BIOE.44

Synthetic Biology Lab

27 April 2010 Lecture / Discussion notes

http://openwetware.org/wiki/Stanford/BIOE44

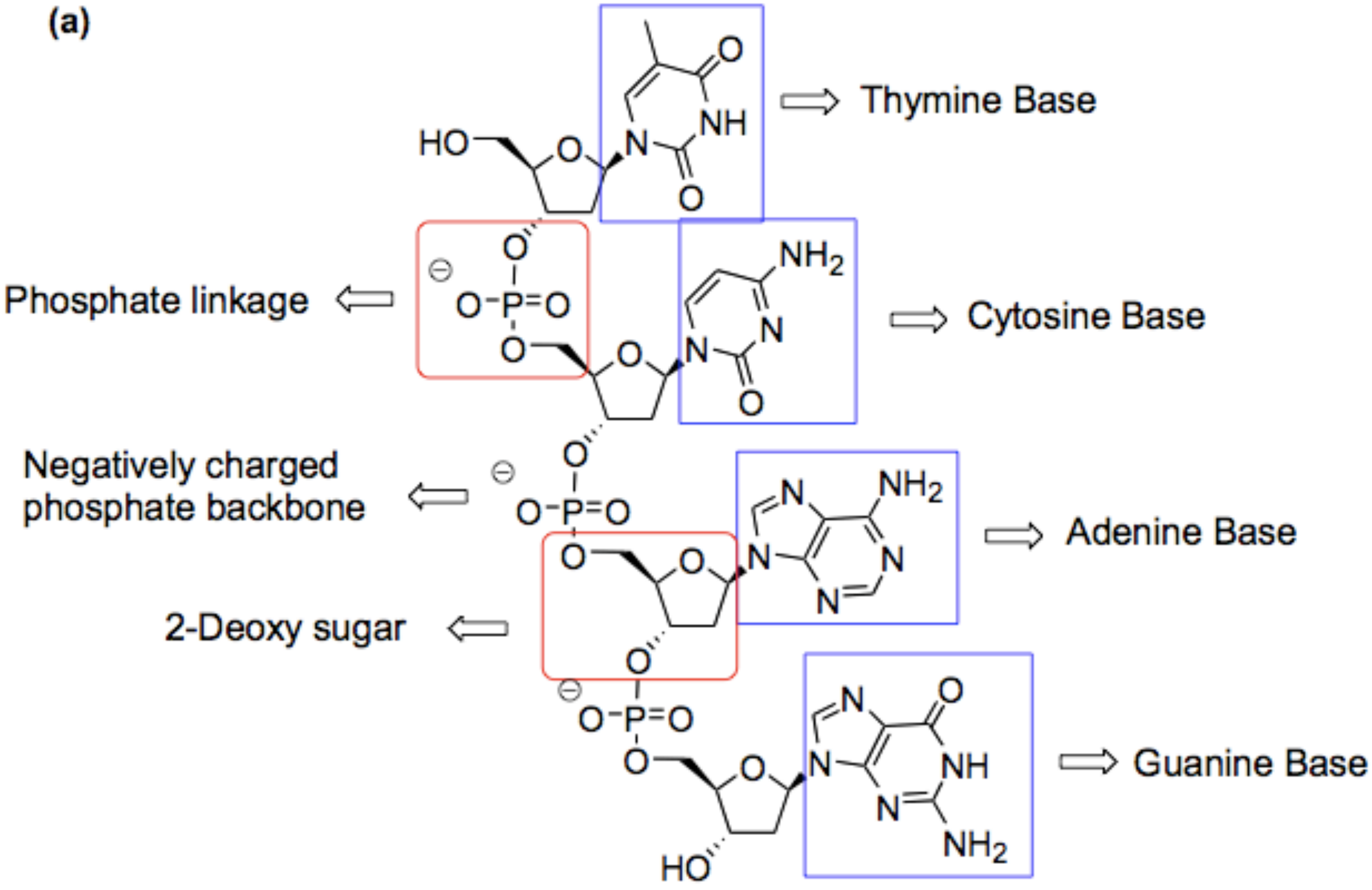
GOALS FOR THIS WEEK

1. *Choosing a pollutant to sense.*
2. *Design and order a novel Pollutant -> PoPS Sensor Device*

GOALS FOR TODAY's CLASS

1. *Understand logic of DNA synthesis chemistry*
2. *Context of DNA synthesis tech. development*
3. *Intro to DNA synthesis governance.*

(a)



from "A Roadmap to the Assembly of Synthetic DNA from Raw Materials" by Sanghvi, Yogesh (http://dspace.mit.edu/handle/1721.1/39657)

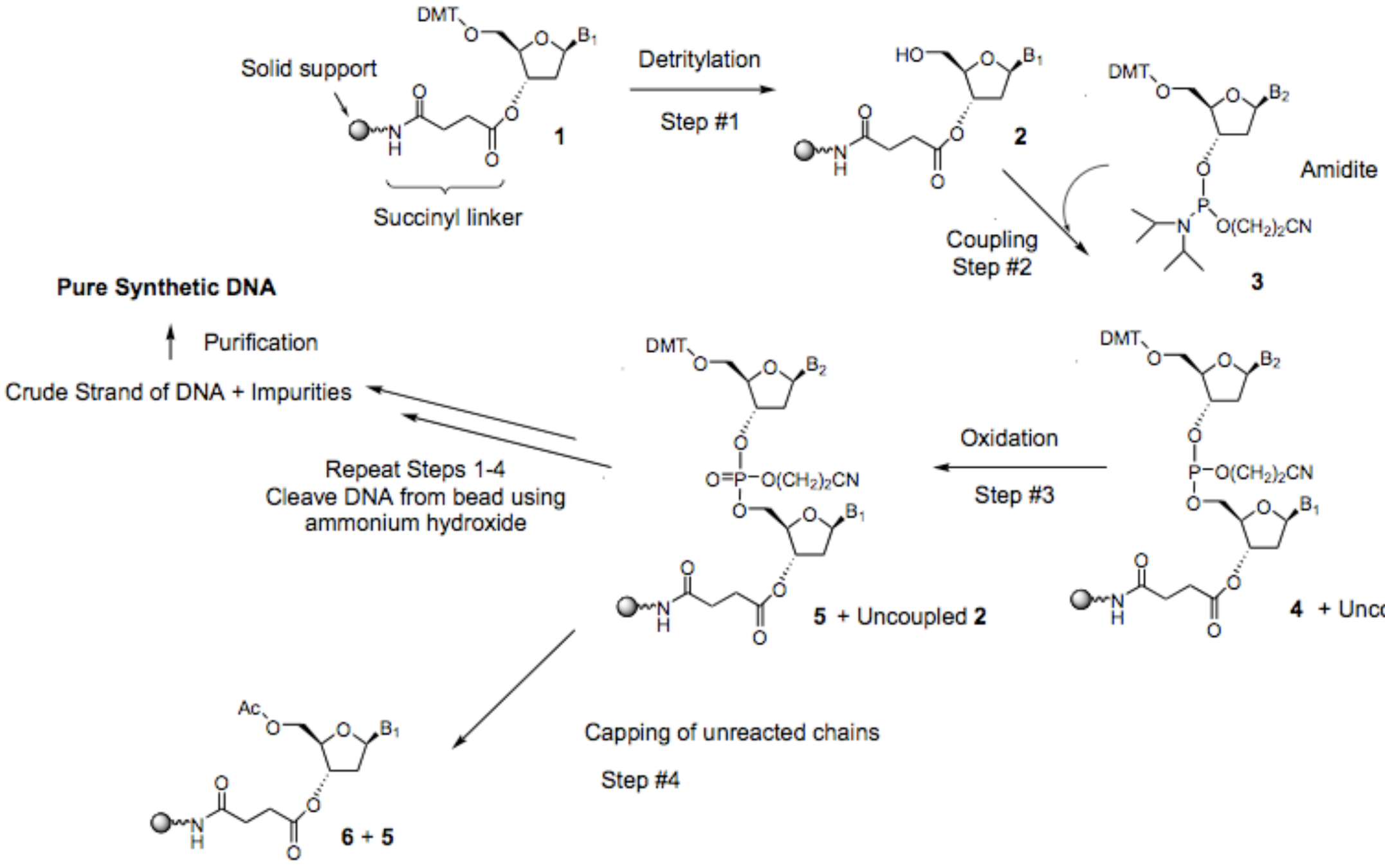


Figure 5: General scheme for automated synthesis of DNA using amidites

from "A Roadmap to the Assembly of Synthetic DNA from Raw Materials" by Sanghvi, Yogesh (http://dspace.mit.edu/handle/1721.1/39657)

Chemistry of DNA defines what must be added (e.g., bases).

Sources of error in synthesis define necessary steps.

- failure to couple leads to capping step in order to prevent deletions from accruing

- propensity to react leads to blocking group in order to prevent poly-As etc.

Coupling efficiency limits length of what can be made from scratch (on conventional DNA synthesizers).

$$\text{yield} = (\text{coupling \%})^{\#\text{bp}}$$

$$36\% = (0.99)^{100\text{mer}}$$

Making longer molecules requires assembly of oligonucleotides via some means (recombination- or ligation-based usually). For example:

6984–6990 *Nucleic Acids Research, 2009, Vol. 37, No. 20* *Published online 10 September 2009*
doi:10.1093/nar/gkp687

Synthesis of DNA fragments in yeast by one-step assembly of overlapping oligonucleotides

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Received January 1, 2009; Revised August 1, 2009; Accepted August 4, 2009

ABSTRACT

Here it is demonstrated that the yeast *Saccharomyces cerevisiae* can take up and assemble at least 38 overlapping single-stranded oligonucleotides and a linear double-stranded vector in one transformation event. These oligonucleotides can overlap by as few as 20 bp, and can be as long as 200 nucleotides in length. This straightforward scheme for assembling chemically-synthesized oligonucleotides could be a useful tool for building synthetic DNA molecules.

fragments could be assembled by yeast into an entire *Mycoplasma genitalium* genome (17). Subsequently, this process was improved and 25 overlapping fragments, between 17 and 35 kb in length, were assembled at once into this genome (18). Later work showed that six smaller fragments were also acceptable (19).

To turn yeast into a 'factory' able to produce whole genomes and large constructs of any reasonable sequence, what remains is to demonstrate the assembly of chemically synthesized oligonucleotides into appropriate dsDNA molecules in yeast (Figure 1). For this to succeed, a single yeast cell would need to take up all of the necessary oligonucleotides as well as a vector that

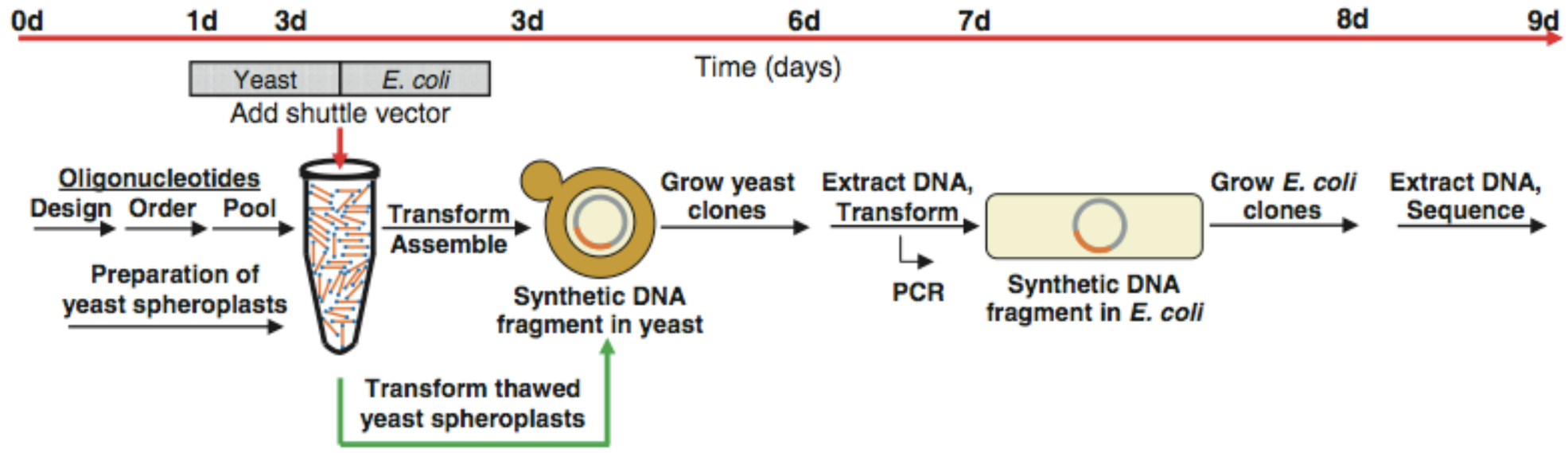
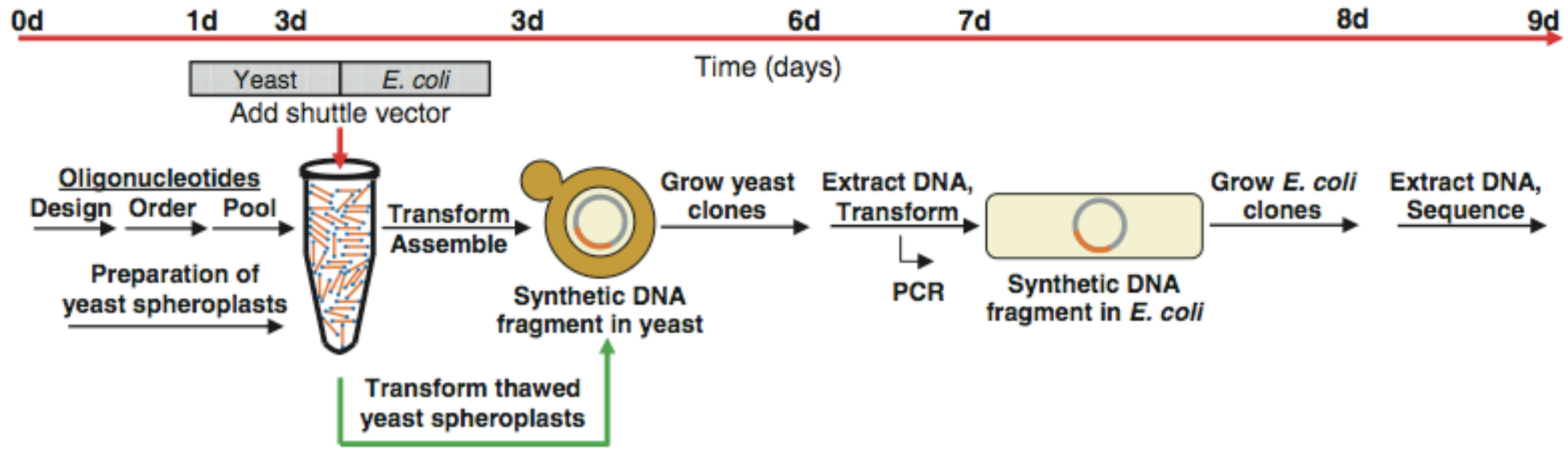


Figure 1. Schematic overview and timeline for the assembly of overlapping ssDNA oligonucleotides (orange lines with blue circles) into a linear dsDNA yeast/*E. coli* shuttle vector (pRS313; grey) within the nucleus of a yeast cell. Following a single transformation event, a synthetic dsDNA fragment (orange) is produced. These fragments are recovered from yeast and then transferred to *E. coli* for more efficient amplification.



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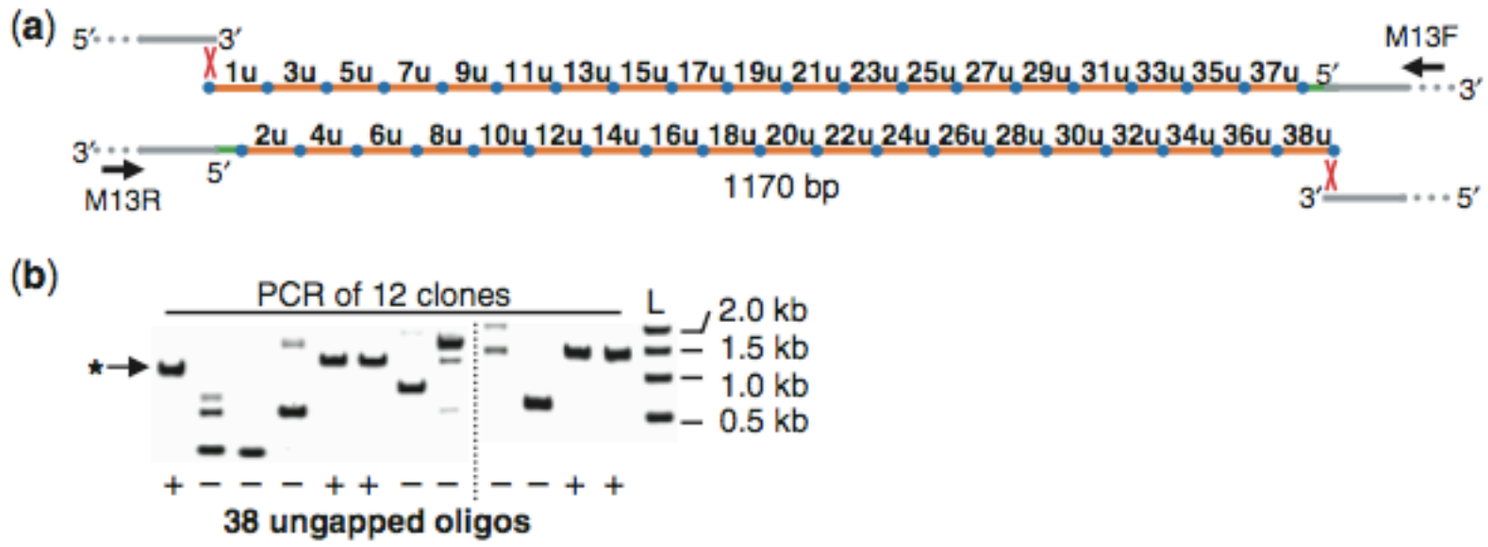
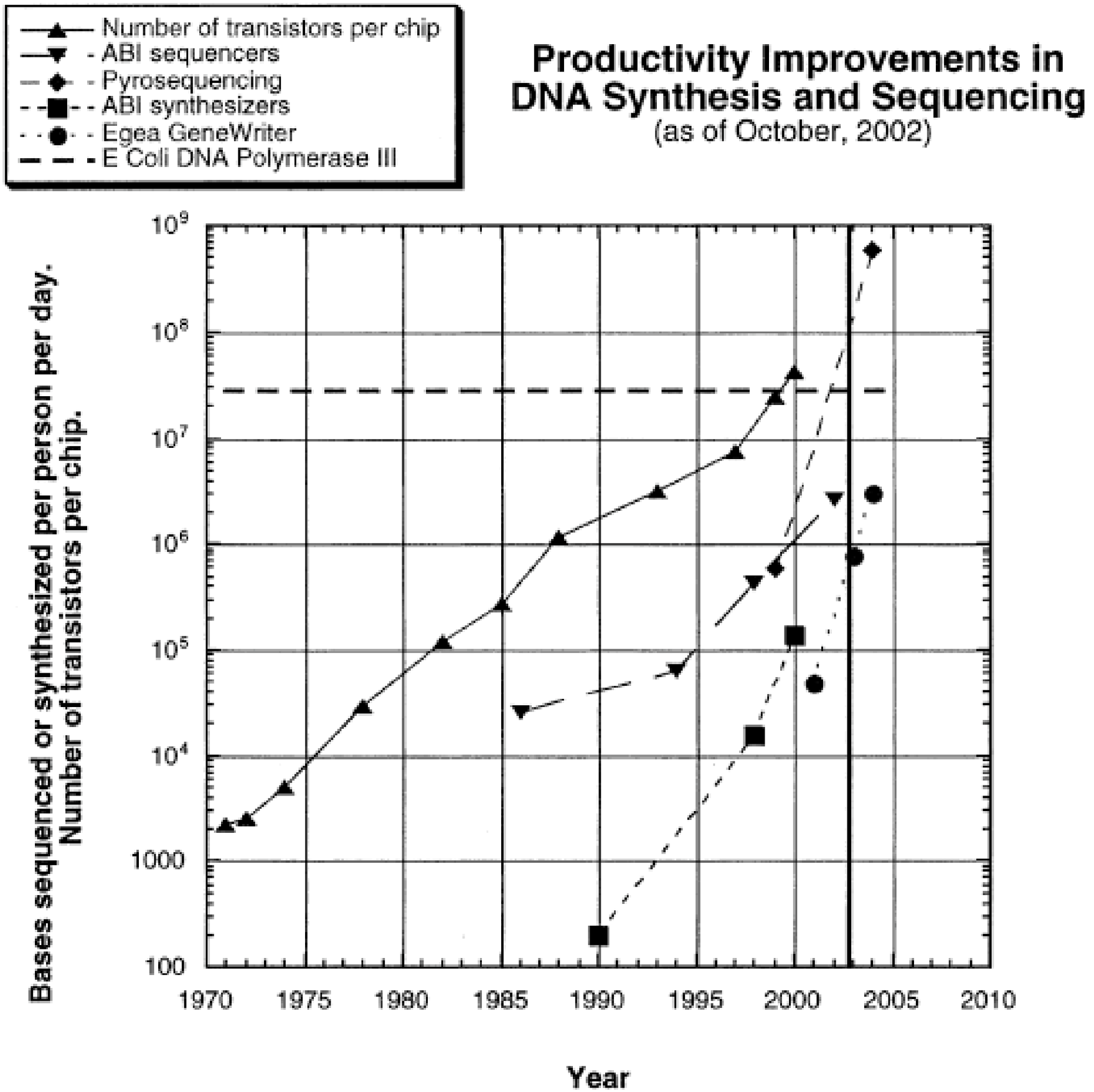


Figure 2. Assembly of 38 overlapping 60-mer oligonucleotides in yeast. (**a**) The 38 oligonucleotides, named 1–38 u, have 30 bp overlaps and produce a 1170 bp synthetic DNA fragment following assembly. The terminal oligonucleotides overlap the vector (grey) by 20 bp (red x). Ten nucleotide gaps (green) are repaired inside the yeast cell. (**b**) PCR analysis of 12 randomly selected yeast clones following transformation and assembly of the oligonucleotides and vector depicted in (a). The primers used for this PCR analysis and for DNA sequencing are M13F and M13R and are shown in (a). The predicted amplicon size for a complete assembly is 1393 bp and is indicated by an asterisk. The presence (+) or absence (−) of the expected product is noted for each clone screened. L indicates the 1 kb DNA ladder (NEB).

Tech. Dev. Context for DNA FAB

“surfing an exponential”



Carlson, Pace & Proliferation of Biological Technologies, Biosec. & Bioterror. 1(3):1 (2003)

Genomes (> 500,000 bp)

Increase process speed and reliability

Pathways (10,000 < 500,000 bp)

Increase process speed and reliability

Characterize reliability and limits

Genes (200 < 100,000 bp)

Increase process speed and reliability

Fab rules that back-propagate to designers
Solve manufacturing control problem
Process specific (sequencers, colony picking)

Oligos (<200 bp)

Move synthesis inside cells?

Reduce scale (1/1000th)
Coupling eff. From 99.3% to 99.99+%

Reagents

Beyond phosphoramidites?

ID & prior. error sources (going up)
Reconsider shift to new chemistry

Raw Materials

From salmon milt to sugarcane

Editing Existing Material

Increase process speed, scale and reliability

Editing Existing Material

Increase process speed, scale and reliability

Positive Actions

Develop & implement strategic products procurement plan
(GC 20-80, length, repeats, modularity)
10kb coupon for all NIH researchers
Low cost guaranteed loan to expand capacity
Targeted grants for process R&D and improvement
Academic research programs (clone by dilution)

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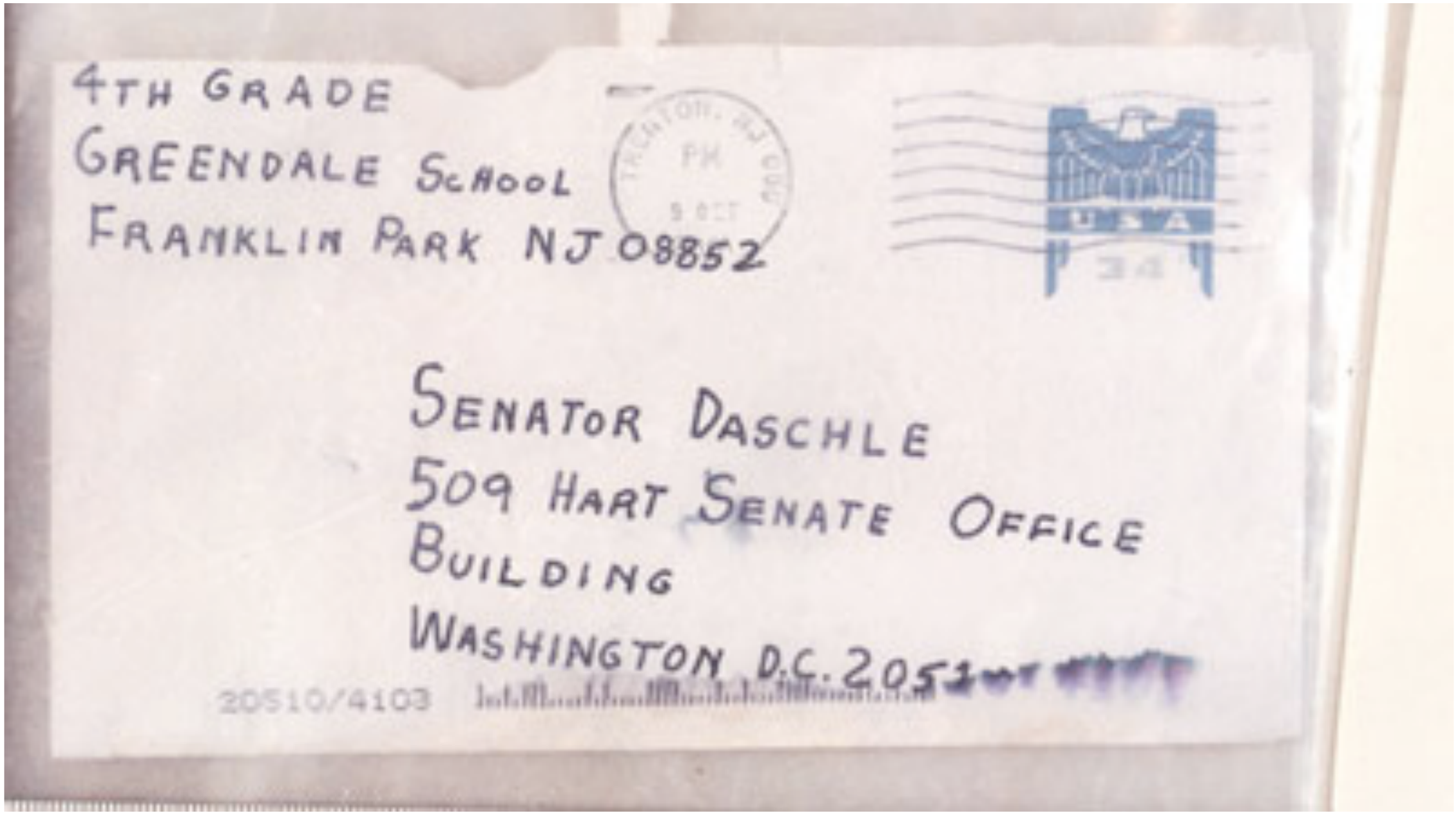
5 Years From Now

Today:
100 mb @ 2kb length, $0.50-$4.00/bp, 5-180 day turn
Future:
3200 mb @ 2kb length, $0.10-$2.00, 3-20 day turn
100 mb @ 1mb length (30 microbial genomes / year)
Impact:
2x improvement in biology and biotech. research

Security Context for DNA FAB

“could synthesis and assembly be misused?



http://en.wikipedia.org/wiki/2001_anthrax_attacks

OP-ED CONTRIBUTORS

Recipe for Destruction

By RAY KURZWEIL and BILL JOY

Published: October 17, 2005

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AFTER a decade of painstaking research, federal and university scientists have reconstructed the 1918 influenza virus that killed 50 million people worldwide. Like the flu viruses now raising alarm bells in Asia, the 1918 virus was a bird flu that jumped directly to humans, the scientists reported. To shed light on how the virus evolved, the United States Department of Health and Human Services published the full genome of the 1918 influenza virus on the Internet in the GenBank database.

This is extremely foolish. The genome is essentially the design of a weapon of mass destruction. No responsible scientist would advocate publishing precise designs for an

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Microbial
Eukaryotic

Archaea
Chromosome
Plasmid
DraftAssembly

Bacteria
Chromosome
Plasmid
DraftAssembly

Display Summary Show 20 Send to

All: 3

Items 1 - 3 of 3

1: NC_004161
Reston Ebola virus, complete genome
ssRNA; linear; Length: **18,891 nt**
Created: **2002/09/04**

2: NC_002549
Zaire ebolavirus, complete genome
ssRNA; linear; Length: **18,959 nt**
Created: **1999/02/10**

3: NC_006432
Sudan ebolavirus, complete genome
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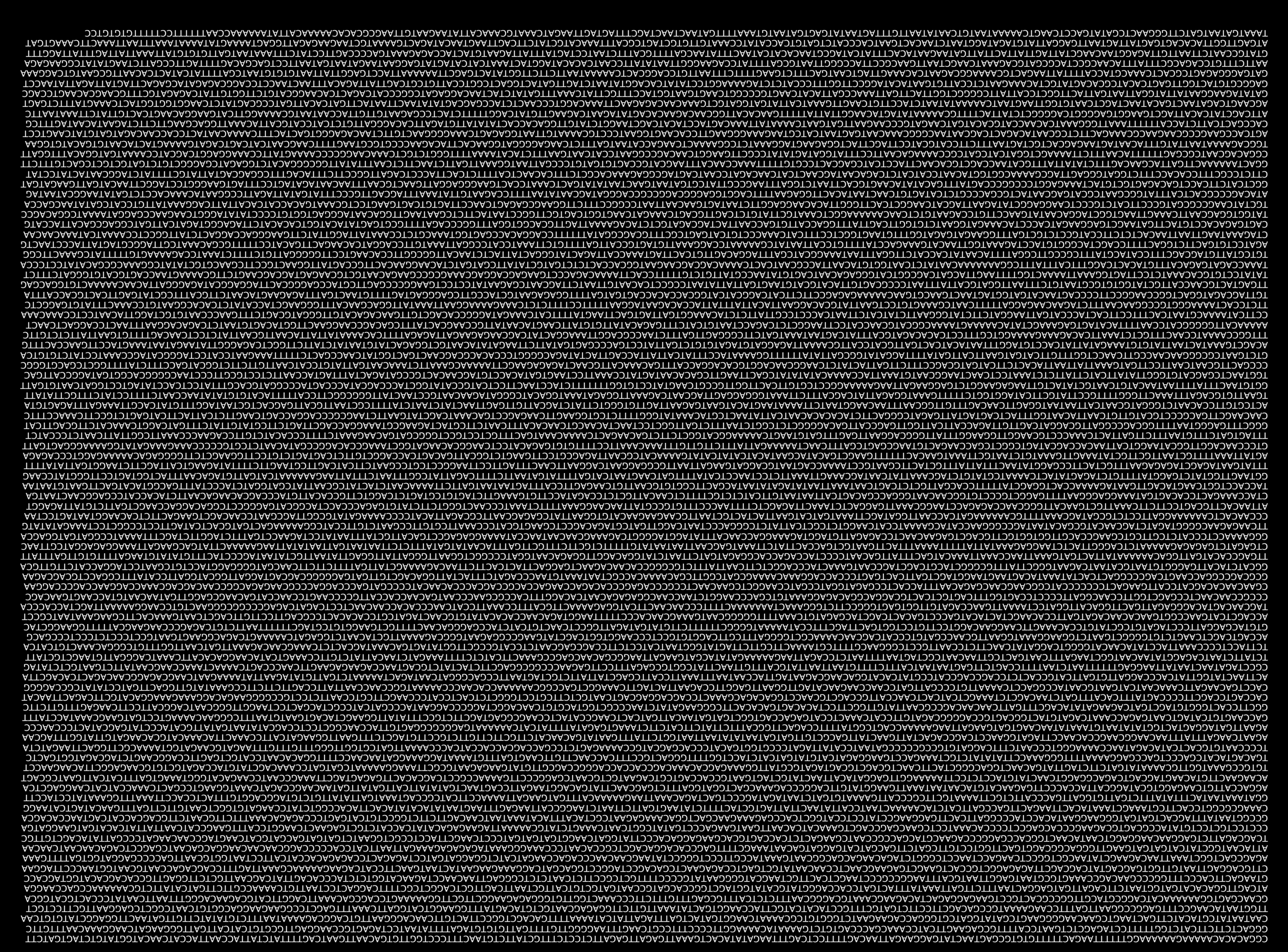
Created: **2004/11/15**



DNA synthesis and biological security

Hans Bügl, John P Danner, Robert J Molinari, John T Mulligan, Han-Oh Park, Bas Reichert, David A Roth, Ralf Wagner, Bruce Budowle, Robert M Scripp, Jenifer A L Smith, Scott J Steele, George Church & Drew Endy

A group of academics, industry executives and security experts propose an oversight framework to address concerns over the security of research involving commercial DNA synthesis.

DNA synthesis allows the direct construction of genetic material starting from information and raw chemicals[1]. Improvements in synthesis technology are accelerating innovation across many areas of research, from the development of renewable energy to the production of fine chemicals, from information processing to environmental monitoring, and from agricultural productivity to breakthroughs in human health and medicine. Like any powerful technology, DNA synthesis has the potential to be purposefully misapplied. Misuse of DNA-synthesis technology could give rise to both known and unforeseeable threats to our biological safety and security. Current government oversight of the DNA-synthesis industry falls short of addressing this unfortunate reality.

Here, we outline a practical plan for developing an effective oversight framework for the DNA-synthesis industry[2]. The resulting framework serves three purposes. First, it promotes biological safety and security. Second, it encourages the further responsible development of synthetic biology technologies and their continued, overwhelmingly constructive application. And third, it is designed to be international in scope. Our plan is informed by past and ongoing discussions of biological security issues associated with DNA-synthesis technology[3–5] and represents the collective views of all founding members of the

Hans Bügl, John P. Danner, Robert J. Molinari, John T. Mulligan, David A. Roth & Ralf Wagner are members of the International Consortium for Polynucleotide Synthesis; Hans Bügl and Ralf Wagner are at GENEART; John P. Danner, George Church & Drew Endy are at Codon Devices; Robert J. Molinari & David A. Roth are at CODA Genomics; John T. Mulligan is at Blue Heron Biotechnology; Han-Oh Park is at Bioneer; Bas Reichert is at BaseClear B.V.; Ralf Wagner is at the University of Regensburg Molecular Virology & Gene Therapy Unit, Institute of Medical Microbiology and Hygiene; Bruce Budowle, Robert M. Scripp, Jenifer A. L. Smith & Scott J. Steele are at the US FBI; George Church is in the Department of Genetics, Harvard Medical School; Drew Endy is in the Department of Biological Engineering, MIT; George Church & Drew Endy are at the multi-institution US National Science Foundation Synthetic Biology Engineering Research Center. e-mail: endy@mit.edu

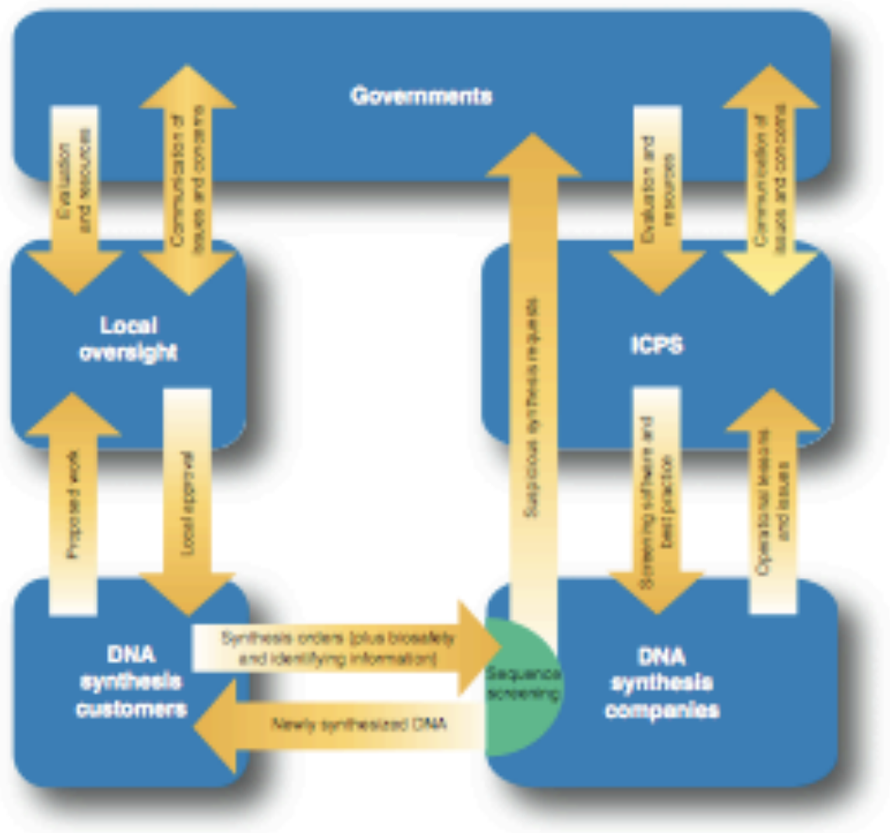


Figure 1 Our framework calls for the immediate and systematic implementation of a tiered DNA synthesis order screening process. To promote and establish accountability, individuals who place orders for DNA synthesis would be required to identify themselves, their home organization and all relevant biosafety information. Next, individual companies would use validated software tools to check synthesis orders against a set of select agents or sequences to help ensure regulatory compliance and flag synthesis orders for further review. Finally, DNA synthesis and synthetic biology companies would work together through the ICPS, and interface with appropriate government agencies (worldwide), to rapidly and continually improve the underlying technologies used to screen orders and identify potentially dangerous sequences, as well as develop a clearly defined process to report behavior that falls outside of agreed-upon guidelines. ICPS, International Consortium for Polynucleotide Synthesis.

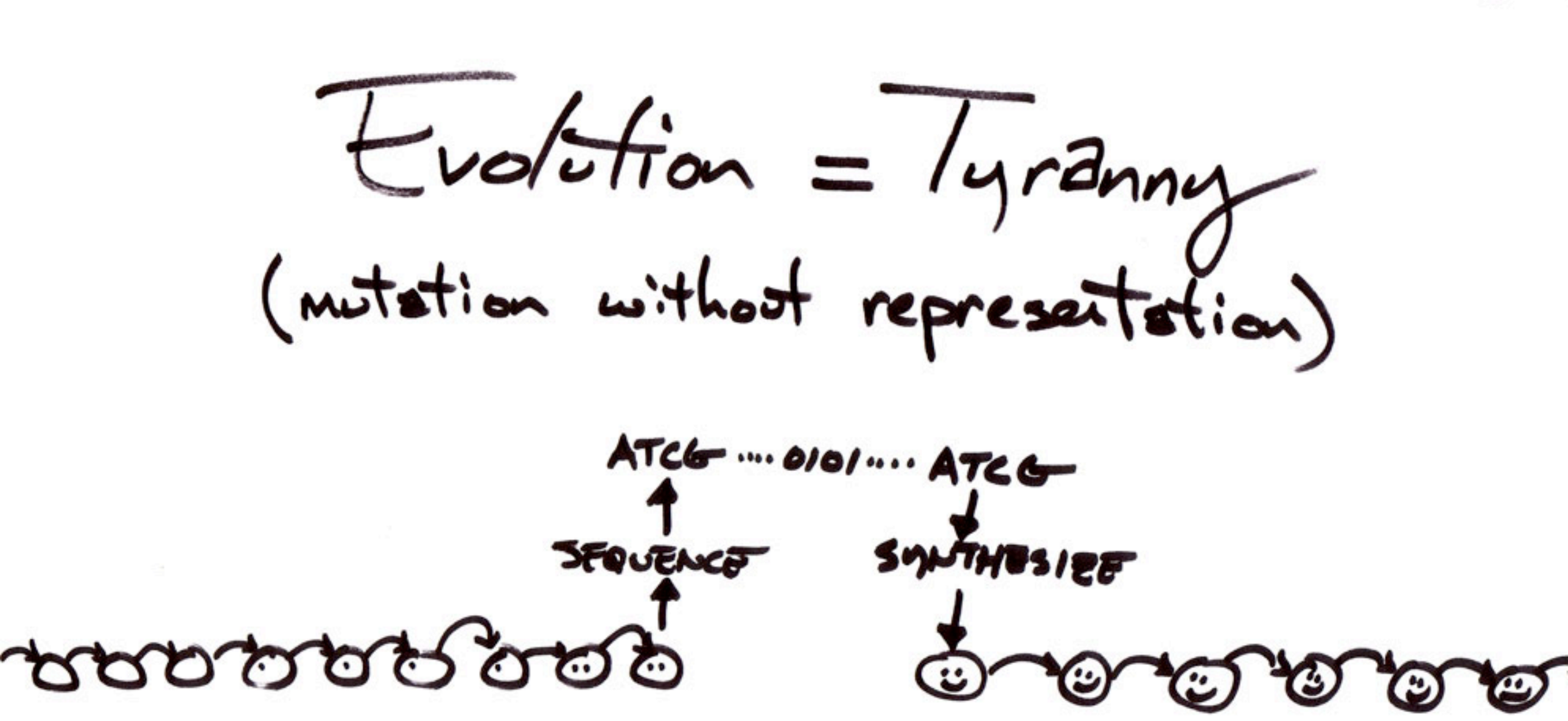
Bonus

Evolution = Tyranny

(mutation without representation)

Evolution = Tyranny

(mutation without representation)



Evolution = Tyranny

(mutation without representation)

Sufficiently mature DNA sequencing & synthesis technology will allow us to decouple the "designs" of life from the constraints of direct descent & replication with error. — D. Endy