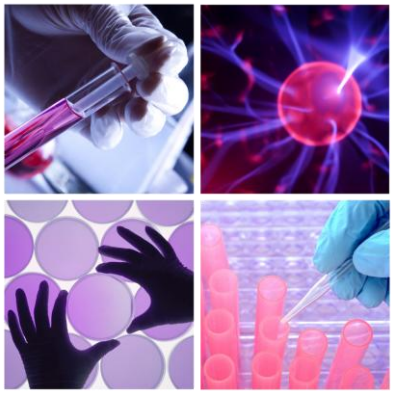

# Genetic Synthesis and Assembly Tools for Synthetic Biology

BIOEN Workshop on Synthetic Biology
October 26-27, 2010
São Paulo, Brazil

Todd Peterson, Ph.D.
VP
Genomics Technologies R&D

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# Presentation Overview

- **Emerging field of synthetic biology**
  - **Technology Drivers/Applications**
  - **Integrated technologies**
- **Gene synthesis**
  - **Process**
  - **Market considerations**
- **Error correction**
- **Assembly technologies and genetic editing**
  - **Micro-editing**
  - ***In vitro* assembly**
  - **High order assembly in yeast**

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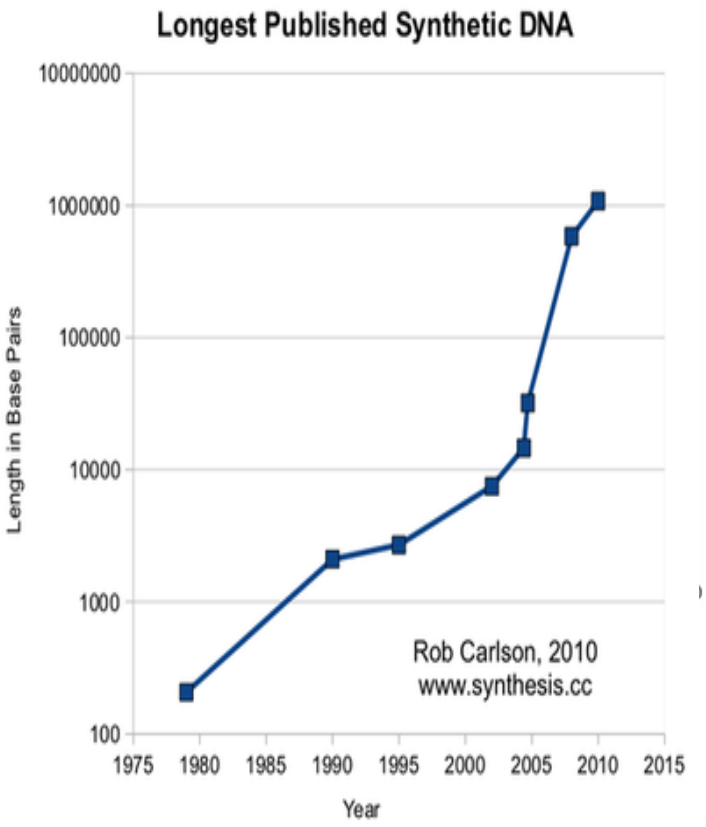
# Technology Drivers & Synthetic Biology

## Understanding & Design Ability to Read & Write DNA




## Converging Technologies

Molecular/Cell Biology
Microbiology

DNA Sequencing
Meta-Genomics

DNA Synthesis

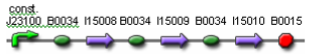
**Tools Revolution**

Engineering

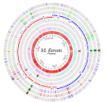
Bioinformatics
Systems and
Computational Biology

Industrial Microbiology
Chemical Engineering
Fermentation Science

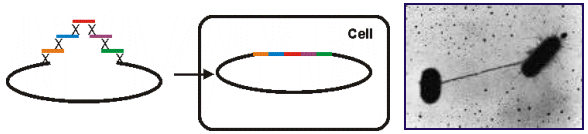
## Synthetic Biology



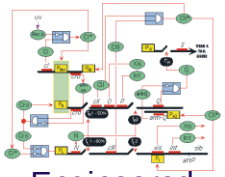

Standardized Genetic Parts/Devices



Engineered Hosts

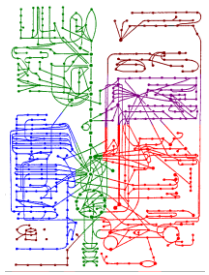



Gene/Chromosome Assembly/Transfer Tools

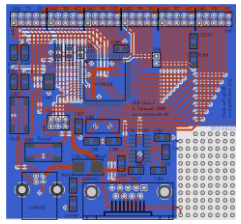


Engineered Genetic Circuits

Predictive BioCAD/FAB Optimized Applications



Genetic/Biochemical Pathways



Design Engineering

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# Synthetic Biology

- Engineering life for useful purposes
- A rapidly growing field of research: a new approach to life sciences
- Multi-disciplinary: Engineering, biology & informatics converge
- Cutting edge research and development tools
- Enable broad industrial applications

Standardized Parts
Engineered Hosts
Synthesis & Assembly Tools
Computational Design Software
Analytical Tools

**Healthcare**

**Energy**

**Chemicals**

**Agriculture**

**Bio-Remediation**

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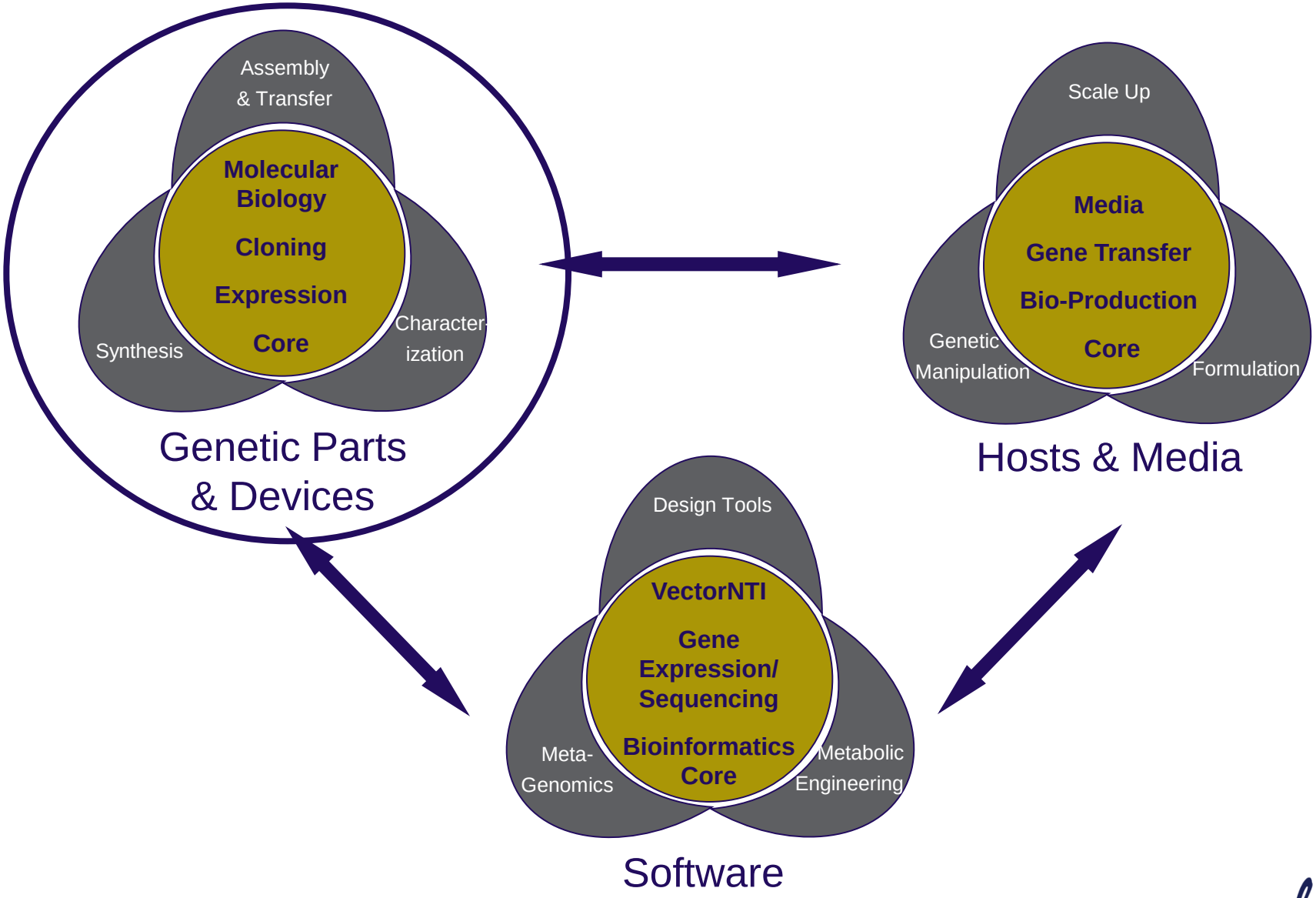
# Synthetic Biology Workflow Integration



# Gene Synthesis:
## Foundational Technology for Synthetic Biology

### Key Considerations

- Highest quality
- Ability to synthesize difficult sequences
- State-of-the-art design/optimization
- Cost effective
- Delivery time
- Scale and capacity
- Market and brand leadership
- Synthetic biology vision

genomeweb | GenomeWeb Daily News

**Life Technologies Takes Majority Stake in GENEART**
April 09, 2010

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Press Releases

**Life Technologies Completes Tender Offer for Synthetic Biology Firm Geneart**
Establishes Leadership Position in Emerging Field
May 28, 2010

Anticipate acquisition will be completed Q4 2010

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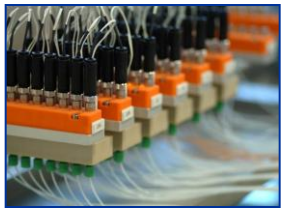
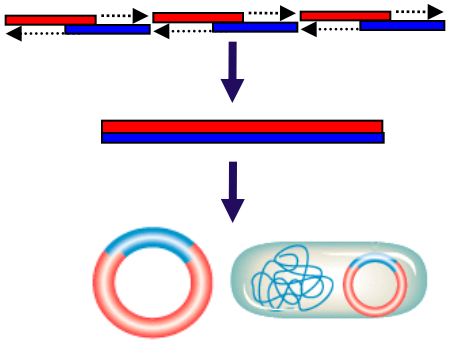
# Technology and Process Overview

**Bioinformatics**
- Optimization/CAD
- Design fragments and oligos
- Alignment and simulation *in silico*

**Oligosynthesis**
- Nano scale synthesis
- High throughput/quality

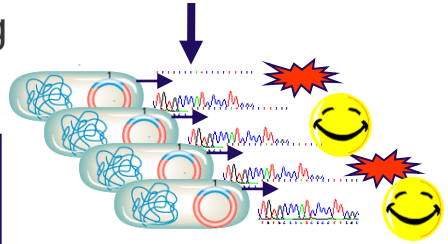


**Gene Assembly**
- Several approaches
- High throughput
- Up to 2.0 kb/fragment



**Cloning**
- High throughput
- Semi-automated

**Sequencing**
- automated CE sequencing
- automated alignment



**Final Documentation**
- Product report
- Ship overnight
- ISO 9001

LIMS

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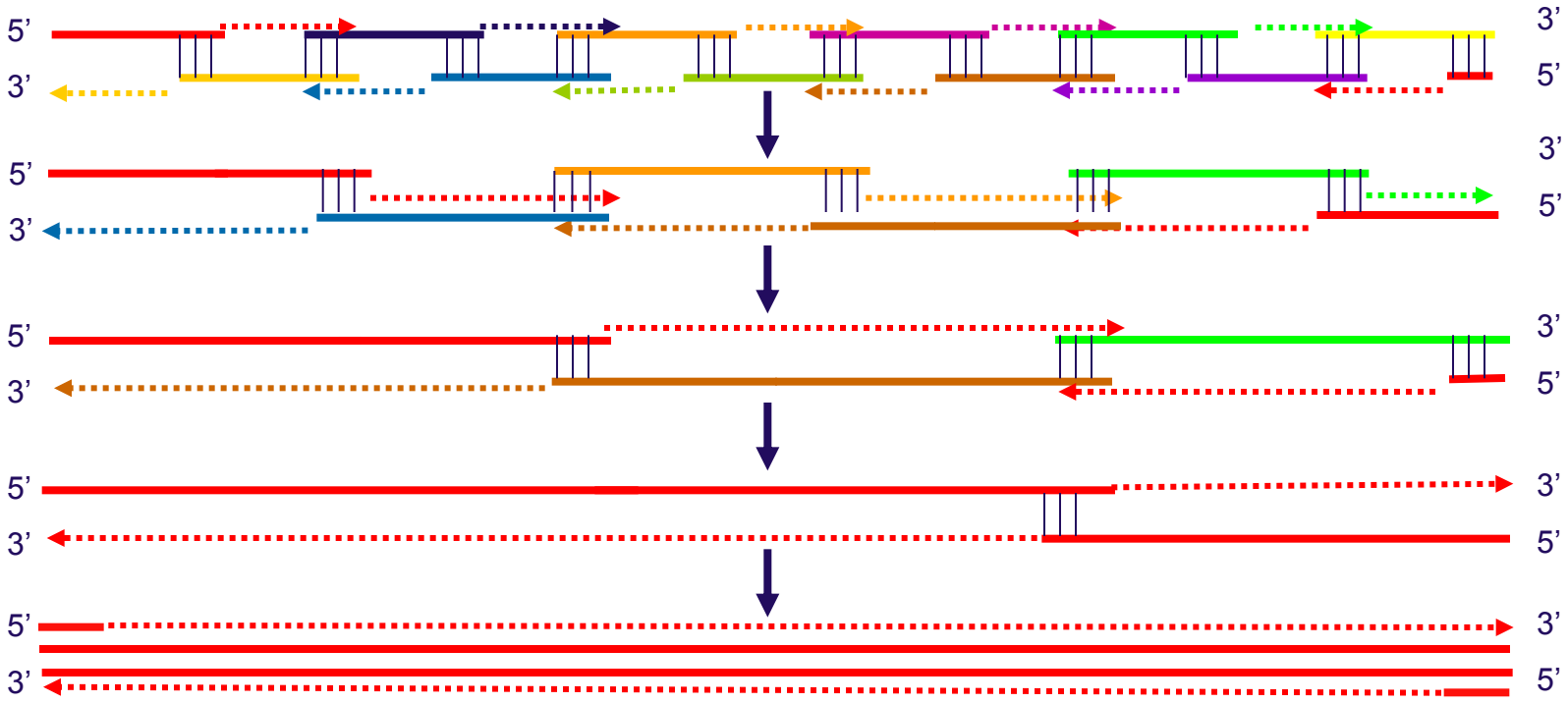
# GENEART PCR-Extension Gene Synthesis:

- Primary gene sequence from customer, optimization options, vector of choice
- Single-strand oligonucleotide ~40-45mer tiles are designed, synthesized & pooled
- Tile pools are amplified by PCR in two rounds:
  - All oligonucleotide tiles
  - Terminal Primers
- Assembled fragment is cloned and sequenced




Double Stranded DNA Fragment up to 2.0 kb

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# Gene (and Larger) Synthesis Considerations

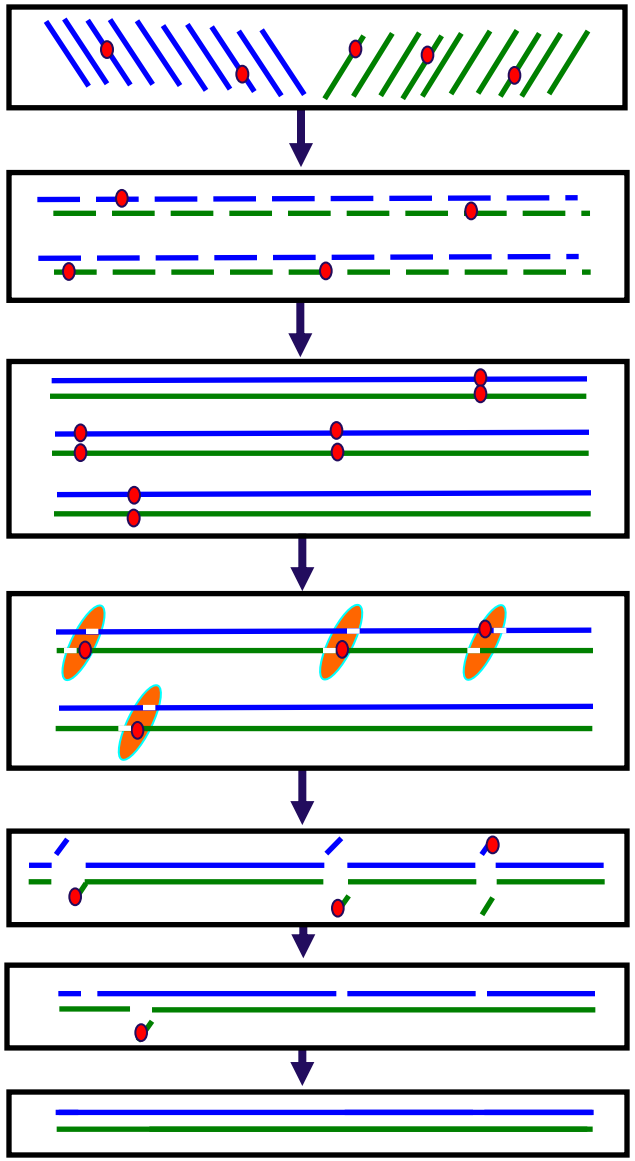
- The quality bar is already set – ordered DNA is sequence verified
- Trending toward larger/complex constructs and “limited libraries”
- Parameters influencing quality, capacity and cost at scale
  - Reagents and chemicals
  - Process consumables
  - Automation and LIMS
  - Leveraging gene-synthesis associated Services
- Cost efficiencies and capacity driven by:
  - Oligonucleotide synthesis quality and low scale
  - Gene and construct assembly methods
  - Sequencing and reducing error rate

**Life Technologies Signs Exclusive License Agreement for DNA Error-Correction Technology from Novici Biotech LLC**
**October 11, 2010**

NOVICI BIOTECH

life technologies™

# ErrASE Synthetic Gene Assembly Method



Oligos designed & mixed.
Some oligos have errors.

PCR assembled 1st round

PCR assembled 2nd round
(Can also use assembly by ligation)

DNA denatured & reannealed
Errase nicks at errors

HiFi Polymerase exonuclease
blunts ends, removing errors

Long “corrected oligos” are PCR re-assembled in 3rd round, Remaining errors removed by HiFi polymerase

Corrected gene

NOVICI BIOTECH

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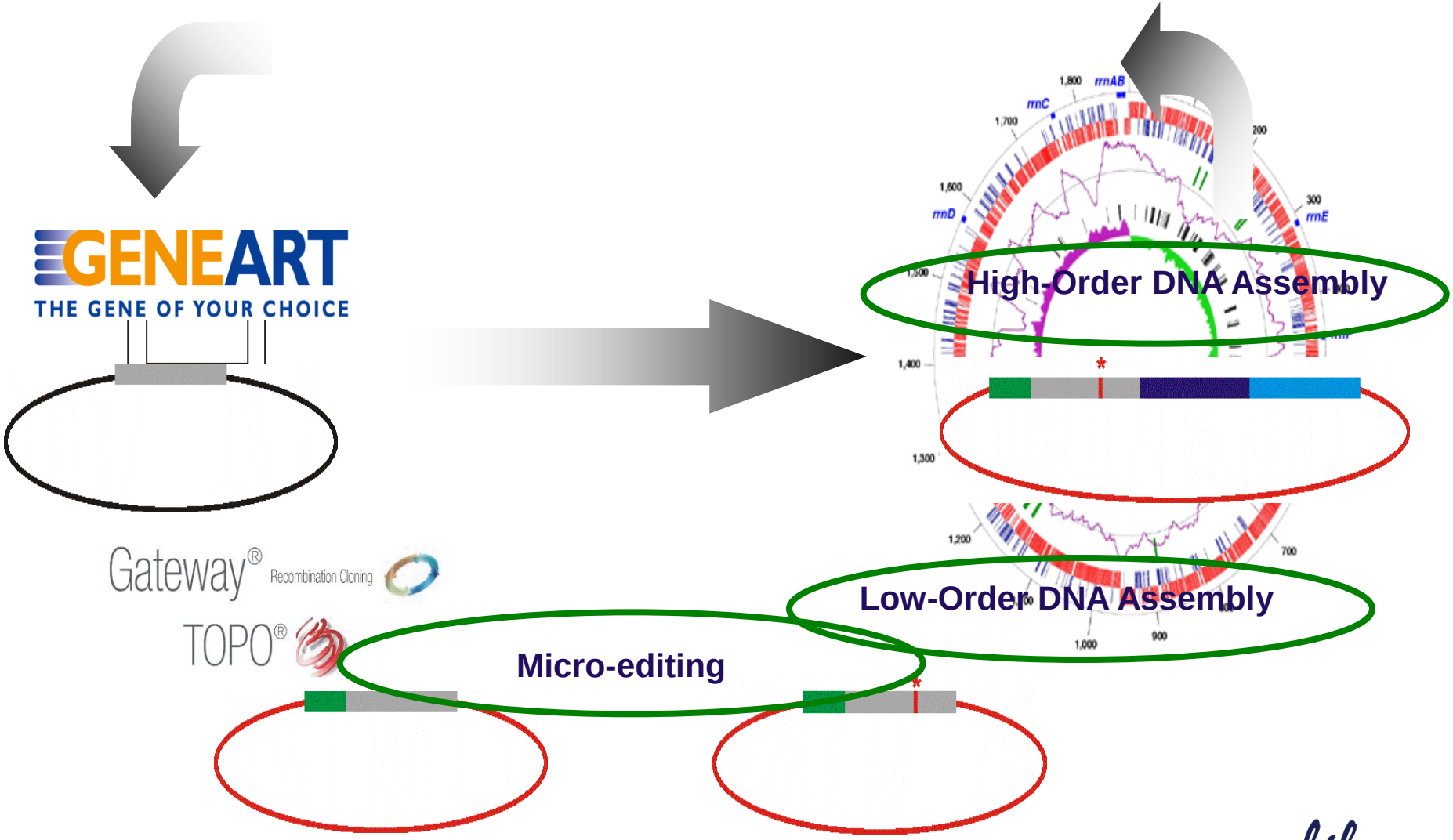
# Errase Synthetic Gene Error Correction

| Gene | Length | # Bases Sequenced | Deletion | Insertion | Base change | Error Rate (bases/error) |
|---|---|---|---|---|---|---|
| 1 | 717 | 10,870 | 2 | 0 | 0 | 5,435 |
| 2 | 291 | 6,024 | 1 | 0 | 0 | 6,024 |
| 3 | 1,048 | 7,336 | 1 | 0 | 1 | 3,668 |
| 4 | 1,158 | 28,650 | 6 | 1 | 4 | 2,605 |
| 5 | 1080 | 13,960 | 1 | 0 | 2 | 4,653 |
| 6 | 891 | 8,370 | 4 | 2 | 0 | 1,395 |
| 6 (no Errase) | 891 | 25,740 | 52 | 2 | 7 | 422 |
| GFP | 732 | 16,790 | 4 | 0 | 0 | 4,198 |
| GFP (no Errase) | 732 | 16,790 | 44 | 1 | 5 | 336 |

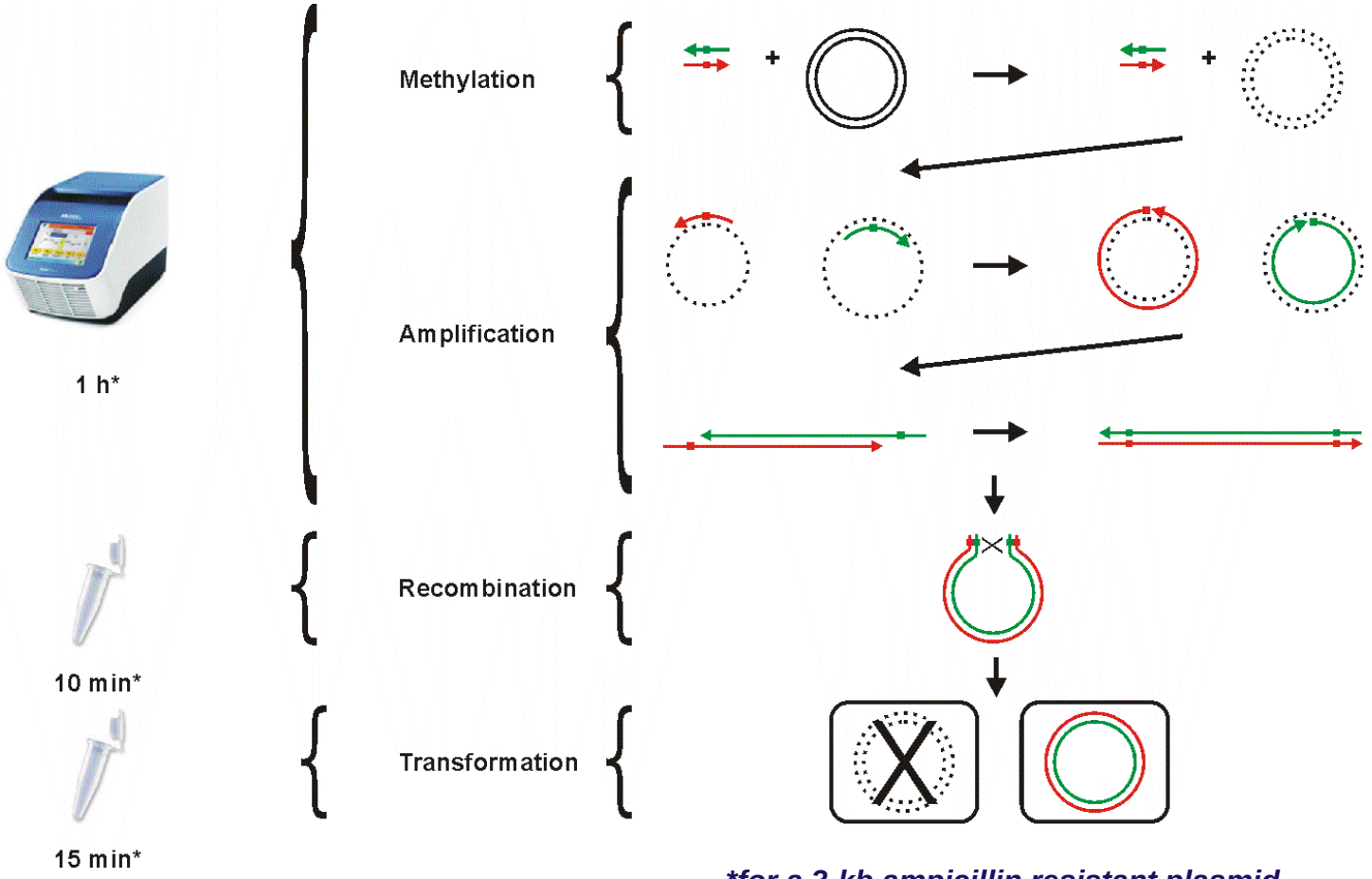
No detectable preference or bias to error correction

# Genetic Assembly & Editing



# Micro-Editing: Improved Site-Directed Mutagenesis

## GENEART® Site Directed Mutagenesis - Workflow




**for a 3-kb ampicillin resistant plasmid*

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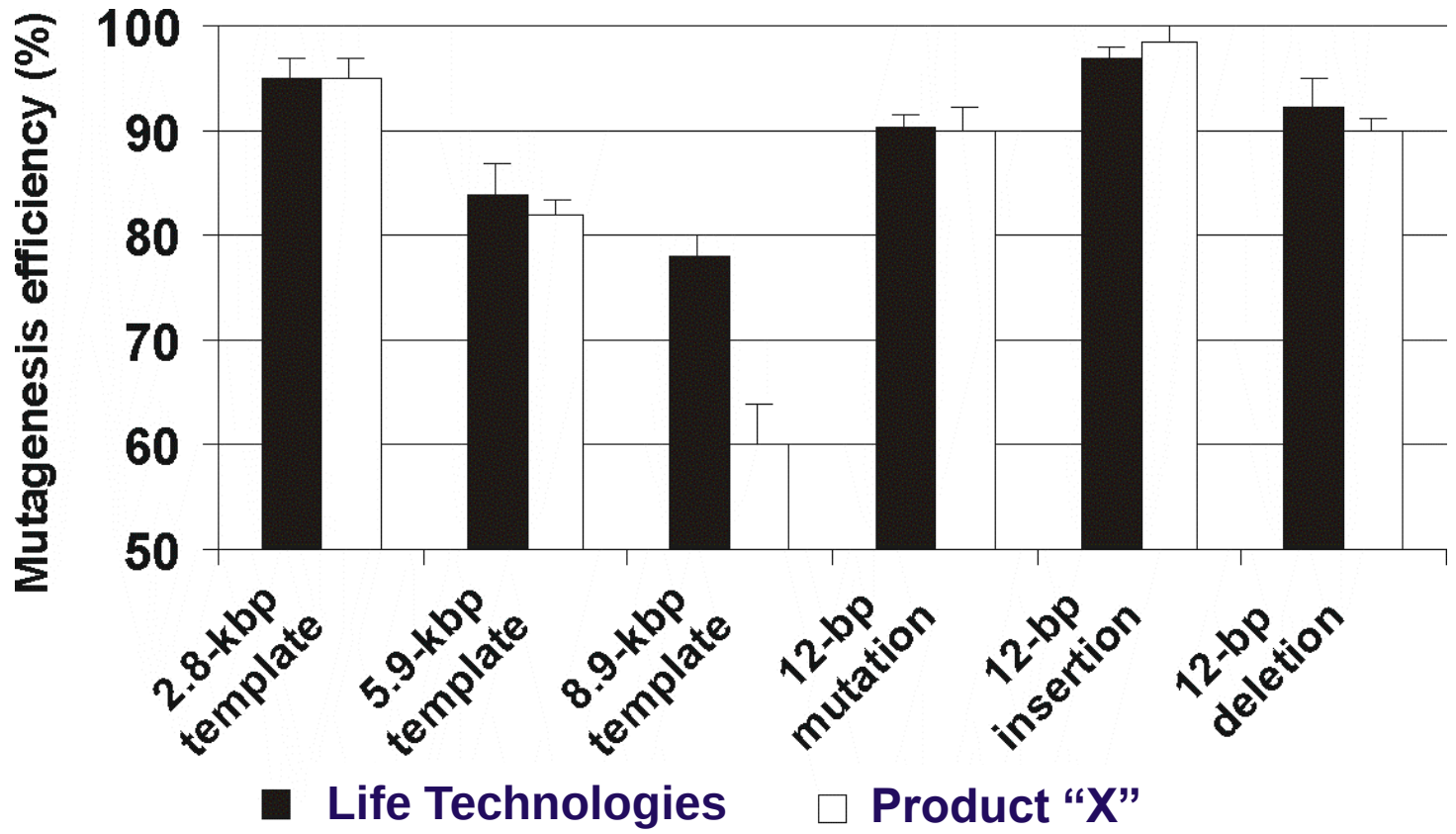
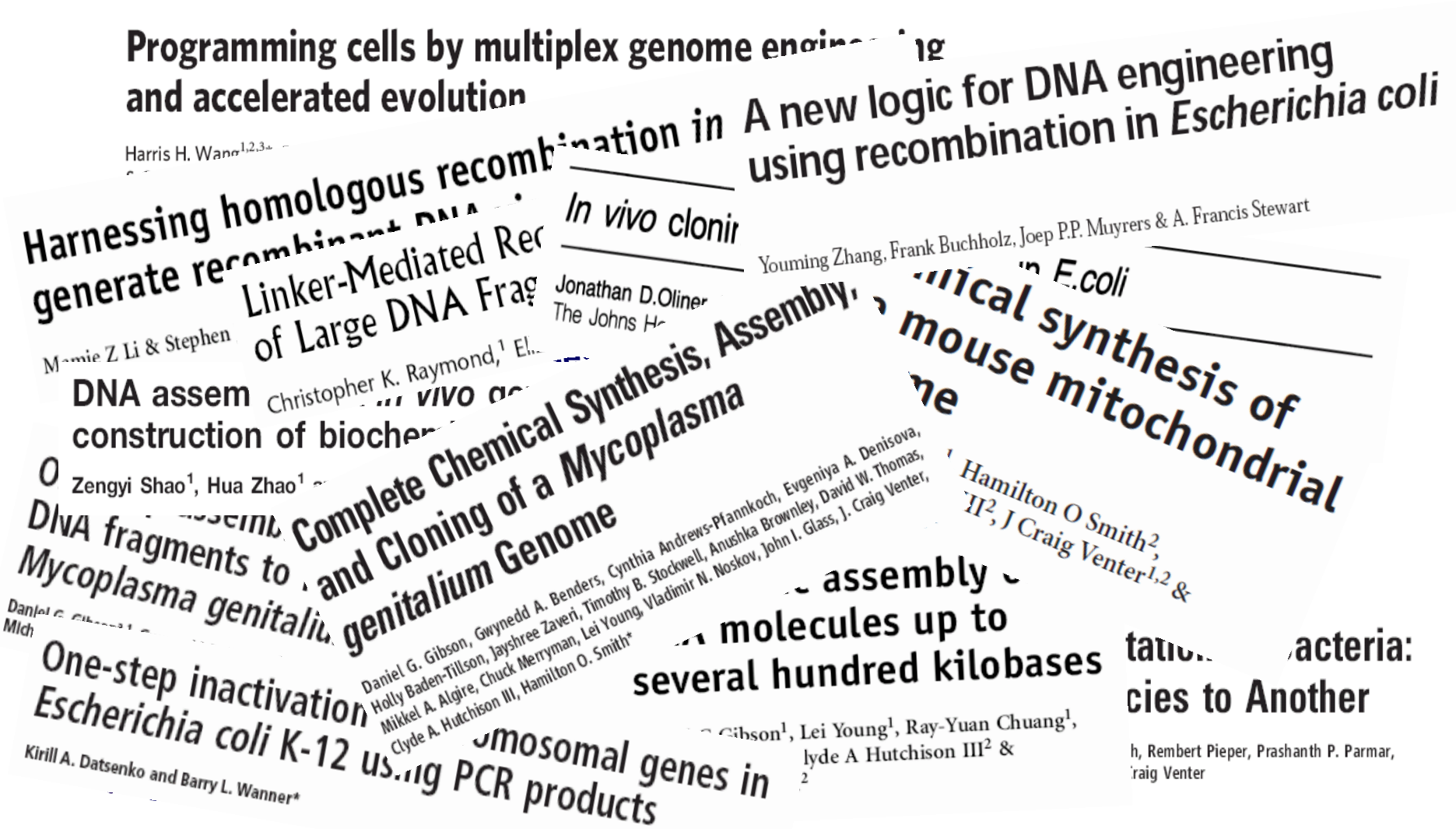
# GENEART® Site Directed Mutagenesis: Performance

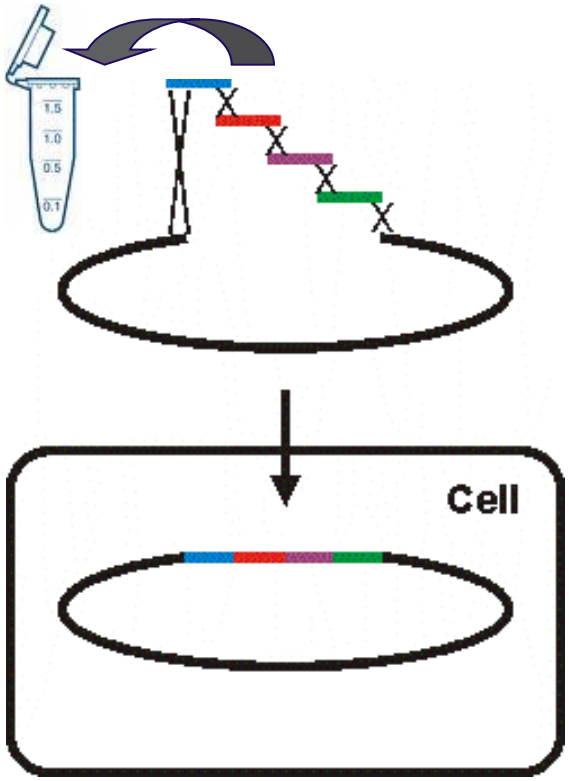


# Assembly/Engineering Technologies for Synthetic Biology

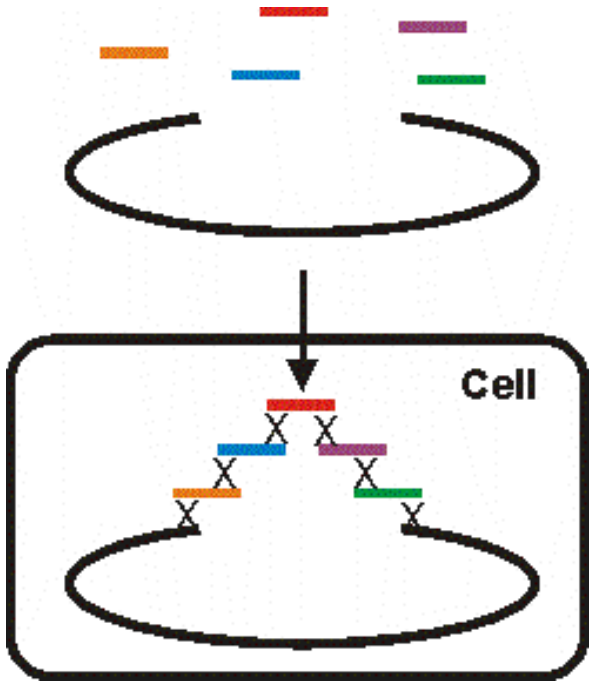


# Simple Version of Assembly Solutions

- Design, fabricate or prepare fragments for assembly with terminal homology
- *In vitro* assembly:  creation/stabilization of hybridized termini and transformation
- *In vivo* assembly:  high-fidelity/efficiency homologous recombination




*In vitro* assembly




*In vivo* assembly

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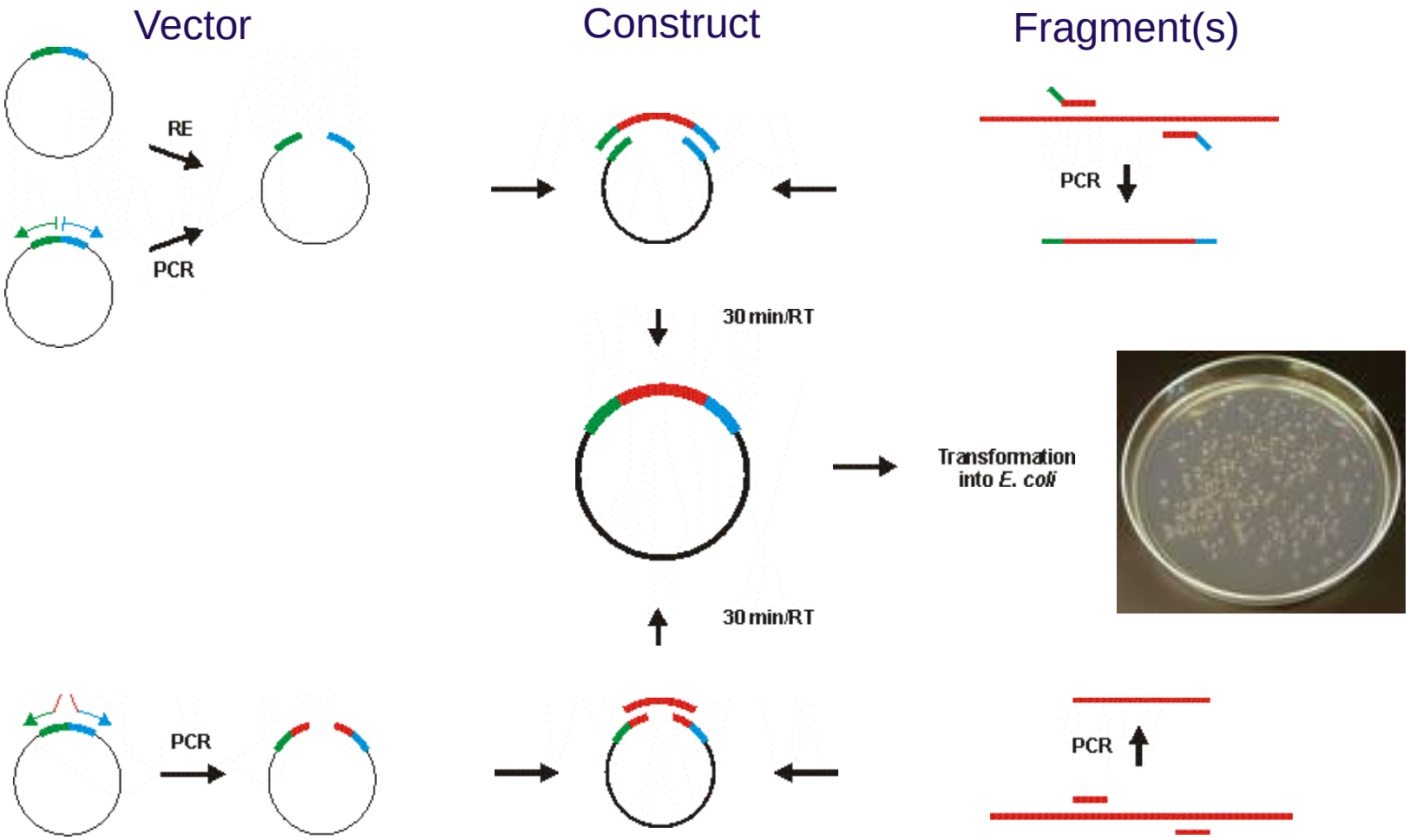
# *In Vitro* Assembly Approach




Only 15 bp of terminal homology required

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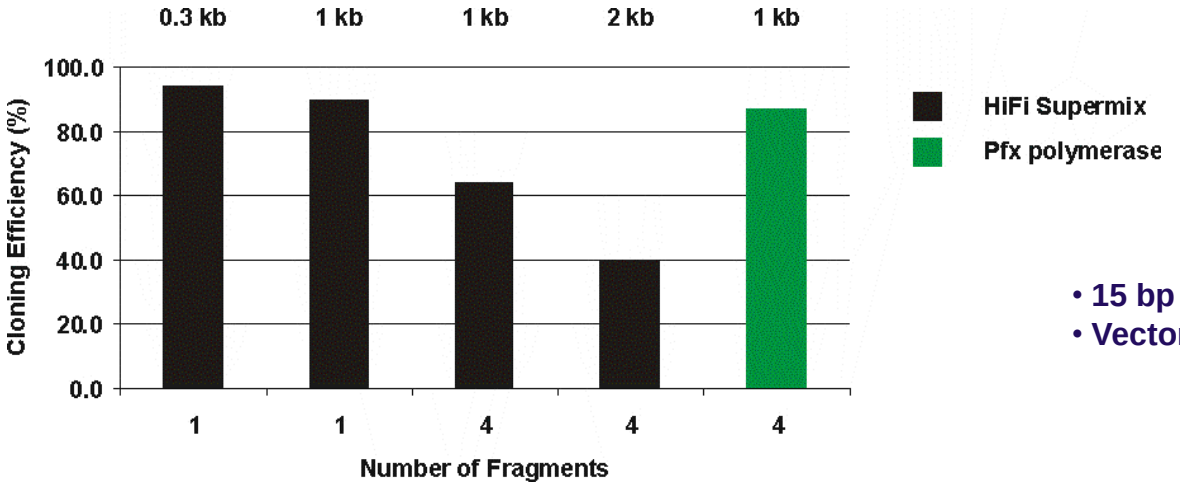
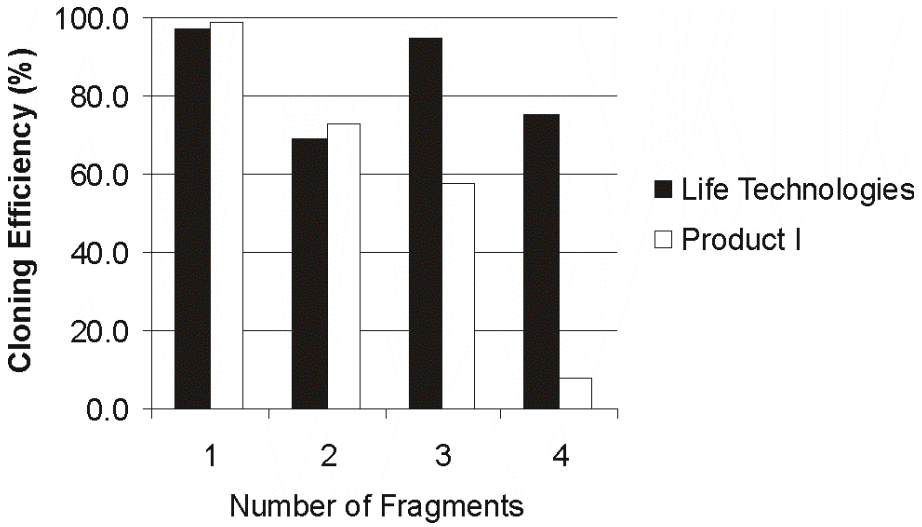
# Seamless Cloning: 4 Fragments into a Vector




- 15 bp overlap
- Vector Size: 2.6 kbp




- Fragment length: 1 kbp each
- Overlap between fragments: 15 bp
- Polymerase: Accuprime Pfx Supermix

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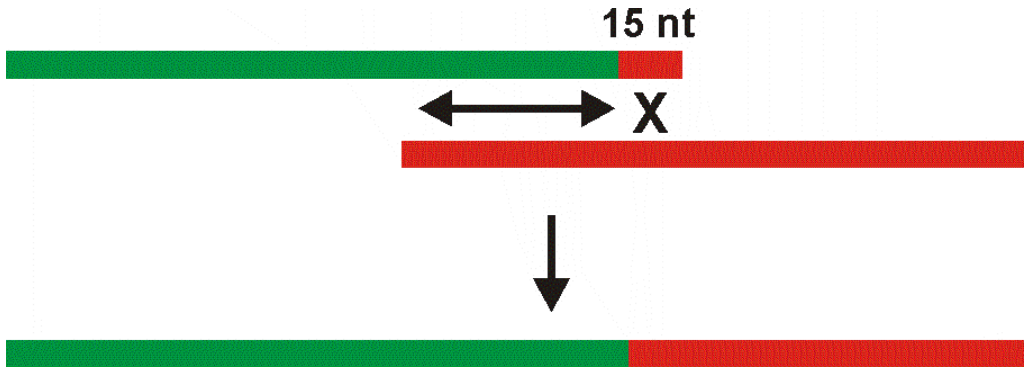
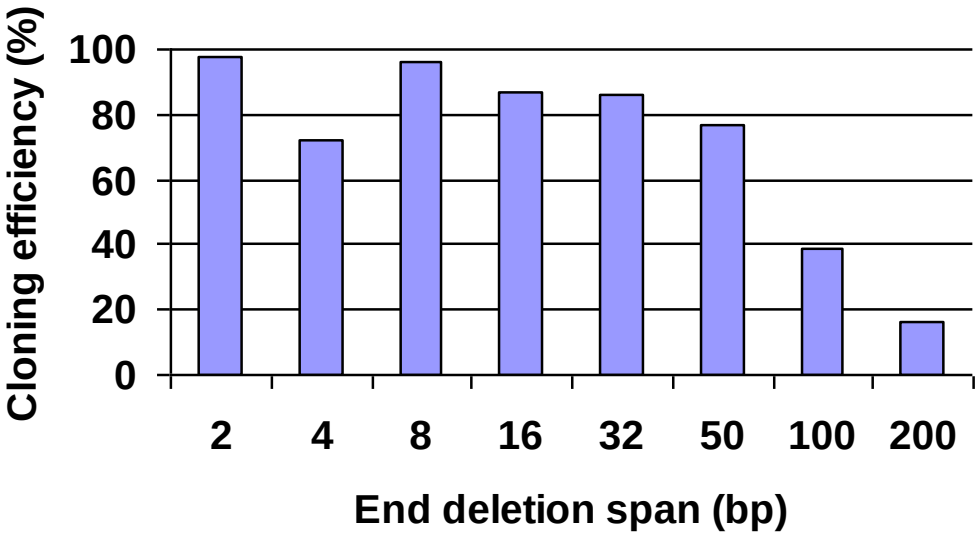
# *In Vitro* Recombination: End Deletions/Editing

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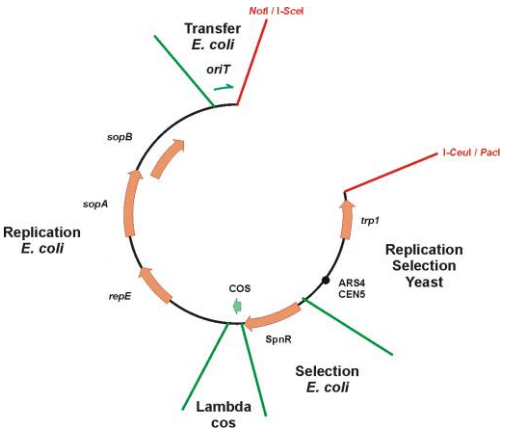
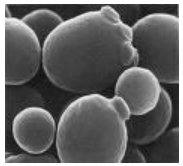
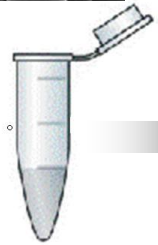
# *In Vivo* Assembly Approach

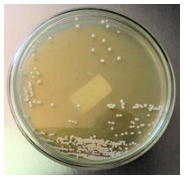
## Transformation-Associated Recombination (TAR) in *S.cerevasiae*

Yeast. **10**:93, 1994
Proc Natl Acad Sci **92**:11701, 1995
Proc Natl Acad Sci **93**:491, 1996
Plasmid **38**:91, 1997
Nucleic Acids Res. **29**:E32, 2001
US Patent 6391642, 2002
Genome Res **12**:190, 2002
FEBS Lett **576**:291, 2004
Mol Plant Microbe Interact **17**:571, 2004
Nucleic Acids Res **33**:e130, 2005
BioTechniques **40**:79, 2006
Science 319:**1215**, 2008
Proc Natl Acad Sci **105**:20404, 2008
Science **325**:1693, 2009
Nucleic Acids Res **37**:6984, 2009
*Science* **329**, 52, 2010



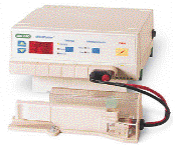



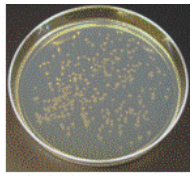




+ 3 ul glass beads
+ Vortex 5 min

Electroporation

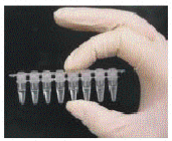




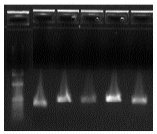
*E.coli*

Pick Colonies

Colony-PCR



Electrophoresis



+

**Transform Plate**

**Mix vector & DNA fragments with homologous termini (30 bp)**

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# Multiple Fragment Assembly in Yeast

| No fragments | No/size preexisting fragments | No/size amplified fragments | Overlap | Amt Insert (ng) | Colony output | Cloning efficiency |
|---|---|---|---|---|---|---|
| 3 | 3 x 30 kb | 0 | 80 bp | 100 | 4000 | 100% |
| 5 | 5 x 50 kb | 0 | 80 bp | 100 | 1400 | 100% |
| 10 | 10 x 10 kb | 0 | 80 bp | 100 | 670 | 50% |
| 20 | 8 x 10 kb | 12 x 0.5-2.5 kb | 80 bp | 100 | 660 | 58% |
| 20 | 8 x 10 kb | 12 x 0.5-2.5 kb | 80 bp | 200 | 770 | 83% |

| Assembly Type | Fragment Overlap | Colony Output | Cloning Efficiency |
|---|---|---|---|
| 1 x 10 kb | 80 bp | 1140 | 100% |
| 1 x 10 kb | 30 bp | 1160 | 100% |
| 10 x 5 kb | 30 bp | 1850 | 92% |

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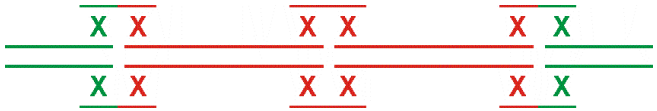
# Bridging Oligos:

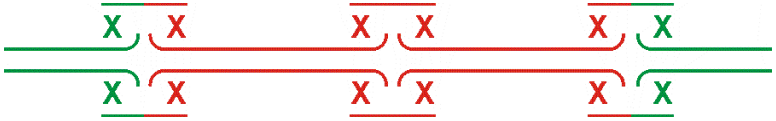
## Perfect/Imperfect Junction Assembly

- No homology between adjacent fragments to be joined
- Homology provided in *trans* by designed bridge oligonucleotides
- Allows for reuse of fragments in a new sequence context
- Allows for junction editing

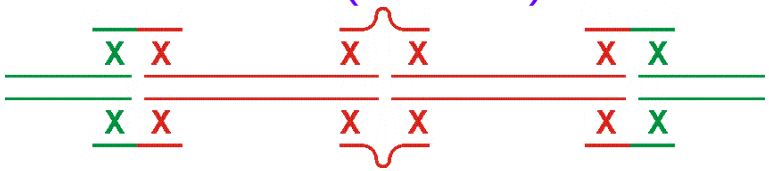
### Perfect Junctions



### Deletion Junctions (12 bases)



### Insertion Junctions (30-X-30)



| Oligo | Colony # | Cloning Efficiency |
|---|---|---|
| 60mer | 2685 | 94% |
| 80mer | 1240 | 75% |
| 60mer | 520 | 25% |
| 10 bp | 460 | 63% |
| 20 bp | 430 | 50% |

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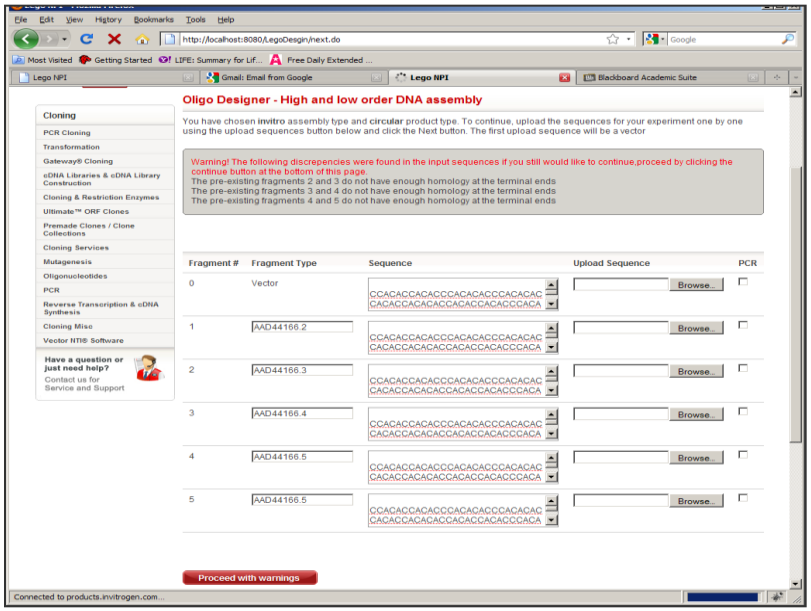
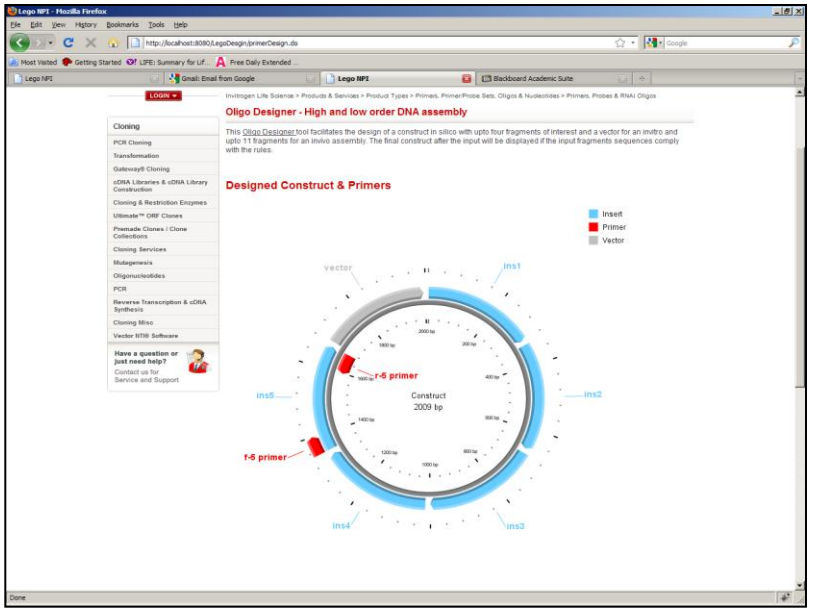
# Web Assembly Design Tools

- Designs DNA oligonucleotides for PCR primers and/or junction bridging
- Automated checks for potential homology issues during assembly
- Delivers final construct maps & the DNA oligonucleotides for assembly




www.invitrogen.com/DNAassembly

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# Assembly Technologies Summary

## *In vitro* DNA assembly

- Up to 4 fragments plus vector
- Seamless (no scars)
- Terminal homology, 15 bp overlaps
- Unmodified oligos/primers
- Constructs up to 15 kb
- Vector-independent
- Isothermal
- 30 minute
- Single tube reaction
- High-fidelity DNA polymerase
- Kitted controls & comp *E. coli* cells
- Web design tools

## *In vivo* (high order) DNA assembly

- Up to 20 fragments plus vector
- Seamless (no scars)
- Terminal homology of 30 bp overlaps
- Unmodified oligos/primers
- High-fidelity DNA polymerase
- Constructs up to 100 kb
- Patented yeast homologous recombination
- Shuttle vector: yeast assembly/*E. coli* propagation
- Bridging oligonucleotide option allows reuse of fragments and junction editing
- Yeast-*E. coli* transfer (10 min), no liquid culture
- Kitted controls, comp yeast & electrocomp *E.coli*
- Web design tools

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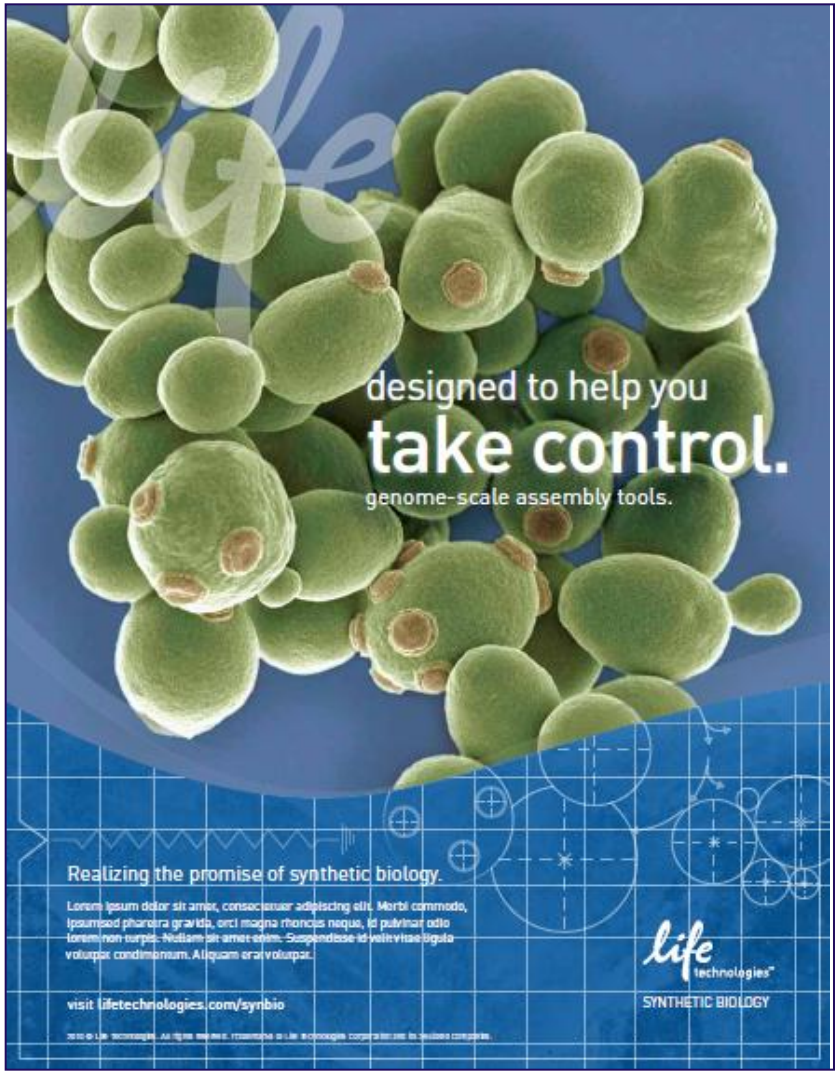
# Acknowledgements


- **Gene Synthesis**
  - Ralf Wagner/Geneart
- **Error Correction**
  - Jason Potter
  - Hal Padgett/Novici
- **Site Directed Mutagenesis**
  - Xiquan Liang
- ***In Vitro* Assembly**
  - Billyanna Tsvetanova
  - Federico Katzen
- ***In Vivo* Assembly**
  - Lansha Peng
  - Ke Li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