Arresting Evolution

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Abstract

Evolution in the form of selective breeding has long been harnessed as a useful tool by humans. However, rapid evolution can also be a danger to our health and a stumbling block for biotechnology. Unwanted evolution can underlie the emergence of drug and pesticide resistance, cancer, and weeds. It makes live vaccines and engineered cells inherently unreliable and unpredictable, and therefore potentially unsafe. Yet, there are strategies that have been and can possibly be used to stop or slow many types of evolution. We review and classify existing population genetics-inspired methods for arresting evolution. Then, we discuss how genome editing techniques enable a radically new set of approaches to limit evolution.

Evolution Is Not Inevitable

Short-term evolution has been both good and bad for humankind. Fueled by artificial selection, it has been critical to the development of modern agriculture, providing high-yielding crops and improved animal breeds [1,2]. On a smaller physical scale, the practice of directed evolution – the biochemical equivalent of artificial selection – has generated molecules and microbial strains useful to industry and medicine. However, evolution is not assured of working toward human goals, and rapid evolution also causes many problems (Table 1, Key Table). Being able to curtail evolution under these circumstances would have considerable practical benefits.

Evolution may indeed be unavoidable if a population is allowed to reproduce unfettered, but there are interventions that can misdirect or slow evolution, block it completely, or even erase it. Some of these approaches mostly require only an understanding of population genetics and assume little to nothing about the underlying molecular biology of a system. They include centuries-old practices and others introduced recently as ostensibly ‘evolution-proof’ interventions for biocontrol [3]. Other strategies have only become evident or possible with recent advances in genomics and genetic engineering. In this review, we

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classify existing approaches for arresting evolution and describe new methods that are now becoming feasible.

**Classical Approaches Informed by Population Genetics**

There is a long history of selective breeding in agriculture, and civilization has been the beneficiary. A close coupling of population (and quantitative) genetics theory with breeding programs provided the foundations for classical evolutionary theory [4]. A dominant issue with selective breeding was obtaining a strong and appropriate response to the human-applied, artificial selection to improve the yield of a desired trait (e.g., livestock weight or fruit size). Progress was slow or nonexistent if there was too little selectable variation for the trait of interest, or when variation had been exhausted during early phases of selection.

The contexts for halting unwanted evolution are different and are not ones in which an absence of variation is expected. For example, an engineered cell may produce a useful protein or chemical compound at the expense of its own growth. We wish to avoid the loss in yield that will occur as cells evolve to jettison this burdensome function over many generations in a bioreactor. Or we may be trying to suppress a population of pests, and individuals evolve that escape our lethal treatment. We want the population to retain its sensitivity so that we can continue to suppress it in the future. Genetic variation to return the organism to its previous (unengineered) state or for it to develop resistance will either already be present or will be easily accessed by mutation.

An understanding of how natural selection works (Box 1) has led to various protocols for reducing unwanted evolution (Figure 1A). What we classify as traditional approaches are those that rely on manipulating population dynamics, breeding, or selection to thwart evolution. They require and are justified by an understanding of these principles, but one does not need to understand the genetic basis of the desirable property or its loss to use them. In fact, our own bodies have evolved to use several of these mechanisms to prevent cancer (Box 2).

**Box 1**

**The Speed of Adaptive Evolution**

The process of adaptive evolution has been studied for over a century, and the key components are now well understood [61,62]. Arresting evolution requires interfering with one or more of them. Among the most important are:

**Mutation**

Evolutionary change requires genetic variation. If those variants can be prevented from arising, there will be no raw material for evolution. There is natural variation in mutation rates, and there has been some success with reducing mutation rates of microbes. Alternatively, subpopulations with genetic changes can sometimes be discarded.

**Selection**

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Adaptive evolution requires fitness differences among alternative genotypes/alleles. If selective differences can be eliminated, then evolution occurs only from mutation and neutral processes, which are slow. Reducing or altering selection is often a viable approach to controlling evolution. Owing to genetic drift and other stochastic processes, small populations are much less prone to respond to natural selection than are large populations.

**Inheritance**

In this category, we include a wide range of influences, such as population size and structure, mating system, recombination, and epistasis. Although these have important consequences for the speed of evolution, they are not always easily manipulated. Population size and mating system (e.g., inbreeding vs. outbreeding) can sometimes be controlled.

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**Box 2**

**How Multicellular Organisms Limit Evolution within**

Organisms are endowed with mechanisms that have been interpreted as limiting the potential for deleterious evolution within somatic cells (i.e., cancer) and in the germ line. Several of these strategies are conceptually similar to the ones noted in Figure 1A.

Cairns [63] analyzed the cellular organization of the body from the perspective of preventing cancer. His proposed mechanisms include stem cells that undergo limited cell division (because this is a mutagenic process) and the physical loss of cells derived from stem cells from the body, so that any mutations making them cancer prone would be discarded with them. He also proposed that asymmetric inheritance of newly synthesized DNA strands to derivatives of stem cells would help maintain low mutational input to the resident stem cell population. Programmed cell death (apoptosis) is another mechanism that helps limit evolution of stem cell progeny.

Multicellular organisms prevent undesirable somatic mutation from contaminating the germ line by going through a single-cell bottleneck at each generation (e.g., most cancers and other forms of selfish cells are excluded, [64]). Likewise, the germ line of vertebrates typically undergoes few cell divisions. If a gamete is defective in being able to produce a viable organism, this is often detected early in development and it is discarded.

In a similar vein, it has been suggested that some viruses have evolved to limit their own evolution when growing within the host [65]. During long-lasting, chronic infections, within-host evolution can accumulate to the point that the changes thwart transmission to new hosts. By limiting within-host evolution, a larger fraction of viruses will retain the capacity for transmission.

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**Maintain Seed Stocks or Limit Growth**

The simplest traditional approach is to discard the evolving population and start anew with the ancestral stock – all mutations are erased. But discarding the final population after it has performed its function is possible only in specific applications, typically in industrial
cultures, where one controls the cells that go into a bioreactor, or in agriculture, when using seeds that are planted in a field and entirely harvested after a single generation for consumption (Figure 1B).

A related strategy is to subdivide or bottleneck a population of cells and grow each subpopulation separately, discarding those that experience unwanted evolution and continuing from the ones that do not. Extreme bottlenecking is the goal when streaking out bacteria to colonies derived from single cells on agar to create stocks with as little genetic variation as possible. Scale-up of industrial cell-based processes involves developing ‘seed trains’ that limit the total number of cell divisions before full-scale production [5]. These stocks can be individually tested for function, discarding any that are suboptimal, thus preventing mutants from contaminating the full-scale production culture. A recent update to this method, proposed especially for stem cells, is to sequence cell populations after the passages needed to expand their cell number to determine whether they are compromised through mutations in tumor-suppressor genes that can give a growth advantage in the laboratory [6].

When the population cannot be fully replaced, adding back the ancestral genotype may achieve nearly the same effect. The original genotype may be able to outcompete and displace some of the evolved population under certain circumstances [7]. Either that or the ancestor may be able to hybridize with enough evolved individuals, in essence breeding back to the seed, such that a desirable trait or ancestral state replaces one that evolved in the population. Again, many contexts preclude this approach.

Returning to a seed stock is a form of limiting the number of generations of growth in cultivation. The opportunity for evolution typically increases with time or number of generations, so limiting these will restrict many types of evolution (Figure 1C). This is one factor in designing seed trains to populate industrial bioreactors. Another interesting application of this principle concerns methods for DNA amplification: compared to isothermal (continuous) DNA amplification methods, PCR amplifies its template with higher fidelity by limiting the number of amplification opportunities for every DNA molecule to a fixed number of generations (cycles) [8].

Apply Appropriate Selection

At the other end of the traditional approach spectrum, selection is altered. Selection may be weakened to delay evolution or increased to kill intermediates along an evolutionary trajectory to prevent a lineage from reaching an unwanted extreme (Figure 1D). Both approaches are used or advocated in blocking drug-resistance evolution in bacteria. It has been suggested that weakened selection for resistance be achieved by only using antibiotics in appropriate medical situations and by limiting their use in agriculture [9–11]. Increased selection uses combinations of antibiotics to treat infections, so that genotypes resistant to single drugs are killed, and resistance to all drugs is too improbable to arise in a single step [12,13].

More elaborate schemes may be applied to insect vectors and pests. Limiting selection to old individuals within a vector population (e.g., mosquitoes) can theoretically reduce the
magnitude of selection while effectively preventing disease transmission (e.g., of malaria, [3]). A version of changing selection that also incorporates the breed-back-to-seed approach is used to delay the ascent of insect resistance to Bt toxin in crops: ‘refuges’ of nontoxic crops are intermingled with the Bt crops to ensure that any resistant (necessarily homozygous) pests outbreed to produce heterozygous offspring – the latter being sensitive to the toxin [14].

Add Competitors

Manipulating ecology by introducing competitors may also delay evolution in at least two ways. First, if multiple species are competing in the same niche, a competitor can restrict the population size of the focal species, making it less likely to sample adaptive mutations. Second, a competitor can occupy a niche that might otherwise be invaded by descendants of the focal species and lead to undesired evolution (Figure 1E). In an experimental system, evolution of bacterial resistance to viruses depended on whether a competitor was also present for this reason [15]. In the same vein, monocultures of bacteria are known to evolve substrains that specialize on the high concentrations of waste metabolites of the parent strain [16]. Presumably, addition of a competitor that already specialized on the waste (and could not utilize the main nutrient) would prevent this evolution.

Competitive interactions like these may normally impede evolution in host-associated microbiomes. For example, there is evidence that evolve more slowly in the guts of immunodeficient Rag2−/− mice than in wild-type mice, which has been interpreted as being due to Rag2−/− mice having a more diverse gut microbiota [17]. Probiotics can be added as competitors to a microbiome to occupy niches that might be left vacant after a disturbance, such as antibiotic treatment. These types of probiotics will directly protect against the establishment of pathogens [18]. They may also preclude the evolution of surviving native species into opportunistic pathogens that exploit a newly unoccupied niche. It remains to be seen if this secondary effect of slowing evolution is important for any protective effects of probiotics.

Engineering Fitness Landscapes to Suppress Evolution

The ability to create genomes of arbitrary composition, when combined with the knowledge of how those genomes function and evolve, opens new avenues for blocking evolution. These approaches may be applied to constrain evolution of a genome that we construct, but it is also possible to release a small number of engineered individuals and have them suppress or reverse evolution in a larger nonengineered population. Evolution may not be totally prevented, but either the rate of fitness increase or the final fitness itself is suppressed. Many of these strategies require that the engineered genome be asexual, or at least that it not recombine with other genomes in ways that could simply replace the engineered genome sections with their corresponding wild-type sequences.

Reduce the Fitness Limit

There are limits to adaptive evolution, at least in the near term. The concept of a fitness limit may be little more than a fuzzy upper boundary to the fitness trajectory in a particular
environment, or a scaling down of the trajectory of fitness increase over time [19]. It arises from the simple fact that organisms are not infinitely adaptable, even when the same selective pressure is applied gradually and for long periods of time. Genetic engineering allows one to edit genomes to lower this fitness limit, meaning that modified organisms cannot evolve to the same fitness level as would the wild type on a given timescale (Figure 2A).

One application is the design of live attenuated vaccines (Box 3). Such vaccines use variants of a microbe with a lower growth rate or reduced pathogenicity [20,21]. They need to be designed to elicit protection while minimizing the chances that they evolve to be harmful after being administered to a patient. By engineering viruses with genome rearrangements or deletions of nonessential but useful regions, it has been possible to not only lower initial fitness [22,23] but also to limit the fitness they attain on sustained adaptation [22,24]. These genomes will typically evolve a fitness above their starting value, but they cannot catch up with wild type.

### Box 3

**Methods to Engineer Attenuated Viruses Stable against Evolutionary Reversion**

Attenuated viruses are live viruses with a reduced capacity to grow compared to their wild-type counterparts. Historically, methods to attenuate viruses were haphazard processes of adaptation to novel environments, on the hope that adaptation to the new conditions would compromise growth in the original host. Genome engineering now enables a variety of attenuation methods that provide greater control and repeatability. Achieving attenuation – growth rate reduction – is now routine, but a more challenging issue is whether the attenuated virus undergoes evolutionary reversion and can recover higher fitness when grown for long periods of time. Different methods of attenuation have different consequences for reversion.

**Codon (Pair) Deoptimization**

This method engineers viral protein coding sequences to have different codons while retaining the same amino acid sequence [27,28]. Viral growth rate can be adjusted by recoding longer or shorter sequences, but typically hundreds of codons are changed. On the assumption that each codon change has a small fitness effect, full evolutionary reversion should require reversing all the changes and thus be very slow and possibly incomplete. In practice, reversion is faster than predicted and occurs with relatively few changes, but reversions are still acceptably slow [66].

**Genome Rearrangements**

Reordering a viral genome has regulatory effects that lower fitness in the few viruses in which this method has been attempted. On extended adaptation, the engineered genomes do not re-establish the wild-type gene order. Partial evolutionary reversion is possible, but it falls short of returning to wild-type levels [24,67].

**Deletions**
Deleting nonessential genes or parts of genes is easily used to cause fitness reductions. However, this method has not proven especially recalcitrant to evolutionary recovery [66]. Genomes are prone to evolve in response to the deletion, offsetting its effect by adjusting gene expression or even by replacing deleted sequences.

Likewise, if we observe that most mutations yielding an undesirable trait require one or a few nonessential genes, we can delete those genes from a genome. This strategy has been used to delay the evolution of yeast that aggregate and clog continuous culture systems (e.g., chemostats). Most mutants yielding this phenotype were found to activate one key regulatory gene, either directly or indirectly. Removing it delayed the evolution of aggregation [25].

Change Pathways of Evolution

Closely related to the preceding case is the design of low-fitness genomes in which the genetic pathway back to higher fitness is forced through particular intermediates that slow the process.

This approach can be used when the adaptive pathways are few and known. Engineering may block those pathways by making the intermediate states deleterious so that there is a valley in the fitness landscape that impedes evolution, or it can lengthen those pathways such that more mutational steps are required to reach high fitness from the engineered starting point (Figure 2B).

In the latter category, we can engineer genomes to low fitness by ‘a thousand cuts’. That is, introduce many changes of individually small, deleterious effects so that the pathway to higher fitness must reverse each change individually and thus slowly. One such example is wholesale codon deoptimization in viral genomes, which not only attenuates (reduces viral growth rate) but also slows evolutionary recovery [26–28]. In these studies, single mutations can sometimes compensate for many cuts in one step to take shortcuts back to high fitness that are still slowed as planned, but not by as much as expected if each change had to be repaired separately.

Another example occurs in cells engineered for bioproduction. Here we can delay the loss of a costly protein or metabolic enzyme required for production by adding redundant copies of that sequence to a genome [29]. As long as the copies are added in a configuration in which one mutation cannot simultaneously eliminate all of them, each one will have to be inactivated by a separate mutation before the strain becomes a nonproducer.

Limit Expression of Costly Functions to Weakly Selected Life Cycle Phases

When a trait is deleterious to fitness, engineering can limit its expression to certain phases or tissues to weaken negative selection. Avoiding costly gene expression during growth phases of a culture should help ensure that the final population has not lost those functions when they are eventually expressed. For example, delayed selection can be achieved in cell culture by adding a chemical that activates a heterologous metabolic pathway late in the growth phase [30]. This method has obvious parallels to the traditional approach of limiting an
intervention to late ages to weaken selection [3], but many new methods for engineering cells to implement this type of spatiotemporal control are now available.

**Reduce the Mutation Rate**

Reducing the mutation rate to delay the origin of unwanted mutations will likewise delay the ascent of those mutations. Simply engineering DNA sequences that are less prone to recombination (and deletions) or less prone to polymerase errors can be effective [31]. One can also use genome engineering to eliminate selfish DNA elements such as transposons that cause mutations. In microorganisms, there are multiple examples of such ‘clean-genome’ strains that have significantly reduced mutation rates [32,33]. Engineering more robust error-correction or error-prevention systems into cells may also ultimately be feasible.

**Immutable Sequences**

It is sometimes possible to pile up multiple functional constraints on a single DNA sequence to slow unwanted evolution. Such architectures can evolve naturally. Co-regulation of genes (e.g., in operons in bacteria) will prevent mutations that alter the expression of the entire set of genes if a change to any one regulated gene is lethal. Engineering can lend more precision to this approach. For example, a rationally designed bidirectional promoter sequence was used to slow the loss of expression of a gene of interest by making mutations that inactivated the promoter lethal because they also resulted in loss of expression of an essential gene [34].

Some genomes, mostly viruses, contain protein-coding genes that overlap on different strands or in different reading frames. If one of these genes is essential for survival of the organism, then it causes the sequence of the other to evolve more slowly because what would normally be entirely silent changes in one sequence now disrupt its other meaning [35,36]. If one could engineer this type of overlap, then evolution would be slowed (Figure 2C). It is possible that other types of immutable sequences with even more constraints exist, but that they are inaccessible to natural evolution and will come to exist only through gene synthesis.

**Ensure Genome Extinction**

Populations that progressively produce fewer offspring than their parents are destined to die out, and any evolution dies with them. A solution to ensuring extinction of a genome is thus to engineer it so that the population cannot replace itself in the long term. This approach is chiefly one of demography and has roots in the sterile insect technique as well as the release of triploid strains [37]. Those examples take this principle to the extreme of the first generation failing to produce any offspring. It may be possible to engineer a more gradual die out, for example, using synthetic gene regulatory networks that count bacterial cell divisions [38], although any production of viable offspring gives an opportunity for evolution to escape by restoring self-sustaining fertility.

Similarly, conditional lethals may be engineered, so that an organism can grow only in special environments [39,40], such as requiring particular nutrient supplements (e.g., the bacterial auxotrophs of early genetics work). Recent engineering of microorganisms with novel genetic codes provides a unique implementation of this approach that can potentially
force them to be dependent on a chemical that is not found in nature [41]. This type of approach is also suggested in pest control, where one can try to breed a future lethality into a wild population over several generations before it becomes activated by a change in conditions [42].

A subtle case of engineered extinction makes use of lethal mutagenesis [43–45]. A potentially high-fitness genome is engineered to have a high enough mutation rate that extinction occurs gradually through the inevitable accumulation of deleterious mutations. Whether a sufficiently high mutation rate can be engineered and maintained is not clear, particularly since the high mutation rate also increases the opportunity for compensatory mutations that might reduce the mutation rate below lethal levels.

Most of these approaches operate on timescales measured in generations. Therefore, extinction will happen faster in absolute time for an organism with a short generation time than for one with a long generation time. Since absolute time is what is most relevant for real-world applications, these interventions may be too slow to be practical for some organisms.

**Infectious and Inherited Reversal of Evolution**

The preceding cases used engineering to create genomes with limited evolutionary capacity. Engineered constructs can instead be introduced to populations, spread into other genomes and ultimately affect the entire population. This mechanism is a form of using evolution in one part of a genome to block evolution in a different part. Some of these approaches have been implemented experimentally, or partly so.

**Reversing Drug Resistance Evolution in Bacteria**

Temperate phages can infect their bacterial hosts without killing; merely inserting their viral genomes into the bacterial chromosome. Some temperate phages have been engineered to carry genes that suppress or obliterate drug resistance encoded by other parts of the genome. The sensitive bacterial converts remain alive, able to compete ecologically with remaining drug-resistant strains, suppressing the overall levels of resistance in the population [46,47]. This specific technology is undoubtedly of limited scope, but the general strategy of combining engineering and ecology to drive resistant mutants extinct in new ways may have broad utility.

**Gene Drives to Counteract Evolution or Cause Extinction**

Gene drives are genetic elements that exhibit super-Mendelian inheritance. They operate by biasing heterozygote transmission in their favor (Figure S1); the extreme case being that an offspring organism that is (at least initially) a heterozygote for the drive transmits only the drive allele to its offspring. This advantage is potentially so strong that gene drives can spread through a population despite causing harm to the individual carriers [48–51].

Gene drives were first discovered in nature [52,53]. With the advent of RNA-guided nuclease technologies (e.g., CRISPR-Cas9), a specific type of homing endonuclease gene drive can now be engineered into virtually any region of a genome and in any species [54–
The engineered drive can be designed arbitrarily to convert a nonessential part of the genome to almost any designed sequence. Gene drives can thus be targeted to sequences that are themselves the basis of unwanted evolution. The result would be a gene drive evolving on top of (and destroying) prior evolution in the same population. Esvelt et al. [57] proposed targeting one gene drive to destroy a previous gene drive, but there are likely many possibilities. Gene drive systems can also be used to carry a genetic cargo that will spread with the drive through the population. This cargo could express a gene that thwarts some kind of evolution at a different locus.

Closely related to this approach is to combine population inundation with genetic engineering. A two-part ‘killer–rescue’ system can be engineered, the killer portion destroying any genome that lacks the rescue. When introduced into the population at even a low level, the killer and rescue bootstrap themselves to high frequency [50,58]. When these constructs target regions of the genome responsible for unwanted evolution, they can reverse it.

An extreme use of a gene drive is to cause population extinction [48,49,59]. A recessive-lethal gene drive can in theory spread throughout a population and drive it extinct: a population that has evolved undesirable traits encoded by or linked to a specific genomic sequence could be targeted for eradication, and the lethal gene drive could even be manipulated to control the timing of the extinction. Success requires that resistance does not evolve against the gene drive. We have little experience with these systems to know how easily resistance evolves.

**Concluding Remarks**

Current approaches for arresting evolution need to be more thoroughly evaluated in real-world situations, and we predict that creative uses of genome engineering will continue to enable new antievolution designs (see Outstanding Questions). Much like drug combination therapies, it will be possible to stack together multiple systems for arresting evolution to further delay inconvenient or dangerous outcomes. Strategies that blend engineering with classical population-genetics approaches are likely to be the most successful. For example, there are countless possibilities for developing antievolution systems in the context of species-specific ecologies, especially once one not only adds back or takes out natural community members but uses custom-engineered competitors with smart weapons that target or reverse the unwanted phenotypes that result from mutations.

**Outstanding Questions**

Will the ability to easily sequence genomes and find rare variants in populations lead to a greater ability to control evolution? Screening individuals for the presence of adaptive mutations may inform control strategies. Such designs are already implemented in some viral treatment programs, but the approach can be used more widely.

To what extent can cellular mutation rates be reduced by genome engineering? It is possible to alter DNA replication and repair processes to have higher fidelity, but at some
point the chemical stability of DNA will become limiting. Can synthetic approaches that utilize other types of molecules as the genetic material surpass these limits?

Is the introduction of antievolution elements into populations feasible? Recent uses of CRISPR technologies to engineer gene drives have met with short-term success in experimental populations, but the long-term responses of populations are unknown. The evolution of resistance to gene drives is a potentially serious hurdle.

Will manipulating ecology and competition open a new frontier in this quest? Altering resources and adding competitors may offer the most robust approaches, at least in the near future, for populations that are not easily engineered.

In the longer term, innovative leaps in our capabilities for arresting evolution are most likely to come on a few fronts. First, as with the recent CRISPR-Cas9 gene drive work, we are likely to continue to find new molecular biology tools in nature that can be adapted to control evolution. Second, more types of antievolution strategies may remain as yet undiscovered or unrecognized in nature; perhaps among organisms with slow replication times or long quiescent stages, for example. Third, chemical biologists are currently building non-natural DNA bases into the genomes of cells [60]. Such xenobiotic genetic materials could surpass what we now view as fundamental limitations on the fidelity of DNA-encoded information to arrest the evolution of synthetic organisms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Evolution is sometimes unwanted or harmful to our purposes, especially in organisms we want to control.

An understanding of evolutionary principles, specifically of natural selection, can be used to limit evolution. These traditional approaches can restrict the opportunity for evolution even without a molecular understanding of a system.

Our multicellular bodies seem to have evolved many of these same strategies for restricting evolution within our bodies (e.g., cancer) and in the germ line.

Genetic engineering opens fundamentally new avenues for limiting evolution. It can be used to design genomes that have limited evolutionary capacity or to introduce elements that spread and reverse evolution in populations.
Figure 1.
Traditional Methods for Arresting Evolution. (A) Many methods for slowing or preventing undesirable evolution are based on understanding population genetic principles, as described in the main text. Some have been applied for decades or even longer. One need not have a detailed understanding of the molecular biology of a particular organism to use them. To arrest evolution one can: (B) maintain seed stocks, (C) limit growth, (D) apply appropriate selection, or (E) add competitors.
Figure 2.
Methods for Arresting Evolution Enabled by Genome Engineering. The ability to arbitrarily make large-scale edits to genomes has recently made new methods for preventing evolution possible or easier. To prevent unwanted evolution one can, for example, engineer genome sequences to: (A) reduce the fitness limit, (B) change pathways of evolution, or (C) create immutable sequences.
Table 1, Key Table

Problems Caused by Short-Term Evolution

| Context                     | Problem                                                        |
|-----------------------------|----------------------------------------------------------------|
| Industrial cultures and strains | Production decays during large-scale or long-term propagation |
| Live vaccines               | Vaccine strains evolve back to high virulence (poliovirus)     |
| Drug resistance             | Bacteria and viruses evolve to resist drugs                    |
| Pesticide resistance        | Crop pests evolve to resist chemical control agents             |
| Weeds                       | Weeds evolve to mimic crops, leading to greater crop contamination |
| Cancer                      | Cells evolve through multiple stages to escape controls on cell division and programmed cell death |
| Stem cells                  | Cells potentially used for therapy evolve cancerous properties after culture in the laboratory |
| Opportunistic infections    | Pathogens sometimes evolve to invade new species or locations in the body |