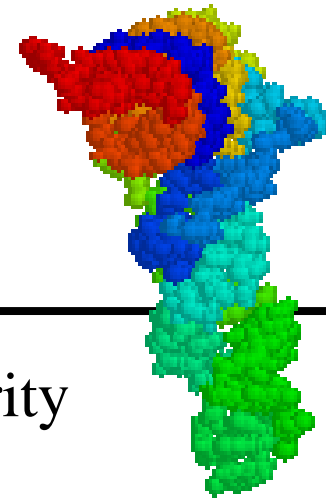


# Risks and Rewards of Synthetic Biology



National Science Advisory Board for Biosecurity  
NSABB 1-Jul-2005 morning

*Thanks to:*

Washington U, Harvard-MIT

**Broad Inst., DARPA-BioSpice, [DOE-GTL](#), EU-MolTools,  
[NGHRI-CEGS](#), NHLBI-PGA, NIGMS-SysBio, PhRMA,  
Lipper Foundation**

[Agencourt](#), Ambergen, [Atactic](#), BeyondGenomics, Caliper,  
Genomatica, Genovoxx, [Helicos](#), MJR, NEN, [Nimblegen](#),  
[SynBioCorp](#), ThermoFinnigan, Xeotron/Invitrogen

**For more info see: [arep.med.harvard.edu](http://arep.med.harvard.edu)**

# Defensive options

- Inexpensive monitoring -- **bio-weather-map** (air-born & medical fluids).
- International bio-supply-chain **licensing** (min research impact, max surveillance)
- Multi-epitope vaccines & drugs.
- Cells resistant to most existing viruses via codon changes

For more info see: [arep.med.harvard.edu](http://arep.med.harvard.edu)

# Risks & security

**1956:** "Anthrax 836 .. after another accident..disinfected the sewer but ..one of the rodents captured in the Kirov sewers.. more virulent than the original. The army immediately ordered him to cultivate the new strains." --Ken Alibek in "Biohazard"

**1995:** The cult Aum Shinrikyo, aerosolize anthrax & botulinum in Tokyo on 8 occasions.

**2000:** Computer Viruses and Hacking Take \$1.6 Trillion Toll on Worldwide Economy <http://seclists.org/lists/isn/2000/Jul/0070.html>

**2001:** US Anthrax attacks

**2001:** Immunized genetically resistant mice are remarkably susceptible to expressing IL-4 mousepox." Jackson et al. J Virol. 75:1205.

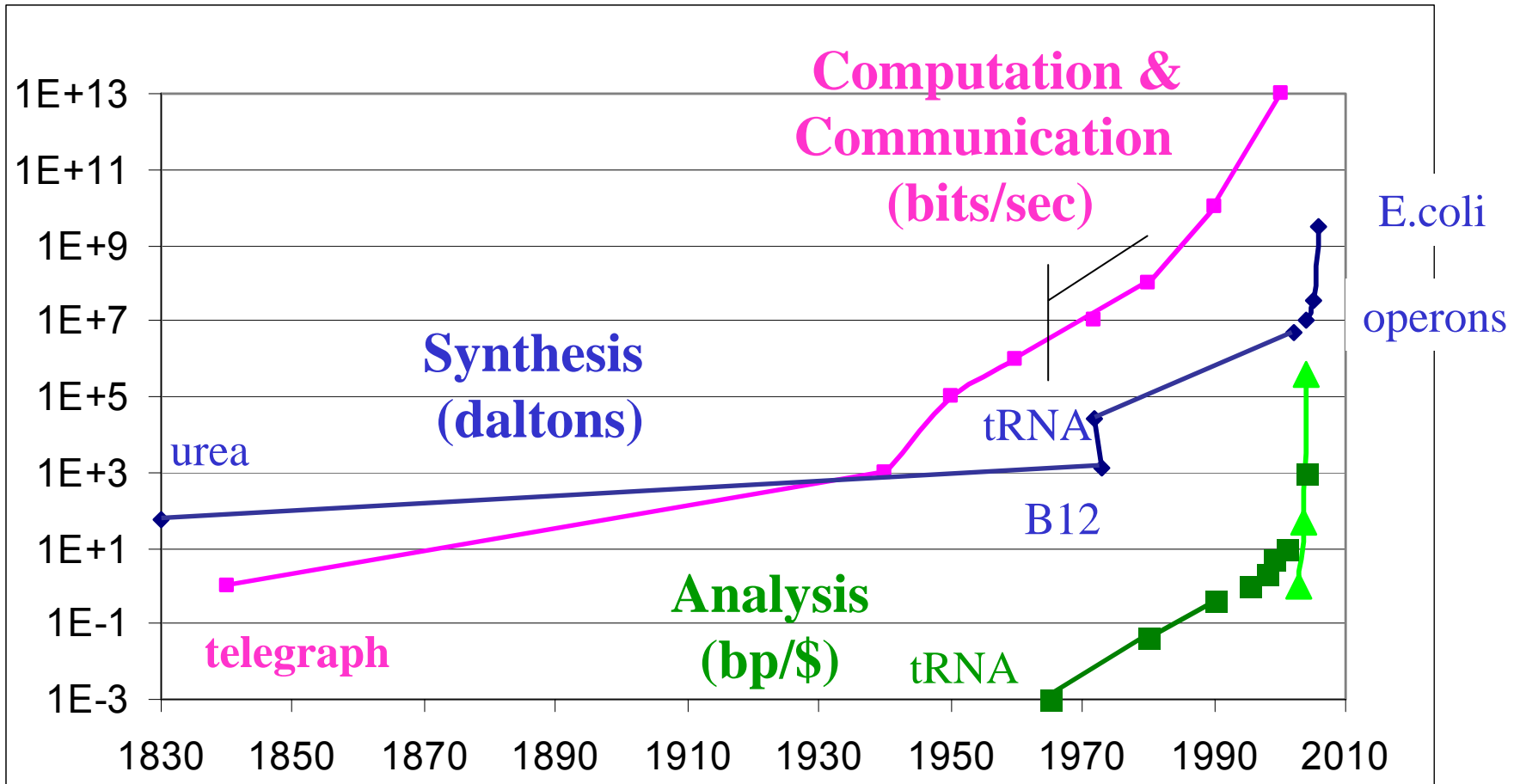
# Vaccines

**Are adverse reactions avoidable?**

**What limits the number of antigens/year?**

- Flu: egg allergy
- Denque: 2nd strain hemorrhagic fever.
- Sabin Polio: reversion
- Preservatives like thimerosal
- Genetic predisposition

# 3 Exponential technologies (synergistic)



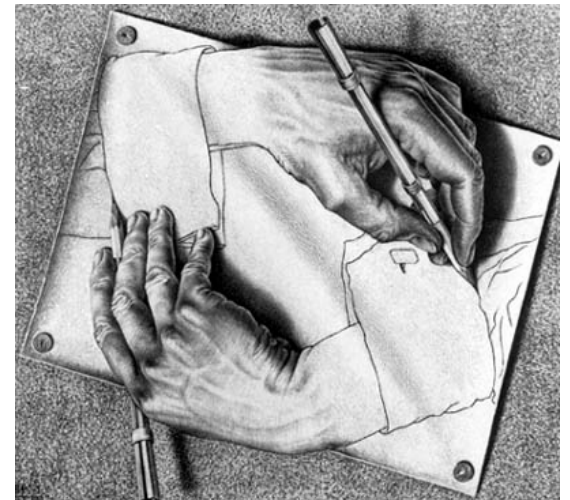
Shendure J, Mitra R, Varma C, Church GM, 2004 Nature Reviews of Genetics.  
Carlson 2003 ; Kurzweil 2002; Moore 1965

# Safer Synthetic Biology

Church, G.M. (2004) A synthetic biohazard non-proliferation proposal.

<http://arep.med.harvard.edu/SBP>

- Monitor oligo synthesis via expansion of Controlled substances, Select Agents, Recombinant DNA
- Computational tools are available; small number of reagent, instrument & synthetic DNA suppliers at present.
- System modeling checks for synthetic biology projects
- Metabolic dependencies & novel genetic codes prevent functional transfer of DNA to other cells.



# Encourage responsibility in the entire bio-supply-chain via licensing

1. Chemicals: Phosphoramidites
2. Instruments
3. Synthetic oligonucleotides
4. Synthetic genes/genomes
5. Design & ordering software (check for select agents)
6. Educational goals (defense not offense)
7. Intellectual property & know-how
8. Engineering societies & code of ethics
9. Network of trainees (no trainee left behind)

# Constructing new genetic codes (two examples)

1. Codons: UAG stop > UAA stop
2. Delete RF1
3. Codons: AGY Ser > UCX Ser
4. tRNAs: AGY Ser > AGY Leu
5. Codons: UUR/CUX Leu > AGY Ser
6. tRNAs: UUR Leu > UUR Ser
7. Codons: UCX Ser > UUR Ser  
(Leu & Ser now switched)



# Constructing new genetic codes

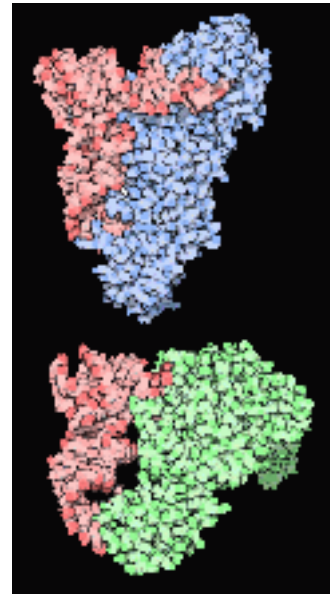
Forster & Church

		2nd base						
		U	C	A	G			
5' base	U	Phe GAA <sub>ms2i6A</sub>	Ser GGAA	Tyr GUA <sub>ms2i6A</sub>	Cys GCA <sub>ms2i6A</sub>	CUA	3' base	
		Leu * cmnm5UAA <sub>ms2i6A</sub>	Ser ms2i6A VGA	RF1	Trp CCA <sub>ms2i6A</sub>			UAG
		Leu GAG <sub>m1G</sub>	Pro GGG <sub>m1G</sub>	His GUGA	Arg ICGA			CUA
	C	* Leu UAGG	Pro VGG <sub>m1G</sub>	Gln cmnm5s2UUGA	Arg CCG <sub>m1G</sub>	UAG		
		Ile GAU <sub>t6A</sub>	Thr GGU <sub>t6A</sub>	Asn GUU <sub>t6A</sub>	Ser GCU <sub>t6A</sub>	CUA		
		* Ile k2CAU <sub>t6A</sub>	Thr VGU <sub>t6A</sub>	Lys SUU <sub>t6A</sub>	Arg * mnm5UCU <sub>t6A</sub>	UAG		
	A	fMet CAUA	Met CAU <sub>t6A</sub>					
		Val GACA	Ala GGCA	Asp GUCA	Gly GCCA	CUA		
		* Val VACA	Ala VGCA	Glu SUCA	Gly U*CCA	UAG		

*M. luteus*  
missing 6  
codons:  
UUA(L),  
CUA(L),  
AUA(I),  
GUA(V),  
CAA(Q),  
AGA(R).

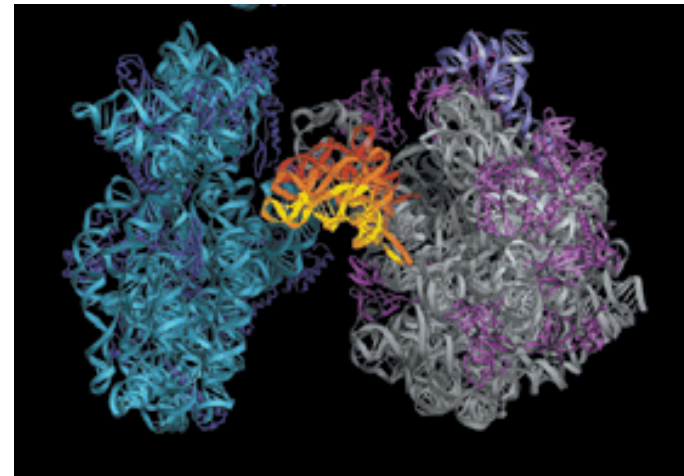
# Why Synthetic Genomes & Proteomes?

- Test or engineer cis-DNA/RNA-elements
- Optimal biosynthesis e.g. artemisinin (malaria)
- Epitopes & vaccines.
- Unnatural aa & post-translational modifications
- *De novo* protein design & selection.
- Humanizing aa, colizing codon usage
- 20 bit *in vivo* counters



## • Why whole genomes?

Changing the genetic code,  
safety, genome stability.



# Up to 760K Oligos/Chip

18 Mbp for \$1K raw (6-18K genes)

~5000 lower oligo costs

<1K Oxamer Electrolytic acid/base

8K Atactic/Xeotron/Invitrogen

Photo-Generated Acid

Sheng, Zhou, Gulari, Gao (U.Houston)

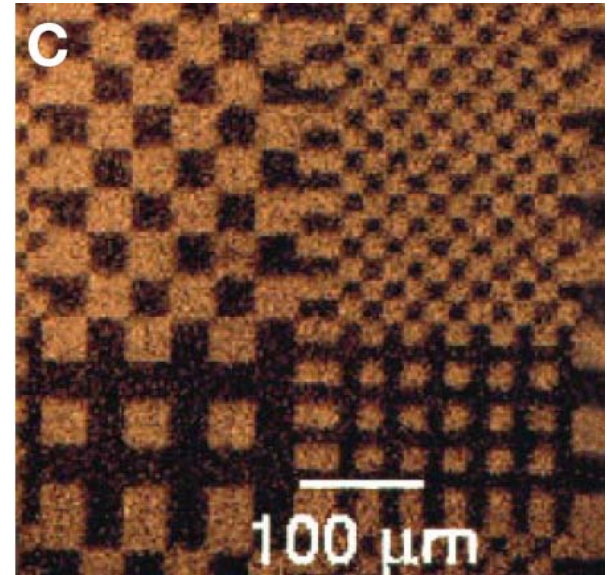
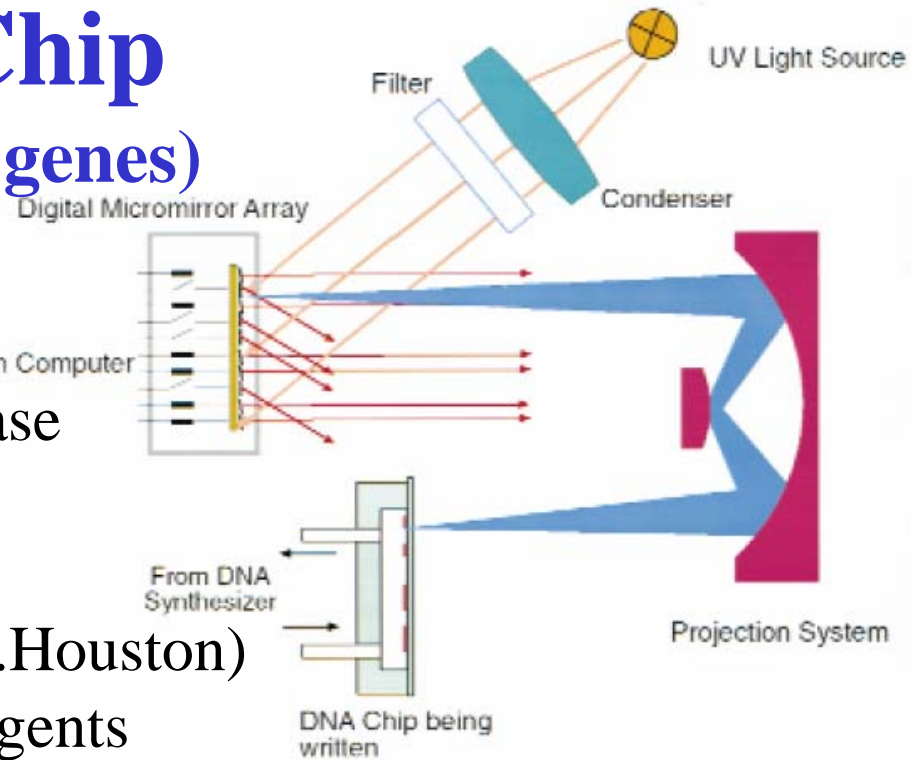
24K Agilent Ink-jet standard reagents

48K Febit

100K Metrigen

380K Nimblegen Photolabile 5'protection

Nuwaysir, Smith, Albert



Tian, Gong, Church

# Improving DNA synthesis accuracy

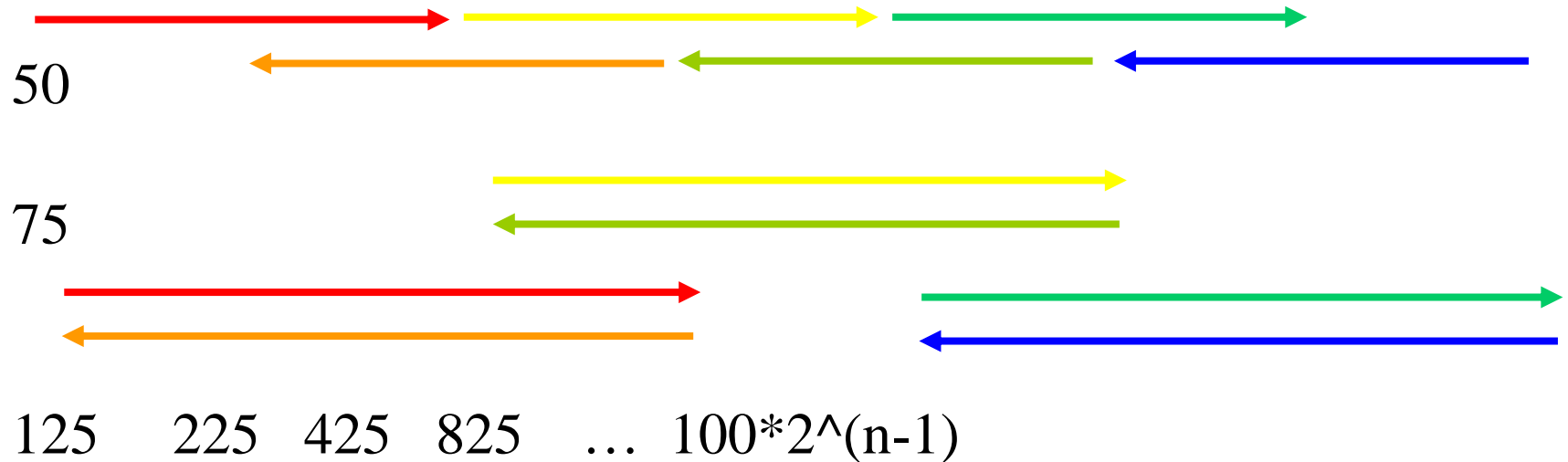
Method	Bp/error
Chip assembly only	160
Hybridization-selection	1,400
MutS-gel-shift	10,000
PCR 35 cycles	10,000
MutHLS cleavage	100,000
<i>In vivo</i> replication	1,000,000,000

Tian & Church 2004 Nature

Carr & Jacobson 2004 NAR

Smith & Modrich 1997 PNAS

# CAD-PAM: Computer aided Design - Polymerase Assembly Multiplexing

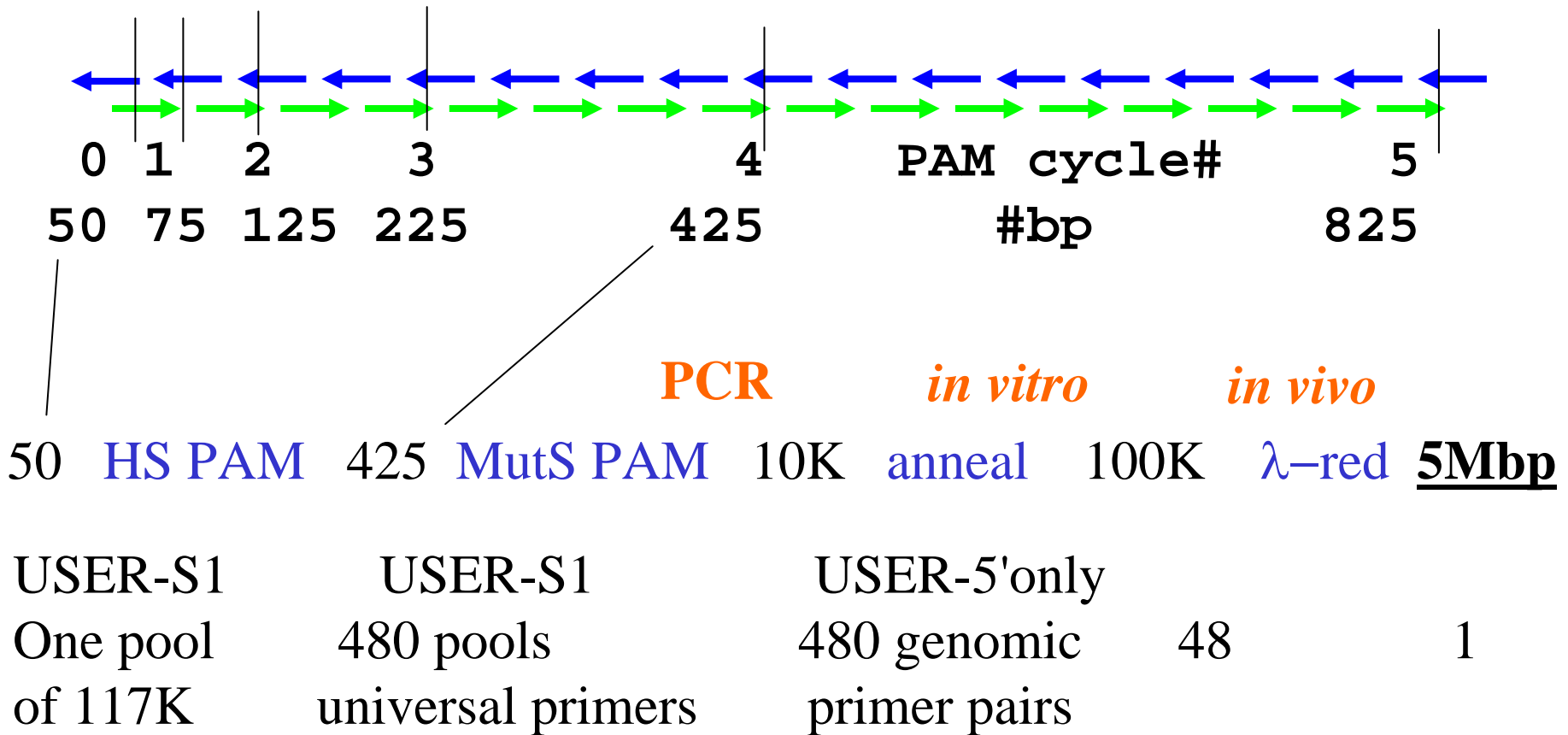


**For tandem, inverted and dispersed repeats:**

Focus on 3' ends, hierarchical assembly,  
size-selection and scaffolding.

Mullis 1986 CSHSQB, Dillon 1990 BioTech, Stemmer 1995 Gene  
Tian et al. 2004 Nature, Kodumal et 2004 PNAS

# Genome assembly

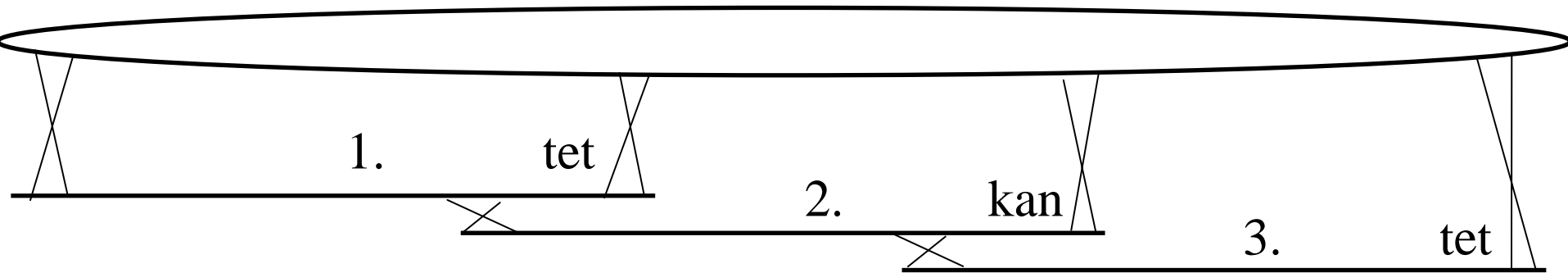


HS=Hybridization-Selection

USER=Uracil DNA glycosylase & EndoVIII  
to remove flanking primer pairs

Isaacs, Carr, Emig,  
Gong, Tian,  
Jacobson, Church

# Genome assembly alternatives

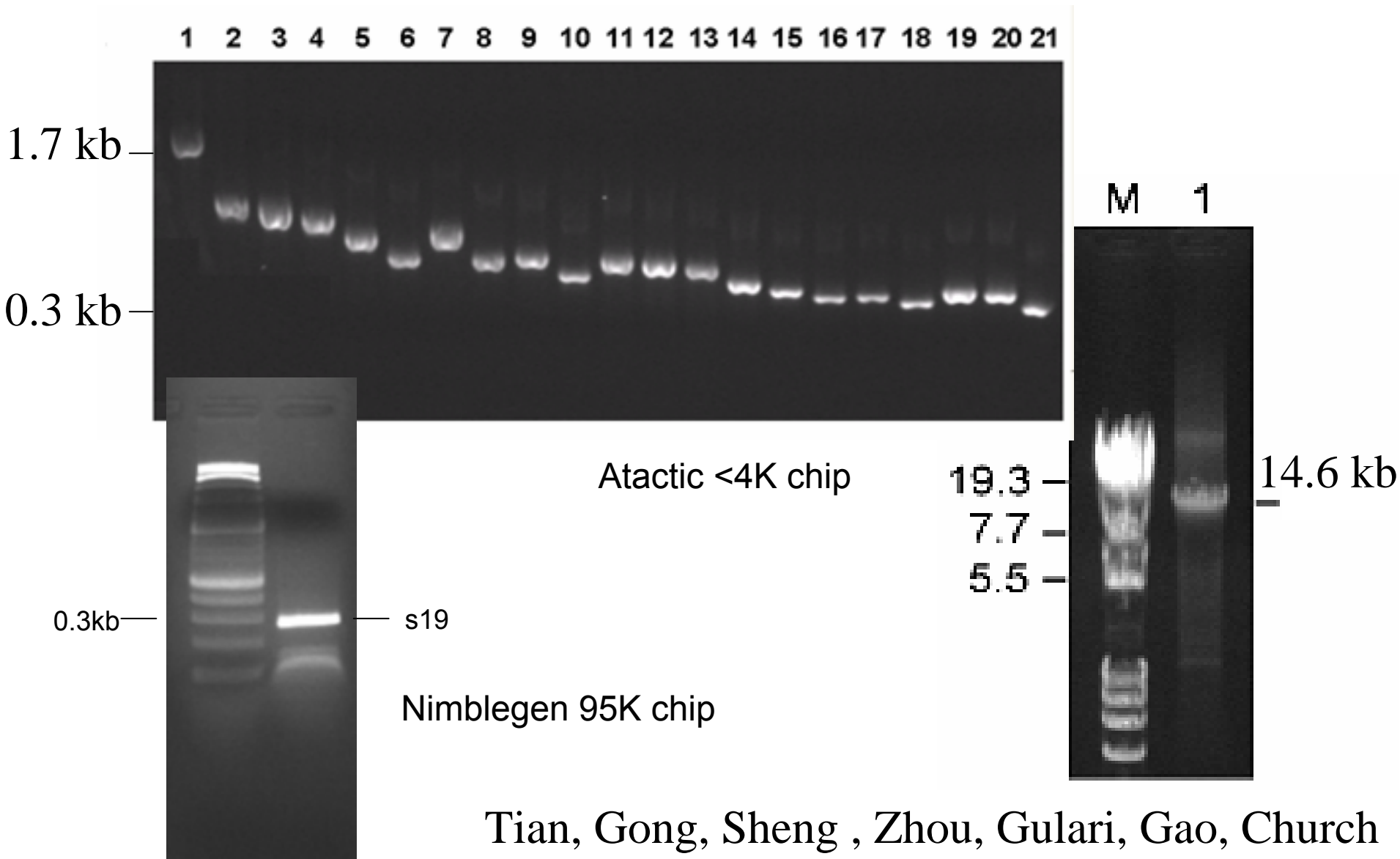


Serial electroporation: **48 stages**: 1 strain (1.6 days/stage)

vs.

Conjugation: **7 stages**:  $48 > 24 > 12 > 6 > 3 > 2 > 1$  strains

# All 30S-Ribosomal-protein DNAs (codon re-optimized)



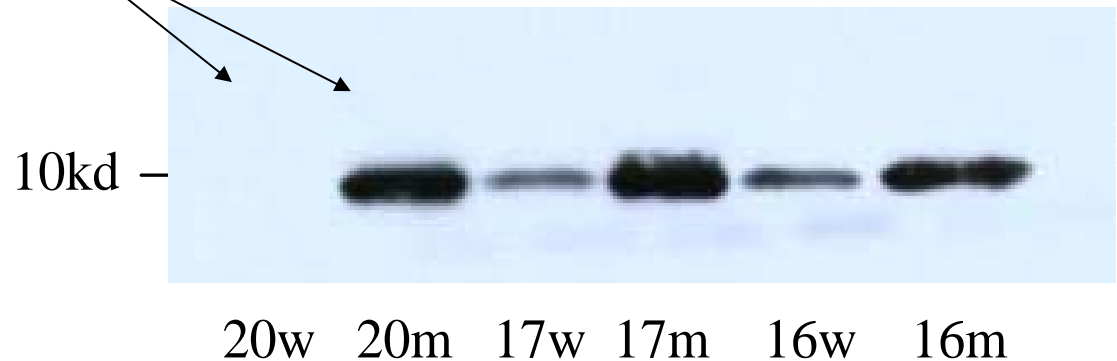


# Extreme mRNA makeover for protein expression *in vitro*

RS-2,4,5,6,9,10,12,13,15,16,17,and 21 detectable initially.

RS-1, 3, 7, 8, 11, 14, 18, 19, **20** initially weak or undetectable.

**Solution:**  
Iteratively resynthesize  
all mRNAs with less  
mRNA structure.



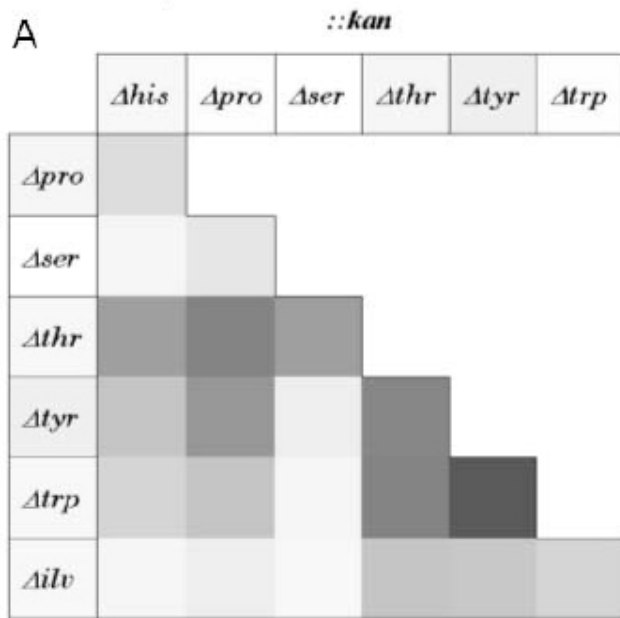
Western blot based on His-tags

W: wild-type

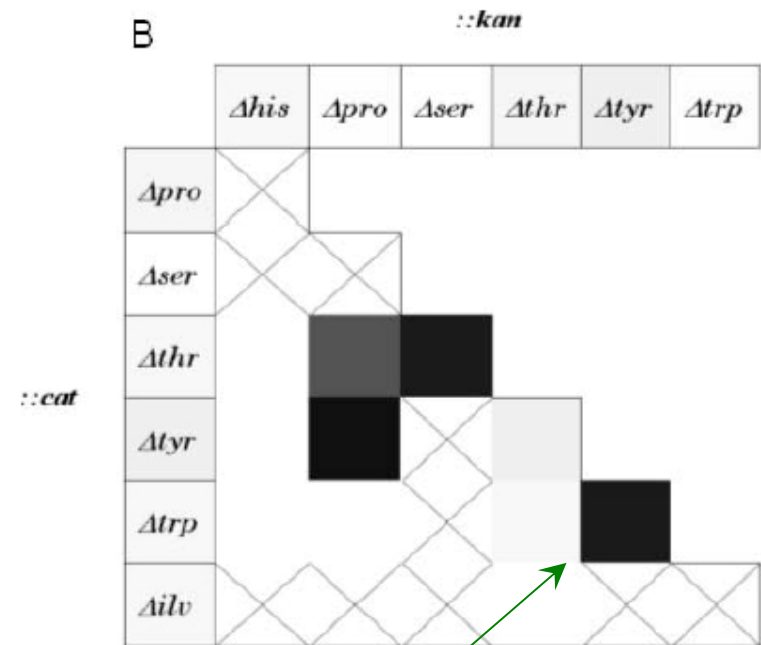
M: modified

# Genome engineering & evolution: Cross-feeding Metabolic Mutants

First Passage



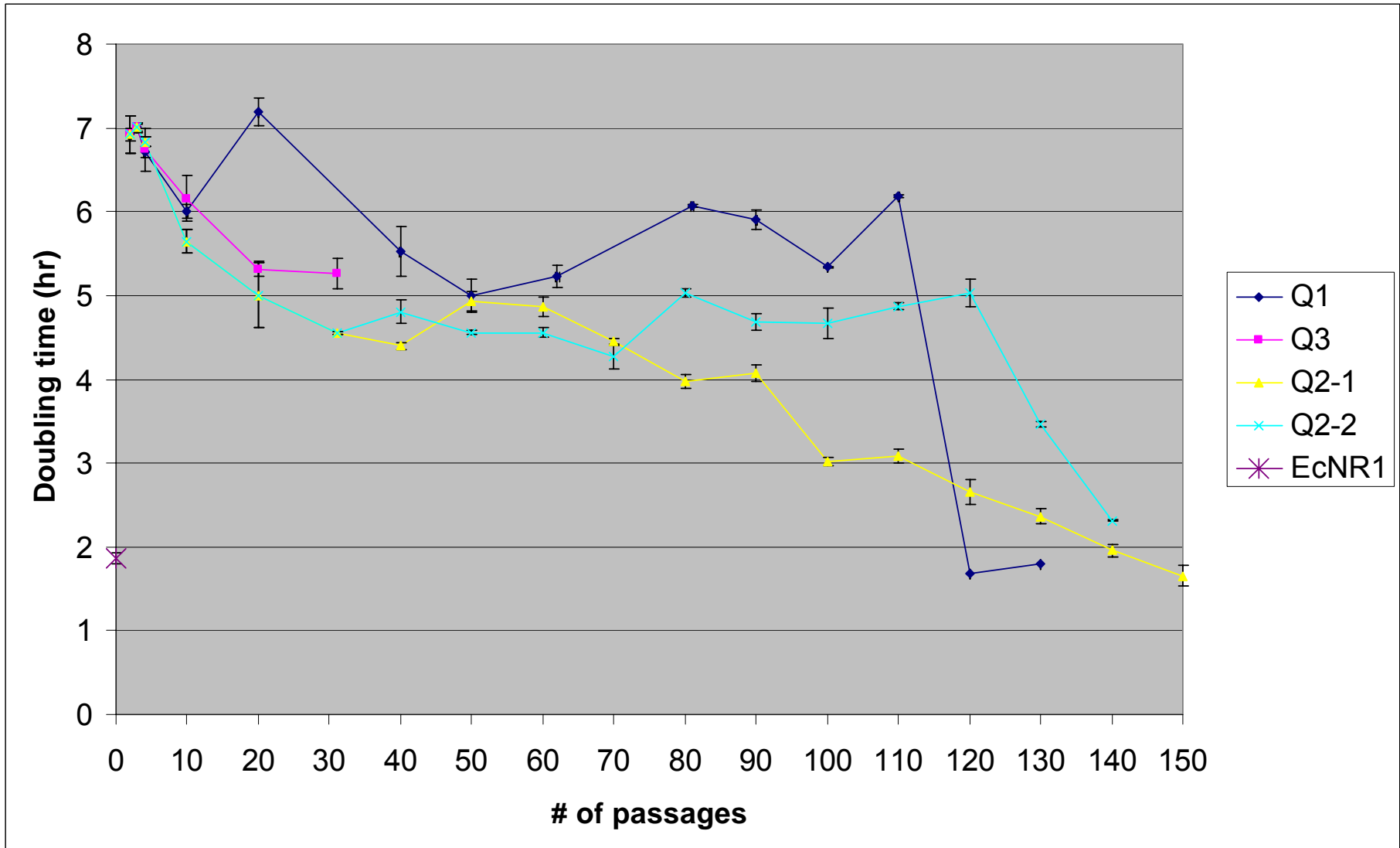
Second Passage



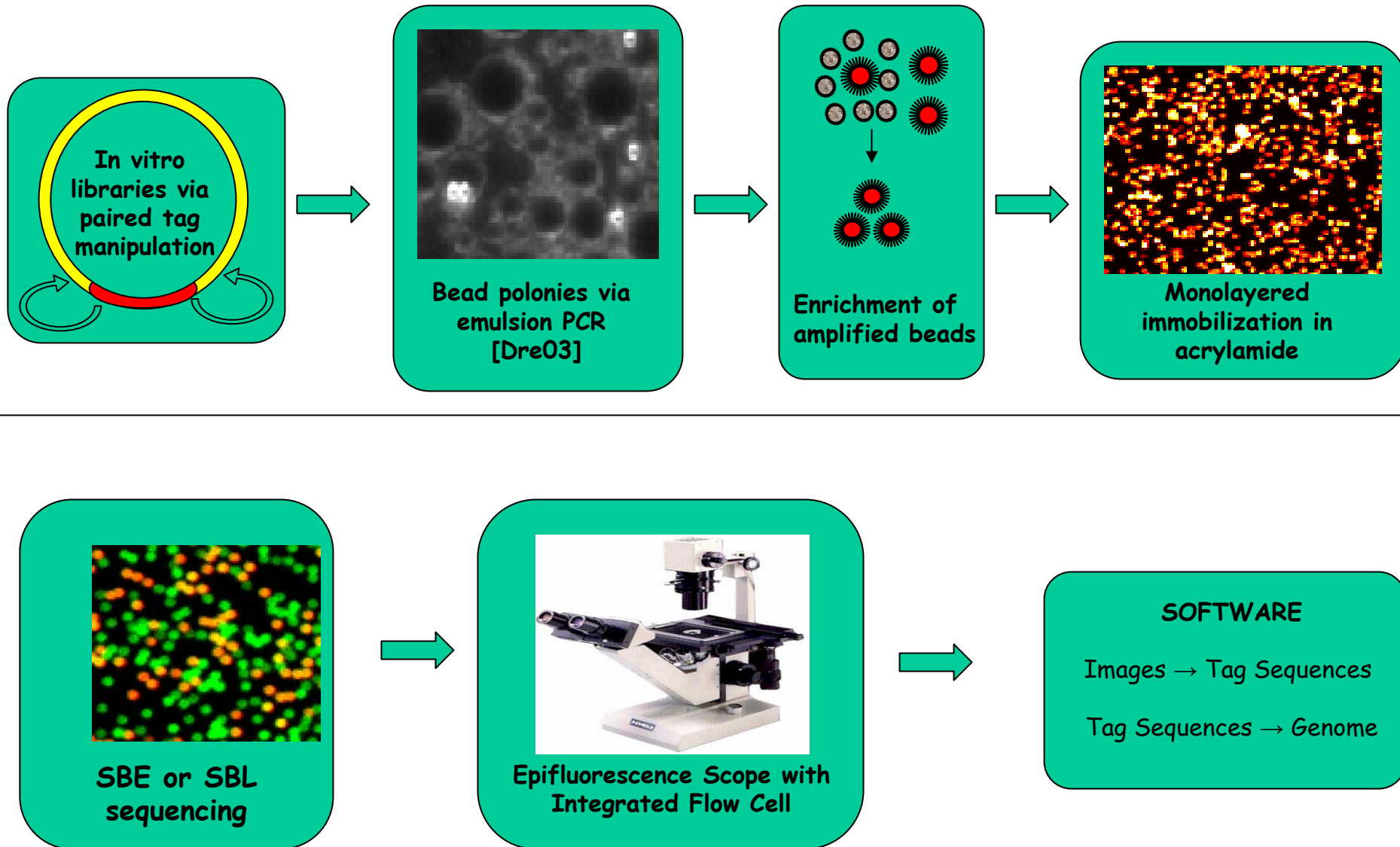
**$\Delta trp/\Delta tyrA$  pair of genomes shows the best co-growth**

Reppas, Lin & Church unpublished.

# Co-evolution of syntrophic Trp-/Tyr- genome pair



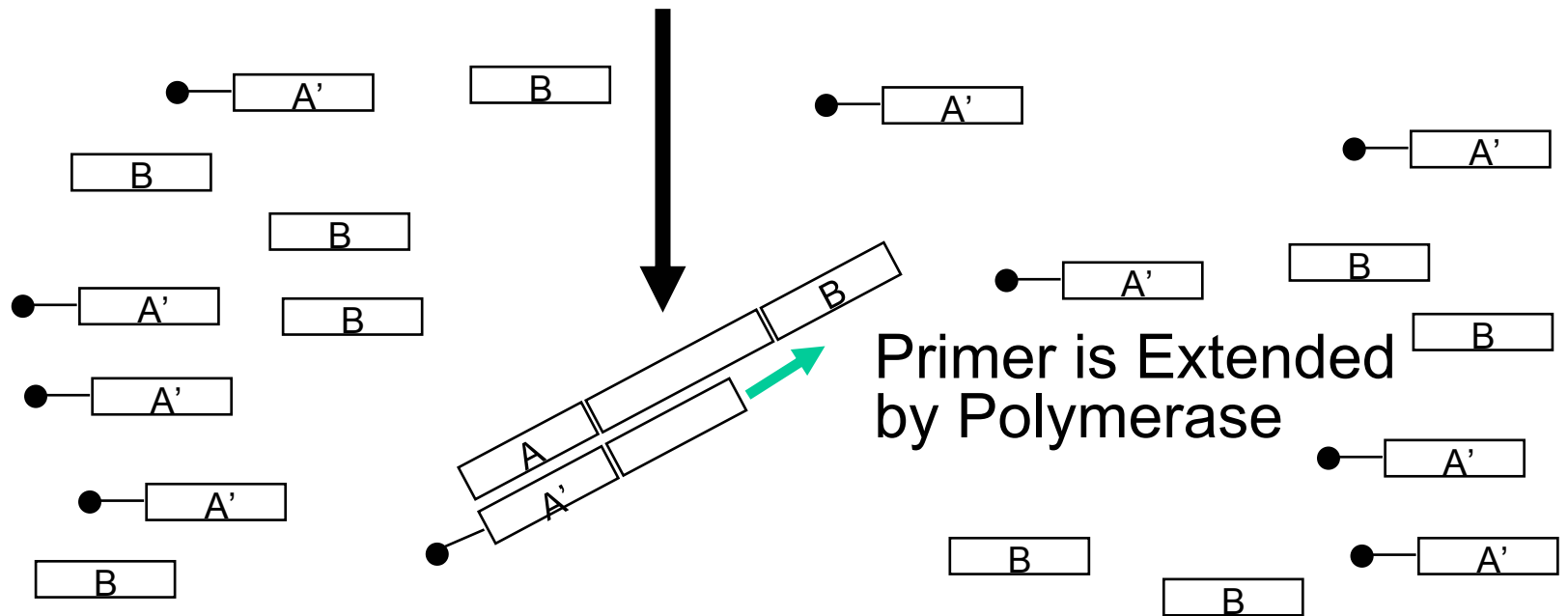
# Plone Bead Sequencing Pipeline



Mitra, Shendure, Porreca, Rosenbaum, Church unpub.

# Polymerase colony (colony) PCR in a gel

Single molecule from a library or population



Primer A has 5' immobilizing Acrydite

# Plone-bead FISSeq SBE SBL

Consider amplification , homopolymer, context errors?

• # of bases sequenced (total Mbp)	<b>23 (no)</b>	<b>10.8 (<u>yes</u>)</b>
• # bases sequenced (unique)	<b>73 b</b>	<b><u>4.7 Mb</u> (72%)</b>
• Average fold coverage	<b>324,000</b>	<b>2.3</b>
• Pixels used per bead (analysis)	<b>3.6</b>	<b>3.6</b>
• Read Length (bp)	<b>14</b>	<b>24</b>
• Indels	<b>0.6%</b>	<b>?</b>
• Substitutions (raw error-rate)	<b>4e-5</b>	<b>1e-2</b>
• Throughput (kb/min)	<b>360</b>	<b>10</b>
• Speed/cost ratio relative to current ABI capillary sequencing @ <b><u>0.75</u> kb/min/device</b>	<b>1100</b>	<b>32</b>

# Plone **analysis** of **synthetic** evolved *E.coli* Tyr- / Trp- pair

374,449	G>A	MhpT	5'UTR	3-hydroxyphenylpropionic acid transport
986,327	T>G	OmpF	Promoter-10	Outer membrane porin
1,990,682	G>A	PgsA	Arg>Cys	Essential phospholipid synthesis protein
3,599,626	G>C	FtsX	Leu>Val	Essential cell division protein
3,957,957	C>T	PpiC	5'UTR	Peptidyl-prolyl cis-trans isomerase (stress)
4,002,444	G>C	YigI	Arg->Gly	Unknown conserved protein

Reppas, Shendure, Porreca

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