

Low-Cost DNA Production Roadmap.

(George Church, Dept of Genetics, Harvard Medical School, Draft 10-Aug-2007)

A. Motivations and status of applications

The first commercial application for DNA nanostructures has finally arrived (Apr 2007) in the form of large 6-helix bundle rods needed in gram quantities for NMR. This invention has been licensed to Codon Devices of Cambridge, MA. DNA has also been shown capable of being a ligand sensitive mechanical switch, catalyst, and/or actuator/motor (without need for proteins). The possibility of using such devices in bulk as (biocompatible smart materials/plastics) is motivation for bring the cost of DNA down closer to other biopolymers (see topic B below).

Douglas SM, Chou JJ, Shih WM. Proc Natl Acad Sci U S A. 2007 Apr 17;104(16):6644-8. DNA-nanotube-induced alignment of membrane proteins for NMR structure determination.

Mao C, Sun W, Shen Z, Seeman NC. A nanomechanical device based on the B-Z transition of DNA. Nature. 1999 Jan 14;397(6715):144-6.

Garibotti AV, Knudsen SM, Ellington AD, Seeman NC. Functional DNazymes organized into two-dimensional arrays. Nano Lett. 2006 Jul;6(7):1505-7.

B. Precedents for expected costs, and milestones for polymers

1. Raw lignocellulose (wood) \$23/ton.
2. Protein: Raw Silk cocoons \$2/kg <http://www.fao.org/docrep/x5326e/x5326e0c.htm>
3. Protein Casein \$30/ kg
<http://www.sinopia.com/index.asp?PageAction=VIEWCATS&Category=156>
3. ds-DNA: raw, high complexity: \$200 / 5 g
<http://www.biocompare.com/matrix/13835/Salmon-Testes-DNA.html>
4. ss-DNA: pure M13 phage derived -- suitable for DNA Origami: \$15K / g.

While it is evident that there is currently a huge range in cost of production of biopolymers, nothing fundamental or theoretical prevents DNA production costs from being comparable to those of the least expensive polymers (see topic C below).

C. Key requirements for implementation

1. Engineering of nucleotide synthesis
We are collaborating with Philippe Marliere on optimizing metabolic pathways to the synthesis of the four dNTPs in vivo.
2. DNA secretion : This is a natural process in some bacteria, could be enhanced to prevent (potentially toxic) levels of DNA in vivo.

Whitchurch, et al. (2002) Extracellular DNA Required for Bacterial Biofilm Formation. Science 295:1487

Renelli, et al. (2004) DNA-containing membrane vesicles of *Pseudomonas aeruginosa* PAO1 and their genetic transformation potential. *Microbiology* 150: 2161-2169

D. Micro meets nano: We need to also focus on ways to integrate photolithographic microfabrication or nano-imprinting with atomically precise-self-assembly. One option is to use the 6-helix bundles (mentioned in topic A, above) using 6 BACs (100 kbp = 30 microns) and staples (optionally made by PCR from cloned junction DNAs). Staples for DNA origami typically exploit single-stranded complementarity, but recA proteins permit strand invasion of a ss-oligos into long ds DNAs. This will permit binding of long, stiff DNAs to each other and to microfabricated oligo-arrays (center-to-center spacing of about 1 micron). We also need error-correcting and/or fault-tolerant 2D & 3D DNA-nanostructure “bricks” for repetitive and non-repetitive structures.

Rigas, B, A A Welcher, D C Ward, and S M Weissman *Proc Natl Acad Sci U S A.* 1986 December; 83(24): 9591–9595. Rapid plasmid library screening using RecA-coated biotinylated probes.

Taidi-Laskowski, B, D Tyan, S M Honigberg, C R Radding, and F C Grumet *Nucleic Acids Res.* 1988 August 25; 16(16): 8157–8169. Use of RecA protein to enrich for homologous genes in a genomic library.

E. Extension of DNA chemistry. Unlike proteins, ds-DNA is commonly thought to have a very uniform (nearly sequence independent) surface. Unlike carbon allotropes (carbon-nanotubes & diamonds), current DNA lacks desirable mechanical properties. However, we aren't stuck with the standard form of DNA for a variety of future applications. A wide range of DNA “side-chains” exist, both natural (e.g. primary amines - putrescine) and synthetic (biotinyl). There are also multiple ways crosslink and/or intercalate to modify stiffness and conductance.

Gerhard B, Warren RA. *Biochemistry.* 1982 Oct 26;21(22):5458-62. Reactivity of the alpha-putrescinylythymine amino groups in phi W-14 deoxyribonucleic acid.

Richmond TJ, Steitz TA. *J Mol Biol.* 1976 May 5;103(1):25-38. Protein-DNA interaction investigated by binding *Escherichia coli* lac repressor protein to poly(d(A-U-HgX)).

Rocha MS, Viana NB, Mesquita ON. *J Chem Phys.* 2004 Nov 15;121(19):9679-83. DNA-psoralen interaction: a single molecule experiment.

Inouye M, Ikeda R, Takase M, Tsuru T, Chiba J. *Proc Natl Acad Sci U S A.* 2005 Aug 16;102(33):11606-10. Single-nucleotide polymorphism detection with "wire-like" DNA probes that display quasi "on-off" digital action.

Ma, X, J. Huang, F. Lombardi 25th IEEE VLSI Test Symposium (VTS'07) pp. 131-140. Error Tolerance in DNA Self-Assembly by $(2k-1) \times (2k-1)$ Snake Tile Sets