EXPLORING TEMPLATE-INDEPENDENT POLYMERASES FOR AUTOMATED DNA SYNTHESIS

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Proposal

We seek to learn whether electrically-controlled, template independent enzymatic DNA synthesis of DNA is feasible. If proof-of-concept is successful, further development could quickly lead to ultra low-cost, extremely rapid, and highly accurate microbial genome-scale DNA synthesis – on the order of an *E. coli* genome (4.6 million bases) for $100 in reagent costs, produced in under 1 hour.

Current limits

At present, DNA is synthesized in an iterative chemical process. Chemical synthesis requires specialized reagents and equipment, is error prone and slow (minutes/base), and relatively expensive – currently between $0.40 and $1.00 per base. With current technology, *E. coli* would cost between $2M - $4M, well outside the reach of most genomic investigators. Wikipedia has excellent overviews of chemical oligonucleotide[1] synthesis and artificial gene/genome synthesis[2].

New approach and significance

We believe enzyme-based DNA synthesis could produce step change in DNA synthesis technologies. Enzymes that assemble DNA are called polymerases. They are present in all living cells and work to copy genetic material at a rate of about 1000 bases per second. Typically, one strand of DNA is used as a "template" for the polymerase to produce a complimentary matching strand, forming the double helix.

In 1997, it was discovered that archaebacterial polymerase could generate long DNA repeats (50,000 bp or more) spontaneously, even in the absence of template DNA[3]. Sequences are in effect programmed directly into the polymerase structure. We seek to harness this synthetic ability and dictate the sequences this polymerase can generate.

Our selection and screening strategy is as follows:

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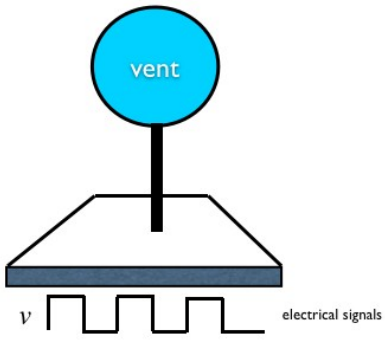
1 http://en.wikipedia.org/wiki/Oligonucleotide_synthesis

2 http://en.wikipedia.org/wiki/Gene_synthesis

3 Biochem. J. (1997) 324, 667–671

We start with *Thermococcus litoralis* polymerase known to have template-independent synthetic activity. Cloned and characterized it is 774 amino acids (see GenBank: AAA72101.1) and is commercially available as "Vent" polymerase[4].
Using a suitable protein linker (*to be determined, comments required*), the polymerase molecule is attached to a conductive element to which specific electrical signals can be applied.



The goals of the experiment are as follows:

1. Ensure the tethered polymerase remains capable of producing template-independent repeat sequences in the appropriate reaction conditions. This can be detected by gel electrophoresis.

2. Explore electrical signals that "brake" this polymerase activity, producing an off state. Results can be analyzed by gel electrophoresis.

3. Explore electrical signals that instruct the polymerase which of the four DNA bases to add. Results can be analyzed by gel electrophoresis, PCR with specific primers, and DNA sequencing.

If these results can be achieved, further characterization and optimization of the system would follow.

Risks and Payoffs
Should the template-independent DNA synthesis capability of vent polymerase prove amenable to be electronically control, rapid and inexpensive DNA synthesis using common reagents will be possible at scale – a true step change in DNA synthesis technology. The tool would be highly sought by the scientific community and generate

4 The source organism lives near deep-sea "smoker" vents

significant commercial device and reagent sales. Importantly, it would also broadly accelerate the genetic engineering of complex metabolic pathways and small genomes, addressing much larger markets.

The technical risk here is limited to failure to deliver proof-of-concept results. The greater risk is to lose technical leadership in the area of DNA synthesis, which could have widespread ramifications.

Costs and timelines

Proof-of-concept requires an experienced molecular biologist and electrical engineer, access to a basic laboratory facility, and sufficient operational funding for 6 months -- about $200,000 in total. This assumes the basic strategy is sound and no significant technical hurdles are encountered.

If proof of concept is successful, R&D towards optimization would require 18-24 months, 5-10 personnel (both technical and organizational), and approximately $2M in funding.