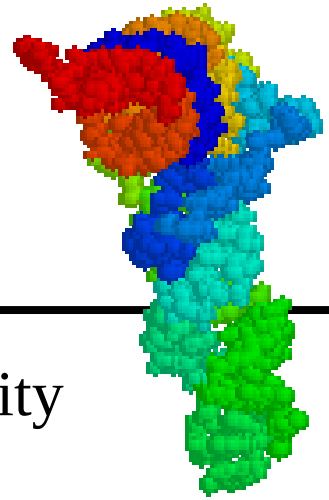
Risks and Rewards of Synthetic Biology



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

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

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.

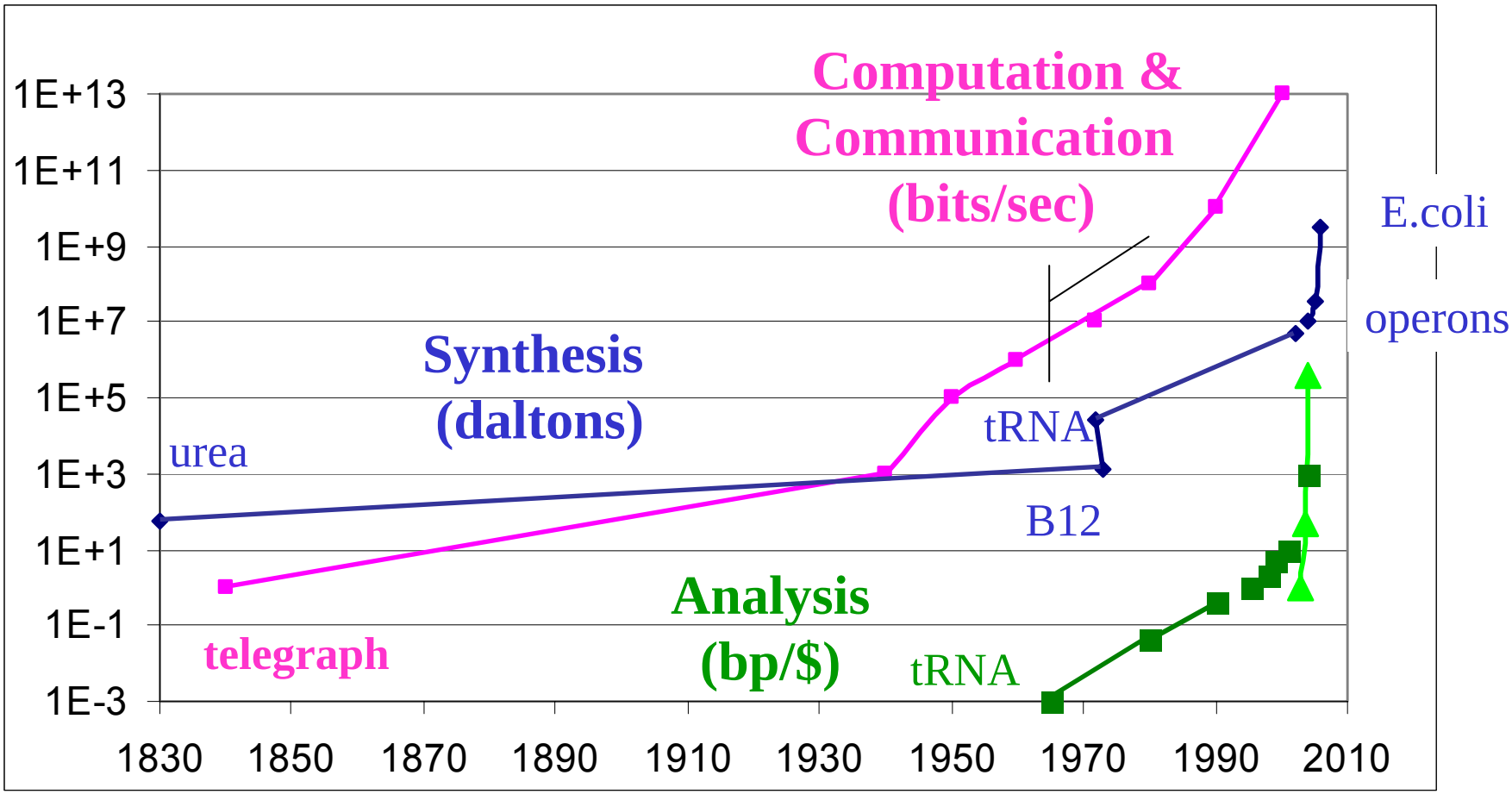
Vaccines

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.

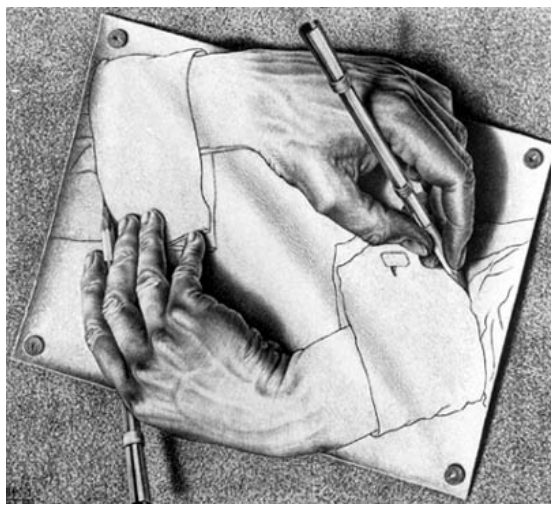
*http://arep.med.harvard.edu/**SBP***

• 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)

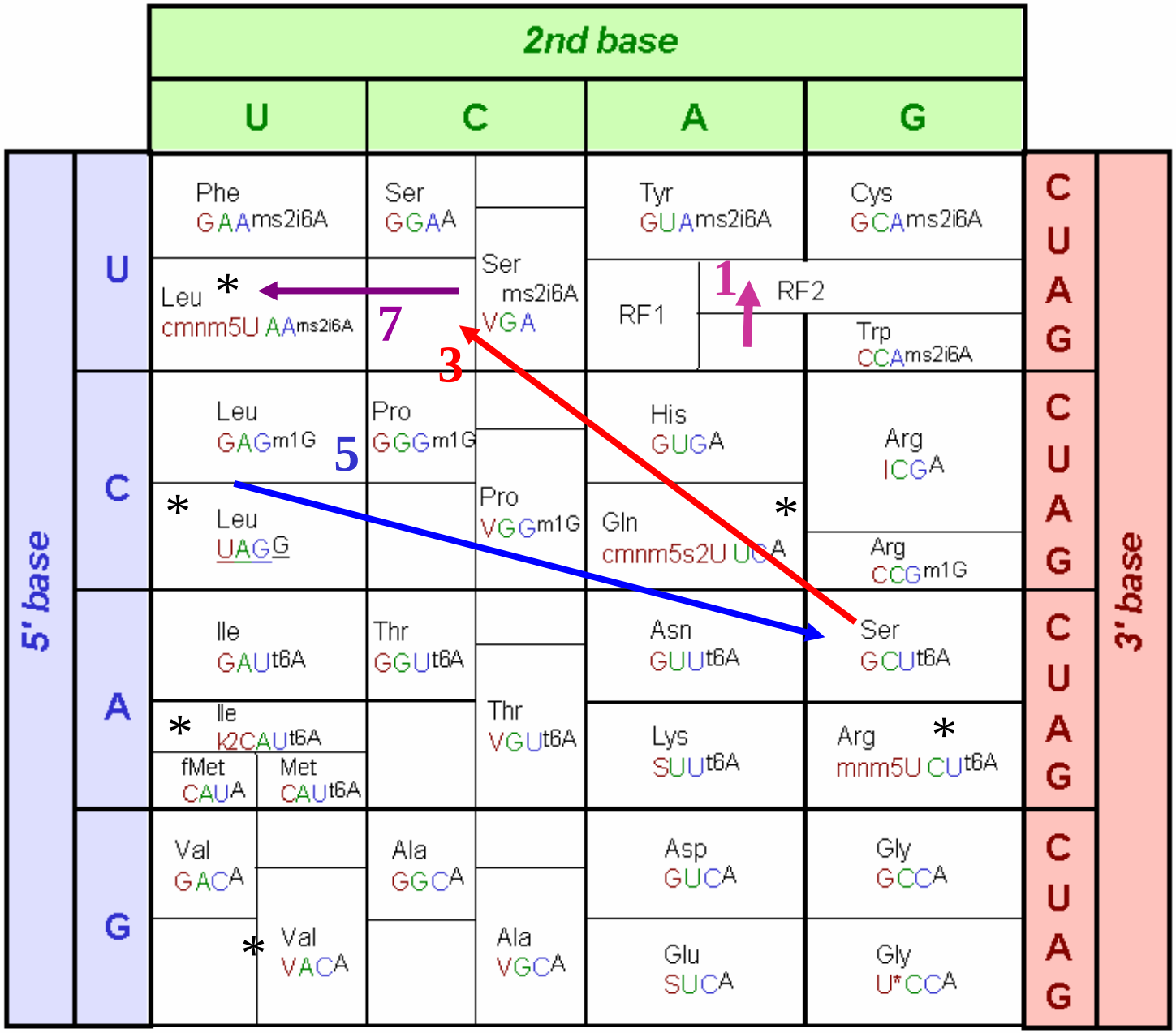
Constructing new genetic codes
(two examples)

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

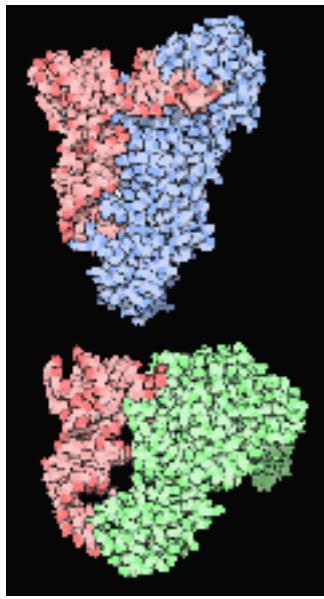
Forster & Church

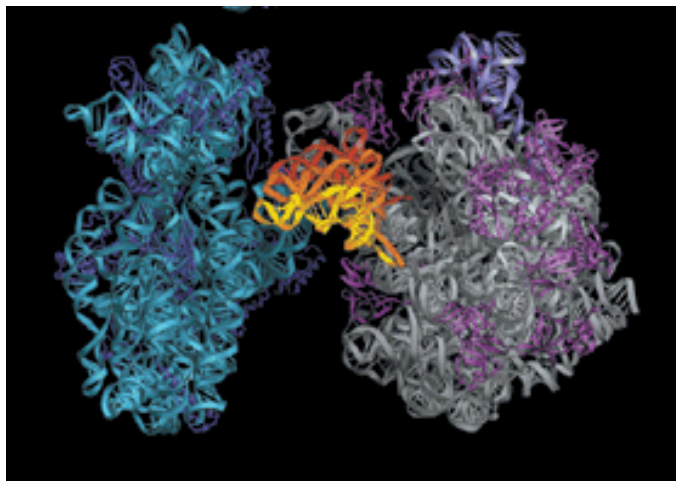


M. luteus missing 6 codons: UUA(L), CUA(L), AUA(I), GUA(V), CAA(Q), AGA(R).

Why Synthetic Genomes & Proteomes?

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





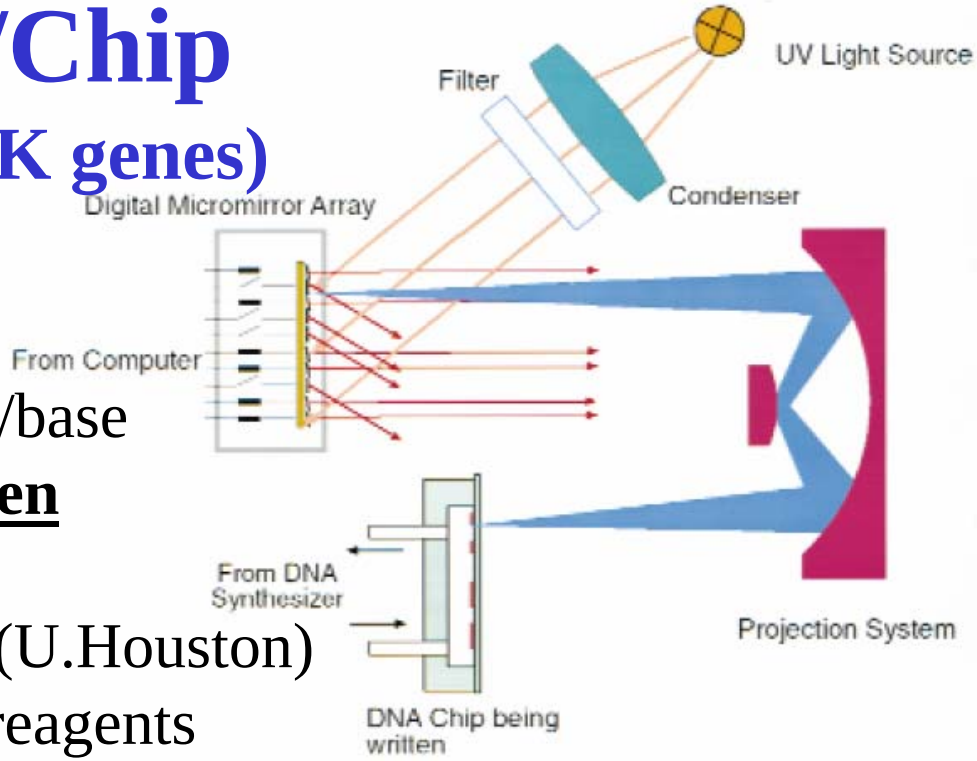
•Why whole genomes?

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

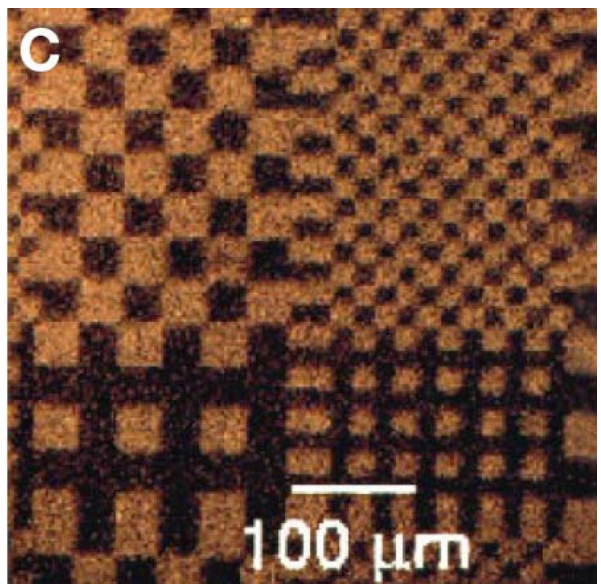
8K <u>Atactic/Xeotron/Invitrogen</u>
Photo-Generated Acid
Sheng , Zhou, Gulari, Gao (U.Houston)

24K Agilent Ink-jet standard reagents

48K Febit

100K Metrigen

380K <u>Nimblegen</u> Photolabile 5'protection
Nuwaysir, Smith, Albert



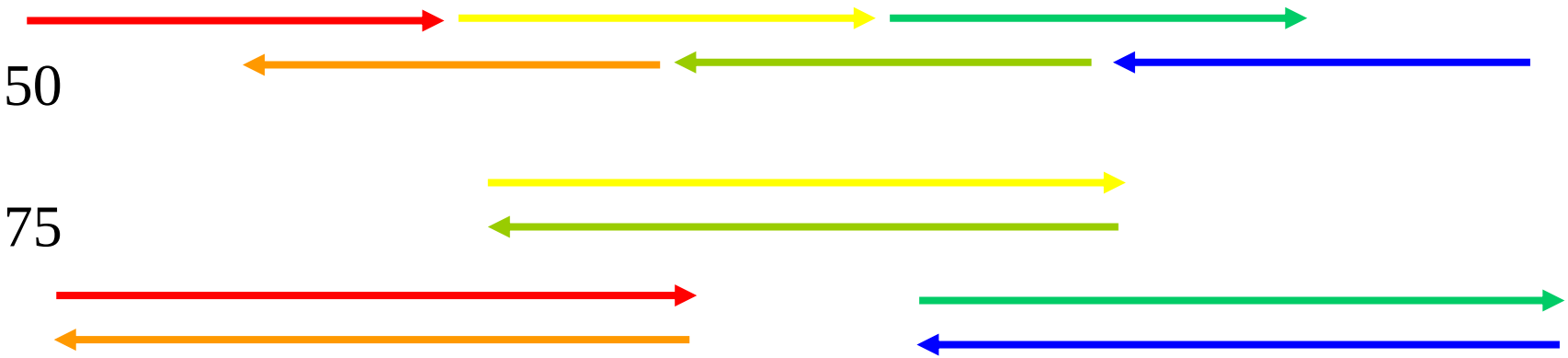
Tian, Gong, Church

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**
***In vivo* 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



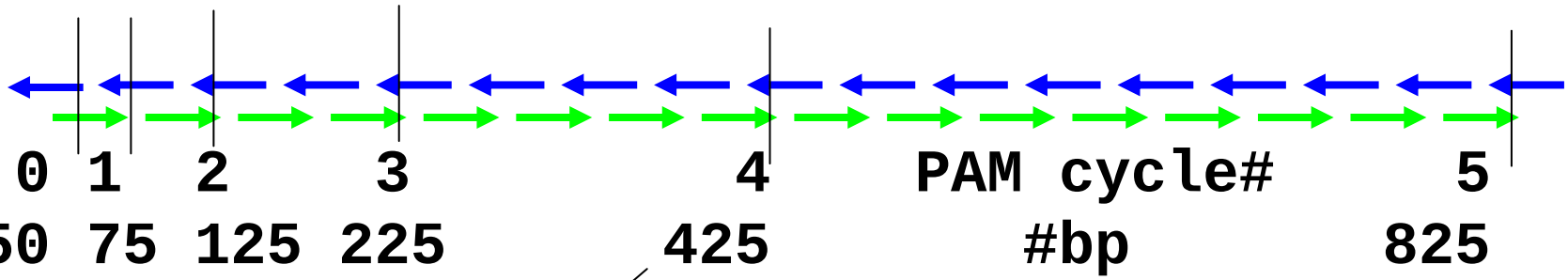
125 225 425 825 … 100*2^(n-1)

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



PCR *in vitro* *in vivo*

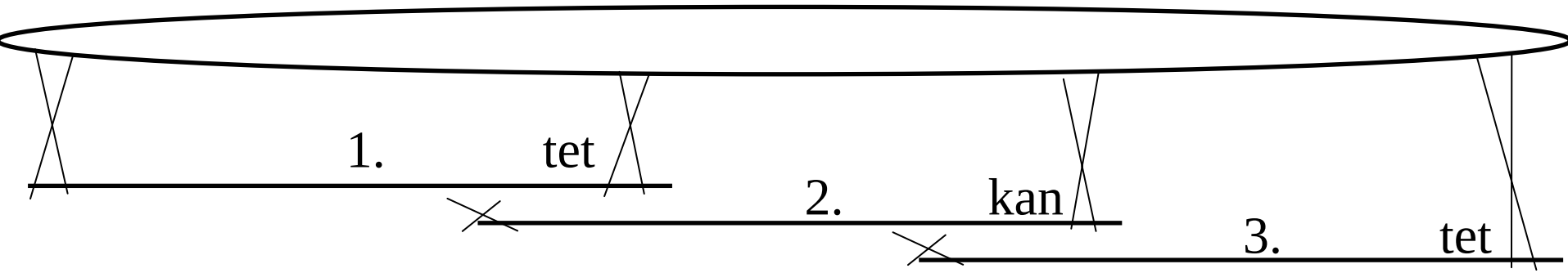
50 HS PAM 425 MutS PAM 10K anneal 100K λ–red **5Mbp**

USER-S1 One pool of 117K	USER-S1 480 pools universal primers	USER-5'only 480 genomic primer pairs	48	1

HS=Hybridization-Selection
USER=Uracil DNA glycosylase &EndoVIII to remove flanking primer pairs

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

Genome assembly alternatives



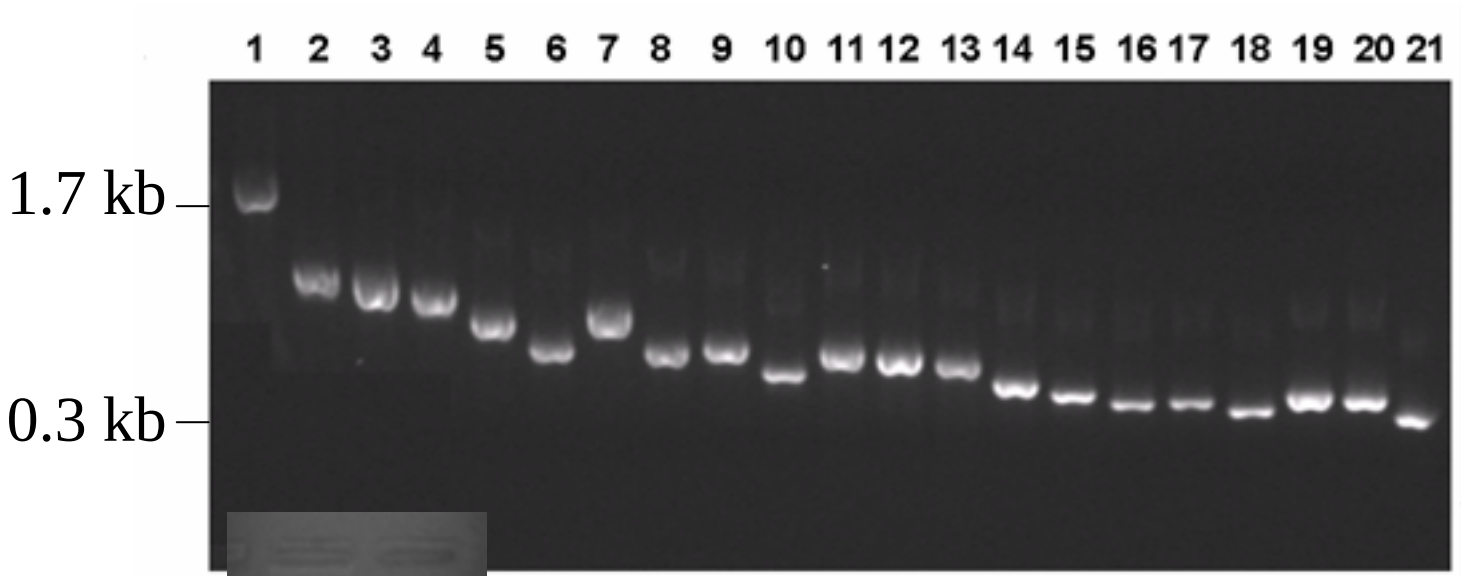
Serial electroporation: **48 stages**: 1 strain (1.6 days/stage)

vs.

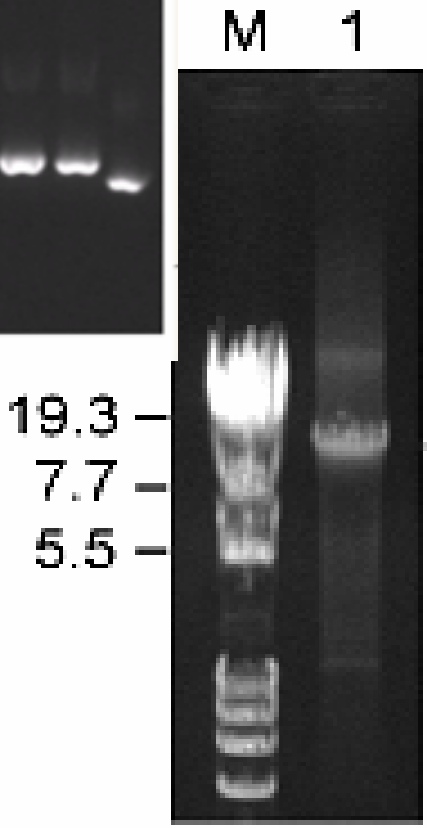
Conjugation: **7 stages**: 48 > 24 > 12 > 6 > 3 > 2 > 1 strains

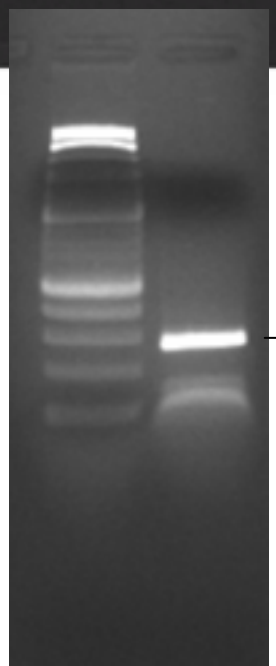
Reppas & Church

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



Atactic <4K chip





Nimblegen 95K chip

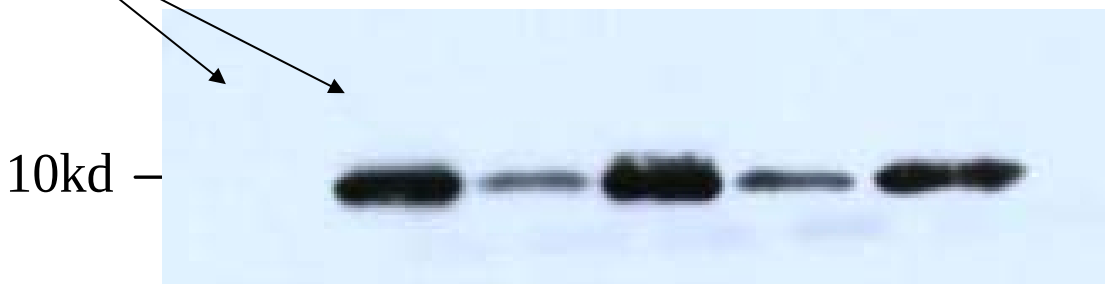
Tian, Gong, Sheng , Zhou, Gulari, Gao, Church

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

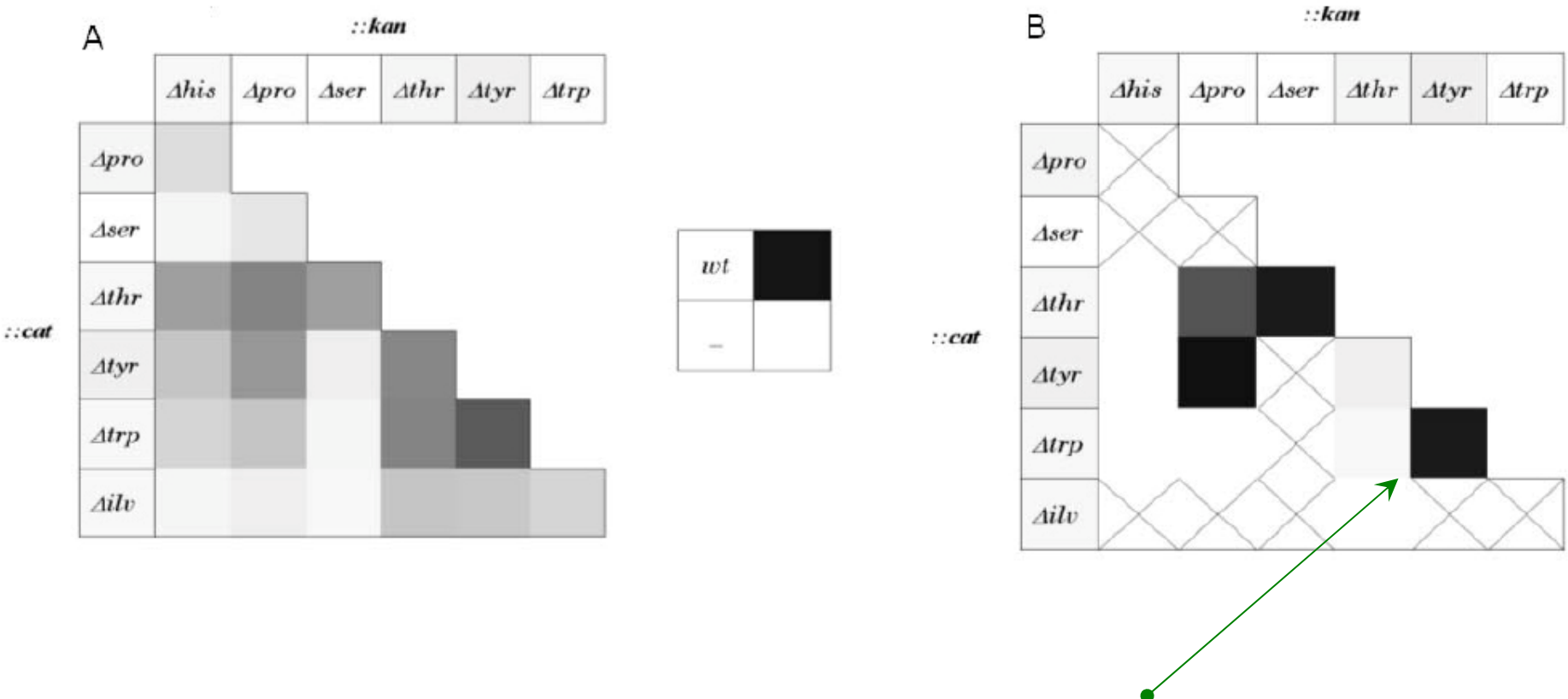
W: wild-type
M: modified

Tian & Church

Genome engineering & evolution: Cross-feeding Metabolic Mutants

First Passage

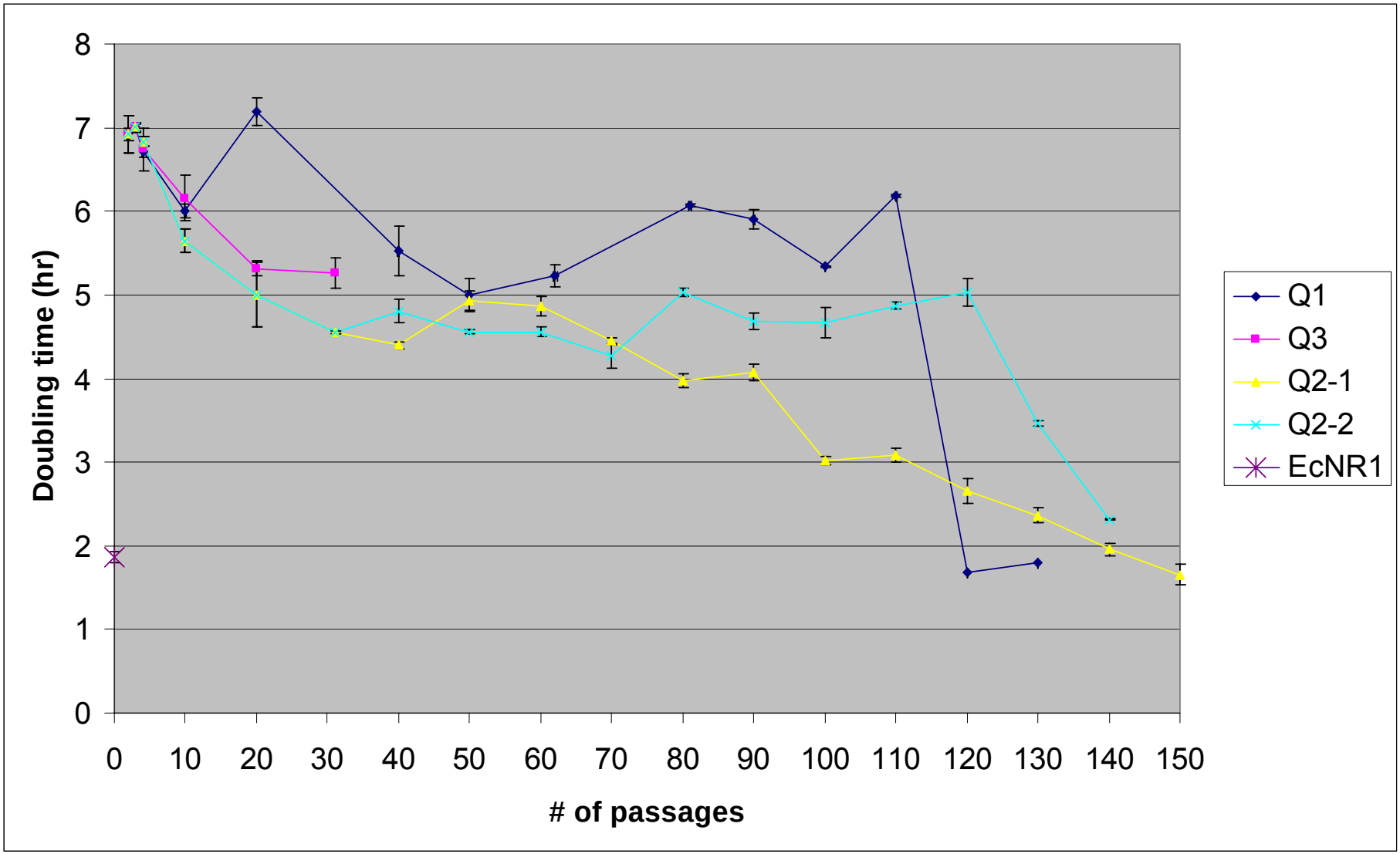
SecondPassage



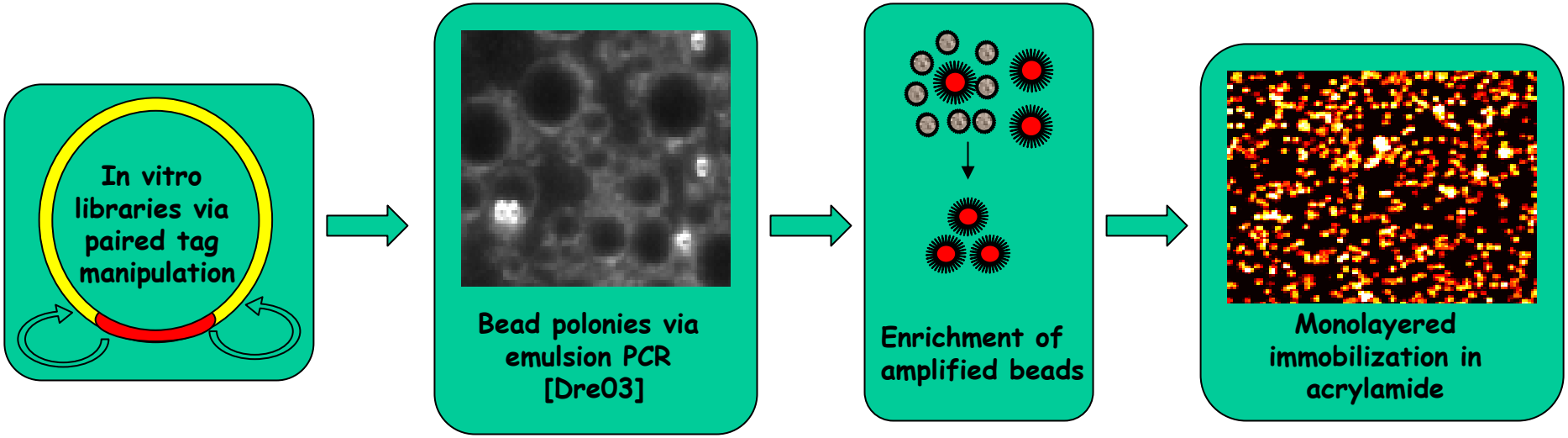
***Δtrp/ΔtyrA* pair of genomes shows the best co-growth**

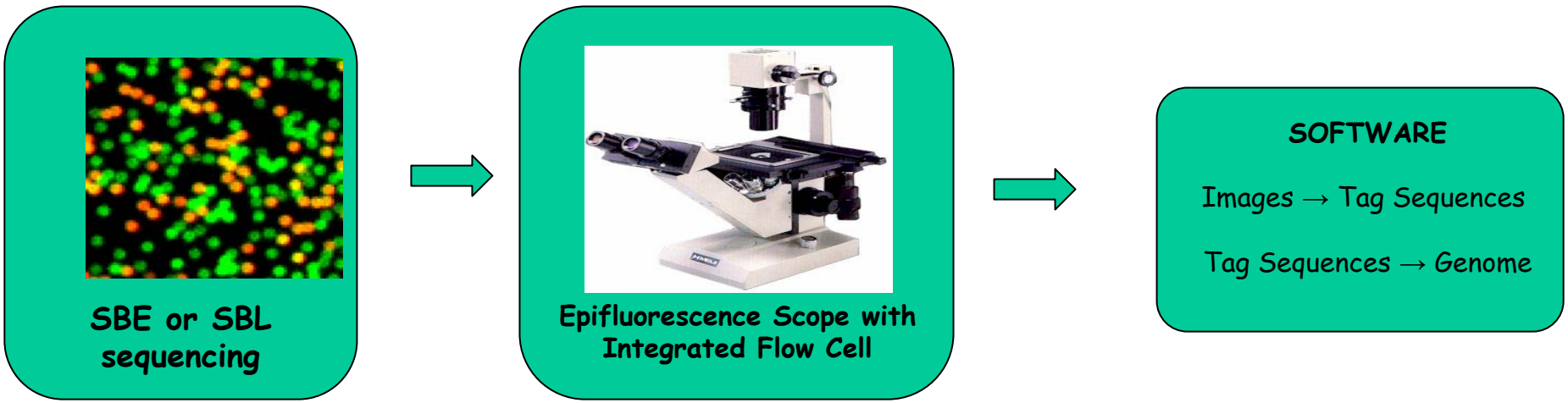
Reppas, Lin & Church unpublished.

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



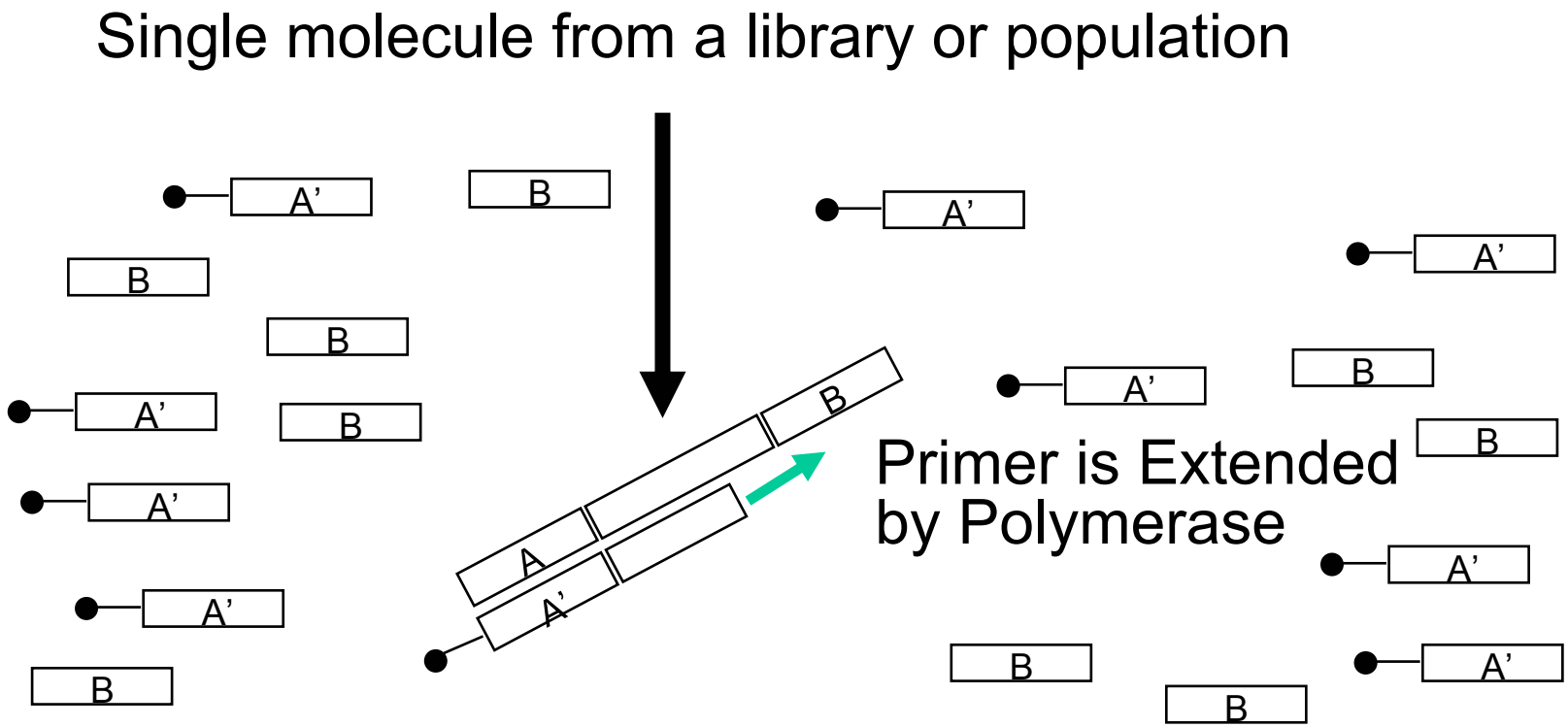
Plone Bead Sequencing Pipeline





Mitra, Shendure, Porreca, Rosenbaum, Church unpub.

Polymerase colony (polony) PCR in a gel



Primer A has 5' immobilizing Acrydite

Mitra & Church Nucleic Acids Res. 27: e34

Plone-bead FISSeq SBE SBL

Consider amplification , homopolymer, context errors?

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

current ABI capillary sequencing @ **0.75 kb/min/device**

Shendure & Porreca

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

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