2.89	-2.48	8
YML034W	SRC1	mitotic sister chromatid separation	-2.30	-2.27	9
YKL052C	ASK1	mitotic spindle assembly (sensu Saccharomyces)	-2.13	-2.06	E
YNL164C	IBD2	mitotic spindle checkpoint	-2.44	-2.44	2
YOR178C	GAC1	mitotic spindle checkpoint	-8.86	-4.88	0
YNL065W	AQR1	monocarboxylic acid transport	-6.47	-5.30	0
YLR070C	XYL2	monosaccharide metabolism	-2.27	-2.93	0
YJL094C	KHA1	monovalent inorganic cation transport	-5.13	-5.02	0
YNL232W	CSL4	mRNA catabolism	-2.15	-2.28	E
YOL142W	RRP4C	mRNA catabolism	-2.43	-2.62	E
YKL059C	MPE1	mRNA cleavage	-3.46	-3.79	E
YOR035C	SHE4	mRNA localization, intracellular	-2.22	-2.59	13
YPL119C	DBP1	mRNA processing	-7.89	-10.71	0
YPL151C	PRP46	mRNA splicing	2.54	3.18	E
YOR319W	HSH49	mRNA splicing	-4.11	-5.17	E
YDR088C	SLU7	mRNA splicing	-2.58	-2.50	E
YDL209C	CWC2	mRNA splicing	-2.95	-3.27	E
YLR298C	YHC1	mRNA splicing	-2.22	-2.46	E
YPR182W	SMX3	mRNA splicing	-2.03	-2.32	E
YPL213W	LEA1	mRNA splicing	-2.58	-3.13	0
YBR119W_ex2	MUD1	mRNA splicing	-2.41	-2.24	0
YOR098C	NUP1	mRNA-nucleus export	-3.23	-3.27	E
YMR255W	GFD1	mRNA-nucleus export	-2.27	-2.63	0
YOR328W	PDR10	multidrug transport	-8.39	-9.94	0
YPL167C	REV3	mutagenesis	-3.33	-3.21	19
YJL153C	INO1	myo-inositol metabolism	-9.24	-11.56	0
YDR497C	ITR1	myo-inositol transport	-2.00	-2.28	0
YGL062W	PYC1	NADPH regeneration	-3.49	-3.75	0
YIL112W	HOS4	negative regulation of meiosis	-2.28	-2.29	0
YER159C	BUR6	negative regulation of transcription from Pol II promoter	-4.00	-4.26	E
YDR464W	SPP41	negative regulation of transcription from Pol II promoter	-5.66	-4.35	E

Table 11A ORF	Gene	Function	WT FC	*mgtFC*	Sensitivity
YHL027W	RIM101	negative regulation of transcription from Pol II promoter	-2.51	-2.70	23
YOR140W	SFL1	negative regulation of transcription from Pol II promoter	-3.21	-3.09	7
YPR065W	ROX1	negative regulation of transcription from Pol II promoter	-10.00	-11.75	6
YER161C	SPT2	negative regulation of transcription from Pol II promoter	-2.20	-2.48	6
YER169W	RPH1	negative regulation of transcription from Pol II promoter	-4.81	-4.13	0
YML113W	DAT1	negative regulation of transcription from Pol II promoter	-3.05	-2.70	0
YOR348C	PUT4	neutral amino acid transport	-64.15	-84.46	0
YJR078W	BNA2	nicotinamide adenine dinucleotide biosynthesis	-19.89	-24.67	0
YKL201C	MNN4	N-linked glycosylation	-9.46	-8.40	0
YMR017W	SPO20	nonselective vesicle fusion	-9.75	-12.08	0
YER015W	FAA2	N-terminal protein myristoylation	-5.28	-7.06	E
YDR150W	NUM1	nuclear migration (sensu Saccharomyces)	-3.43	-3.08	4
YJL019W	MPS3	nuclear migration during conjugation with cellular fusion	-2.59	-2.79	E
YJL019W	MPS3	nuclear migration during conjugation with cellular fusion	-2.15	-2.41	E
YOR023C	AHC1	nucleosome disassembly	-3.86	-3.64	6
YDR460W	TFB3	nucleotide-excision repair	-2.31	2.04	E
YJL090C	DPB11	nucleotide-excision repair	-2.30	-2.14	E
YER162C	RAD4	nucleotide-excision repair, DNA damage recognition	-3.39	-3.15	11
YOR065W	CYT1	oxidative phosphorylation	-3.52	-4.07	4
YHR001W-A_ex2	QCR10	oxidative phosphorylation, ubiquinone to cytochrome c	-3.49	-3.58	0
YDR256C	CTA1	oxygen and reactive oxygen species metabolism	-58.65	-66.50	4
YBR117C	TKL2	pentose-phosphate shunt	-75.32	-87.13	E
YPR049C	CVT9	peroxisome degradation	-4.16	-3.88	0
YDR244W	PEX5	peroxisome organization and	-3.26	-3.14	9

Table 11A ORF	Gene	Function	WT FC	*mgtFC*	Sensitivity
		biogenesis			
YHR150W	NA	peroxisome organization and biogenesis	-3.37	-3.24	7
YOL147C	PEX11	peroxisome organization and biogenesis	-2.40	-2.81	0
YBR296C	PHO89	phosphate transport	-6.26	-13.54	0
YJR077C	MIR1	phosphate transport	-3.39	-4.01	0
YJR073C	OPI3	phosphatidylcholine biosynthesis	-2.61	-2.84	0
YDR147W	EKI1	phosphatidylethanolamine biosynthesis	-3.66	-3.56	0
YOL011W	PLB3	phosphatidylserine catabolism	-6.47	-7.35	0
YHL020C	OPI1	phospholipid biosynthesis	-4.25	-5.46	23
YOL108C	INO4	phospholipid biosynthesis	-3.23	-3.14	0
YDR123C	INO2	phospholipid biosynthesis	-4.03	-4.01	0
YDR173C	ARG82	phosphorylation	-2.53	-2.65	4
YIL031W	ULP2	plasmid maintenance	-2.55	-2.56	E
YOR273C	TPO4	polyamine transport	-2.14	-2.18	4
YKL198C	PTK1	polyamine transport	-4.07	-4.45	0
YKL198C_alt	PTK1	polyamine transport	-3.72	-4.05	0
YJL089W	SIP4	positive regulation of gluconeogenesis	-5.07	-5.38	0
YOR344C	TYE7	positive regulation of glycolysis	-2.53	-2.59	0
YAL032C	PRP45	positive regulation of transcription from Pol II promoter	-3.27	-3.38	E
YMR021C	MAC1	positive regulation of transcription from Pol II promoter	-2.54	-2.71	15
YBR182C	SMP1	positive regulation of transcription from Pol II promoter	-3.58	-5.03	5
YMR039C	SUB1	positive regulation of transcription from Pol II promoter	-2.55	-2.17	5
YNL199C	GCR2	positive regulation of transcription from Pol II promoter	-2.85	-2.78	5
YHR006W	STP2	positive regulation of transcription from Pol II promoter	-3.07	-2.92	0
YMR164C	MSS11	positive regulation of transcription from Pol II promoter	-3.97	-3.65	0
YPL089C	RLM1	positive regulation of transcription	-3.22	-3.32	0

Table 11A ORF	Gene	Function	WT FC	*mgt FC*	sitivity
		from Pol II promoter			
YIR018W	YAP5	positive regulation of transcription from Pol II promoter	-5.45	-6.74	0
YMR280C	CAT8	positive regulation of transcription from Pol II promoter	-21.67	-22.79	0
YKL020C	SPT23	positive regulation of transcription from Pol II promoter	-3.03	-2.98	0
YKR050W	TRK2	potassium ion homeostasis	-2.18	-2.90	2
YHL024W	RIM4	premeiotic DNA synthesis	-3.29	-5.09	2
YPR002W	PDH1	propionate metabolism	-7.39	-7.42	0
YER054C	GIP2	protein amino acid dephosphorylation	-4.80	-3.89	7
YOL110W	SHR5	protein amino acid palmitoylation	-2.85	-3.34	4
YHR082C	KSP1	*protein amino acid phosphorylation*	-2.35	-2.84	12
YDR247W	NA	*protein amino acid phosphorylation*	-6.07	-5.29	6
YPL026C	SKS1	*protein amino acid phosphorylation*	-20.99	-18.47	0
YDL079C_ex1	MRK1	*protein amino acid phosphorylation*	-3.25	-2.55	0
YDL159W	STE7	*protein amino acid phosphorylation*	-2.79	-3.01	0
YDL079C_ex2	MRK1	*protein amino acid phosphorylation*	-3.35	-4.14	0
YOL100W	PKH2	*protein amino acid phosphorylation*	-2.75	-2.70	0
YOL128C	YGK3	*protein amino acid phosphorylation*	-2.48	-2.55	0
YDR041W	RSM10	protein biosynthesis	-5.25	-3.75	E
YDR405W	MRP20	protein biosynthesis	-3.12	-2.74	14
YJR113C	RSM7	protein biosynthesis	-2.24	-2.29	9
YBL038W	MRPL16	protein biosynthesis	-2.64	-2.38	0
YDR462W	MRPL28	protein biosynthesis	-2.60	-2.50	0
RDN5-1	RDN5-1	protein biosynthesis	-8.69	-8.91	0
RDN5-3	RDN5-3	protein biosynthesis	-3.19	-3.95	0
YLR106C	MDN1	protein complex assembly	2.24	-2.22	E
YDR069C	DOA4	protein deubiquitination	-2.77	-2.90	25
YER144C	UBP5	protein deubiquitination	-2.29	-2.18	0
YPL240C	HSP82	protein folding	-2.17	-2.75	2
YOL067C	RTG1	protein localization	-2.62	-3.51	6
YLR121C	YPS3	protein metabolism	-2.05	-2.28	0
YGL045W	RIM8	protein processing	-3.38	-3.44	14
YDR409W	SIZ1	protein sumoylation	-3.32	-3.88	0
YDR313C	PIB1	protein ubiquitination	-4.22	-5.04	2

Table 11A ORF	Gene	Function	WT FC	*mgtFC*	Sensitivity
YLR417W	VPS36	protein-Golgi retention	-2.72	-2.15	30
YNR006W	VPS27	protein-Golgi retention	-2.01	-2.32	7
YEL030W	ECM10	protein-mitochondrial targeting	-3.33	-4.32	30
YBR165W	UBS1	protein-nucleus export	-2.32	-2.09	0
YJR074W	MOG1	protein-nucleus import	-2.27	-3.12	7
YAL055W	PEX22	protein-peroxisome targeting	-2.51	-2.43	21
YOL044W	PEX15	protein-peroxisome targeting	-2.42	-2.42	20
YHR160C	PEX18	protein-peroxisome targeting	-10.05	-9.60	11
YDL065C	PEX19	protein-peroxisome targeting	-2.64	-2.59	7
YDR142C	PEX7	protein-peroxisome targeting	-2.49	-2.64	4
YDR329C	PEX3	protein-peroxisome targeting	-2.96	-2.60	4
YML041C	VPS71	protein-vacuolar targeting	-2.06	-2.41	15
YFL016C	MDJ1	proteolysis and peptidolysis	-4.29	-5.96	0
YJL172W	CPS1	proteolysis and peptidolysis	-3.22	-2.71	0
YNL142W	MEP2	pseudohyphal growth	-6.62	-7.83	4
YMR316W	DIA1	pseudohyphal growth	-3.87	-3.29	0
YDL024C	DIA3	pseudohyphal growth	-10.02	-13.61	0
YOR032C	HMS1	pseudohyphal growth	-6.77	-6.02	0
YER020W	GPA2	pseudohyphal growth	-3.78	-3.44	0
YKL166C	TPK3	pseudohyphal growth	-2.65	-2.68	0
YKL043W	PHD1	pseudohyphal growth	-4.41	-4.64	0
YMR016C	SOK2	pseudohyphal growth	-7.85	-5.94	0
YKL216W	URA1	pyrimidine base biosynthesis	-3.28	-3.13	2
YOL081W	IRA2	RAS protein signal transduction	-2.62	-2.01	23
YLL016W	SDC25	RAS protein signal transduction	-2.40	-2.26	0
YIL122W	POG1	re-entry into mitotic cell cycle after pheromone arrest	-3.01	-2.43	0
YDR216W	ADR1	regulation of carbohydrate metabolism	-4.82	-3.68	18
YGL237C	HAP2	regulation of carbohydrate metabolism	-2.43	-2.49	0
YKL109W	HAP4	regulation of carbohydrate metabolism	-3.83	-2.52	0
YMR036C	MIH1	regulation of CDK activity	-7.31	-6.85	0
YOL078W	AVO1	regulation of cell growth	-2.33	-2.05	E
YMR068W	AVO2	regulation of cell growth	-2.83	-2.95	0
YLR403W	SFP1	regulation of cell size	-4.76	-4.83	15

Table 11A ORF	Gene	Function	WT FC	*mgt FC*	sitivity
YNL197C	WHI3	regulation of cell size	-2.70	-3.10	4
YGL134W	PCL10	regulation of glycogen catabolism	-3.80	-3.73	E
YPL219W	PCL8	regulation of glycogen catabolism	-2.37	-2.78	8
YBR212W	NGR1	regulation of growth	-2.26	-2.46	13
YJL106W	IME2	regulation of meiosis	-3.03	-2.63	7
YFL021W	GAT1	regulation of nitrogen utilization	-3.60	-2.69	5
YJL110C	GZF3	regulation of nitrogen utilization	-2.41	-2.35	2
YKR034W	DAL80	regulation of nitrogen utilization	-4.14	-3.96	0
YPL036W	PMA2	regulation of pH	-4.58	-5.90	0
YBR049C	REB1	regulation of transcription from Pol II promoter	-2.21	-2.24	E
YFL024C	EPL1	regulation of transcription from Pol II promoter	-2.73	-2.92	E
YOR244W	ESA1	regulation of transcription from Pol II promoter	-2.75	-2.59	E
YMR043W	MCM1	regulation of transcription from Pol II promoter	-2.28	-2.36	E
YGL162W	SUT1	regulation of transcription from Pol II promoter	-14.09	-11.90	0
YIR017C	MET28	regulation of transcription from Pol II promoter	-3.14	-4.92	0
YJL056C	ZAP1	regulation of transcription from Pol II promoter	-2.49	-2.78	0
YNL278W	CAF120	regulation of transcription from Pol II promoter	-6.46	-6.11	0
YOR213C	SAS5	regulation of transcription, DNA-dependent	-2.21	-2.33	0
YMR037C	MSN2	regulation of transcription, DNA-dependent	-2.61	-2.95	0
YPL223C	GRE1	response to dessication	-164.51	-200.56	5
YMR175W	SIP18	response to dessication	-67.73	-65.22	2
YMR216C	SKY1	response to drug	-3.44	-3.56	15
YOR028C	CIN5	response to drug	-14.89	-15.13	4
YOR031W	CRS5	response to metal ion	-18.76	-13.25	4
YPL059W	GRX5	response to oxidative stress	-2.10	-2.28	5
YCR083W	TRX3	response to oxidative stress	-4.83	-5.64	0
YCR021C	HSP30	response to stress	-6.94	-8.03	0

Table 11A ORF	Gene	Function	WT FC	*mgtFC*	Sensitivity
YLL010C	PSR1	response to stress	-2.29	-2.35	0
YPL133C	RDS2	response to xenobiotic stimulus	-3.53	-2.99	4
YNL180C	RHO5	Rho protein signal transduction	-4.89	-5.21	E
YBR279W	PAF1	RNA elongation from Pol II promoter	-2.04	-2.07	0
YOL054W	PSH1	RNA elongation from Pol II promoter	-2.75	-3.09	0
YNL230C	ELA1	RNA elongation from Pol II promoter	-2.06	-2.47	0
YLR107W	REX3	RNA processing	-4.54	-3.73	0
Q0115	BI3	RNA splicing	-3.60	-3.62	0
YDR534C	FIT1	siderochrome transport	-6.31	-7.03	0
YKL203C	TOR2	signal transduction	-2.31	-2.03	E
YOR371C	GPB1	signal transduction	-4.62	-3.70	7
YDR277C	MTH1	signal transduction	-5.88	-5.70	2
YPR040W	TIP41	signal transduction	-4.57	-4.40	0
YAL056W	GPB2	signal transduction	-2.32	-2.22	0
YIL047C	SYG1	signal transduction	-8.20	-7.87	0
YOR134W	BAG7	small GTPase mediated signal transduction	-3.67	-2.54	0
YLL061W	MMP1	S-methylmethionine transport	-2.10	-6.61	0
YDR039C	ENA2	sodium ion transport	-8.09	-8.00	E
YDR038C	ENA5	sodium ion transport	-5.27	-6.55	E
YPL057C	SUR1	sphingolipid biosynthesis	-5.15	-4.16	0
YMR240C	CUS1	spliceosome assembly	-3.62	-3.60	E
YBR180W	DTR1	spore wall assembly (sensu Saccharomyces)	-2.04	-2.15	6
YDR273W	DON1	spore wall assembly (sensu Saccharomyces)	-5.02	-4.91	4
YLR399C	BDF1	sporulation (sensu Saccharomyces)	-3.40	-4.34	19
YLR189C	UGT51	sterol metabolism	-2.15	-2.25	0
YJR095W	SFC1	succinate transport	-18.05	-20.49	0
YKR069W	MET1	sulfate assimilation	-2.01	-3.07	0
YBR294W	SUL1	sulfate transport	-2.81	-5.04	0
YNL103W	MET4	sulfur amino acid metabolism	-4.36	-3.97	E
YDR253C	MET32	sulfur amino acid metabolism	-4.10	-6.16	2
YDR499W	LCD1	telomerase-dependent telomere maintenance	-2.68	-2.43	E
YNL216W	RAP1	telomerase-dependent telomere maintenance	-2.35	-2.18	E

Table 11A ORF	Gene	Function	WT FC	*mgt FC*	sitivity
YDR082W	STN1	telomere capping	-2.14	-2.12	E
YGR144W	THI4	thiamin biosynthesis	-6.73	-9.62	10
YPL258C	THI21	thiamin biosynthesis	-3.15	-4.00	4
YMR096W	SNZ1	thiamin biosynthesis	-5.31	-4.81	0
YPR006C	ICL2	threonine catabolism	-2.65	-2.85	0
YHR206W	SKN7	transcription	-3.47	-3.66	7
YMR070W	MOT3	transcription	-2.45	-2.50	0
YKL125W	RRN3	transcription from Pol I promoter	-2.12	-2.32	E
YOL135C	MED7	transcription from Pol II promoter	-2.27	-2.71	E
YBL093C	ROX3	transcription from Pol II promoter	-3.11	-2.89	19
YGL127C	SOH1	transcription from Pol II promoter	-4.25	-4.46	7
YPR104C	FHL1	transcription from Pol III promoter	-2.58	2.70	E
YKR062W	TFA2	transcription initiation from Pol II promoter	-2.51	-2.21	E
YPR025C	CCL1	transcription initiation from Pol II promoter	2.94	-3.76	E
YPR005C	HAL1	transcription initiation from Pol II promoter	-4.81	-6.23	0
YGL166W	CUP2	transcription initiation from Pol II promoter	-8.34	-7.97	0
YOL089C	HAL9	transcription initiation from Pol II promoter	-3.68	-3.98	0
YPR008W	HAA1	transcription initiation from Pol II promoter	-2.56	-2.29	0
YOL068C	HST1	transcriptional gene silencing	-2.98	-3.06	7
YNL014W	HEF3	translational elongation	-6.91	-7.08	0
YOR204W	DED1	translational initiation	-3.74	-5.08	E
YNL006W	LST8	transport	-2.01	-2.55	E
YDR384C	ATO3	transport	-3.54	-4.04	7
YNL125C	ESBP6	transport	-2.28	-2.22	4
YKR039W	GAP1	transport	-4.94	-6.45	4
YKL221W	MCH2	transport	-2.14	-2.45	2
YDR536W	STL1	transport	-91.20	-110.14	0
YDR406W	PDR15	transport	-2.72	-2.76	0
YNR070W	PDR18	transport	-3.70	-4.71	0
YCL025C	AGP1	transport	-4.50	-2.57	0
YBR001C	NTH2	trehalose catabolism	-2.33	-2.04	0

Table 11A ORF	Gene	Function	WT FC	*mgtFC*	Sensitivity
YKL141W	SDH3	tricarboxylic acid cycle	-2.62	-2.81	E
YLL041C	SDH2	tricarboxylic acid cycle	-3.05	-3.47	0
YDR148C	KGD2	tricarboxylic acid cycle	-2.52	-2.73	0
YDR178W	SDH4	tricarboxylic acid cycle	-2.09	-2.36	0
YPR001W	CIT3	tricarboxylic acid cycle	-16.21	-16.66	0
RPR1	RPR1	tRNA processing	-4.78	-7.20	0
YDR463W	STP1	tRNA splicing	-3.46	-3.63	4
YDL020C	RPN4	ubiquitin-dependent protein catabolism	-4.45	-4.81	15
YBR021W	FUR4	uracil transport	-10.81	-14.27	0
YBL042C	FUI1	uridine transport	-9.07	-8.95	0
YBR105C	VID24	vesicle-mediated transport	-4.48	-3.70	0
YBL033C	RIB1	vitamin B2 biosynthesis	-2.91	-3.25	7
YPR192W	AQY1	water transport	-16.56	-15.19	0
YJL221C	FSP2	NA	-4.54	-9.17	E
YKL095W	YJU2	NA	-2.38	-2.38	E
YKR071C	DRE2	NA	-2.25	-2.63	E
YOR077W	RTS2	NA	-3.88	-4.57	E
YGL128C	CWC23	NA	-2.03	-2.26	E
YDL139C	SCM3	NA	-2.22	-2.14	E
YOR353C	SOG2	NA	-2.30	-2.12	E
YNL036W	NCE103	NA	-4.33	-5.97	E
YLR323C	CWC24	NA	-2.71	-3.32	E
YPL024W	NCE4	NA	-2.89	-3.13	27
YDR334W	SWR1	NA	-4.27	-3.80	15
YDR482C	CWC21	NA	-5.03	-5.25	13
YER033C	ZRG8	NA	-2.55	-2.32	10
YBR059C	AKL1	NA	-2.28	-2.60	6
YOR141C	ARP8	NA	-2.53	-2.20	5
YLR136C	TIS11	NA	-3.20	-3.50	5
YJR108W	ABM1	NA	-2.04	-2.12	4
YKR046C	PET10	NA	-2.91	-3.63	4
YLR099C	ICT1	NA	-2.29	-2.31	4
YDR505C	PSP1	NA	-2.39	-2.87	4
YPL221W	BOP1	NA	-2.59	-2.21	4
YKL076C	PSY1	NA	-2.34	-2.03	3

Table 11A ORF	Gene	Function	WT FC	*mgt FC*	sitivity
YDR525W-A	SNA2	NA	-2.23	-3.35	2
YPR106W	ISR1	NA	-2.51	-2.64	2
YNL077W	APJ1	NA	-7.02	-10.48	0
YPL064C	CWC27	NA	-2.52	-2.73	0
YHR146W	CRP1	NA	-4.84	-4.15	0
YPR154W	PIN3	NA	-3.36	-5.36	0
YGR236C	SPG1	NA	-56.63	-50.00	0
YBR150C	TBS1	NA	-6.86	-6.53	0
YAL034C	FUN19	NA	-5.14	-4.95	0
YGR268C	HUA1	NA	-2.51	-2.92	0
YBL066C	SEF1	NA	-4.12	-4.74	0
YBL067C	UBP13	NA	-2.20	-2.64	0
YGL056C	SDS23	NA	-4.27	-4.62	0
YFL013C	IES1	NA	-2.45	-2.42	0
YML118W	NGL3	NA	-7.28	-6.50	0
YDL123W	SNA4	NA	-2.26	-2.80	0
YOR034C	AKR2	NA	-3.18	-3.70	0
YBR054W	YRO2	NA	-10.52	-6.04	0
YER047C	SAP1	NA	-2.99	-2.70	0
YLR213C	CRR1	NA	-2.12	-2.08	0
YNL237W	YTP1	NA	-6.90	-5.40	0
YNL196C	SLZ1	NA	-12.56	-13.53	0
YER039C	HVG1	NA	-4.79	-6.32	0
YMR135C	DCR1	NA	-5.04	-4.84	0
YKL168C	KKQ8	NA	-2.32	-2.08	0
RDN37-1_3	RDN37-1	NA	-2.55	-5.14	0
ARS_CEN12	CEN12	NA	-2.44	-2.90	0
YMR322C	SNO4	NA	-13.14	-14.68	0
YDL169C	UGX2	NA	-2.68	-2.89	0
YBR222C	PCS60	NA	-2.36	-2.68	0
YDL048C	STP4	NA	-3.63	-2.49	0
YIL130W	GIN1	NA	-2.64	-2.18	0
YBR157C	ICS2	NA	-15.06	-16.77	0
YML017W	PSP2	NA	-2.17	-2.26	0
YNL240C	NAR1	NA	-2.48	-2.66	0

Table 11B: ESR subset of genes that are repressed in both WT and *mgt1*.

There were 60 genes in this category. Genes which had a known function are included here. The essential genes are highlighted in red.

ORF	Gene	Function	WT FC	*mgtFC*	Sensitivity
YMR081C	ISF1	aerobic respiration	-6.91	-5.06	0
YKL093W	MBR1	aerobic respiration	-19.55	-15.41	0
YGL180W	APG1	autophagy	-2.49	-2.40	6
YBL078C	AUT7	autophagy	-2.48	-2.89	0
YMR170C	ALD2	beta-alanine biosynthesis	-2.38	-2.18	0
YLR074C	BUD20	bud site selection	-2.47	-2.29	11
YGL156W	AMS1	carbohydrate metabolism	-3.71	-3.63	0
YML042W	CAT2	carnitine metabolism	-5.32	-6.01	4
YLR001C	NA	cell adhesion	-3.16	-3.27	0
YGR023W	MTL1	cell wall organization and biogenesis	-2.28	-2.14	0
YBR183W	YPC1	ceramide metabolism	-2.58	-2.63	9
YER035W	EDC2	deadenylation-dependent decapping	-6.15	-7.15	10
YOR374W	ALD4	ethanol metabolism	-2.17	-2.44	4
YKR009C	FOX2	fatty acid beta-oxidation	-5.33	-6.24	2
YKL188C	PXA2	fatty acid transport	-4.30	-4.22	0
YIL107C	PFK26	fructose 2,6-bisphosphate metabolism	-2.61	-2.76	0
YJL155C	FBP26	gluconeogenesis	-2.39	-2.26	0
YGR256W	GND2	glucose metabolism	-9.74	-10.02	0
YHR096C	HXT5	hexose transport	-57.40	-56.14	0
YMR053C	STB2	histone deacetylation	-2.92	-3.49	0
YDL022W	GPD1	intracellular accumulation of glycerol	-2.49	-2.60	0
YDR096W	GIS1	intracellular signaling cascade	-2.79	-2.84	0
YMR302C	PRP12	mitochondrial genome maintenance	-2.80	-2.17	4
YNL009W	IDP3	NADPH regeneration	-9.74	-10.28	0
YOR317W	FAA1	N-terminal protein myristoylation	-3.30	-2.65	0
YLR142W	PUT1	proline catabolism	-9.98	-12.33	0
YLL019C	KNS1	protein amino acid phosphorylation	-5.19	-6.42	5
YJL141C	YAK1	protein amino acid phosphorylation	-2.92	-2.31	0
YOR145C	PNO1	protein complex assembly	-2.59	-2.30	E
YBR169C	SSE2	protein folding	-3.17	-2.86	0
YEL012W	UBC8	protein monoubiquitination	-4.06	-3.50	0
YKL026C	GPX1	response to oxidative stress	-4.70	-4.51	0
YMR169C	ALD3	response to stress	-13.63	-5.26	0
YMR169C	ALD3	response to stress	-6.08	-4.26	0
YGR088W	CTT1	response to stress	-9.82	-4.01	0
YIL101C	XBP1	response to stress	-11.27	-10.31	0
YGL121C	GPG1	signal transduction	-3.73	-2.36	0
YGL208W	SIP2	signal transduction	-3.06	-2.50	0
YIL099W	SGA1	sporulation (sensu Saccharomyces)	-5.44	-5.09	0
YIL099W	SGA1	sporulation (sensu Saccharomyces)	-6.85	-7.07	0
YBL075C	SSA3	SRP-dependent cotranslational membrane targeting, translocation	-16.90	-25.55	0
YMR136W	GAT2	transcription	-3.86	-3.64	0
YKR025W	RPC37	transcription from Pol III promoter	-2.77	-2.17	E
YNR002C	FUN34	transport	-6.43	-6.51	0
YPR026W	ATH1	trehalose catabolism	-4.04	-4.45	0
YMR174C	PAI3	vacuolar protein catabolism	-8.11	-9.37	0
YPL054W	LEE1	NA	-10.74	-14.53	0
YBL049W	MOH1	NA	-7.77	-9.77	0
YJL066C	MPM1	NA	-4.35	-3.63	0
YIL136W	OM45	NA	-7.29	-4.72	0
YOL084W	PHM7	NA	-77.47	-81.45	4
YIL045W	PIG2	NA	-4.10	-2.28	2
YDR255C	RMD5	NA	-2.03	-2.90	6
YDL204W	RTN2	NA	-5.01	-2.58	0
YGR161C	RTS3	NA	-4.89	-4.65	5
YER150W	SPI1	NA	-4.97	-3.09	0
YDR169C	STB3	NA	-3.07	-3.17	0
YDR350C	TCM10	NA	-2.05	-2.22	0
YGL096W	TOS8	NA	-2.90	-2.85	0
YPL186C	UIP4	NA	-5.15	-3.72	0

Table 12: Genes involved in DNA replication and repair.

Table 12A lists several genes that are induced in WT and *mgt1* (except CCE, PRI2, UNG1, SIR2). Table 12C lists 20 genes that are repressed in WT and *mgt1*. Table 12C includes genes that are a part of the ESR subset of the same category. A total of 133 genes were selected from the Affymetrix annotation file (which is derived from SGD). While most genes did not have an appreciable fold change (induction >2 or repression <0.5), there were 25 genes that were induced (Table 12A) and 20 genes that were repressed.

ORF	Gene	Function	Sensit	Basal *mgt1*	WT FC	*Mgt1* FC
YDR489W	NA	DNA dependent DNA replication	E	1.05	3.01	2.67
YEL055C	POL5	DNA dependent DNA replication	E	1.19	3.18	3.41
YJL072C	NA	DNA dependent DNA replication	E	-1.13	2.24	2.00
YDL164C	CDC9	DNA recombination	E	1.28	3.70	3.95
YKL011C	CCE1	DNA recombination	10	-1.37	1.77	2.14
YCR066W	RAD18	DNA repair	30	1.16	2.55	2.61
YHR120W	MSH1	DNA repair	0	1.31	2.18	2.20
YJR069C	HAM1	DNA repair	0	1.16	9.17	8.81
YLR383W	RHC18	DNA repair	E	1.18	2.29	3.54
YML021C	UNG1	DNA repair	0	1.27	2.02	1.84
YKL045W	PRI2	DNA repair synthesis	E	-1.34	1.96	2.00
YGR180C	RNR4	DNA replication	9	1.05	3.14	3.06
YIL066C	RNR3	DNA replication	0	1.29	4.18	6.16
YNL273W	TOF1	DNA topological change	15	1.62	4.95	6.19
YGL251C	HFM1	DNA unwinding	0	-1.18	2.58	2.27
YBL023C	MCM2	DNA unwinding	E	1.18	2.88	2.77
YLR274W	CDC46	DNA unwinding	E	-1.35	4.83	5.30
YDL042C	SIR2	DSB repair via NHEJ	4	1.20	2.07	1.79
YML032C	RAD52	DSB repair via synthesis-dependent strand annealing	23	-1.00	2.32	2.00
YNL312W_ex2	RFA2	nucleotide-excision repair	0	1.14	4.69	4.27
YAR007C	RFA1	nucleotide-excision repair	E	1.21	3.96	4.13
YBR088C	POL30	nucleotide-excision repair	E	1.09	4.05	3.42
YDL102W	CDC2	nucleotide-excision repair	E	1.09	2.32	2.44
YJL173C	RFA3	nucleotide-excision repair	E	1.05	2.12	2.31
YPR175W	DPB2	nucleotide-excision repair	E	-1.44	2.11	2.28

Table 12B: DNA repair genes that are repressed in *mgt1* upon MNNG treatment.

ORF	Gene	Function	Sensit	Basal *mgt1*	WT FC	*Mgt1* FC
YDL200C	MGT1	DNA dealkylation	12	-26.49	-2.05	-23.61
YFR023W	PES4	DNA dependent DNA replication	0	1.14	-2.23	-2.58
YCR010C	ADY2	DNA metabolism	0	1.12	-36.50	-44.54
YER116C	SLX8	DNA recombination	11	-1.01	-4.19	-3.13
YDL013W	HEX3	DNA recombination	8	-1.03	-3.80	-3.34
YKL032C	IXR1	DNA repair	5	-1.55	-4.21	-4.35
YLR007W	NSE1	DNA repair	0	1.38	-2.52	-2.51
YDR030C	RAD28	DNA repair	4	1.14	-2.80	-1.57
YHR031C	RRM3	DNA replication	17	-1.09	-3.32	-2.88
YJR060W	CBF1	DNA replication and chromosome cycle	9	-1.18	-1.89	-2.26
YJR046W	TAH11	DNA replication licensing	E	-1.49	-4.95	-7.41
YMR106C	YKU80	DSB repair via NHEJ	5	-1.32	-3.44	-3.19
YNL133C	FYV6	DSB repair via NHEJ	3	1.45	-2.97	-3.40
YDR227W	SIR4	DSB repair via NHEJ	2	-1.09	-2.28	-2.29
YHR081W	LRP1	DSB repair via NHEJ	0	-1.07	-2.46	-2.55
YDR460W	TFB3	nucleotide-excision repair	E	-1.21	-2.31	-2.04
YJL090C	DPB11	nucleotide-excision repair	E	1.19	-2.30	-2.14
YLR005W	SSL1	nucleotide-excision repair	E	1.27	-1.93	-2.27
YER162C	RAD4	NER, DNA damage recognition	11	1.11	-3.39	-3.15
YBR114W	RAD16	NER, DNA damage recognition	0	-1.04	-2.58	-1.56

Table 12C: ESR subset of the DNA repair genes.

ORF	Gene	Function	Sensit	Basal *mgt1*	WT FC	*Mgt1* FC
YER142C	MAG1	base-excision repair	30	-1.15	1.37	1.44
YML060W	OGG1	BER, AP site formation	0	-1.09	4.85	4.95
YJL208C	NUC1	DNA recombination	0	-1.44	1.45	1.59
YMR173W	DDR48	DNA repair	0	-1.10	1.42	1.50
YNL102W	POL1	DNA repair synthesis	E	1.44	2.75	3.26
YER070W	RNR1	DNA replication	5	-1.13	23.03	22.30
YDR097C	MSH6	mismatch repair	0	1.26	1.98	2.21
YJR043C	POL32	nucleotide-excision repair	30	-1.22	1.13	1.05

Table 13: Genes that are incrementally induced in both WT and *mgt1*

The net fold-change (FC) over time, was calculated from ratio of mean expression at the 60th minute to mean expression at the 10th minute. A cut-off of FC>2 (for induction) was used to select genes that have been included in this representation. There were 39 genes induced incrementally. Only 2 (out of 24 genes with known function, 8%) were essential.

Yname	Gene	Functional group	Sensitivity	Avg WT10	Avg WT20	Avg WT30	Avg WT 40	Avg WT 50	Avg WT 60	wt60/wt10	Avg mgt 10	Avg mgt 20	Avg mgt 30	Avg mgt 40	Avg mgt 50	Avg mgt 60	mgt60/mgt10
YDL243C	AAD4	aldehyde metabolism	10	423.5	729.3	792.4	937.6	644.3	1195.9	2.82	273.83	463.21	511.48	542.11	420.49	600.34	2.19
YFL056C	AAD6	aldehyde metabolism	3	160.7	250.2	229.8	339.1	384.5	398.6	2.48	50.03	100.60	91.68	109.99	98.94	180.67	3.61
YFL057C	NA	aldehyde metabolism	-5	7650.0	11532.1	9717.4	14634.7	14098.1	16622.5	2.17	2983.01	6985.08	10176.58	8394.84	7827.92	10050.09	3.37
YHR033W	NA	amino acid biosynthesis	0	111.9	126.7	136.3	205.4	243.5	234.7	2.10	83.79	159.57	198.48	301.26	288.55	340.60	4.06
YMR042W	ARG80	arginine metabolism	0	121.4	197.8	173.7	246.7	267.8	414.4	3.41	81.64	161.68	246.65	234.30	214.98	221.79	2.72
YML058w-a	HUG1	cell cycle arrest	0	830.7	2239.6	3511.9	5185.8	5718.7	7118.5	8.57	470.02	1346.56	3375.48	3790.19	5247.58	5847.35	12.44
YER096W	SHC1	chitin biosynthesis	0	81.5	117.9	145.3	170.8	175.2	209.5	2.57	40.23	83.82	135.31	126.85	133.90	141.61	3.52
YIL066C	RNR3	DNA replication	0	1440.2	2484.8	2417.8	3218.4	3591.1	3032.7	2.11	1308.17	2807.29	3752.06	4698.10	5072.30	6225.60	4.76
YGL209W	MIG2	glucose metabolism	0	398.4	324.8	267.4	676.0	734.8	846.6	2.13	205.33	590.58	614.32	604.96	535.09	431.35	2.10
YCR005C	CIT2	glutamate biosynthesis	0	342.4	517.8	483.8	756.4	662.2	852.9	2.49	212.22	864.02	1743.23	1305.01	1773.16	1649.19	7.77
YJL116C	NCA3	mitochondrion organization and biogenesis	0	90.3	109.8	113.0	148.6	185.7	232.1	2.57	97.00	118.93	192.07	314.79	303.04	204.88	2.11
YJL088W	ARG3	ornithine metabolism	0	54.0	83.6	96.6	142.2	160.6	164.5	3.05	72.86	85.00	211.63	175.24	133.42	248.12	3.41
YBR296C	PHO89	phosphate transport	0	655.0	945.9	1313.1	2044.6	1776.4	1703.6	2.60	251.08	369.45	763.52	361.38	1081.11	1075.80	4.28
YOR120W	GCY1	salinity response	0	1552.6	2195.4	2533.5	3871.6	4050.6	3727.5	2.40	2850.67	3689.45	4011.68	4208.10	4389.40	5949.84	2.09
YMR095C	SNO1	thiamin biosynthesis	0	99.4	211.4	280.2	432.2	397.4	469.9	4.73	76.46	154.61	344.48	236.06	253.88	488.60	6.39
YMR096W	SNZ1	thiamin biosynthesis	0	301.4	764.1	976.3	1305.0	1158.6	1244.5	4.13	262.80	569.14	1438.37	913.24	1115.98	2048.97	7.80

Table 13B: ESR subset of Genes that are incrementally induced in both WT and *mgt1*

Yname	Gene	Functional group	Sensitivity	Avg WT10	Avg WT20	Avg WT30	Avg WT 40	Avg WT 50	Avg WT 60	wt60/wt10	Avg mgt 10	Avg mgt 20	Avg mgt 30	Avg mgt 40	Avg mgt 50	Avg mgt 60	mgt60/mgt10
YLR267W	BOP2	NA	0	107.6	169.1	231.9	307.1	374.8	407.5	3.79	64.27	274.66	625.14	357.24	575.23	499.22	7.77
YGR161C	RTS3	NA	5	843.6	1080.3	1152.4	1460.6	1853.3	2083.2	2.47	549.72	1064.81	1714.70	1755.88	1678.78	2134.98	3.88
YBL054W	NA	NA	8	84.8	144.1	101.7	176.3	209.3	216.4	2.55	52.30	154.91	288.23	247.98	252.49	253.60	4.85
YDL222C	NA	NA	0	141.1	215.4	269.8	394.6	308.1	333.2	2.36	301.14	559.44	493.51	720.33	936.17	1056.03	3.51

Table 14 Genes that are incrementally induced specifically in WT

The net fold-change (FC) over time, was calculated from ratio of mean expression at the 60th minute to mean expression at the 10th minute. A cut-off of FC>2 (for induction) was used to select genes that have been included in this representation. There were 61 genes in this category. Genes that have a known function are indicated here. The genes that are essential are highlighted in red.

Yname	Gene Symbol	Functional group	Av WT/WT0	MMS, viability	Avg WT10	Avg WT20	Avg WT30	Avg WT 40	Avg WT 50	Avg WT 60	WT60/WT10
YML054C	CYB2	electron transport	0.07	0	99.13	203.26	238.41	238.78	256.01	220.98	2.23
YDL085W	NDE2	ethanol fermentation	0.04	0	144.10	184.81	238.17	265.54	269.26	325.05	2.26
YOR100C	CRC1	fatty acid metabolism	0.09	0	161.05	238.13	252.13	339.38	311.16	394.92	2.45
YGL035C	MIG1	glucose metabolism	0.21	7	126.07	133.19	156.80	218.41	251.11	298.64	2.37
YAL062W	GDH3	glutamate biosynthesis	0.04	0	281.74	428.32	460.85	472.53	535.90	573.41	2.04
YLL060C	GTT2	glutathione metabolism	4.01	7	1771.32	2721.67	2488.43	2979.29	2854.34	4535.04	2.56
YFL030W	NA	glyoxylate cycle	0.22	2	615.69	791.77	742.85	1090.96	1076.14	1343.21	2.18
YLR205C	HMX1	iron ion homeostasis	0.98	0	206.71	257.03	254.06	372.90	324.28	455.81	2.21
YMR177W	MMT1	iron ion homeostasis	4.24	0	271.52	328.19	298.04	300.36	341.23	581.03	2.14
YOR226C	ISU2	iron-sulfur cluster assembly	0.90	0	1022.65	1197.71	1083.34	1132.19	1165.73	2268.71	2.22
YOR058C	ASE1	mitotic anaphase B	0.28	4	175.89	217.26	210.68	202.42	194.98	381.23	2.17
YCL026C-A	FRM2	negative regulation of fatty acid metabolism	4.33	0	259.66	377.95	310.93	349.26	287.34	568.32	2.19
YKL029C	MAE1	pyruvate metabolism	1.76	4	951.71	1629.09	1822.08	1584.07	1886.66	2290.81	2.41
YBR008C	FLR1	response to toxin	3.32	2	1860.31	2924.27	2831.04	4072.04	3361.60	6093.18	3.28
YOR119C	RIO1	S phase of mitotic cell cycle	1.03	-5	79.87	135.66	126.31	166.09	132.27	168.18	2.11
YIL168W	SDL1	serine family amino acid metabolism	1.23	0	86.10	105.89	102.16	138.24	120.22	219.88	2.55
YPL093W	NOG1	NA	1.25	-5	231.33	333.06	335.78	359.10	399.49	486.67	2.10
YIL164C	NIT1	NA	3.13	0	751.69	1311.47	1385.40	1584.14	1600.41	1617.29	2.15
YPL171C	OYE3	NA	2.43	4	2957.80	4349.72	4090.99	6722.06	6444.98	7782.61	2.63
YKR046C	PET10	NA	0.34	4	960.20	1303.97	1703.99	1782.36	1939.97	2113.00	2.20

Table 14B ESR subset of genes that are induced specifically in WT

Yname	Gene Symbol	Functional group	Av WT/WT0	MMS, viability	Avg WT10	Avg WT20	Avg WT30	Avg WT 40	Avg WT 50	Avg WT 60	WT60/WT10
YLR074C	BUD20	bud site selection	0.41	11	268.72	441.26	400.32	416.72	458.51	591.42	2.20
YKR076W	ECM4	cell wall organization and biogenesis	0.65	2	740.90	850.44	1087.85	1278.07	1313.60	1881.47	2.54
YNR002C	FUN34	transport	0.16	0	391.12	457.98	594.61	935.55	839.85	993.69	2.54
YFL014W	HSP12	response to dessication	0.43	4	3372.85	6607.11	7848.80	8372.66	8140.08	8758.40	2.60

Table 15: Genes that are incrementally induced specifically in *mgt1*

The net fold-change (FC) over time, was calculated from ratio of mean expression at the 60th minute to mean expression at the 10th minute. A cut-off of FC>2 (for induction) was used to select genes that have been included in this representation. There were 210 genes in this category. Genes that are not a part of the ESR are included in Table 15A. The genes that are incrementally induced specifically in *mgt1* but are a part of the ESR are included in table 15B

Table 15A

Yname	Gene Symbol	Functional group	Sensit	Avg mgt 10	Avg mgt 20	Avg mgt 30	Avg mgt 40	Avg mgt 50	Avg mgt 60	mgt60/mgt10
YLR452C	SST2	adaptation to pheromone during conjugation with cellular fusion	0	535.84	1189.53	1061.18	1368.06	898.91	1108.25	2.07
YGL032C	AGA2	agglutination during conjugation with cellular fusion	0	1156.57	1572.32	1600.66	2038.59	1719.50	2442.50	2.11
YER024W	YAT2	alcohol metabolism	0	176.02	321.47	508.51	304.97	405.29	422.34	2.40
YDR309C	GIC2	axial budding	2	447.75	473.49	703.90	839.48	710.11	1012.98	2.26
YKR063C	LAS1	bud growth	-5	108.40	165.29	239.29	199.82	227.87	226.67	2.09
YDR006C	SOK1	cAMP-mediated signaling	0	223.62	276.11	430.32	437.23	367.69	478.14	2.14
YIL046W	MET30	cell cycle	-5	135.63	181.78	206.90	239.95	225.18	283.71	2.09
YGR032W	GSC2	cell wall organization and biogenesis	0	705.35	958.25	1177.00	1324.83	1623.21	1697.65	2.41
YGL178W_ex2	MPT5	cell wall organization and biogenesis	0	949.12	1710.98	1678.09	1835.80	1947.02	2006.64	2.11
YDL223C	HBT1	cellular morphogenesis during conjugation with cellular fusion	0	222.27	415.99	402.08	373.65	476.12	503.29	2.26
YPL156C	PRM4	conjugation with cellular fusion	0	273.84	450.16	567.68	554.08	516.00	554.65	2.03
YIL117C	PRM5	conjugation with cellular fusion	0	1644.14	3106.99	3972.19	2667.34	2895.99	3509.93	2.13
YDR030C	RAD28	DNA repair	4	172.86	286.26	412.05	371.45	413.01	527.46	3.05
YFR028C	CDC14	exit from mitosis	-5	186.25	307.10	282.50	358.03	339.11	381.43	2.05
YJL098W	SAP185	G1S transition of mitotic cell cycle	0	95.92	198.27	270.67	195.22	205.64	218.20	2.27
YKR099W	BAS1	histidine biosynthesis	0	61.87	123.49	228.52	154.09	148.25	152.60	2.47
YHL040C	ARN1	iron-siderochrome transport	0	173.35	442.71	559.06	394.39	356.55	362.35	2.09
YPL135W	ISU1	iron-sulfur cluster assembly	7	811.84	1207.80	1567.37	1583.01	1343.19	1657.97	2.04
YIL026C	IRR1	mitotic sister chromatid conesion	-5	77.52	158.40	178.61	180.67	173.41	188.15	2.43
YDR021W	FAL1	mRNA splicing	-5	26.46	34.08	58.12	61.11	49.26	60.75	2.30
LSR1_0	LSR1	mRNA splicing	0	293.43	568.81	494.57	551.71	547.21	639.08	2.18
YNR053C_ex1	NOG2	mRNA splicing	0	38.26	48.71	78.62	101.24	79.94	84.24	2.20
YNR053C_ex2	NOG2	mRNA splicing	0	355.63	422.71	886.09	906.71	662.12	752.72	2.12

Yname	Gene Symbol	Functional group	Sensit	Avg mgt 10	Avg mgt 20	Avg mgt 30	Avg mgt 40	Avg mgt 50	Avg mgt 60	mgt60/mgt10
YML113W	DAT1	negative regulation of transcription from Pol II promoter	0	103.27	263.23	455.26	434.78	273.80	286.74	2.78
YPR175W	DPB2	nucleotide-excision repair	-5	161.01	255.32	454.76	424.68	354.83	370.46	2.30
YNL279W	PRM1	plasma membrane fusion	0	123.52	215.43	210.81	310.69	257.61	352.05	2.85
YGR138C	TPO2	polyamine transport	0	212.01	269.46	386.15	577.69	545.78	623.60	2.94
YOR078W	BUD21	processing of 20S pre-rRNA	0	128.72	241.50	350.43	335.94	263.00	306.34	2.38
YHR205W	SCH9	protein amino acid phosphorylation	-5	238.67	413.50	455.03	476.89	471.06	517.77	2.17
YPL026C	SKS1	protein amino acid phosphorylation	0	65.88	128.16	185.92	200.14	113.32	135.20	2.05
YOR056C	NOB1	protein complex assembly	-5	213.54	326.29	507.79	534.51	532.25	672.08	3.15
YGR239C	PEX21	protein-peroxisome targeting	0	77.91	101.48	151.15	131.93	122.36	192.95	2.48
YHR187W	IKI1	regulation of transcription from Pol II promoter	-5	68.79	129.86	150.29	117.18	140.67	144.32	2.10
YHR023W	MYO1	response to osmotic stress	-5	62.02	136.94	151.44	122.15	138.73	131.45	2.12
YHR178W	STB5	response to xenobiotic stimulus	18	80.82	138.28	149.74	150.48	154.32	179.96	2.23
YGL145W	TIP20	retrograde (Golgi to ER) transport	-5	66.37	111.36	121.10	112.12	125.64	138.08	2.08
YGR159C	NSR1	ribosomal small subunit assembly and maintenance	2	718.77	1014.86	1492.63	1679.17	1411.31	1449.98	2.02
YHR085W	IPI1	rRNA processing	-5	46.36	79.40	149.02	118.70	93.36	103.27	2.23
YOL144W	NOP8	rRNA processing	-5	127.31	196.94	273.96	258.70	260.83	264.37	2.08
YBR257W	POP4	rRNA processing	-5	85.36	109.57	178.43	162.65	151.04	183.74	2.15
YOL010W	RCL1	rRNA processing	-5	60.09	89.39	187.41	176.05	125.07	174.79	2.91
YGR055W	MUP1	sulfur amino acid transport	0	364.89	563.47	983.59	976.29	779.42	1075.34	2.95
YHR124W	NDT80	transcription	0	67.34	107.72	142.09	121.87	123.07	143.62	2.13
YOR337W	TEA1	transcription	0	136.41	226.25	376.04	367.70	263.19	323.40	2.37
YKL125W	RRN3	transcription from Pol I promoter	-5	131.44	181.94	280.60	287.73	237.07	284.92	2.17
YER028C	MIG3	transcription initiation	0	144.30	312.65	355.47	308.67	379.82	407.84	2.83
YPL202C	AFT2	transcription initiation from Pol II promoter	0	225.87	437.58	518.65	471.65	448.07	543.32	2.41
YGL243W	TAD1	tRNA modification	0	242.44	443.35	639.68	475.38	528.97	598.55	2.47
YNL077W	APJ1	NA	0	72.90	81.46	90.50	97.50	131.96	158.21	2.17
YPL250C	ICY2	NA	0	489.29	1161.48	2085.00	1376.20	1456.37	2160.60	4.42
YHR156C	LIN1	NA	0	76.30	124.46	142.05	128.88	131.00	171.54	2.25

Table 15B: ESR subset of genes that are incrementally *mgt1*

Yname	Gene Symbol	Functional group	Sensit	Avg mgt 10	Avg mgt 20	Avg mgt 30	Avg mgt 40	Avg mgt 50	Avg mgt 60	mgt60/mgt10
YHR169W	DBP8	35S primary transcript processing	-5	68.86	110.14	168.25	174.38	143.42	139.09	2.02
YGR158C	MTR3	mRNA catabolism	-5	121.55	275.23	279.80	280.19	265.57	260.10	2.14
YLR129W	DIP2	processing of 20S pre-rRNA	-5	233.64	464.57	633.84	626.48	553.49	515.36	2.21
YLR222C	UTP13	processing of 20S pre-rRNA	-5	139.65	258.53	430.32	384.16	345.47	377.06	2.70
YML093W	UTP14	processing of 20S pre-rRNA	-5	127.02	219.35	356.51	298.95	267.95	276.32	2.18
YMR093W	UTP15	processing of 20S pre-rRNA	-5	83.62	144.76	275.57	246.58	150.70	182.60	2.18
YDR398W	UTP5	processing of 20S pre-rRNA	-5	231.09	276.21	420.64	389.90	371.88	466.21	2.02
YDR449C	UTP6	processing of 20S pre-rRNA	-5	41.81	64.58	120.76	104.82	86.62	103.70	2.48
YER082C	UTP7	processing of 20S pre-rRNA	-5	87.69	116.60	176.64	140.72	155.32	176.84	2.02
YHR196W	UTP9	processing of 20S pre-rRNA	-5	47.43	84.42	137.08	148.50	96.77	122.52	2.58
YOR145C	PNO1	protein complex assembly	-5	274.33	352.01	624.30	600.34	632.53	647.92	2.36
YHR088W	RPF1	ribosomal large subunit assembly and maintenance	-5	73.38	153.45	235.87	189.68	240.99	293.72	4.00
YNL308C	KRI1	ribosome biogenesis	-5	242.25	350.99	555.31	527.63	497.87	571.39	2.36
YDL060W	TSR1	ribosome biogenesis and assembly	-5	425.12	621.10	959.29	937.47	884.10	937.44	2.21
YHR148W	IMP3	rRNA modification	-5	81.45	117.42	149.50	137.99	168.08	166.53	2.04
YLL011W	SOF1	rRNA modification	-5	84.65	171.27	349.76	329.96	213.21	250.61	2.96
YHR197W	IPI2	NA	-5	208.42	302.67	431.26	404.92	445.96	438.12	2.10
YNL182C	IPI3	NA	-5	46.32	69.30	110.25	110.04	92.08	93.80	2.03
YGL248W	PDE1	cAMP-mediated signaling	0	857.73	1515.46	1984.02	1475.29	1777.83	1938.20	2.26
YGL156W	AMS1	carbohydrate metabolism	0	267.04	363.48	458.83	396.14	487.11	593.61	2.22
YHR096C	HXT5	hexose transport	0	128.78	224.38	273.32	255.55	278.39	296.70	2.30
YGL078C	DBP3	ribosomal large subunit assembly and maintenance	0	177.58	277.67	375.11	321.56	350.27	358.39	2.02
YKR056W	TRM2	tRNA modification	0	153.85	219.86	375.37	299.41	345.23	367.08	2.39
YDR496C	PUF6	NA	0	101.08	150.90	240.93	207.74	219.70	249.32	2.47
YGL180W	APG1	autophagy	6	219.66	365.16	347.27	310.44	334.26	443.67	2.02
YKR024C	DBP7	ribosomal large subunit assembly and maintenance	6	48.51	85.74	138.75	143.84	99.38	117.27	2.42
YHR066W	SSF1	ribosomal large subunit assembly and maintenance	6	47.26	124.74	244.82	242.50	148.78	190.84	4.04

Table 16: Genes that incrementally repressed in both WT and *mgt1*

The net fold-change (FC) over time, was calculated from ratio of mean expression at the 60th minute to mean expression at the 10th minute. A cut-off of FC<0.5 (for repression) was used to select genes that have been included in this representation. There are 35 genes in this category. Genes included in Table 16B are the ones that are in this category but are a part of the ESR.

Table 16A

ORF	Gene	Function	Sensitivity	AvWT0	AvWT10	Av WT 20	Av WT30	Av WT40	Av WT50	Av WT60	Avg mgt0	Av mgt 10	Av mgt 20	Av mgt 30	Av mgt40	Av mgt 50	Avg mgt60	WT60/WT10	mgt60/mgt10
YBR158W	AMN1	null	0	177.6	467.3	429.7	371.1	253.5	213.4	206.9	185.3	473.4	416.2	346.6	303.3	236.6	231.3	0.44	0.49
YNR067C	DSE4	cytokinesis, completion of separation	0	52.4	123.5	93.0	77.5	30.2	28.1	30.1	66.4	133.2	84.7	79.9	50.6	28.5	32.0	0.24	0.24
YJL157C	FAR1	cell cycle arrest	0	87.4	87.7	72.4	57.3	42.5	42.8	35.0	88.4	149.2	126.2	69.6	110.2	71.3	54.9	0.40	0.37
YBL002W	HTB2	chromatin assembly disassembly	0	175.2	233.9	191.4	162.5	104.3	108.1	109.0	188.1	255.0	150.3	136.3	150.9	152.8	113.3	0.47	0.44
YDL179W	PCL9	cell cycle	0	54.3	45.3	30.3	27.0	17.8	16.8	17.9	71.7	40.7	40.1	31.7	24.5	19.4	15.0	0.40	0.37
YKL164C	PIR1	cell wall organization and biogenesis	0	1310.6	1740.8	1266.2	1280.5	626.8	631.9	421.9	875.7	1840.0	###	915.9	514.0	481.7	284.9	0.24	0.15
YJL078C	PRY3	NA	0	423.6	1064.6	1032.4	930.7	506.4	453.6	473.1	376.5	1094.5	852.4	768.7	511.1	462.3	328.0	0.44	0.30
YDR055W	PST1	NA	0	645.3	600.6	555.4	558.6	360.2	302.5	267.3	566.5	949.0	939.4	873.9	481.8	449.0	354.7	0.45	0.37
YLR367W_ex1	RPS22B	protein biosynthesis	0	242.9	626.5	510.7	726.1	386.2	336.2	297.0	271.4	717.7	389.5	418.5	354.8	319.0	221.2	0.47	0.31
YDR502C	SAM2	methionine metabolism	0	381.7	518.5	369.5	376.1	220.7	288.9	253.5	371.9	357.5	153.7	123.5	144.8	129.7	123.8	0.49	0.35
YGL028C	SCW11	cytokinesis, completion of separation	0	39.7	94.7	81.8	73.0	35.8	36.4	28.0	48.6	136.9	134.8	93.6	74.6	56.2	54.0	0.30	0.39
TLC1_0	TLC1	telomerase-dependent telomere maintenance	0	34.7	134.8	122.4	112.4	88.6	79.7	59.0	35.9	271.1	194.0	118.0	154.1	172.6	111.6	0.44	0.41

Table 16B: ESR subset of genes that are repressed in both WT and *mgt1*

ORF	Gene	Function	Sensitivity	AvWT0	AvWT10	Av WT 20	Av WT30	Av WT40	Av WT50	Av WT60	Avg mgt0	Av mgt 10	Av mgt 20	Av mgt 30	Av mgt40	Av mgt 50	Avg mgt60	WT60/WT10	mgt60/mgt10
YNL066W	SUN4	mitochondrion organization and biogenesis	0	144.3	822.5	747.8	734.0	410.3	387.8	398.0	166.6	734.2	633.9	501.5	384.2	334.4	261.7	0.48	0.36

Table 17: Genes that are incrementally repressed specifically in *mgt1*

The net fold-change (FC) over time, was calculated from ratio of mean expression at the 60th minute to mean expression at the 10th minute. A cut-off of FC<0.5 (for repression) was used to select genes that have been included in this representation. There are 118 genes in this category. The genes that are in this category and are a part of ESR are included in table 17B.

ORF	Gene	Function	Sensitivity	Avg mgt0	Av mgt 10	Av mgt 20	Av mgt 30	Av mgt40	Av mgt 50	Avg mgt60	mgt60/mgt10
YKL190W_ex1	CNB1	adaptation to pheromone during conjugation with cellular fusion	0	204.7	390.6	202.0	192.8	195.5	214.4	166.0	0.42
YNL098C	RAS2	adenylate cyclase activation	0	383.8	549.2	335.1	270.8	326.1	316.5	265.8	0.48
YPR138C	MEP3	ammonium transport	0	91.3	62.7	44.5	46.1	54.4	33.6	28.4	0.45
YBR085W	AAC3	ATP ADP exchange	0	21.6	87.5	50.1	46.5	50.7	36.6	34.0	0.39
YDL181W	INH1	ATP synthesis coupled proton transport	0	963.4	165.1	81.3	54.6	58.0	51.2	59.3	0.36
YKL163W	PIR3	cell wall organization and biogenesis	0	2330.1	478.9	460.9	405.0	177.0	290.9	232.4	0.49
YBR093C	PHO5	cellular response to phosphate starvation	0	111.1	533.0	258.9	257.9	231.9	226.7	230.7	0.43
YNR075W_0	COS10	endocytosis	0	18.9	50.0	36.6	21.7	24.3	28.0	20.1	0.40
YLR300W	EXG1	glucan metabolism	0	239.7	842.2	442.2	374.6	408.6	313.5	319.6	0.38
YOR375C	GDH1	glutamate biosynthesis, using glutamate dehydrogenase (NAD(P)+)	0	1309.1	1012.1	490.2	419.4	406.3	428.5	465.1	0.46
YMR006C	PLB2	glycerophospholipid metabolism	0	175.0	326.1	236.7	176.3	190.2	204.8	159.0	0.49
YFR015C	GSY1	glycogen metabolism	0	563.8	1521.5	978.6	467.4	438.9	671.7	535.0	0.35
YMR319C	FET4	intracellular copper ion transport	4	109.8	268.6	169.3	153.8	151.0	126.2	117.4	0.44
YKL140W	TGL1	lipid metabolism	0	73.7	241.6	160.9	102.4	130.5	128.4	115.3	0.48
YBR041W	FAT1	lipid transport	5	26.3	146.3	89.4	75.3	66.3	71.9	68.0	0.46
YLR180W	SAM1	methionine metabolism	0	2391.0	3974.1	1477.4	1505.2	1828.0	2329.0	1922.5	0.48
YDR381W_ex1	YRA1	mRNA-nucleus export	0	637.9	1029.1	478.7	316.5	359.0	414.8	349.8	0.34
YDR381W_ex2	YRA1	mRNA-nucleus export	0	202.1	307.9	165.6	107.7	119.1	125.6	114.7	0.37
YNR016C	ACC1	nuclear membrane organization and biogenesis		864.2	1168.7	883.7	662.9	753.6	769.5	566.3	0.48

ORF	Gene	Function	Sensitivity	Avg mgt0	Av mgt 10	Av mgt 20	Av mgt 30	Av mgt40	Av mgt 50	Avg mgt60	mgt60/mgt10
YKR093W	PTR2	peptide transport	6	460.4	599.2	364.3	293.4	282.0	284.1	253.2	0.42
YAR071W	PHO11	phosphate metabolism		118.8	705.1	389.6	310.3	303.7	387.2	316.1	0.45
YNL185C	MRPL19	protein biosynthesis		71.0	96.9	55.6	59.7	58.4	61.8	44.7	0.46
YNL328C	MDJ2	protein folding	0	23.8	35.0	27.5	24.2	23.2	23.2	15.6	0.44
YLL024C	SSA2	protein folding	8	201.9	2736.6	736.6	571.5	884.3	815.1	869.4	0.32
YJR045C	SSC1	protein folding		441.0	448.7	290.6	211.6	249.9	250.7	213.4	0.48
YJR126C	VPS70	protein-vacuolar targeting	0	544.2	591.0	317.4	273.4	243.8	247.6	244.3	0.41
YKL216W	URA1	pyrimidine base biosynthesis	2	906.4	703.0	265.4	233.4	233.3	279.4	235.3	0.33
YML010W	SPT5	regulation of transcription, DNA-dependent		144.2	135.9	64.4	56.2	58.2	77.1	59.5	0.44
YBR189W_ex2	RPS9B	regulation of translational fidelity	0	76.7	705.2	192.3	170.9	231.8	219.2	214.9	0.30
YLR006C	SSK1	response to hydrogen peroxide	18	197.9	115.0	105.9	88.4	89.2	55.6	52.9	0.46
YHL028W	WSC4	Rho protein signal transduction	4	57.4	117.1	76.0	43.3	65.9	47.5	45.2	0.39
YJL212C	OPT1	sulfur metabolism	0	69.4	331.3	178.7	167.7	197.9	195.7	152.8	0.46
YKL194C	MST1	threonyl-tRNA aminoacylation	0	10.8	53.2	32.0	34.8	30.7	30.8	23.6	0.44
YKL081W_ex1	TEF4	translational elongation	0	57.5	398.9	221.8	128.8	206.0	226.8	155.0	0.39
YKL081W_ex2	TEF4	translational elongation	0	84.3	518.6	274.0	221.4	252.2	292.2	206.0	0.40
YJR047C	ANB1	translational initiation	0	61.3	391.6	165.7	137.8	166.5	186.0	135.8	0.35
YMR260C	TIF11	translational initiation		116.1	266.9	128.4	126.9	107.6	119.8	116.7	0.44
YNR008W	LRO1	triacylglycerol biosynthesis	0	46.9	122.5	76.2	56.7	66.4	56.6	41.9	0.34
YKL085W	MDH1	tricarboxylic acid cycle	0	1641.9	1852.4	1336.2	677.9	856.0	905.4	837.6	0.45
RPR1	RPR1	tRNA processing	0	327.6	120.5	56.5	45.5	55.8	58.4	54.2	0.45
YMR119W	ASI1	ubiquitin-dependent protein catabolism	4	91.2	213.3	119.3	91.9	93.8	100.8	93.1	0.44
YJR118C	ILM1	NA	4	61.8	265.5	180.9	165.9	157.2	163.1	126.7	0.48
YOL002C	PHO36	NA	7	127.7	859.8	543.0	441.4	371.8	409.7	393.9	0.46
RDN37-1_2	RDN37-1	NA	0	878.6	1001.5	87.5	38.9	101.4	278.7	404.4	0.40
RDN37-1_3	RDN37-1	NA	0	381.6	256.9	26.6	16.7	32.0	31.6	41.1	0.16
YMR266W	RSN1	NA	0	477.2	619.6	353.6	268.2	245.1	284.7	264.5	0.43
YBR054W	YRO2	NA	0	600.0	208.8	86.0	50.0	85.1	63.0	86.6	0.41

17B: ESR subset of genes that are specifically repressed in *mgt1*

ORF	Gene	Function	Sensitivity	Avg mgt0	Av mgt 10	Av mgt 20	Av mgt 30	Av mgt40	Av mgt 50	Avg mgt60	mgt60/mgt10
YAR073W	IMD1	GTP biosynthesis		34.4	117.8	58.7	41.7	48.8	56.9	43.3	0.37
YLR372W	SUR4	fatty acid biosynthesis	0	58.8	1116.1	669.2	669.1	670.6	659.0	541.9	0.49
YLR258W	GSY2	glycogen metabolism	0	495.1	1743.8	1239.9	763.5	730.1	885.3	773.5	0.44
YDL022W	GPD1	intracellular accumulation of glycerol	0	2091.3	1398.7	902.1	565.7	744.5	639.5	696.1	0.50
YJL080C	SCP160	mRNA localization, intracellular	10	63.9	734.4	507.4	446.6	460.5	487.0	365.3	0.50
YJL183W	MNN11	protein amino acid glycosylation	0	60.1	122.1	68.8	74.4	65.9	62.0	60.4	0.49
YBR031W	RPL4A	protein biosynthesis	0	18.7	101.4	25.1	25.4	22.3	28.6	38.9	0.38
YNL067W	RPL9B	protein biosynthesis	0	423.4	1150.2	635.1	480.5	530.8	578.3	470.5	0.41
YOL097C	WRS1	tryptophanyl-tRNA aminoacylation		112.2	730.7	519.4	436.7	458.9	435.8	361.9	0.50
YDL204W	RTN2	NA	0	284.8	178.1	141.8	77.0	77.1	83.8	77.9	0.44

Table 18 Genes that are incrementally repressed specifically in WT

There are 90 genes in this category. Genes that have a known function are included in Table 18A. 2 of the genes that had a known function and are also a part of the ESR are included in table 18B. The genes that are essential are included in red.

ORF	Gene	Function	Sensitivity	AvWT0	AvWT10	Av WT 20	Av WT30	Av WT40	Av WT50	Av WT60	WT60/WT10
YGR041W	BUD9	bud site selection	0	23.0	74.4	56.5	53.1	37.0	32.9	25.9	0.35
YER124C	DSE1	cell wall organization and biogenesis	0	57.3	122.5	89.0	113.7	50.5	56.5	46.5	0.38
YHR143W	DSE2	cell wall organization and biogenesis	0	612.7	2665.5	2536.6	2417.3	1867.6	1771.1	1247.9	0.47
YMR032W	HOF1	cytokinesis	28	37.1	80.7	59.6	46.4	42.5	33.4	36.8	0.46
YNR075W_1	COS10	endocytosis	0	45.3	195.4	136.6	96.5	91.3	68.5	80.3	0.41
YGR249W	MGA1	filamentous growth	0	34.3	73.0	56.0	56.9	44.1	41.9	36.1	0.49
YDL227C	HO	gene conversion at MAT locus	0	12.5	30.0	27.2	19.7	19.5	14.8	14.8	0.49
YDR432W	NPL3	mRNA-nucleus export	18	799.7	926.2	734.5	703.8	720.8	519.2	362.8	0.39
YHR123W_ex1	EPT1	phosphatidylethanolamine biosynthesis	0	7.3	92.7	51.1	48.6	53.1	40.7	36.1	0.39
YIR033W	MGA2	positive regulation of transcription from Pol II promoter	0	36.6	83.3	50.2	53.2	38.5	45.7	36.6	0.44
YKR080W	MTD1	purine base biosynthesis	2	913.9	1136.0	1138.6	1594.5	719.1	1096.1	472.6	0.42
YGR116W	SPT6	RNA elongation from Pol II promoter	[redacted]	28.6	83.0	42.4	50.0	34.4	47.1	37.6	0.45
YIL074C	SER33	serine family amino acid biosynthesis	2	105.9	524.0	455.8	459.9	397.8	417.1	261.7	0.50
TLC1_1	TLC1	telomerase-dependent telomere maintenance	0	41.5	183.7	153.9	147.5	128.8	119.3	82.6	0.45
YIL119C	RPI1	thiamin biosynthesis	0	174.4	89.9	61.3	67.0	58.5	79.9	44.5	0.49
YCL064C	CHA1	threonine catabolism	0	23.8	166.3	154.4	138.9	94.8	72.0	70.9	0.43
YGL077C	HNM1	transport	0	87.0	185.6	130.8	132.4	119.0	127.9	91.6	0.49
YEL003W	GIM4	tubulin folding	6	37.4	93.6	72.4	70.9	64.5	79.7	46.5	0.50

Table 18B: ESR subset of genes that are incrementally repressed specifically in WT

ORF	Gene	Function	Sensitivity	AvWT0	AvWT10	Av WT 20	Av WT30	Av WT40	Av WT50	Av WT60	WT60/WT10
YEL040W	UTR2	cell wall organization and biogenesis	0	22.6	359.0	318.2	251.2	205.0	230.2	178.2	0.50
YGL147C	RPL9A	protein biosynthesis	0	105.8	561.1	171.1	232.5	271.6	382.2	222.3	0.40

Total RNA synthesis

RNeasy Mini Protocol for Isolation of Total RNA from Yeast
Enzymatic Lysis Protocol — standard version

Use an appropriate number of yeast cells

Important notes before starting

• For RNA isolation from yeast, cells should be harvested in log-phase growth. Use only freshly harvested cells for the enzymatic lysis protocol.

• Prepare Buffer Y1

Buffer Y1 1 M sorbitol
0.1 M EDTA, pH 7.4

Just before use, add:
0.1% -mercaptoethanol
50 U lyticase/zymolase per 1 x 10^7 cells

Depending on the yeast strain and enzyme used, the incubation time, enzyme concentration, and composition of Buffer Y1 may vary. Please adhere to the guidelines of the enzyme supplier.

Important

• β-Mercaptoethanol (β-ME) must be added to Buffer RLT before use. β -ME is toxic; dispense in a fume hood and wear appropriate protective clothing. Add 10 µl β -ME per 1 ml Buffer RLT. Buffer RLT is stable for 1 month after addition of -ME.

• Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%), as indicated on the bottle, to obtain a working solution.

• Buffer RLT may form a precipitate upon storage. If necessary, redissolve by warming, and then place at room temperature. Buffer RLT and Buffer RW1 contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. Guanidine is an irritant. Take appropriate safety measures and wear gloves when handling.

• After enzymatic lysis, all steps of the RNeasy protocol should be performed at room temperature. During the procedure, work quickly.

• After harvesting the cells, all centrifugation steps should be performed at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.

1. Harvest yeast cells in a 12 ml or 15 ml centrifuge tube by centrifuging at 1000 x *g* for 5 min at 4°C. (Do not use more than 5 x 107 yeast cells.) Decant supernatant, and carefully remove remaining media by aspiration. After centrifuging, heat the centrifuge to 20–25°C if the same centrifuge is to be used in the following centrifugation steps of the protocol.

Incomplete removal of the supernatant will affect digestion of the cell wall in step 2.

Note: Freshly harvested cells must be used.

2. Resuspend cells in 2 ml freshly prepared Buffer Y1 containing lyticase or zymolase. Incubate for 10–30 min at 30°C with gentle shaking to generate spheroplasts. Spheroplasts must be handled gently.

Depending on the yeast strain used, the incubation time, amount of enzyme and composition of Buffer Y1 may vary. For best results, follow the guidelines of lyticase/zymolase supplier.

Complete spheroplasting is essential for efficient lysis. **Note:** Freshly harvested cells must be used for preparation of spheroplasts.

3. Centrifuge for 5 min at 300 x *g* to pellet spheroplasts. Carefully remove and discard the supernatant.
Note: Incomplete removal of the supernatant will inhibit lysis and dilute the lysate, affecting the conditions for binding of RNA to the RNeasy silica-gel membrane. Both effects may reduce RNA yield.

4. Add 350 µl Buffer RLT to lyse spheroplasts, and vortex vigorously. If insoluble material is visible, centrifuge for 2 min in a microcentrifuge at maximum speed, and use only the supernatant in subsequent steps.
Note: Ensure that β-ME is added to Buffer RLT before use (see "Important notes before starting").

5. Add 1 volume (usually 350 µl) of 70% ethanol to the homogenized lysate, and mix thoroughly by pipetting. Do not centrifuge. Continue immediately with step 6.
A precipitate may form after the addition of ethanol, but this will not affect the RNeasy procedure.

6. Apply the sample (usually 700 µ any precipitate that may have formed, to an RNeasy mini column placed in a 2 ml collection tube (supplied). Close the tube gently, and centrifuge for 15 s at 8000 x *g* (10,000 rpm). Discard the flowthrough.
Reuse the collection tube in step 7.
If the volume exceeds 700 µl, load aliquots successively onto the RNeasy column, and centrifuge as above. Discard the flow-through after each centrifugation step.*

7. Add 700 µl Buffer RW1 to the RNeasy column. Close the tube gently, and centrifuge for 15 s at □ 8000 x *g* (□ 10,000 rpm) to wash the column. Discard the flow-through and collection tube.*

8. Transfer the RNeasy column into a new 2 ml collection tube (supplied). Pipet 500 µl Buffer RPE onto the RNeasy column. Close the tube gently, and centrifuge for 15 s at □ 8000 x *g* (□ 10,000 rpm) to wash the column. Discard the flow-through.
Reuse the collection tube in step 9.
Note: Buffer RPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RPE before use

9. Add another 500 µl Buffer RPE to the RNeasy column. Close the tube gently, and centrifuge for 2 min at □ 8000 x *g* (□ 10,000 rpm) to dry the RNeasy silica-gel membrane. Continue directly with step 10, or, to eliminate any chance of possible Buffer RPE carryover, continue first with step 9a.
It is important to dry the RNeasy silica-gel membrane since residual ethanol may interfere with downstream reactions. This centrifugation ensures that no ethanol is carried over during elution.
Note: Following the centrifugation, remove the RNeasy mini column from the collection tube carefully so the column does not contact the flow-through as this will result in carryover of ethanol.

9a. Optional: Place the RNeasy column in a new 2 ml collection tube (not supplied), and discard the old collection tube with the flow-through. Centrifuge in a microcentrifuge at full speed for 1 min.

** Flow-through contains Buffer RLT or Buffer RW1 and is therefore not compatible with bleach.*

10. To elute, transfer the RNeasy column to a new 1.5 ml collection tube (supplied). Pipet 30–50 µl RNase-free water directly onto the RNeasy silica-gel membrane. Close the tube gently, and centrifuge for 1 min at ≥ 8000 x *g* (≥ 10,000 rpm) to elute.

11. If the expected RNA yield is >30 µg, repeat the elution step (step 10) as described with a second volume of RNase-free water. Elute into the same collection tube.
To obtain a higher total RNA concentration, this second elution step may be performed by using the first eluate (from step 10). The yield will be 15–30% less than the yield obtained using a second volume of RNase-free water, but the final concentration will be higher.

1st Strand Synthesis

Amount of RNA

- Using a 1.5 ml centrifuge tube, mix reagents according to the following Table.

STEP 1

Reagent	Volume (µl)
DEPC-water	x
RNA (1.0 µg/µl)	y
T7 Primer (100 pmol/µl)	1
TOTAL	**12**

- Incubate @ 70°C in a water bath for 10 minutes
- Spin briefly and place on ice till ready to proceed.
- When incubation is done, to the same tube, add reagents according to the Table below.

STEP 2

Reagent	Volume (µl)
Tube from step 1	12
5X 1st strand cDNA buffer	4
0.1 M DTT	2
10 mM dNTP mix	1
TOTAL	**19**

- Mix well with a pipette (carefully, in and out) and incubate at 42 °C, 2 minutes.
- Add variable amount of SS Reverse Transcriptase (final reaction volume =**20 µl**)
- Mix well with a pipette and incubate at 42 °C, 1 hour.

2nd Strand Synthesis

Add the following components to the 1st strand tube from above:

2nd STRAND MIXTURE

Reagent	Volume (µl)
1st Strand Reaction	20
DEPC-Water	91
5X 2nd strand cDNA buffer	30
10 mM dATP, dCTP, dGTP, dTTP	3
DNA Ligase (10 U/µl)	1
DNA Polymerase (10 U/µl)	4
RNase H (2 U/µl)	1
TOTAL	**150**

- Transfer this total amount to a PCR tube and place in the thermocycler at 16°C for 2 hours.
- Add 2 µl of T4 DNA Polymerase (10 U/µl) and incubate at 16°C for 5 minutes.
- Add 10 µl of 0.5 M EDTA. (The total added volume is ~162 µl).
- Proceed to cDNA cleanup or store the reaction at -20°C.

cDNA Clean-up

- Pellet the PLG light in the green tube and set aside.
- Transfer the 2nd strand cDNA solution from previous step back to a 1.5 ml tube.
- Add to the 2nd strand cDNA tube (equal volume, assuming 150 µl recovery):

Phenol 75 µl Chloroform: Isoamyl alcohol (24:1) 75 µl

The total volume is now approximately 300 µl

- Vortex the 2nd strand cDNA tube and transfer the entire amount to the pelleted PLG-light tube.
- Centrifuge at 14,000 rpm for 2 minutes. Transfer the aqueous (top) layer to a new tube.
- Add the following to the aqueous layer (assuming recovery of 150 µl):

Glycogen (5 mg/ml)	1 µl	
NH_4OAc (7.5 M)		75 µl
EtOH (100 %)		562 µl

- Vortex and immediately centrifuge for 20 minutes at 14,000 rpm, RT.
- Remove supernatant and wash pellet twice with 500 µl of 80 % EtOH.
- After the last wash, centrifuge and eliminate EtOH using a micropipettor. Centrifuge again and eliminate EtOH remnants with micropipettor.
- Air dry the pellet (5 min at 37°C and 5 min RT) and resuspend in 12 µl DEPC water

Note: Complete drying is very important. The presence of ethanol will inhibit the IVT reaction

Biotin Labeling by *In Vitro* Transcription Reaction (IVT)

Since we started with 5-8 µg of total RNA, the Affymetrix manual recommends using 10 µl out of the 12 µl cDNA solution to setup a 40 µl IVT reaction (see chart in the manual). Using reagents from the ENZO kit, add the following components to a 1.5 ml centrifuge tube.

IVT Reaction Setup

Reagent	Volume (µl)
cDNA template	10
DEPC-Water	12
10X HY reaction buffer	4
10X biotin labeled NTP	4
10X DTT	4
10X RNase inhibitor	4
20X T7 RNA polymerase	2
TOTAL	**40**

- Mix the reagents and centrifuge briefly
- Incubate @ 37°C for 5 hours in an oven to avoid evaporation.
- Make sure to mix with a pipette every 30-45 minutes.
- Store @ -20°C or go to the clean up step

IVT Clean-up

RNeasy RNA Cleanup Procedure

Notes:

-Buffer RLT may form a precipitate upon storage. If this happens, warm up to redissolve.

-Add 10µl of □ -ME to 1 ml of Buffer RLT before use (stable for 1 month).

-Buffer RPE is provided as a concentrate. Before using for the first time, add 4 volumes of ethanol (96-100%) as indicated on the bottle to obtain a working solution.

-All centrifugations are done at room temperature.

Work in groups of two: each group will process half of the sample for RNA cleanup

1. Measure the volume that you now have in your IVT tube (should be ~40 µl).
2. Split the sample in half and continue with the cleanup procedure on half of the sample. Adjust the volume of your portion (~20 µl) to 100 µl using RNase-free water.
3. Add 350 µl Buffer RLT to the sample, and mix thoroughly.

   Note: Ensure that □ -ME is added to Buffer RLT before use.
4. Add 250µl ethanol (96-100%) to the sample, and mix well by pipetting. Do not centrifuge.
5. Apply sample (now 700 µl) to an RNeasy mini spin column sitting in a collection tube. Centrifuge for 15 seconds at 12,000 rpm.
6. Discard flow-through and collection tube.
7. Transfer RNeasy column into a new 2-ml collection tube. Pipet 500µl of wash Buffer RPE onto the RNeasy column, and centrifuge for 15 sec at 12,000 rpm to wash.

   Ensure that ethanol is added to Buffer RPE before use.
8. Discard flow-through and reuse the collection tube in the following step.
9. Pipet 500 µl of wash Buffer RPE onto the RNeasy column. Centrifuge for 2 min at 14,000 rpm to dry the RNeasy membrane.
10. Following the spin, remove the RNeasy column from the collection tube carefully so that the column does not contact the flow-through as this will result in carryover of ethanol.

    It is important to dry the RNeasy membrane since residual ethanol may interfere with subsequent reactions. The following spin ensures that no ethanol is carried over during elution.
11. Place the RNeasy spin column in a new 1.5-ml collection tube, and discard the old collection tube with the filtrate. Centrifuge at 14,000 rpm for 1 min.
12. Transfer the RNeasy column into a new 1.5-ml collection tube, and pipet 30 µl of RNase-free water directly onto the RNeasy membrane. Centrifuge for 1 min at 12,000 rpm to elute.

    A second elution step can be performed using another 30-50 µl RNase-free water. This might improve yield.
13. Dilute 1 µl of the reaction into 99 µl of water and use this for a spec reading using the Biophotometer (Eppendorf).
14. Store the rest at –20°C or proceed with the next step.

Fragmentation

- Use the spec reading to determine what volume will give you 20 μg for fragmentation.

  Apply the convention that 1 OD at 260 nm equals 40μg/ mL RNA.

  1. Check the OD at 260 nm and 280 nm to determine the sample concentration and purity.
  2. Maintain the A_{260}/A_{280} ratio close to 2.0 for pure RNA (ratios between 1.9 and 2.1 are acceptable).
  3. In our test run, we obtained a post- IVT cleanup concentration of 1.356 μg/μl (32 μl), for a total of 43.4 μg. This means that we need 14.7 μl of IVT cleaned up product for the Fragmentation reaction.

- Set up the reaction. The volumes shown in the Table below are from our example run done before the workshop. Your volumes might vary…

STEP 1

Reagent	Volume (μl)
RNA (20 μg)	14.7
DEPC-Water	17.3
5X Fragmentation Buffer	8
TOTAL	**40**

- Mix well, and incubate at 94°C in a water bath for 35 minutes.
- Place on ice or at -20°C.

Adjusted cRNA calculation

- Calculate the adjusted reaction yield from the Cleanup step of the IVT reaction. This is done using the following formula:

Adjusted cRNA yield= RNA_m- (total RNA_t)(y)
RNA_m = amount of cRNA measured after IVT (μg)
Total RNA_t = starting amount of total RNA (μg)
Y = fraction of cDNA reaction used in IVT

In our example: RNA_m = 43.4μg
Total RNA_t = 7.0 μg
Y = 10/12 (fraction of cleaned up cDNA used in the IVT reaction)

Therefore, our adjusted cRNA yield: 37.6 μg [i.e., 43.4 – 7(10/12)]
The volume was 30 μl (see under Fragmentation)
Hence the adjusted concentration previous Fragmentation is 1.25 μg/μl
Since we used 14.7 μl of the prefragmented cRNA in the Fragmentation step, the adjusted amount was 18.4 μg [i.e., 14.7 μl X (1.25 μg/μl)].

Prepare the Target Hybridization Reaction

The Affymetrix manual recommends using 15 μg of sample in the target hybridization. Use the adjusted concentration to calculate the volume of sample needed for 15 μg.

Amount of adjusted cRNA used in the Fragmentation reaction= 18.4 μg
The volume of the Fragmentation reaction was 40 μl
In order to get 15 μg from the Fragmentation reaction we need: 15 μg X (40 μl/18.4 μg)=32.6 μl

Note: One group of two will perform the Test3 Array Hybridization, the corresponding partner group will perform the Species Array Hybridization.

Step 1: Test3 Array Hybridization

- Set the Test3 chip at RT before setting up the reaction.
- Prepare 85 μl of 1X Hybridization buffer. Add 80 μl of this solution to the Test3 Array Chip (Mini format) to wet it.
- Put it in the oven at 45°C for at least 10 minutes at 40-50 rpm.
- Heat the Eukaryotic Hybridization Controls to 65°C for 5 minutes prior to adding it to the reaction below.
- Set up the Target hybridization reaction according to the following Table:

HYBRIDIZATION COCKTAIL

Reagent	Volume (μl)
Fragmented cRNA (15 μg)	32.6
Control oligonucleotide B2	5
20X Eukaryotic Hybridization Controls	15
Herring Sperm DNA (10 mg/ml)	3
Acetylated BSA (50 mg/ml)	3
2X Hyb. Buffer	150
Water	81.4
TOTAL	**300**

- After setting up the reaction, remove 100 μl from the Hybridization cocktail solution (save the rest of the solution at -20°C) and process it as follows:

Heat at 99°C for 5 minutes
Heat at 45°C for 5 minutes
Centrifuge at 14,000 rpm for 5 minute to clarify the cocktail

- Remove the 1X Hyb solution from the 'Test3 Chip' and then add 80 μl of the Hyb. Cocktail.
- Incubate at 45°C at 60 RPM for 16 hours.

Step 2: Species Array Hybridization

- Set the Species Array chip at RT before setting up the reaction.
- Prepare 210 μl of 1X Hybridization buffer. Add 200 μl of this solution to the Species Array Chip (Standard format) to wet it.
- Put it in the oven at 45°C for at least 10 minutes at 40-50 rpm.
- Heat the Eukaryotic Hybridization Controls to 65°C for 5 min prior to adding it to the reaction below.
- Set up the Target hybridization reaction according to the following Table:

HYBRIDIZATION COCKTAIL

Reagent	Volume (µl)
Fragmented cRNA (15 µg)	32.6
Control oligonucleotide B2	5
20X Eukaryotic Hybridization Controls	15
Herring Sperm DNA (10 mg/ml)	3
Acetylated BSA (50 mg/ml)	3
2X Hyb. Buffer	150
Water	81.4
TOTAL	**300**

- After setting up the reaction, remove 250 µl from the Hybridization cocktail solution (save the rest of the solution at -20°C) and process it as follows:

  Heat at 99°C for 5 minutes
  Heat at 45°C for 5 minutes
  Centrifuge at 14,000 rpm for 5 minute to clarify the cocktail

- Remove the 1X Hyb solution from the 'Species Array Chip' and then add 200 µl of the Hyb Cocktail.
- Incubate at 45°C at 60 RPM for 16 hours.

Washing, Staining and Scanning Probe Arrays

- Shortly before the 16 hour incubation is done, follow the fluidics station setup according to Chapter 4, Section 3.
- For the Test3 Array, prepare the SAPE stain solution under 'Washing and Staining Procedure 1: Single Stain' (Chapter 4, Section 4). Streptavidin Phycoerythrin (SAPE) stocks should be stored in amber tubes at 4°C. Remove SAPE stocks from refrigerator and mix well before preparing stain solution. Do not freeze concentrated SAPE or diluted SAPE stain solution. Always prepare the SAPE stain solution immediately before use.

  For 600 µL of SAPE Stain solution:
  300 µL 2X Stain Buffer
  270 µL water
  24 µL of 50 mg/mL acetylated BSA (final concentration of 2 mg/mL)
  6 µL of 1 mg/mL streptavidin phycoerythrin (SAPE) (final concentration of 10 µg/mL)

- For the Species Array (Human U95A), prepare the SAPE stain solution and Antibody solution under 'Washing and Staining Procedure 2: Antibody Amplification' (Chapter 4, Section 4).

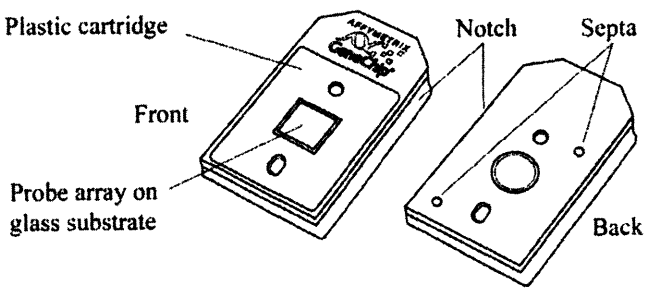
  For 1200 µL SAPEStain solution:
  600 µL of 2X Stain Buffer
  540 µL of water
  48 µL of 50 mg/mL acetylated BSA (final concentration of 2 µg/µL)
  12 µL of 1 mg/mL SAPE (final concentration of 10 µg/mL)

  Mix well and divide into two aliquots of 600 µL each to be used for stains 1 and 3 respectively.

  For 660 µl of Antibody Solution:
  300 µL of 2X Stain Buffer
  266.4 µL of water

24 µL of 50 mg/mL acetylated BSA (final concentration of 2 mg/mL)
6.0 µL of 10 mg/mL normal goat IgG (final concentration of 0.1 mg/mL)
3.6 µL of 0.5 mg/mL biotinylated antibody (final concentration of 3 µg/mL)

- After 16 hours, remove the cocktail and save it (-20°C). Do not let the chip dry out. Immediately add 100 µl of the non-stringent wash buffer to the chip.



GeneChip® Probe Array

RNEasy Cleanup Procedure

Affymetrix recommends an additional cleanup step for the RNA sample before using in a microarray experiment. This is done using the RNeasy reagents as follows (procedure was adapted from user's instructions in the kit):

> *Notes: Do not exceed the RNA binding capacity (100 μg) of the RNeasy mini spin columns.*
>
> *Buffer RLT may form a precipitate upon storage. If this happens, warm up to redissolve.*
>
> *Add 10 μl of□ -ME to 1 ml of Buffer RLT before use (stable for 1 month).*
>
> *Buffer RPE is provided as a concentrate. Before using for the first time, add 4 volumes of ethanol (96-100%) as indicated on the bottle to obtain a working solution.*
>
> *All centrifugations are done at room temperature.*

1. Adjust sample to a volume of 100 μl with RNase-free water, add 350 μl Buffer RLT to the sample, and mix thoroughly.
2. *Note: Ensure that□ -ME is added to Buffer RLT before use.*
3. Add 350 μl Buffer RLT to the sample, and mix thoroughly.
4. *Note: Ensure that□ -ME is added to Buffer RLT before use.*
5. Add 250μl ethanol (96-100%) to the lysate, and mix well by pipetting. Do not centrifuge.
6. Apply sample (now 700 μl) to an RNeasy mini spin column sitting in a collection tube. Centrifuge for 15 seconds at 12,000 rpm.
7. Discard flow-through and collection tube.
8. Transfer RNeasy column into a new 2-ml collection tube. Pipet 500μl of wash Buffer RPE onto the RNeasy column, and centrifuge for 15 sec at 12,000 rpm to wash.
9. *Ensure that ethanol is added to Buffer RPE before use.*
10. Discard flow-through and reuse the collection tube in the following step.
11. Pipet 500 μl of wash Buffer RPE onto the RNeasy column. Centrifuge for 2 min at 14,000 rpm to dry the RNeasy membrane.
12. Following the spin, remove the RNeasy column from the collection tube carefully so that the column does not contact the flow-through as this will result in carryover of ethanol.
13. *It is important to dry the RNeasy membrane since residual ethanol may interfere with subsequent reactions. The following spin ensures that no ethanol is carried over during elution.*
14. Place the RNeasy spin column in a new 2-ml collection tube, and discard the old collection tube with the filtrate. Centrifuge at 14,000 rpm for 1 min.
15. Transfer the RNeasy column into a new 1.5-ml collection tube, and pipet 30 μl of RNase-free water directly onto the RNeasy membrane. Centrifuge for 1 min at 12,000 rpm to elute.
16. *A second elution step can be performed using another 30-50 μl RNase-free water. This might improve yield.*
17. Dilute 1 μl of the reaction into 99 μl of water and use this for a spec reading using the Biophotometer (Eppendorf).

18. Store the rest at –80°C or proceed with the next step.

2g. Spectrophotometric analysis

1. To determine the concentration and purity of the RNA solution, transfer 2 µl of your RNA solution into an RNase-free tube containing 98 µl of DEPC-water. The lab instructors will measure the A_{260}/A_{280}. Pure RNA will give a ratio of approximately 2.0.

   1.0 A_{260}= 40 µg/ml RNA

   Dilution factor in spectrophotometric cuvette= 50

   RNA solution conc. (µg/ml)= (A_{260})(100)(40µg/ml)