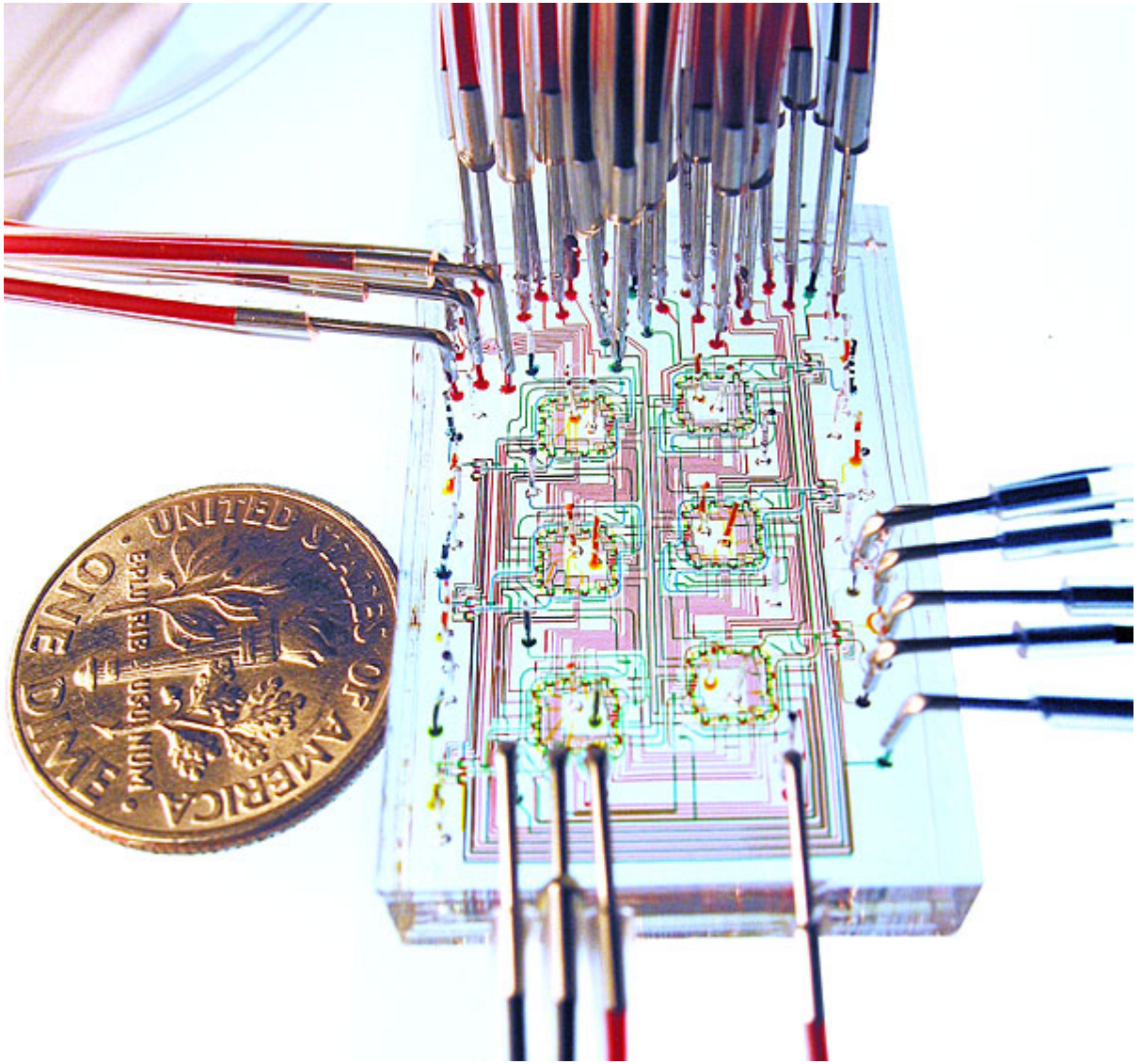
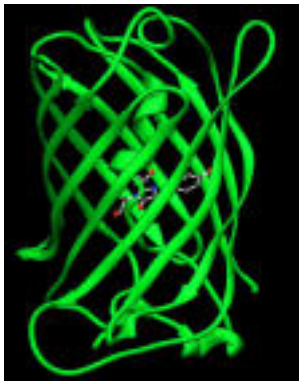
Building a Better, Cheaper Tool for DNA Synthesis

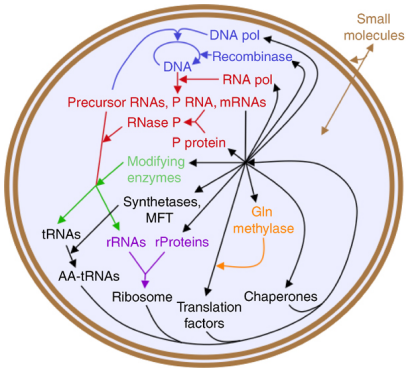


Nucleic Devices

Uses for DNA On-Demand



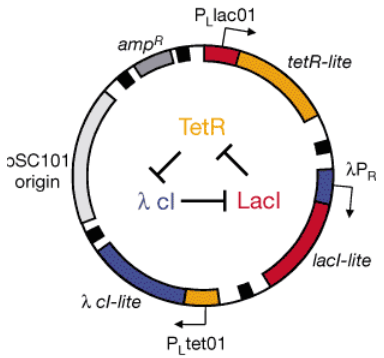
single genes*



minimal life

10^2 10^3 10^4 10^5 10^6 10^7

genetic circuits

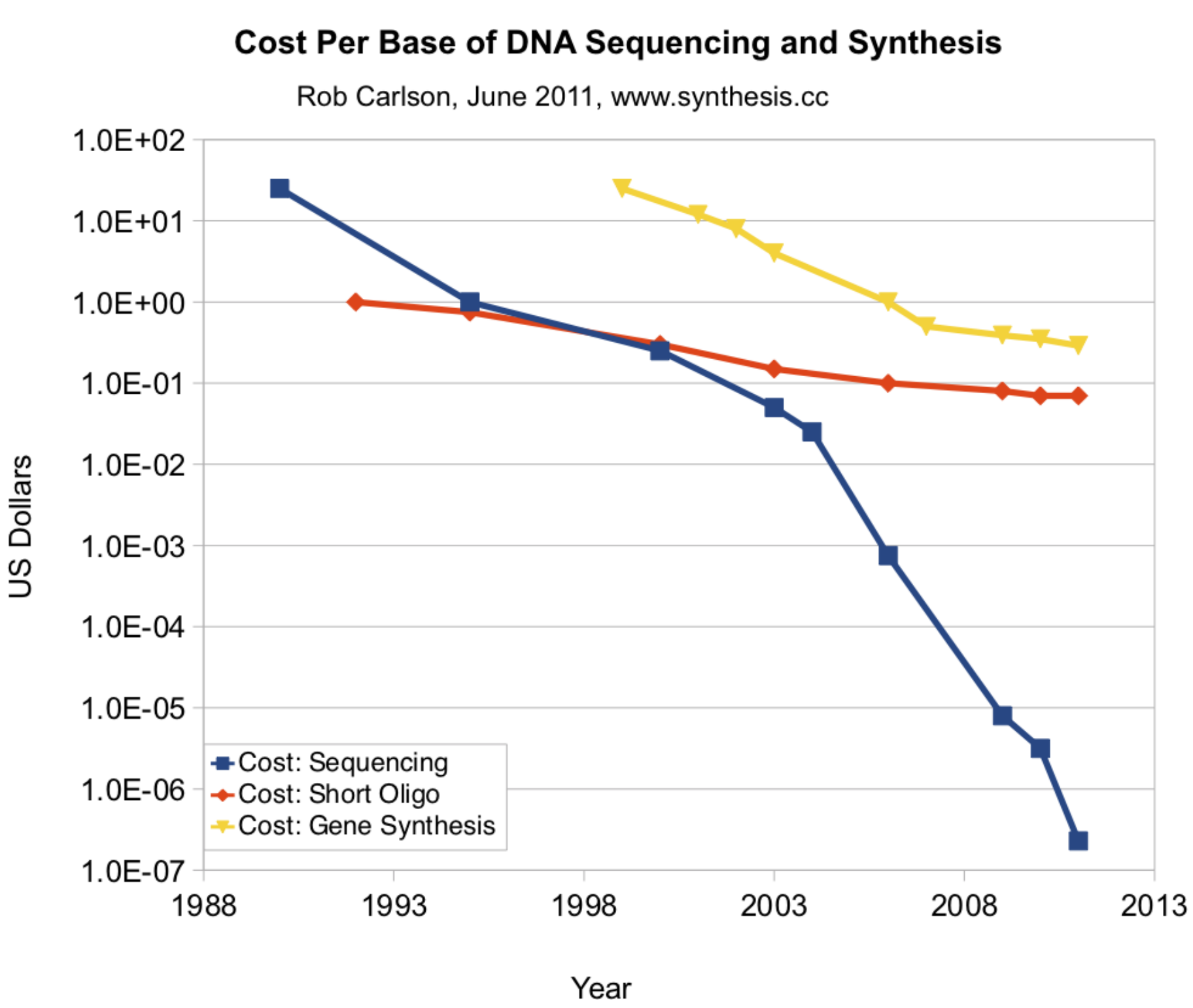


genome rewrite

Microfluidics for Gene Fabrication – Peter Carr, David Kong - MIT

DNA problems

- DNA is fundamental to biotech and research. Labs need primers, genes, plasmids and genomes. Libraries of each. Nucleic acids are in the works as pharmaceutical and disease treatments (RNAi, miRNA, gene therapy)
- Procedure to make DNA is known as oligonucleotide synthesis.
- This chemistry is tedious to do by hand and needs automation.
- “Current” synthesizers are priced too high ($150,000) – prices are stagnant and commercialized tech hasn't changed in 30 years.
- Synthetic DNA cost still high but predicted to fall dramatically.
- Researchers resort to work-arounds to account for costly DNA. Still cheaper to clone.


Cost Per Base of DNA Sequencing and Synthesis
Rob Carlson, June 2011, www.synthesis.cc
US Dollars
1.0E+02
1.0E+01
1.0E+00
1.0E-01
1.0E-02
1.0E-03
1.0E-04
1.0E-05
1.0E-06
1.0E-07
1988
1993
1998
2003
2008
2013
Year
Cost: Sequencing
Cost: Short Oligo
Cost: Gene Synthesis

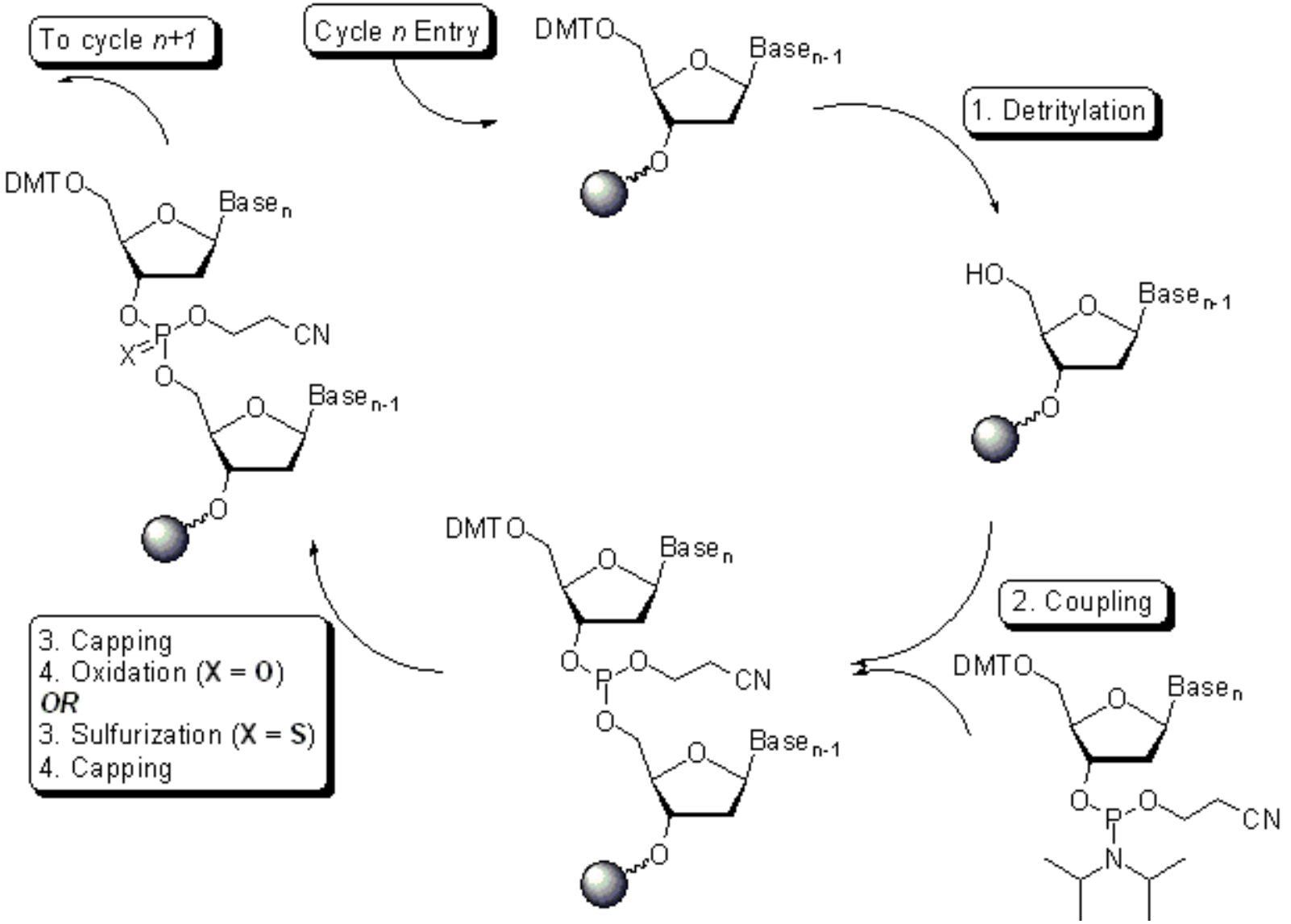
Market Size

- (2004) Synthesis instrument market size - $900 million
- (2006) IDT – 60,000 customers and 25,000 oligos/day
- (2006) Oligo market size - $500 million
- (2006) Synthetic Biology market - $600 million
- (2008) ABI reported $199.8 million in revenue for DNA/RNA equipment (20% mkt share)
- (2010) Oligo market size - $776 million
- (2011) Synthetic Biology market - $1.6B
- (2012) Combinatorial chemistry instruments - $5B
- (2013) Synthetic Biology market - $2.4B and $10.8B in 2016
- Equipment Sales Growth ~ 8%
- Oligo Growth ~ 9%
- Biologists: at least 3,500,000 (I.F.Bio)

Competition

- Synthesizers (none are microfluidic)
  - Azco Biotech, Bioautomation, Biolytic, GE Healthcare, OligoMaker
- Synthesis:
  - IDT, Sigma Aldrich, Gene Link, Bio-Synthesis, Exiqon, Biocorp, OligoFactory, Blue Heron, ...
- Microfluidics
  - Closest relations: Advanced Liquid Logic, Cambridge Biomagnetics, Cyclofluidic, Elveflow, Firefly Bio, GnuBio, Quantalife

Phosphoramidites and the right balance of protective groups



-Public Domain

Their Technology

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Doc No: CA8916.01

One of our High Throughput SuperSAMs in our Production Facility

Biosearch
SAM I
circa 1982

Biosearch Cyclone
circa 1987

DNA Synthesis instruments then and now.

To the left is one of our many SuperSAM robotic synthesizers that automatically manufacture several thousand oligo-nucleotides each day.

Our Technology

- Microfluidic DNA synthesizer using phosphoramidite chemistry
- Cheap - <$5,000 vs. $200,000
- Easy to use
- Small lab footprint
- 100X reduction in reagent volumes (at least)
- Fast reaction times (minutes or less)

Microfluidic Technology

The mess of hoses and tubing is reduced to microscopic traces

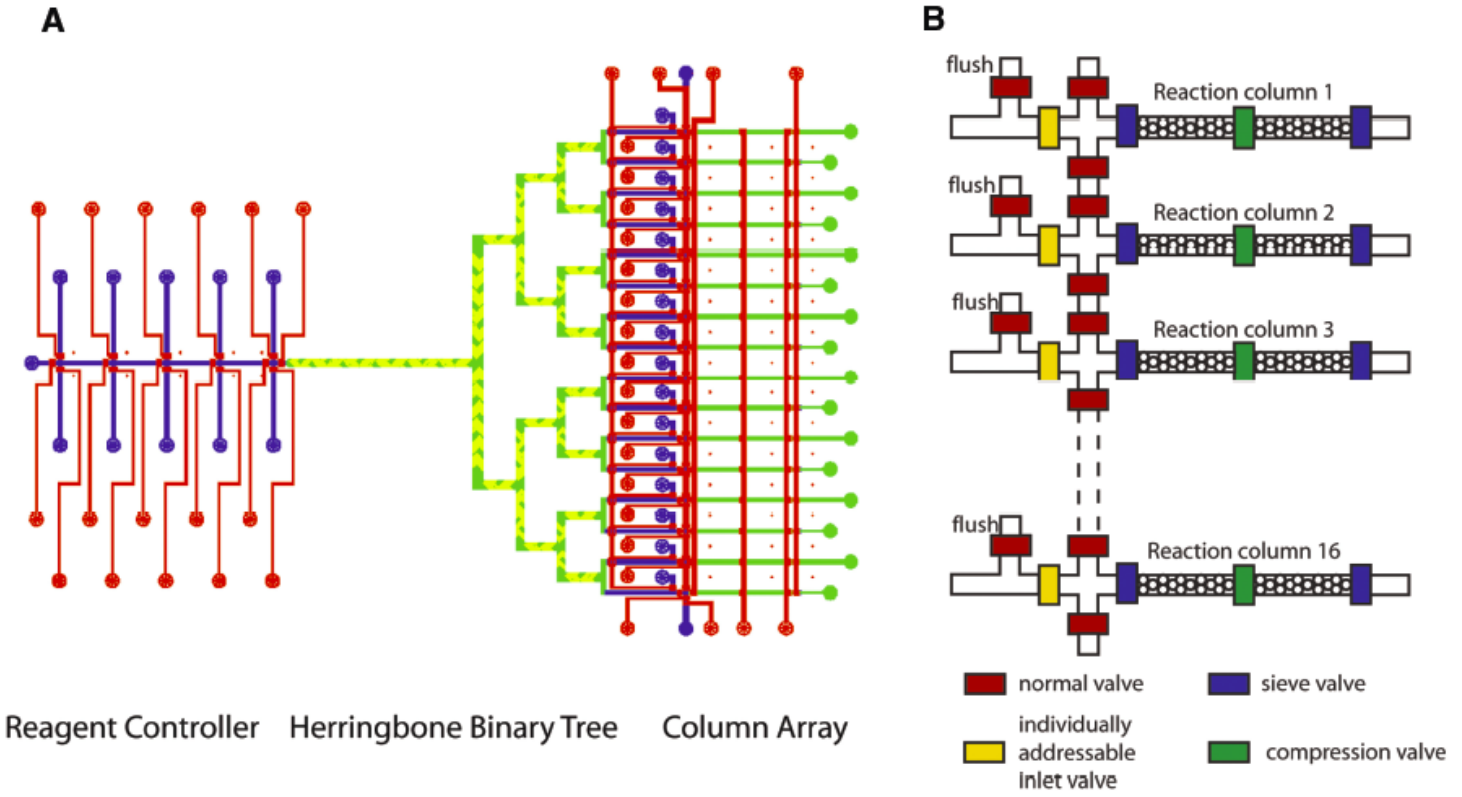
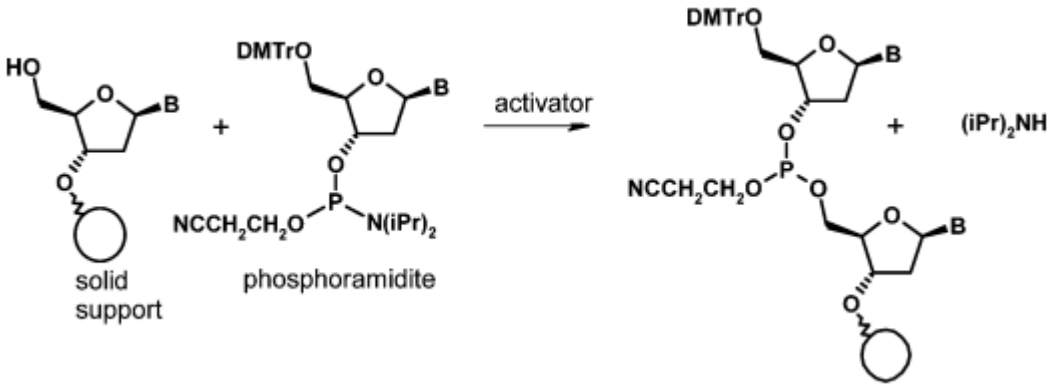
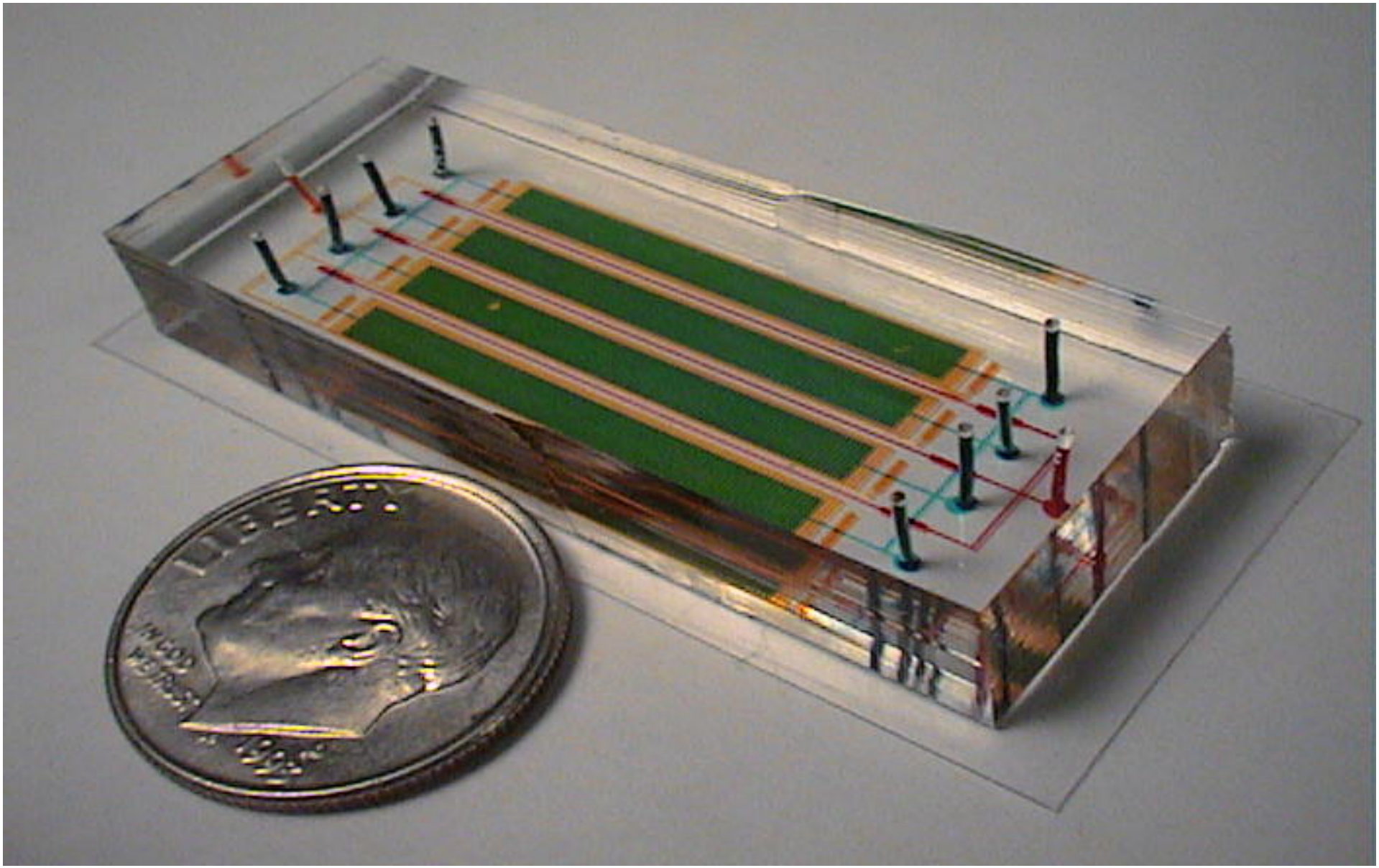


Figure 1. (A) Schematic diagram of a 16-column microfluidic DNA synthesizer.



Microfluidic Technology



Summer Goals

- Use lab space with Singularity University to replicate prior art microfluidic DNA synthesizer
- Build a working product prototype, identify points to optimize for product scale units
- Incorporate
- Build a board of advisors
- Develop business plan and pipeline
- Start the patent process
- By the end of the summer use working prototype to raise additional funding.
- Begin raising additional funding

Prototyping Costs

Reagents

dA-CE (deoxy A - beta cyanoethyl) phosphoramidite	1g	$50
dC-CE phosphoramidite	1g	$50
dG-CE phosphoramidite	1g	$50
dT-CE phosphoramidite	1g	$50
Activator - 0.45M Tetrazole in Acetonitrile	450mL	$200
Diluent - Acetonitrile, Anhydrous (Sigma)	1L	$89.7
Cap Mix A - THF/Pyridine/Ac2O	450mL	$72
Cap Mix B - 16% MeIm in THF	450mL	$96
Oxidizing Solution - 0.02M I2 in THF/Pyridine/H2O	450mL	$72
Deblocking Mix - 3% TCA/DCM	2L	$144
Glen UnySupport 500 Angstrom CPG	1g	$95
DNA synthesis reagent cost	Total	$768.7
Misc solvents, PDMS, Argon		
Mineral Oil, Enzymes, Clean-up kits		
Shipping		
	Total	<$1500

Prototyping Costs

Microfluidic Production

- Initial tests
  - Templateless PDMS structures formed using common CO_2 laser cutter (Liu, H., Gong, H., 2009)
  - Really cheap, Good enough for initial tests, but not ideal due to channel wall roughness
- Prototype quality
  - Stanford Microfluidics Foundry
    - $250-$450 per mold, $50-$75 per chip (~5X less if chips cast in-house)

Prototyping Costs

- Equipment: laser cutter (with high-density focusing optics) $16000, spin coater $1500, vacuum pump + chamber $500, Two 'gold standard' pumps (peristaltic and positive displacement) $1750, several 3-way valves $750, production-quality pump and valve prototyping parts $500, fiber-based UV/vis spectrometer + light $2500, electronics controllers $400, microcontroller $150, PCB fabrication $150, lab stirrer + hot plate $350

  Total $24550

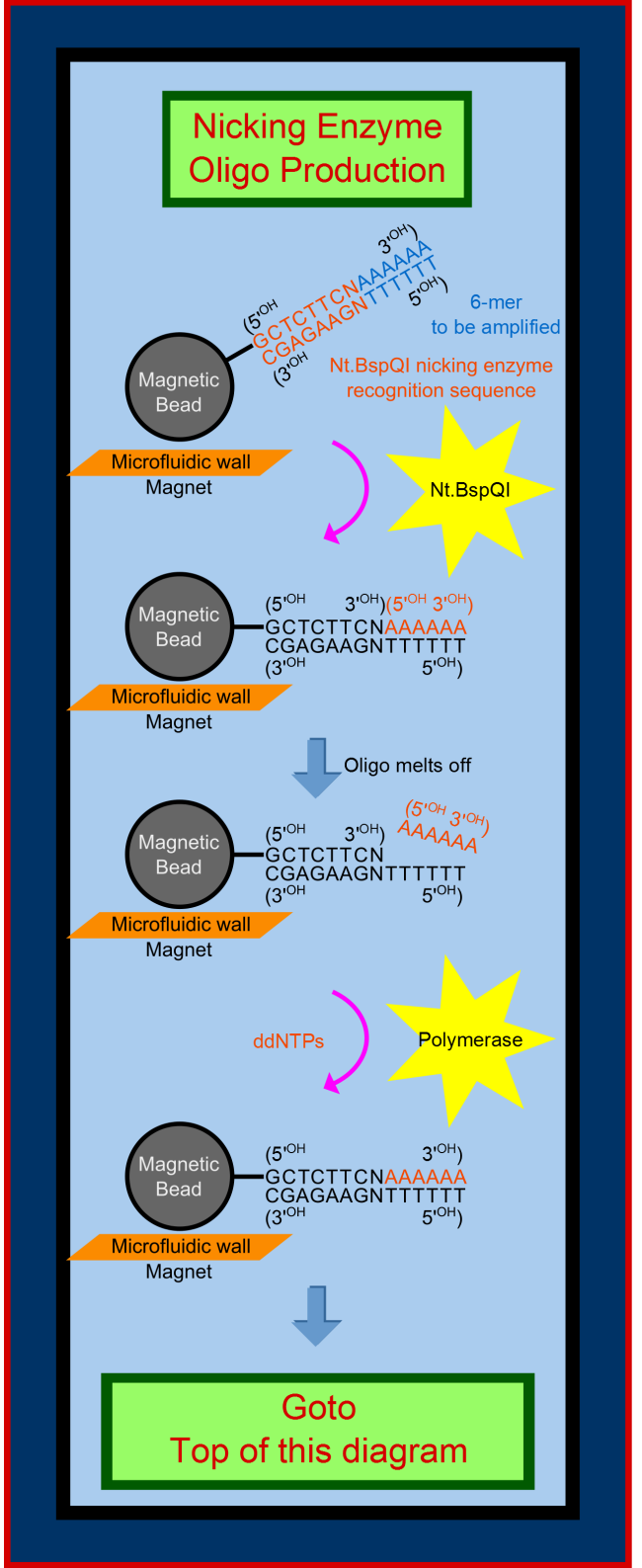
- Living expenses
  - $2000/month/person, 2 people, 4 months
    - Bryan and Nathan, $16000

Future Directions

- Even cheaper DNA chips and components (pumps, valves and reagents)
- More efficient DNA synthesis
- Other microfluidic chips and lab instrumentation
- Ligation and oligo libraries for gene and genomic synthesis
- Water-based synthesis (using enzymes)
- Holy grail: Enzymatic DNA synthesis by control of polymerase

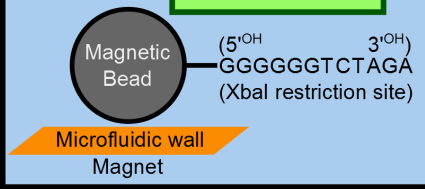
- Water-based oligo production
  - Chicken and egg problem, requires chemical synthesis first time
- No harsh chemicals, uses standard enzymes and easily obtainable ddNTPs
- Avoids water-contamination problems present in chemical synthesis
- Oligos can be ligated in series to form genes and genomes

  (Horspool, Coope, Holt, 2010)

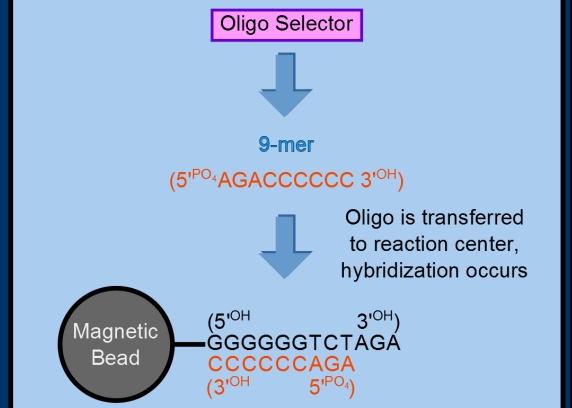


Enzymatic DNA synthesis through sequential hybridization and ligation of short oligos

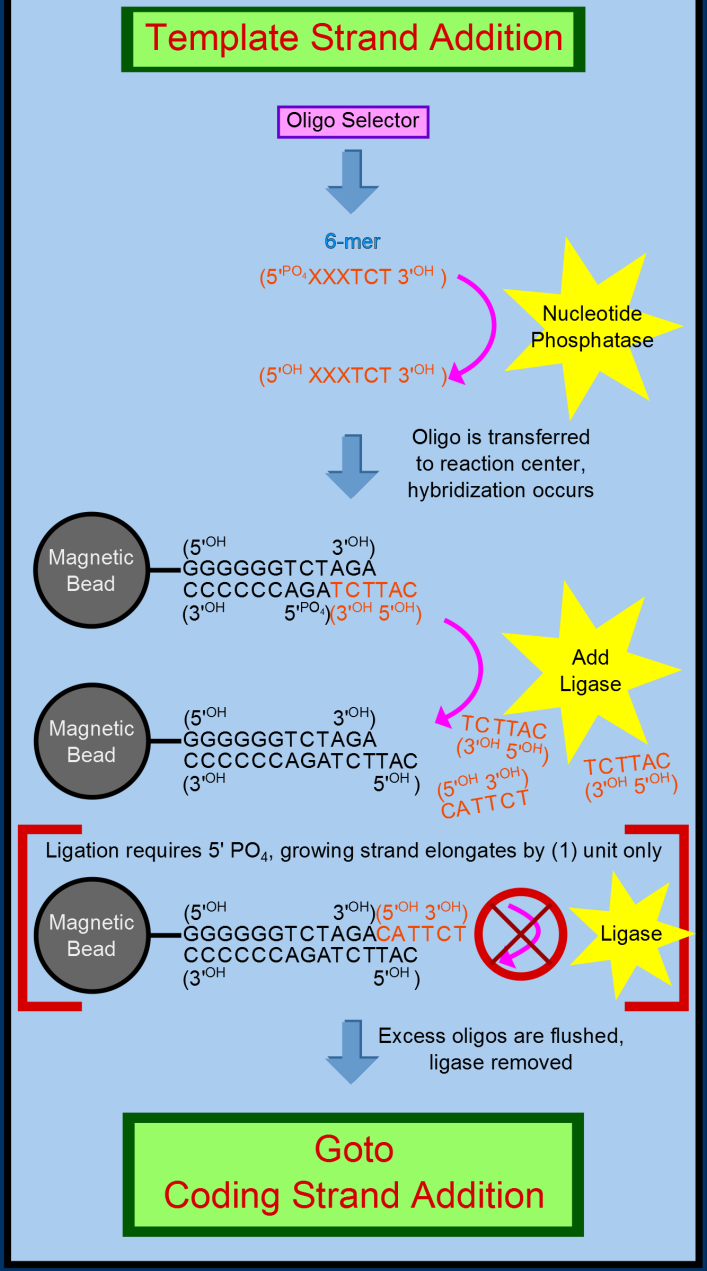
Initial Bead



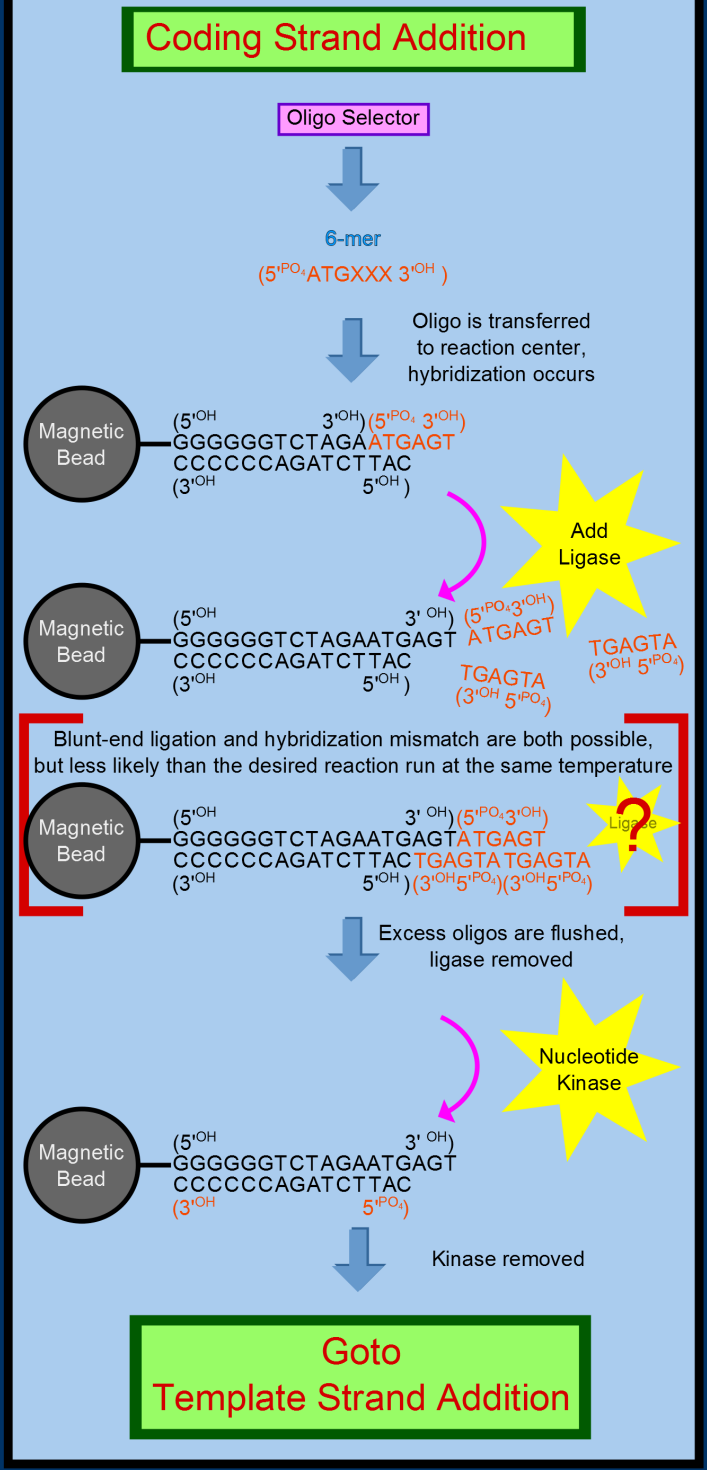
Bead Setup



Template Strand Addition



Coding Strand Addition



Nathan McCorkle
March 2012