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A Processive Protein Chimera Introduces Mutations Across Defined DNA Regions *In Vivo*

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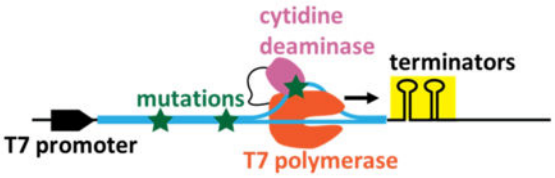
Abstract

Laboratory time scale evolution *in vivo* relies on the generation of large, mutationally diverse gene libraries to rapidly explore biomolecule sequence landscapes. Traditional global mutagenesis methods are problematic because they introduce many off-target mutations that are often lethal and can engender false positives. We report the development and application of the MutaT7 chimera, a potent and highly targeted *in vivo* mutagenesis agent. MutaT7 utilizes a DNA-damaging cytidine deaminase fused to a processive RNA polymerase to continuously direct mutations to specific, well-defined DNA regions of any relevant length. MutaT7 thus provides a mechanism for *in vivo* targeted mutagenesis across multi-kb DNA sequences. MutaT7 should prove useful in diverse organisms, opening the door to new types of *in vivo* evolution experiments.

Graphical Abstract



Traditional *in vivo* mutagenesis strategies, which are critical for studying and using evolution in living systems, rely on exogenous mutagens (e.g., high energy light or chemicals)[1, 2] or expressing mutagenic enzymes (e.g., XL1-Red[3] or the MP6 plasmid[4]). These global mutagenesis strategies can yield high mutation rates and diverse genetic landscapes. However, extensive mutations throughout the genome are problematic in many contexts, especially in directed evolution experiments (Figure 1a). Off-target mutations outside the intended DNA region are often toxic when they occur in essential portions of the genome,[5, 6] a problem that limits library size and engenders rapid silencing of mutagenic plasmids. Global mutagens also introduce “parasite” variants into DNA libraries, originating

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from mutations outside the gene of interest that allow an organism to circumvent selection schemes.[7]

Targeted *in vivo* mutagenesis strategies have the potential to overcome these deficiencies. DNA-damaging enzymes fused to deactivated Cas9 nucleases can edit bases at specific genetic loci,[8–12] but require many gRNAs to tile mutagenic enzymes throughout a target DNA that may be multi-kb in length.[13, 14] Moreover, the guide RNAs must be redesigned after each evolution round introduces new mutations in the target DNA. Another example is the use of an error-prone polI variant to selectively mutagenize genes on ColE1 plasmids, although this method is limited to *Escherichia coli* and can target mutations within only a few kb of the ColE1 origin.[15, 16] Error-prone replication mediated by the Ty1 retrotransposon specifically in yeast can also selectively mutate <5 kb genetic cargoes inserted into the retrotransposon.[17] Other targeted mutation methods in yeast include oligo-mediated genome engineering,[18] which can be labor-intensive, and an orthogonal replication system,[19] which was developed specifically in yeast.

We rationalized that a processive, DNA-traversing biomolecule tethered to a DNA-damaging enzyme could provide a generalizable solution to the problem of targeting mutations across large, yet still well-defined, DNA regions. Monomeric RNA polymerases possess inherently high promoter specificity[20] and processivity.[21] Cytidine deaminases are potent DNA-damaging enzymes that can act on single-stranded DNA substrates during transcription.[22] We envisioned that a chimeric "MutaT7" protein consisting of a cytidine deaminase (rApo1) fused to T7 RNA polymerase (T7-pol) would, therefore, allow us to target mutations specifically to any DNA region lying downstream of a T7 promoter (Figure 1b), provided the T7 promoter is not present elsewhere in the genome.

To begin, we used a lacZ expression assay[23] to show that T7-Pol tolerated an rApo1 N-terminal fusion and still efficiently transcribed tens of kilobases (Figure S1). Next, we integrated the MutaT7 gene under control of a weak promoter into the genome of *E. coli* lacking uracil N-glycosylase (Δung) (Figure S2 and Table S1). Deleting *ung* inhibits repair of deoxyuridine to deoxycytidine and increases mutagenesis rates,[24, 25] especially in the context of cytidine deaminases.[26] We assayed for targeted mutagenesis using a codon reversion assay based on reporter plasmids either having or lacking a T7 promoter sequence upstream of silent drug resistance genes with ACG triplets in place of ATG start codons (Figures 2a and S3-S5a). The kanamycin resistance gene *(Kan^R)* was placed immediately downstream of the T7 promoter. In this assay, successful C → T mutagenesis at the Kan^R start codon yields kanamycin-resistant colonies. Global mutagens such as the MP6 plasmid yielded high levels of kanamycin-resistant colonies regardless of the T7 promoter, consistent with a lack of promoter-based targeting (Figure 2b). In contrast, MutaT7 strains attained significant kanamycin resistance only when reporter plasmids possessed a T7 promoter upstream of the Kan^R gene (Figure 2b and Table S2). Expression of a catalytically dead version of MutaT7 (drApo1-T7)[27] yielded kanamycin resistance frequencies similar to background levels, indicating that T7 activity alone was not responsible for the observed increase in kanamycin resistance (Figure 2b).

T7 promoter-dependent *Kan*^{R} mutagenesis by MutaT7 shows that mutagenesis can be targeted to a desired DNA region near a T7 promoter. Because T7-pol is highly processive, we anticipated mutations would also be introduced further downstream of the T7 promoter. We assayed for MutaT7 processivity by inserting a tetracycline-resistance *(Tet*^{R}*)* gene with an inactive, ACG start codon ~1.6 kb downstream of the *Kan*^{R} gene (Figure 2a). We observed high levels of MutaT7-dependent tetracycline resistance only in reporter strains having the T7 promoter, consistent with targeted and processive introduction of mutations across a lengthy, multi-kb DNA region (Figure 2b). Global mutagens again generated tetracycline-resistant colonies at high frequency in all cases, irrespective of the T7 promoter (Figure 2b).

Targeted mutagenesis using the processive MutaT7 chimera requires not just recruitment to a DNA locus, but also termination at the end of targeted DNA. To address termination, we used *Kan*^{R}*/Tet*^{R} reporter plasmids in which we separated the silent, start codon-defective resistance genes by one or more T7 terminators (Figure 2a). Upon assaying for drug resistance, we found that four copies of the T7 terminator fully constrained mutagenesis to the intended upstream *Kan*^{R} gene (Figure S6). Using this terminator array, we observed tetracycline resistance for MutaT7 strains similar to background levels, whereas kanamycin resistance remained high (Figure 2b). Global mutagens again induced high levels of kanamycin- and tetracycline-resistance, irrespective of the terminator array (Figure 2b).

To further assess whether MutaT7 induces mutagenesis specifically on the target DNA, we evaluated the evolution of resistance to rifampicin[28] and fosfomycin[29]. Resistance can derive from diverse genomic mutations such that the appearance of resistant colonies correlates with off-target mutation rates in the genom[4,28] analogous to cheating parasites in directed evolution schemes. Selection on either rifampicin- or fosfomycin-treated plates revealed that MutaT7-expressing samples displayed drug resistance frequencies comparable to background. In contrast, high frequencies of antibiotic resistance were observed in all global mutagenesis samples (Figures 2c and S5b-c).

We next attempted to use MutaT7 to evolve ectopically expressed *folA* gene variants that confer trimethoprim resistance. The *folA* gene encodes dihydrofolate reductase, and *folA* mutations are just one of many potential routes to trimethoprim resistance.[30] We used either global mutagenesis or MutaT7 to mutagenize *E. coli* carrying a T7-targeted episomal copy of *folA*. We then Sanger-sequenced colonies that grew on trimethoprim plates. We observed that 29 of 44 trimethoprim-resistant colonies mutagenized using MutaT7 had a mutation known to confer resistance[31] in the episomal *folA* promoter (Tables S3 and S4, Figure S7). In contrast, none of the 43 trimethoprim-resistant colonies obtained using the global mutagen contained mutations in the episomal *folA* gene. Instead, they presumably gained trimethoprim resistance via undesired mutations in the *E. coli* genome. The ability of MutaT7 to generate a high rate of true positives in the desired episomal gene target, whereas global mutagenesis exclusively generated cheaters (false positives), highlights a key advantage of MutaT7.

In theory, another benefit of targeted mutagenesis is the capacity to attain much larger library sizes by avoiding toxic mutations in essential, off-target genes. On the basis of the

apparently low off-target mutagenesis rate of MutaT7, we hypothesized that *E. coli* carrying MutaT7 would have significantly higher viability than bacteria treated with global mutagens. Indeed, consistent with prior work,[4] we observed very low viability in all populations treated with global mutagens. In contrast, populations expressing MutaT7 possessed viability similar to untreated cells (Figures 2d and S5d). We also found that the total number of kanamycin-resistant colonies was similar between MutaT7 and globally mutagenized samples (Figure 2e) despite the somewhat lower mutagenesis rate of the MutaT7 construct relative to MP6 (Figure 2b; the average kanamycin resistance frequency for MutaT7 was 6.7 $\times 10^{-6}$ versus 5.7 $\times$ 10^{-5} for MP6). This observation highlights that the use of MutaT7 to maximize on-target mutations while simultaneously minimizing off-target mutations results in larger productive library sizes.

We next turned to DNA sequencing to better understand the processivity and targeting of MutaT7 mutagenesis. We allowed an *E. coli* population expressing MutaT7 and the episomal Kan^R/Tet^R reporter plasmid to drift in the absence of selection pressure for 15 days prior to isolation of episomal DNA from clones (Figure 3a). Sanger sequencing of the target episomal region revealed mutations at multiple loci throughout the Kan^R target gene, independent of selection pressure (Figure S8 and Table S5). In a separate experiment where the target DNA consisted of an episomal *rpsL* allele (initially sensitive to streptomycin) downstream of a T7 promoter (Figure S9a), we further evaluated the processivity of MutaT7. Sanger sequencing of streptomycin-resistant DNA isolated from a MutaT7-expressing strain of *E. coli* again revealed that multiple mutations appeared throughout the targeted *rpsL* gene, with ~90% C → T mutations and ~10% G → A mutations (Figure S9b and Table S6).

Next, we employed Illumina sequencing to identify mutations anywhere in the episomal reporter DNA sequence obtained from clones of the *E. coli* populations in Figure 3a. This experiment assesses on- versus off-target mutagenesis across a ~10 kb stretch of DNA containing only ~1 kb of intended target DNA. MutaT7 samples displayed many mutations throughout the episome when the terminator array was removed but the T7 promoter was maintained (Figure 3b; see also Table S7). Treatment with the MP6 global mutagen also led to mutations throughout the entire episomal DNA. In contrast, mutations in MutaT7 strains appeared almost exclusively within the Kan^R target gene when both a promoter and terminator array were present, even after 15 days of continuous culturing (Figure 3b; see also Table S7). Upon normalizing on- and off-target mutation rates, we observed that the few off-target mutations found on plasmids with a terminator from MutaT7 strains were present only to the same extent as in the control sample not treated with any mutagen (Figure 3c, red striped bars).

A disadvantage of MutaT7 is its limited mutational spectrum and an apparent strand bias observed in our sequencing results showing that we obtained predominantly C → T transitions in the sense strand using a single T7 promoter (Figure 3c and Tables S5–S7). We hypothesized that the mutational spectrum could be doubled by introducing a second T7 promoter that would recruit MutaT7 to the 3’ end of the target DNA and enable processive activity in the opposing direction. Indeed, we found that installing an additional antisense T7 promoter led to the accumulation of both G → A and C → T mutations throughout the target gene during continuous culturing (Figure S10a-b and Table S8). Furthermore, the

average number and range of mutations per clone increased over time (Figure S10c). The latter observation indicates that, in contrast to global mutagenesis methods where the organism often rapidly silences mutagen expression, the high on-target to off-target mutation ratio of MutaT7 enabled long-term maintenance of mutagen expression in cells.

We note that repair of deoxyuridine must be prevented to observe significant mutagenesis with MutaT7 (also observed with other cytidine deaminase-based systems[4,8]). Although we used Δ*ung* cells to address this issue in the aforementioned experiments, a more flexible alternative is to co-express MutaT7 with the uracil glycosylase inhibitor (UGI; a protein that can inhibit UNG activity in many prokaryotes and eukaryotes[4,8,32]). Such co-expression resulted in a high rate of mutagenesis similar to that achieved using Δ*ung* cells (see Figure S11). UGI thus eliminates the need to delete *ung* to achieve efficient mutagenesis with MutaT7, significantly increasing the flexibility of our system.

In summary, the processively acting MutaT7 chimera can selectively direct mutations to large, yet well-defined, regions of DNA *in vivo.* We anticipate that utilizing other base editing enzymes[11] in concert with cytidine deaminase will significantly widen the mutational spectrum of MutaT7 and further enable the creation of rich and diverse DNA libraries *in vivo.* Moreover, DNA-modifiers fused to T7 could facilitate targeted epigenetic studies.[33] The ubiquitous applicability of T7-pol in diverse organisms[34–37] suggests that MutaT7 will prove useful in a broad range of evolutionary and synthetic biology settings.

Supplementary Material

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REFERENCES

[1]. Cupples CG, and Miller JH (1989) A set of lacZ mutations in Escherichia coli that allow rapid detection of each of the six base substitutions, Proc. Natl. Acad. Sci. U.S.A 86, 5345–5349. [PubMed: 2501784]

[2]. Tessman I, Ishiwa H, and Kumar S (1965) Mutagenic effects of hydroxylamine in vivo, Science 148, 507–508. [PubMed: 14263771]

[3]. Greener A, Callahan M, and Jerpseth B (1997) An efficient random mutagenesis technique using an E. coli mutator strain, Mol. Biotechnol 7, 189–195. [PubMed: 9219234]

[4]. Badran AH, and Liu DR (2015) Development of potent in vivo mutagenesis plasmids with broad mutational spectra, Nat. Commun 6, 8425. [PubMed: 26443021]

[5]. Gerdes SY, Scholle MD, Campbell JW, Balazsi G, Ravasz E, Daugherty MD, Somera AL, Kyrpides NC, Anderson I, Gelfand MS, Bhattacharya A, Kapatral V, D'Souza M, Baev MV, Grechkin Y, Mseeh F, Fonstein MY, Overbeek R, Barabasi AL, Oltvai ZN, and Osterman AL (2003) Experimental determination and system level analysis of essential genes in Escherichia coli MG1655, J. Bacteriol 185, 5673–5684. [PubMed: 13129938]

[6]. W ang T, Birsoy K, Hughes NW, Krupczak KM, Post Y, W ei JJ, Lander ES, and Sabatini DM (2015) Identification and characterization of essential genes in the human genome, Science 350, 1096–1101. [PubMed: 26472758]

[7]. Tizei PA, Csibra E, Torres L, and Pinheiro VB (2016) Selection platforms for directed evolution in synthetic biology, Biochem. Soc. Trans 44, 1165–1175. [PubMed: 27528765]

[8]. Komor AC, Kim YB, Packer MS, Zuris JA, and Liu DR (2016) Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage, Nature 533, 420–424. [PubMed: 27096365]

[9]. Nishida K, Arazoe T, Yachie N, Banno S, Kakimoto M, Tabata M, Mochizuki M, Miyabe A, Araki M, Hara KY, Shimatani Z, and Kondo A (2016) Targeted nucleotide editing using hybrid prokaryotic and vertebrate adaptive immune systems, Science 353.

[10]. Komor AC, Zhao KT, Packer MS, Gaudelli NM, W aterbury AL, Koblan LW, Kim YB, Badran AH, and Liu DR (2017) Improved base excision repair inhibition and bacteriophage M u Gam protein yields C:G-to-T:A base editors with higher efficiency and product purity, Sci. Adv 3, eaao4774. [PubMed: 28875174]

[11]. Gaudelli NM, Komor AC, Rees HA, Packer MS, Badran AH, Bryson DI, and Liu DR (2017) Programmable base editing of A*T to G*C in genomic DNA without DNA cleavage, Nature 551, 464471. [PubMed: 29160308]

[12]. Kim D., Lim K, Kim ST, Yoon SH, Kim K, Ryu SM, and Kim JS (2017) Genome-wide target specificities of CRISPR RNA-guided programmable deaminases, Nat. Biotechnol 35, 475–480. [PubMed: 28398345]

[13]. Hess GT, Fresard L, Han K, Lee CH, Li A, Cimprich KA, Montgomery SB, and Bassik MC (2016) Directed evolution using dCas9-targeted somatic hypermutation in mammalian cells, Nat. Methods 13, 1036–1042. [PubMed: 27798611]

[14]. Ma Y, Zhang J, Yin W, Zhang Z, Song Y, and Chang X (2016) Targeted AID-mediated mutagenesis (TAM) enables efficient genomic diversification in mammalian cells, Nat. Methods 13, 1029–1035. [PubMed: 27723754]

[15]. Camps M, Naukkarinen J, Johnson BP, and Loeb LA (2003) Targeted gene evolution in Escherichia coli using a highly error-prone DNA polymerase I ,Proc. Natl. Acad. Sci. U.S.A 100, 9727–9732 [PubMed: 12909725]

[16]. Allen JM, Simcha DM, Ericson NG, Alexander DL, Marquette JT, Van Biber BP, Troll CJ, Karchin R, Bielas JH, Loeb LA, and Camps M (2011) Roles of DNA polymerase I in leading and lagging-strand replication defined by a high-resolution mutation footprint of ColE1 plasmid replication, Nucleic Acids Res 39, 7020–7033. [PubMed: 21622658]

[17]. Crook N, Abatemarco J, Sun J, W agner JM, Schmitz A, and Alper HS (2016) In vivo continuous evolution of genes and pathways in yeast, Nat. Commun 7 , 13051. [PubMed: 27748457]

[18]. DiCarlo JE, Conley AJ, Penttila M, Jantti J, Wang HH, and Church GM (2013) Yeast oligo-mediated genome engineering (YOGE), A C S Synth. Biol 2, 741–749.

[19]. Ravikumar A, Arrieta A, and Liu CC (2014) An orthogonal DNA replication system in yeast, Nat. Chem. Biol 10, 175–177. [PubMed: 24487693]

[20]. Rong M, He B, McAllister WT, and Durbin RK (1998) Promoter specificity determinants of T7 RNA polymerase, Proc. Natl. Acad. Sci. U.S.A 95, 515–519. [PubMed: 9435223]

[21]. Thiel V, Herold J, Schelle B, and Siddell SG (2001) Infectious RNA transcribed in vitro from a cDNA copy of the human coronavirus genome cloned in vaccinia virus, J. Gen. Virol 82, 1273–1281. [PubMed: 11369870]

[22]. Ramiro AR, Stavropoulos P, Jankovic M, and Nussenzweig MC (2003) Transcription enhances AID-mediated cytidine deamination by exposing single-stranded DNA on the nontemplate strand, Nat. Immunol 4, 452–456. [PubMed: 12692548]

[23]. Schaefer J, Jovanovic G, Kotta-Loizou I, and Buck M (2016) Single-step method for beta-galactosidase assays in Escherichia coli using a 96-well microplate reader, Anal. Biochem 503, 56–57. [PubMed: 27036618]

[24]. Nilsen H, Rosewell I, Robins P, Skjelbred CF, Andersen S, Slupphaug G, Daly G, Krokan HE, Lindahl T, and Barnes DE (2000) Uracil-DNA glycosylase (UNG)-deficient mice reveal a

primary role of the enzyme during DNA replication, Mol. Cell 5, 1059–1065. [PubMed: 10912000]

[25]. Alsøe L, Sarno A, Carracedo S, Domanska D, Dingler F, Lirussi L, SenGupta T, Tekin NB, Jobert L, Alexandrov LB, Galashevskaya A, Rada C, Sandve GK, Rognes T, Krokan HE, and Nilsen H (2017) Uracil accumulation and mutagenesis dominated by cytosine deamination in CpG dinucleotides in mice lacking UNG and SMUG1, Sci. Rep 7, 7199. [PubMed: 28775312]

[26]. Petersen-Mahrt SK, Harris RS, and Neuberger MS (2002) AID mutates E. coli suggesting a DNA deamination mechanism for antibody diversification, Nature 418, 99–103. [PubMed: 12097915]

[27]. Navaratnam N, Bhattacharya S, Fujino T, Patel D, Jarmuz AL, and Scott J (1995) Evolutionary origins of apoB mRNA editing: Catalysis by a cytidine deaminase that has acquired a novel RNA-binding m otif at its active site, Cell 81, 187–195. [PubMed: 7736571]

[28]. Garibyan L, Huang T, Kim M, Wolff E, Nguyen A, Nguyen T, Diep A, Hu KB, Iverson A, Yang HJ, Miller JH (2003) Use of the rpoB gene to determine the specificity of base substitution mutations on the Escherichia coli chromosome, DNA Repair 2, 593–608. [PubMed: 12713816]

[29]. Nilsson AI, Berg OG, Aspevall O, Kahlmeter G, and Andersson DI (2003) Biological costs and mechanisms of fosfomycin resistance in Escherichia coli, Antimicrob. Agents Chemother 47, 28502858. [PubMed: 12936984]

[30]. Acar JF, and Goldstein FW (1982) Genetic aspects and epidemiologic implications of resistance to trimethoprim, Rev. Infect. Dis 4, 270–275. [PubMed: 7051229]

[31]. Herrington MB, MacRae TJ, Panagopoulos D, and Wong S-H (2002) A mutation in the folA promoter delays adaptation to minimal medium by Escherichia coli K-12, J. Basic Microbiol 42, 172. [PubMed: 12111744]

[32]. Serrano-Heras G, Ruiz-Maso JA, del Solar G, Espinosa M, Bravo A, and Salas M (2007) Protein p56 from the Bacillus subtilis phage phi29 inhibits DNA-binding ability of uracil-DNA glycosylase, Nucleic Acids Res 35, 5393–5401. [PubMed: 17698500]

[33]. DeNizio JE, Schutsky EK, Berrios KN, Liu MY, and Kohli RM (2018) Harnessing natural DNA modifying activities for editing of the genome and epigenome, Curr. Opin. Chem. Biol 45, 10–17. [PubMed: 29452938]

[34]. McBride KE, Schaaf DJ, Daley M, and Stalker DM (1994) Controlled expression of plastid transgenes in plants based on a nuclear DNA-encoded and plastid-targeted T7 RNA polymerase, Proc. Natl. Acad. Sci. U.S.A 91, 7301–7305. [PubMed: 8041784]

[35]. Lieber A, Sandig V, and Strauss M (1993) A mutant T7 phage promoter is specifically transcribed by T7-RNA polymerase in mammalian cells, Eur. J. Biochem 217, 387–394. [PubMed: 8223577]

[36]. Weinstock MT, Hesek ED, Wilson CM, and Gibson DG (2016) Vibrio natriegens as a fast-growing host for molecular biology, Nat. Methods 13, 849–851. [PubMed: 27571549]

[37]. Dower K, and Rosbash M (2002) T7 RNA polymerase-directed transcripts are processed in yeast and link 3’ end formation to mRNA nuclear export, RNA 8, 686–697. [PubMed: 12022234]

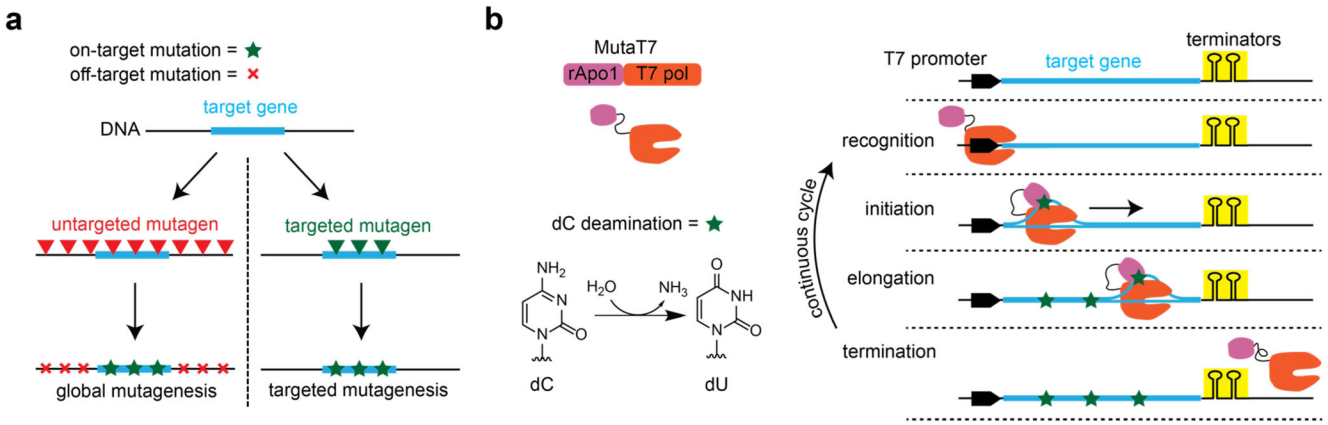

Figure 1.

(a) Schematic illustrating global versus targeted mutagenesis. (b) The MutaT7 construct and the targeted mutagenesis cycle.

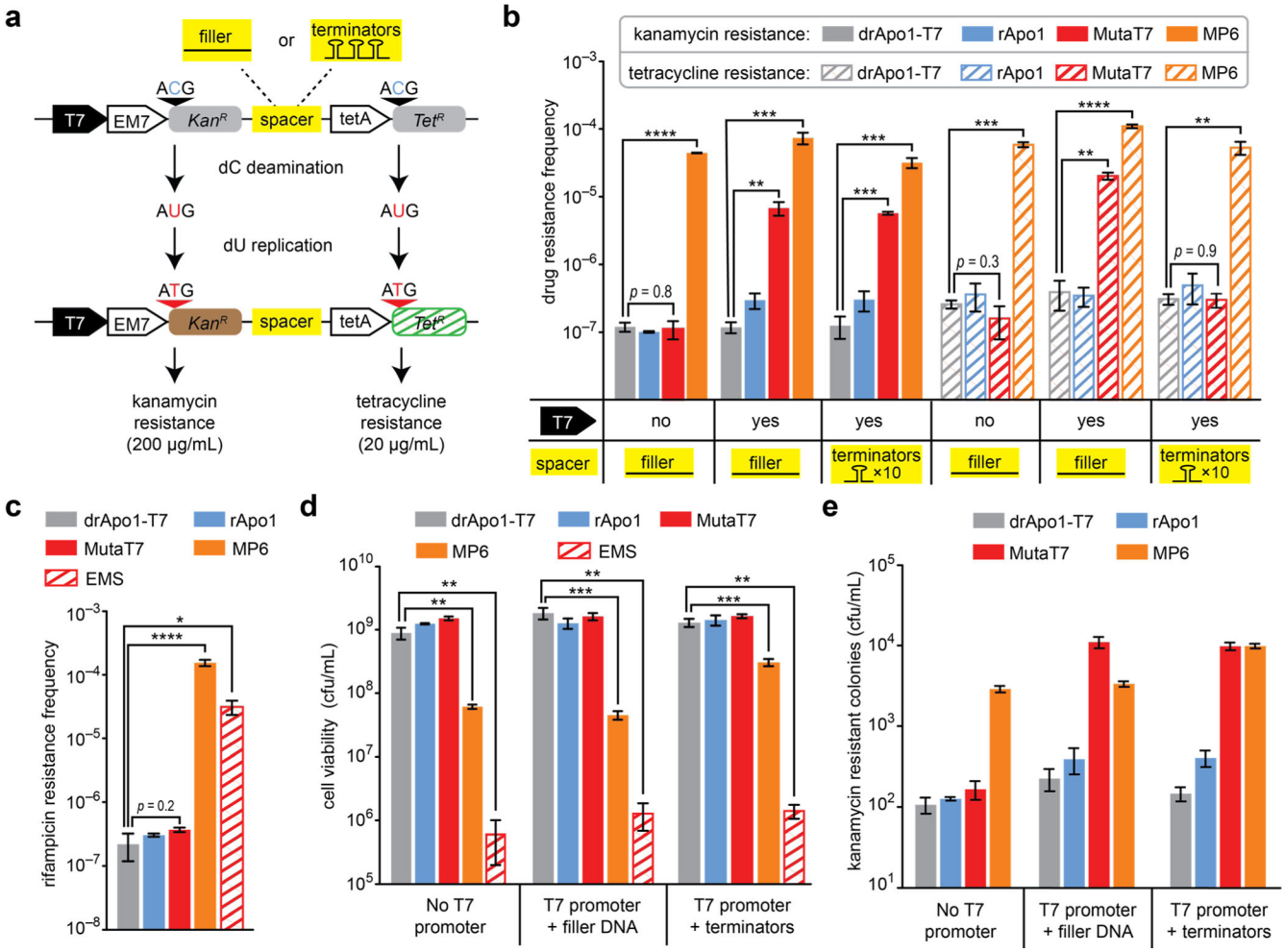

Figure 2.

(a) Drug resistance start codon reversion reporter assay for measuring extent of mutagenesis at specific DNA loci. (b) Codon reversion reporter assay data for combinations of mutagen and reporter plasmids. Mutagens include deactivated rApo1 fused to T7 RNA polymerase (drApo1-T7; negative control), unfused rApo1 (rApo1), targeted mutagen (MutaT7), and global mutagen (MP6). (c) Extent of off-target mutagenesis assessed by rifampicin resistance assay for populations carrying the codon reversion reporter plasmid with a terminator array in Figure 2b (EMS = ethyl methanesulfonate). (d) Viability data for cell populations in panel b, along with drApo1-T7 populations treated with EMS. (e) Total number of kanamycin resistant colonies for populations in panel 2b. Values represent mean of independent experiments ($n = 3$); error bars represent s.e.m.; statistical significance was evaluated by a Student's t-test: *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$; notable non-significant p-values shown; see also Table S2.

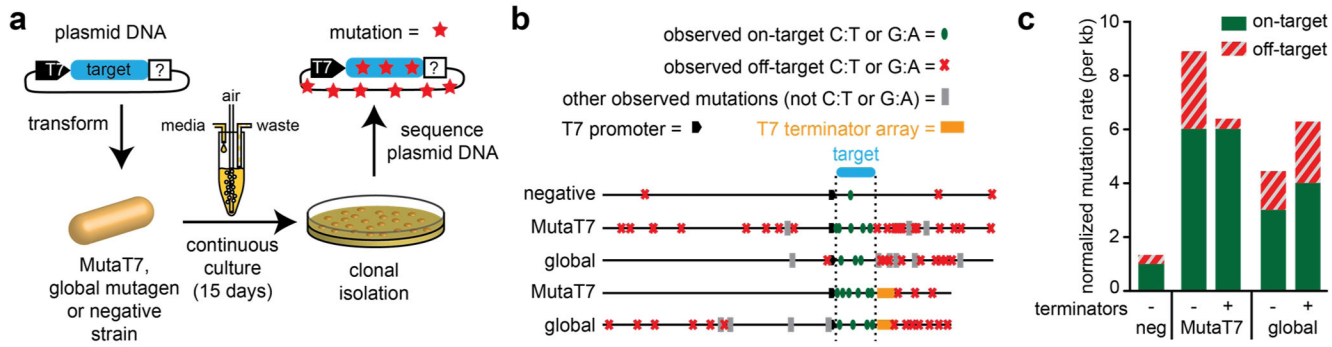

Figure 3.

(a) Reporter construct and continuous culture experiment to assess mutation accumulation under drift conditions. (b) On-target (green oval) and off-target (red x) mutations identified by sequencing episomes propagated in the presence of targeted (MutaT7) and global (MP6) mutagens. (c) Normalized mutation frequency (number of mutations observed divided by kb of DNA sequenced in associated regions) for data in panel b.