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Engineering of bacterial strains and vectors for the production of plasmid DNA

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Abstract

The demand for plasmid DNA (pDNA) is anticipated to increase significantly as DNA vaccines and non-viral gene therapies enter Phase 3 clinical trials and are approved for use. This increased demand, along with renewed interest in pDNA as a therapeutic vector, has motivated research targeting the design of high-yield, cost-effective manufacturing processes. An important aspect of this research is engineering bacterial strains and plasmids that are specifically suited to the production of plasmid biopharmaceuticals. This review will survey recent innovations in strain and vector engineering that aim to improve plasmid stability, enhance product safety, increase yield, and facilitate downstream purification. While these innovations all seek to enhance pDNA production, they can vary in complexity from subtle alterations of the host genome or vector backbone to the investigation of non-traditional host strains for higher pDNA yields.
Introduction

In recent years, great strides have been made toward the development of safe and effective gene therapies and DNA vaccines for many infectious, acquired, and genetic diseases. In the United States, naked plasmid DNA (pDNA)-based therapies for metastatic melanoma, critical limb ischemia, and non-small cell lung cancer in humans are in Phase 3 clinical trials (Vical 2008; Sanofi-Aventis 2008; NovaRx Corporation 2008). Four veterinary DNA therapies have been licensed in the US, Canada, and Australia (Kutzler and Weiner 2008). As more pDNA-based therapies enter late-stage clinical trials and are approved for clinical use, the demand for high-quality, pharmaceutical-grade plasmid DNA is anticipated to increase significantly. Interest in non-viral gene therapy has also been bolstered by disappointing results from a Phase 2 clinical trial of an adenovirus-based HIV DNA vaccine (Moore et al. 2008), along with recent advances in delivery vehicles and adjuvants that have increased the potency of naked pDNA (Green et al. 2007; Pan et al. 2008).

From a manufacturing standpoint, the increasing demand for plasmid DNA is coupled to a need for high-yield, cost-effective production processes. Currently, plasmid DNA is often produced using “off the shelf” strains of *Escherichia coli* and plasmid backbones that are known to be effective producers of recombinant proteins. However, these choices may not be the most favorable when plasmid DNA is the final product. As a result, significant research efforts are in progress to rationally design bacterial strains and plasmids specifically suited to the production of plasmid biopharmaceuticals.

The motivation to design new bacterial strains for plasmid DNA production is due in part to the realization that many common laboratory strains like DH5α and DH10B have undergone a high degree of mutagenesis to improve their performance in cloning, library construction, and/or
recombinant protein production applications. The complex genotypes of many *E. coli* strains in industrial use today also greatly hinder the ability to reliably predict the plasmid yield based on the genotype alone. In a survey of 17 strains of *E. coli* harboring plasmids ranging in size from 5.8 kb to 20 kb, Yau and colleagues (2008) found little correlation between strain genotype and plasmid yield. With these factors in mind, many researchers have used the strategies outlined in Fig. 1 to develop well-characterized strains for pDNA production. These strain engineering efforts often seek to preserve the sequence fidelity of both the plasmid and host genome and to address upstream and downstream process issues. Complementing these efforts are alterations in the vector backbone designed to enhance characteristics such as copy number, genetic fidelity, and segregational stability. Typical plasmid backbones used for gene therapeutics have several common features that fall into two categories: features required for propagation in *E. coli* and features required for therapeutic efficacy. Elements in the first category include a bacterial origin of replication, such as the ColE1 origin, and a selective marker, such as a kanamycin resistance gene. Kanamycin resistance is preferred over a marker that requires addition of β-lactam antibiotics to the culture medium, as these could potentially induce an allergic response in patients (Butler 1996). The second category of plasmid features includes the therapeutic gene and the associated sequences required for its expression *in vivo* such as a eukaryotic promoter and poly-adenylation signal. Many vector engineering efforts focus on modifications to the basic therapeutic backbone as illustrated in Fig. 2. These modifications aim to improve the production process by increasing yield, improving product homogeneity and quality, and/or ensuring the sequence fidelity of the final plasmid product.

This review will describe recent developments in strain and vector engineering for the production of pDNA-based therapies. The state of the field from a clinical perspective has
recently been reviewed (Kutzler and Weiner 2008) as well as vector engineering for increased in vivo expression and potency levels (Mairhofer and Grabherr 2008). Here, we will discuss strain and vector engineering strategies specifically associated with production issues and the resulting product quality.

**Engineering to maintain sequence stability**

The sequence integrity of a plasmid vector must be controlled during the production process because of its enormous impact on product safety, yield, and quality. Modifications in the strain and vector used for production of pDNA can ensure that the sequence and structure of the plasmid is maintained throughout the duration of the process. The complete sequence of *E. coli* DH10B was recently published (Durfee et al. 2008) and underscores the need for re-evaluation of strains used for pDNA production. For example, DH10B was found to have a mutation rate 13.5-fold higher than wild-type *E. coli* (strain MG1655), mostly due to a significantly higher rate of insertion sequence (IS) transposition. When compared to MG1655, the DH10B genome contains five additional copies of IS1A (IS1E)-type transposons which are known to insert into plasmid DNA (Chen and Yeh 1997). These findings are especially relevant for pDNA production where the integrity of the final plasmid preparation is paramount.

Contamination of plasmid DNA by mobile elements is a serious regulatory concern, as these elements can alter the biological properties and safety profile of the vector DNA. This concern is not purely theoretical – IS1-mediated mutagenesis was recently reported in an industrial process for selection of HIV plasmid DNA vaccine candidates. An IS1 insertion in the *E. coli* DH5 genome was postulated to be the cause of the presence of a significant population of low plasmid-producing clones (Prather et al. 2006). Insertion of both an IS1 and an IS5
sequence in the neomycin resistance gene of the therapeutic plasmid was also observed. Prolonged cultivation in minimal medium may have caused the high incidence of IS insertion detected in this study, as transposon activity can often be induced by environmental stressors such as nutrient limitation (Twiss et al. 2005). The population of low-producers was detrimental to process efficiency by necessitating extensive screening for high-producing clones for use in master seed banks.

To address the problem of IS-mediated genetic instability, researchers have constructed multiple-deletion series (MDS) strains of *E. coli* which contain a significantly reduced genome that is about 15% smaller than that of the parent strain, MG1655 (Posfai et al. 2006). These strains have all of the mobile elements removed, and showed no detectable transposon activity when compared to MG1655 and DH10B. Neither the growth rate of the MDS strains nor their capacity for recombinant protein production was compromised by the genome reduction. Without insertion sequences, MDS strains were able to stably maintain plasmids coding for recombinant ectopic fusion proteins and adeno-associated virus-based plasmids that contain hammerhead secondary structure. Both of these vector types are often highly unstable in *E. coli* K-12 strains. Evaluation of the MDS strains in a pDNA production process has not yet been reported, but the advantages of using these strains is clear and they have great potential to streamline the production of high-quality, stable pDNA.

Contamination of gene therapeutics by insertion sequences has also been addressed using vector design solutions. For instance, a plasmid containing the human cystic fibrosis transmembrane conductance regulator (CFTR) gene for cystic fibrosis therapy showed a high level of segregational instability due to the expression of toxic peptides from a cryptic *E. coli* promoter present in the gene (Boyd et al. 1999). An IS1-containing variant of the vector was
discovered in high amounts in some fermentations, presumably due to increased fitness via suppression of toxic peptide expression. To remedy these issues, the researchers inserted an intron near the site of the cryptic promoter that reduced expression of toxic gene products, thus substantially increasing plasmid stability and decreasing contamination by IS1 elements.

Changes in plasmid sequence can also be mediated by factors other than IS elements, even in recombination deficient $recA$ strains. For example, vectors that contain direct sequence repeats are especially vulnerable to $recA$-independent recombination and can form monomers that have lost one of the direct repeats and the intervening sequence, as well as head-to-tail combinations of two or three recombined monomers (Bi and Liu 1996). This type of $recA$-independent recombination is thought to occur through a pathway involving strand mispairing at a stalled replication fork or replication slippage (Bi and Liu 1996). Ribeiro et al. (2008) observed this type of recombination in pCIneo as well as in a pCIneo-derived rabies DNA vaccine candidate cultivated in cells exposed to kanamycin. Both plasmids contained two 28-bp direct repeats, and all three recombination products described above were observed. This recombination conferred kanamycin resistance, possibly by reducing the distance between the neomycin phosphotransferase gene and a cryptic $E. coli$ promoter. The kanamycin resistance gene should not have been expressed in $E. coli$ because it was under the control of the eukaryotic SV40 promoter. These changes in plasmid structure and aberrant expression of an antibiotic resistance gene represent an obvious safety hazard, and emphasize the need for detailed sequence analysis of therapeutic vectors. This study lays the groundwork for future vector engineering efforts to eliminate mutational hot spots. To aid in this work, Oliveira et al. (2008) have developed an algorithm to predict the recombination frequency of a plasmid containing a given set of direct repeats.
Engineering to promote safety

As discussed in the previous section, it is essential to ensure the sequence fidelity of gene-based therapeutics that will ultimately be administered to humans. Two other product safety concerns that can significantly affect the design of a pDNA production process include the use of antibiotics for plasmid selection and the potential for the therapeutic vector to be transferred to other organisms in the environment.

In terms of antibiotics, the FDA has long recommended that β-lactam antibiotics be avoided, as residual contamination in the final product could potentially cause an allergic reaction in sensitive individuals (Butler 1996). Also, antibiotics that are in wide clinical use should be avoided. With these concerns in mind, the development of antibiotic-free selection systems is desirable from both a cost and safety perspective – especially as the emergence of antibiotic-resistant pathogens becomes more common. Several researchers have addressed these concerns by modifying the vector, host, or both to develop alternative plasmid selection systems. In particular, several groups have chosen to manipulate essential E. coli genes to ensure efficient killing of plasmid-free cells. One group chose to target dapD, an essential gene for diaminopimelate and lysine biosynthesis (Cranenburgh et al. 2001). The endogenous dapD locus was disrupted, and an ectopic copy of dapD under the control of a lac promoter was integrated into the chromosome. Transforming this strain with a high copy plasmid containing the lac operator resulted in sufficient titration of lac repressor away from dapD to give expression of the essential gene. As a result, only cells containing pDNA with the lac operator sequence survived in culture. This protocol was later adapted to medium copy number plasmids, like pBR322 (Cranenburgh et al. 2004), and was successfully applied to a plasmid DNA production process for an HIV DNA vaccine candidate (Hanke and McMichael 2000).
In other work, Hägg et al. (2004) created a host/vector system where the chromosomal \textit{infA} gene, coding for an essential translation initiation factor, was deleted, and was complemented by a copy of the gene on the expression vector of interest. A similar system was developed by Vidal et al. (2008) using \textit{glyA}, an essential gene for glycine biosynthesis, which allowed only plasmid-bearing cells to grow in minimal medium without glycine. In both of these studies, the implementation of the antibiotic-free selection system did not significantly affect the growth rate of the strains. Goh and Good (2008) overexpressed the essential \textit{fabI} gene (enoyl ACP reductase) from a plasmid, and selected for plasmid-bearing cells using triclosan, a biocide that chemically inhibits \textit{fabI}. While this technique has yet to be investigated at the large scale, it has the advantage of not requiring an engineered host strain. Use of an antidote/poison system has also been investigated in the context of antibiotic-free recombinant protein or DNA production. Szpirer and Milinkovitch (2005) constructed an \textit{E. coli} B strain carrying a chromosomal copy of the \textit{ccdB} poison gene and selected for plasmid-bearing cells using vectors containing the \textit{ccdA} antidote gene. (\textit{E. coli} B strains are non-pathogenic laboratory strains that can be used for many of the same applications as K-12 strains, and the differences between the two strains that are relevant to pDNA production will be discussed below.) The \textit{ccd} system is an example of a class of natural plasmid maintenance strategies, known as post-segregational killing systems, which have been reviewed by Zielenkiewicz and Ceglowski (2001).

Alternative selection systems based on antisense RNA have also recently been investigated. Dryselius et al. (2003) used a synthetic peptide-nucleic acid complex to inactivate the mRNA from the chromosomal copy of the essential \textit{acpP} gene. The strain was rescued by a plasmid containing a copy of \textit{acpP} with a mutation that rendered it resistant to the antisense nucleic acid, thus allowing for selection of plasmid-bearing cells. This selection technique was
effective, but is likely to be cost prohibitive at the large scale owing to the expense of synthesizing the peptide nucleic acid. Mairhofer and colleagues (2008) avoided the issue of expense by developing a system that uses the RNA I antisense transcript that is produced during ColE1 plasmid replication to regulate expression of an essential host gene. They integrated an expression cassette containing the essential murA gene (UDP-N-acetylglucosamine enolpyruvyl transferase) under the control of the pLtetO operator into the chromosome of various E. coli strains. The tet repressor (tetR) was modified to contain a sequence complementary to RNA I. In the presence of a plasmid with a ColE1 replicon, tetR was inactivated, allowing expression of the essential gene. In the absence of a replicating ColE1 plasmid, murA expression was repressed, resulting in cell death. A major advantage of these systems is that the antibiotic resistance gene can be removed from the plasmid, resulting in a smaller vector and a reduction in the number of immunostimulatory unmethylated CpG sequences. Antibiotic-free selection systems can also be used in conjunction with well-known natural plasmid stability systems that act by resolving multimers or facilitating plasmid partitioning instead of killing plasmid-free cells. For example, cer sequences present in natural plasmids like ColE1 act with E. coli host proteins to resolve plasmid multimers in order to prevent multimer accumulation which can significantly reduce plasmid segregational stability (Summers 1998). Also, plasmids expressing Par-family partitioning proteins can help ensure that all daughter cells receive at least one plasmid (Pogliano 2002).

A novel vector/host system developed by Soubrier and colleagues (1999) combined antibiotic-free selection with a plasmid replicon that is dependant on a specifically-engineered host for replication. This class of plasmids, called pCOR, contains an R6K-derived origin of replication that requires the π initiator protein encoded by the pir gene. In the system developed
by Soubrier et al. (1999) the gene encoding \( \pi \) was removed from the plasmid and integrated into the *E. coli* genome. This host-dependent replication improves the safety profile of the pCOR vectors by limiting the plasmid host range and thus significantly reducing the chance of plasmid dissemination to the environment. The pCOR plasmids also rely on an antibiotic-free, tRNA amber suppressor system for selection, along with a *cer* sequence to enhance plasmid stability.

In terms of yield, the first-generation pCOR plasmids, which contain a point mutation in the *pir* gene to increase copy number, gave yields on the order of 100 mg pDNA/L (Soubrier et al. 1999). Later work increased the copy number further by additional mutagenesis of *pir*, producing yields of 421 mg pDNA/L in a 7-L fermentor (Soubrier et al. 2005). The pCOR plasmids represent the only known published investigation of plasmids containing a non-ColE1 origin of replication for use in gene therapy or DNA vaccine applications.

Another method of addressing the safety concerns surrounding bacterial elements in plasmid biopharmaceuticals is the use of minicircle DNA. Minicircles contain only the transcription unit required for expression of the therapeutic gene(s) with the bacterial backbone sequences required for propagation in *E. coli* removed by site-specific, intramolecular recombination. The construction of minicircle DNA has been studied for about a decade (Darquet et al. 1997), but feasible production processes for these vectors have only recently been proposed and with them a new category of vector and strain engineering challenges. The vector must be engineered to contain the elements necessary for efficient intramolecular recombination. Two recombination systems currently under investigation include the phage \( \Phi C31 \) integrase gene (Chen et al. 2005) and the *parA* resolvase (Mayrhofer et al. 2008) with their associated recognition sites. Both of these systems have the required enzyme integrated into the backbone of the parent plasmid. In contrast, Tolmachov et al. (2006) have designed a minicircle
production system consisting of an *E. coli* strain with an arabinose-inducible copy of the Cre recombinase gene integrated into the chromosome along with a plasmid vector with *loxP* sites flanking the eukaryotic transcription unit. Overall, minicircle DNA represents an attractive alternative to plasmid DNA because the product ultimately delivered to the patient lacks an antibiotic resistance gene, immunostimulatory CpG motifs, and other bacterial elements that could be inadvertently expressed from cryptic eukaryotic promoter elements. However, until the production of minicircle DNA can be scaled up and streamlined, plasmid DNA is likely to remain the preferred vector for non-viral gene therapy.

**Engineering to increase plasmid DNA yield**

The art of high-density cell culture for pDNA production has been an active area of process research, often focusing on the development of seed trains (Okonkowski et al. 2005), media (Danquah and Forde 2007; O’Kennedy et al. 2000; Wang et al. 2001), and induction strategies (Carnes et al. 2006). However, strain engineering efforts have also recently begun to address upstream process issues such as increasing plasmid DNA yield. In an attempt to design an organism better suited to production of plasmid DNA, researchers have investigated both *E. coli* B and K-12 strains. B and K-12 strains are genetically similar, but analysis of mobile elements in a particular B strain showed a very different profile of insertion sequences. Notably, *E. coli* B lacks a copy of IS5, and contains 20 copies of IS1 versus the six to eight usually found in K-12 (Schneider et al. 2002). The lipopolysaccharides (LPS) of K-12 and B strains both lack the O-antigen. B strains also lack the distal part of the LPS core consisting of D-galactose, D-glucose, and N-acetyl-D-glucosamine residues (Nikaido 1996). However, we are not aware of any studies demonstrating that this difference in polysaccharide content significantly affects the
endotoxin levels in a pDNA production process. Metabolically, derivatives of *E. coli* B differ from *E. coli* K-12 in that B strains typically produce significantly less acetate, even when grown in medium with a high glucose concentration. This phenomenon has been attributed to a more active glyoxylate shunt and consequently, more active acetate uptake pathways (Phue et al. 2005).

BL21, a B strain derivative, has been used with great success for overexpression of recombinant proteins. However, until recently, BL21 has not been considered to be a viable host for pDNA production because its genome contains intact *endA* and *recA*, resulting in sub-optimal plasmid preparations. Deleting *recA* in BL21(DE3) has been shown to improve plasmid segregational stability (Zhao et al. 2007), but this investigation was conducted in the context of recombinant antibody production. A recent communication from Phue et al. (2008) reported that with glucose as the carbon source, BL21Δ*recA* and BL21Δ*recAΔendA* were superior producers of pDNA when compared to DH5α. Under these conditions, BL21-derived strains showed significantly less acetate production and improved glucose utilization, as expected for a B strain. With glycerol as a carbon source, BL21Δ*recAΔendA* performed significantly better than DH5α in terms of volumetric yield (1904 mg/L versus 991 mg/L), but the differences were less substantial in terms of specific yield (10.07 mg versus 7.03 mg pDNA per g wet cell weight).

While the study described above found that a strain possessing a more active glyoxylate shunt (BL21) fared better in high density cell culture and produced more pDNA than a K-12 strain, other groups have found that disrupting a positive regulator of the glyoxylate shunt, the global transcriptional regulator *fruR*, also led to increased pDNA production. Ow et al. (2006) conducted global transcription and proteomic analyses to study the differences in metabolism between plasmid-bearing and plasmid-free *E. coli* DH5α cells. As expected, they found that
plasmid-bearing cells had a slower growth rate and an altered profile of central metabolic gene expression when compared to plasmid-free cells. Based on this work, Ow and colleagues (2007) chose to disrupt the \textit{fruR} gene in DH5\textalpha and found that this modification increased growth rate and glycolytic enzyme activity of plasmid-bearing cells along with a concomitant reduction in gluconeogenesis. The ability to recover a growth rate comparable to plasmid-free cells is particularly useful in pDNA production, as it reduces the selective pressure against plasmid-bearing cells and thus increases plasmid stability. In the context of pDNA production, a recent study (Ow et al. 2009) found that disruption of \textit{fruR} led to a 21\% increase in specific pDNA yield from a fed-batch fermentation.

**Engineering to address downstream processing and purification issues**

A key challenge in the development of cost-effective manufacturing processes is improving the yield of downstream purification steps. Large-scale purification of pDNA is difficult due to the complex, dynamic structure of pDNA (Prazeres and Ferreira 2004), viscous process streams (Ciccolini et al. 1998), and the presence of impurities (e.g. RNA, genomic DNA) with similar properties to the desired product (Ferreira et al. 2000). One method to improve downstream purification yields is the development of fermentation processes with high specific pDNA yields which improve overall process yield by increasing the ratio of plasmid DNA to contaminating biomass. A recent study reported a yield of 17.5 mg/L/OD$_{600}$ – a significant improvement over previously reported values (Carnes et al. 2006).

Several researchers have taken a different approach and sought to improve downstream purification by engineering the \textit{E. coli} host strain to reduce the amount of contaminating genomic DNA (gDNA) and RNA in the cell lysate. The FDA has yet to set formal lot release
criteria for gene-based therapeutics; however, a generally-accepted specification for product
purity is that any residual RNA is not present in a high enough concentration to be visualized on
a 0.8% agarose gel (Horn et al. 1995). The separation of RNA from DNA is particularly
challenging because of the similar physicochemical properties of both nucleic acids. One
strategy for RNA removal is digestion of cell lysates with bovine RNase A, which allows the
resulting small RNA fragments to be more easily separated from DNA. However, recent
concerns surrounding prion-based diseases have precluded the use of animal-derived enzymes.
Cooke et al. (2001) addressed this problem by integrating the gene coding for RNase A into the
chromosome of E. coli JM107 under the control of an IPTG-inducible promoter. After
induction, pre-RNase A was targeted to the periplasm where it folded into its active form and
was sequestered from host nucleic acids. Upon cell lysis, the enzyme was released and
efficiently degraded host RNA. This strategy was particularly successful because the RNase A
enzyme is robust enough to withstand the conditions of high pH encountered during alkaline
lysis.

To address removal of host genomic DNA as well as RNA, Nature Technology
Corporation has developed E. coli hosts expressing periplasmic chimeric proteins that degrade
both nucleic acids (Hodgson and Williams 2006). These chimeras include the plasmid-safe
phage T5 D15 exonuclease linked to RNase A or S. The exonuclease is “plasmid-safe” in that it
specifically degrades linear and denatured DNA while not affecting the fidelity of supercoiled
plasmid DNA. The chimeric proteins can be reintroduced into the cytoplasm before lysis by
inner membrane permeabilization techniques, or can begin to degrade host nucleic acids
immediately after being released by cell lysis. Both of these schemes significantly reduce the
viscosity of the lysate, easing the later stages of purification. This approach to strain engineering
also incorporated autolytic host strains, which have been used previously for recombinant protein production applications.

While efforts to improve downstream processing focus on strain engineering, they have addressed many of the major challenges of pDNA production, like separating plasmids from genomic DNA and host RNA contamination. Continued work in this area has the potential to lower manufacturing costs by increasing the yield from downstream purification steps.

Conclusions and perspectives

This review describes a wide range of strain and vector engineering solutions to problems facing the production of plasmid DNA for gene therapies and DNA vaccines. As these technologies mature, they are likely to continue to positively impact the way gene-based therapeutics are produced. However, there are several other interesting options for improved strains and vectors that have yet to be investigated. For example, a potential strategy for increasing pDNA yield at an early stage in the manufacturing process is to increase plasmid copy number. However, surprisingly little work has been reported in this area with respect to strain or vector engineering. With the exception of the work on pCOR (Soubrier et al. 1999, 2005) published pDNA production processes exclusively use high copy number pUC-based plasmids with ColE1 origins of replication. Often, plasmid copy number is modulated using external factors such as temperature (Carnes et al. 2006) to balance the metabolic burden effects of maintaining high copy number plasmids with the desire for high-yield fermentations. These external, sometimes resource-intensive, modulations could potentially be eliminated through rational engineering of the plasmid backbone to increase copy number, or by investigating alternative origins of replication. In terms of strain engineering, areas of research that could
improve production include removing auxotrophies of existing high pDNA producing strains, as well as engineering a strain that can maintain high concentrations of supercoiled pDNA. Engineering the structure of the bacterial outer membrane to reduce endotoxin contamination could also have a significant impact on streamlining downstream purification of pDNA.

Overall, the strain and vector engineering efforts described in this review demonstrate the improved processes that can result from re-evaluation of existing technologies and consideration of process concerns during the basic research phase of product development. While much of the infrastructure is similar for both recombinant protein and pDNA production, there are many issues that are specific to a plasmid DNA final product and these issues have been addressed in many original and innovative ways. As new, engineered strains and vectors continue to be characterized and gain greater acceptance, implementation of these technologies has great potential to result in more productive plasmid DNA manufacturing processes.

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Fig. 1 Strain engineering strategies. The parent strain at the center of the diagram represents any *E. coli* K-12 strain, and the potential modifications to this strain are illustrated. The strain’s genome can be modified subtly by single gene knockouts or implementation of an antibiotic-free selection system. More dramatic modifications, such as genome reduction, can be used to remove mobile elements like insertion sequences. Other strategies aim to ease downstream processing, like expression of heterologous periplasmic nucleases to degrade host genomic DNA and RNA after cell lysis. Changing the parent strain from K-12 to B has also been investigated to improve pDNA production. Note that the engineering strategies are shown as independent of one another, but applying several strategies in parallel to a single strain is possible as well.
Fig. 2 Vector engineering strategies. A generic plasmid DNA vaccine or gene therapy vector is shown with the important features labeled. The bacterial elements required for propagation of the plasmid in *E. coli* are the selectable marker (kanamycin resistance gene, *Kan*<sup>R</sup>) and the pUC origin of replication. The eukaryotic elements of the vector required for *in vivo* efficacy include the eukaryotic promoter/enhancer (*P*<sub>euk</sub>), therapeutic or antigenic gene, and polyadenylation signal (*polyA*). The goal of each vector engineering strategy is described next to the vector feature targeted for modification.