

Genetic Synthesis and Assembly Tools for Synthetic Biology

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

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VP
Genomics Technologies R&D

Presentation Overview

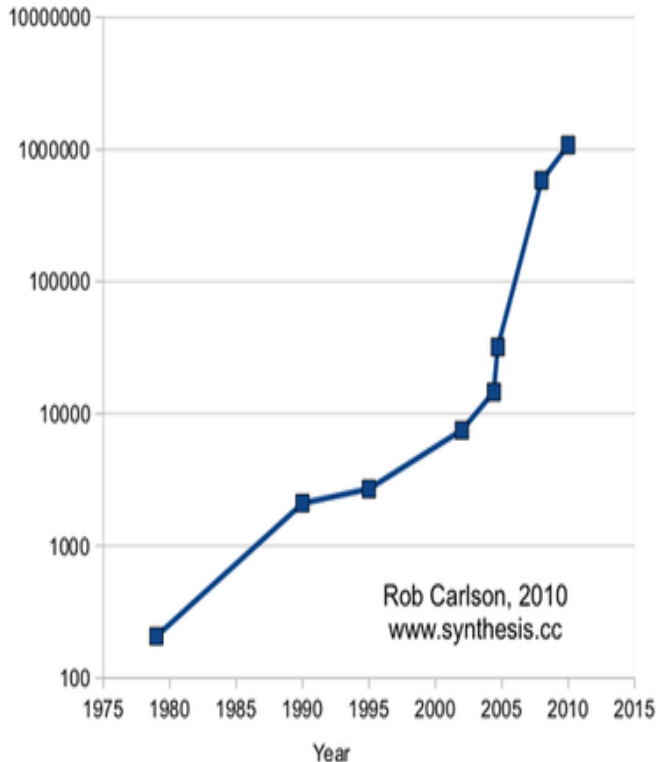
- **Emerging field of synthetic biology**
 - Technology Drivers/Applications
 - Integrated technologies
- **Gene synthesis**
 - Process
 - Market considerations
- **Error correction**
- **Assembly technologies and genetic editing**
 - Micro-editing
 - *In vitro* assembly
 - High order assembly in yeast

Technology Drivers & Synthetic Biology

Converging Technologies

Understanding & Design Ability to Read & Write DNA

Longest Published Synthetic DNA



Molecular/Cell Biology
Microbiology

DNA Sequencing
Meta-Genomics

DNA Synthesis

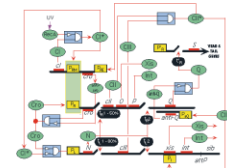
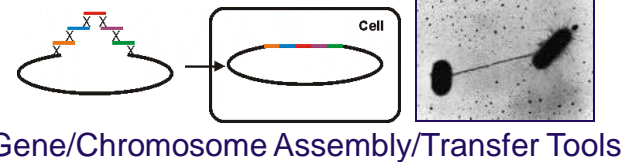
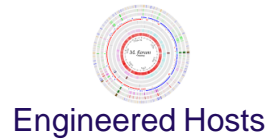
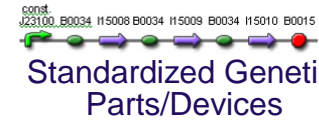
Tools Revolution

Engineering

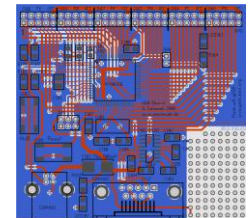
Bioinformatics
Systems and
Computational Biology

Industrial Microbiology
Chemical Engineering
Fermentation Science

Synthetic Biology



Predictive
BioCAD/FAB
Optimized Applications



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



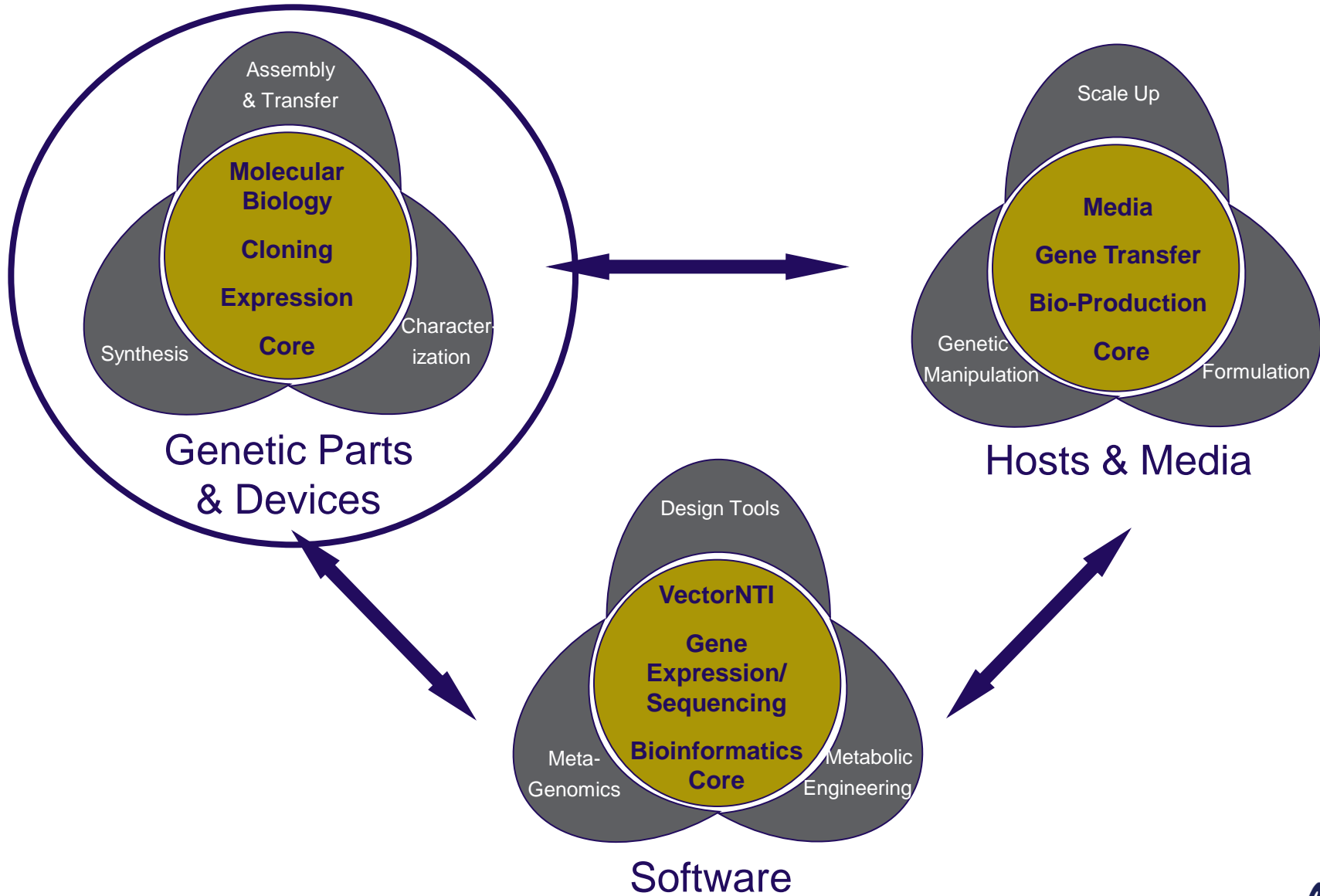
Agriculture



Bio-Remediation



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 Daily News

Life Technologies Takes Majority Stake in GENEART

April 09, 2010



Press Releases

Life Technologies Completes Tender Offer for Synthetic Biology Firm Geneart

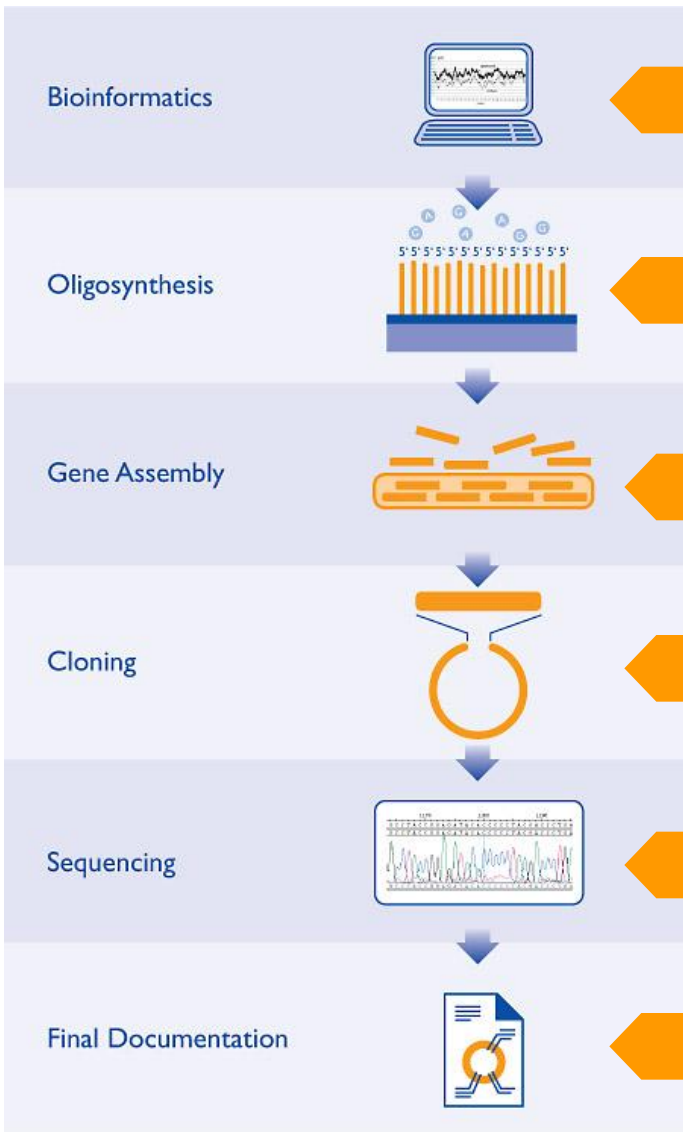
Establishes Leadership Position in Emerging Field

May 28, 2010

Anticipate acquisition will be completed Q4 2010

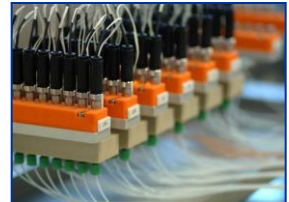


Technology and Process Overview

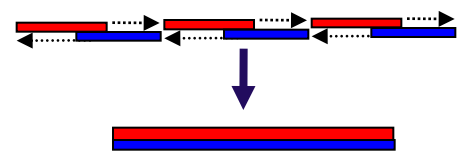


- Optimization/CAD
- Design fragments and oligos
- Alignment and simulation *in silico*

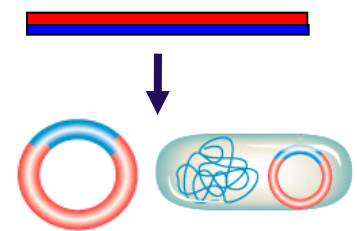
- Nano scale synthesis
- High throughput/quality



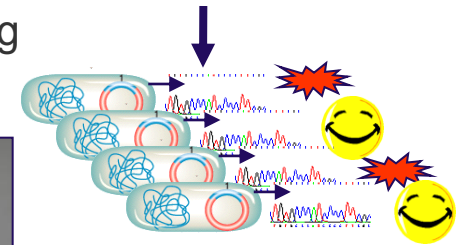
- Several approaches
- High throughput
- Up to 2.0 kb/fragment



- High throughput
- Semi-automated



- automated CE sequencing
- automated alignment



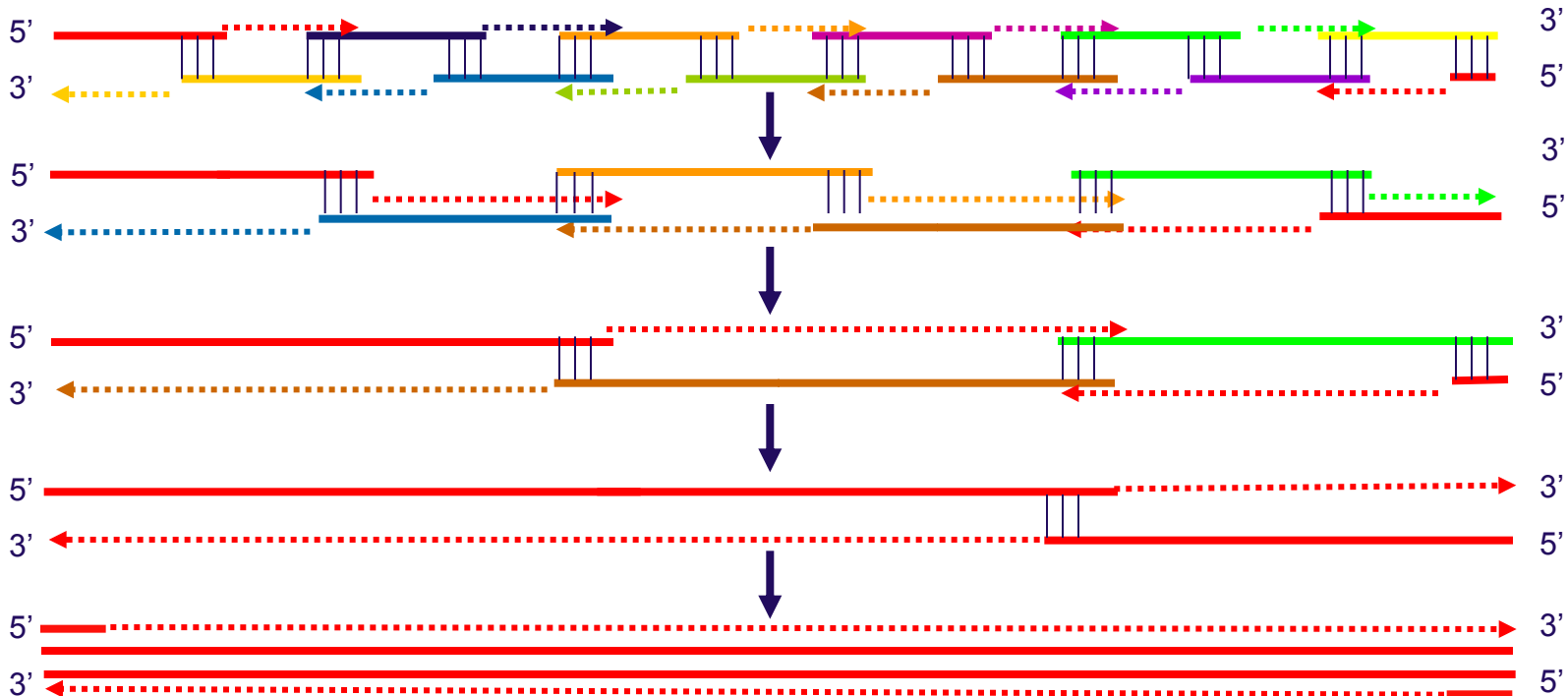
- Product report
- Ship overnight
- ISO 9001



LIMS

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

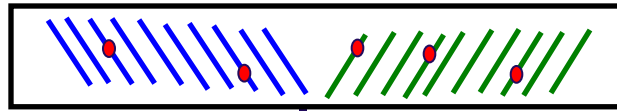
Gene (and Larger) Synthesis Considerations

- 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

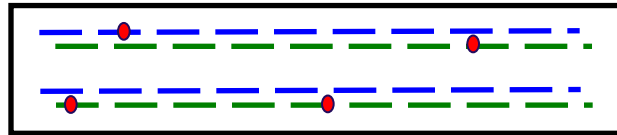
Life Technologies Signs Exclusive License Agreement for DNA Error-Correction Technology from Novici Biotech LLC
October 11, 2010



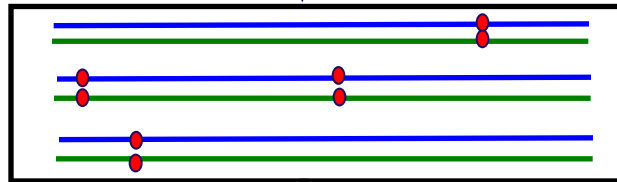
ErrASE Synthetic Gene Assembly Method



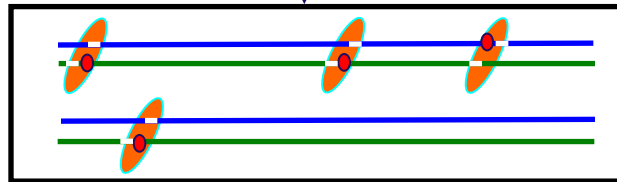
Oligos designed & mixed.
Some oligos have errors.



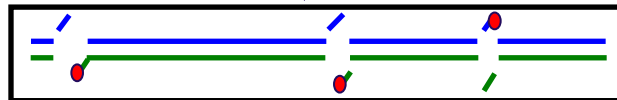
PCR assembled 1st round



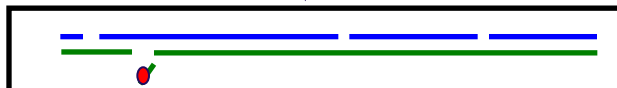
PCR assembled 2nd round
(Can also use assembly by ligation)



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

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

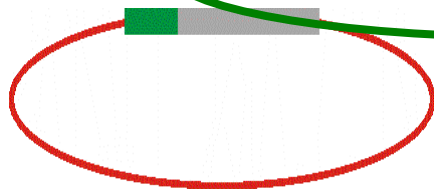
No detectable preference or bias to error correction

Genetic Assembly & Editing

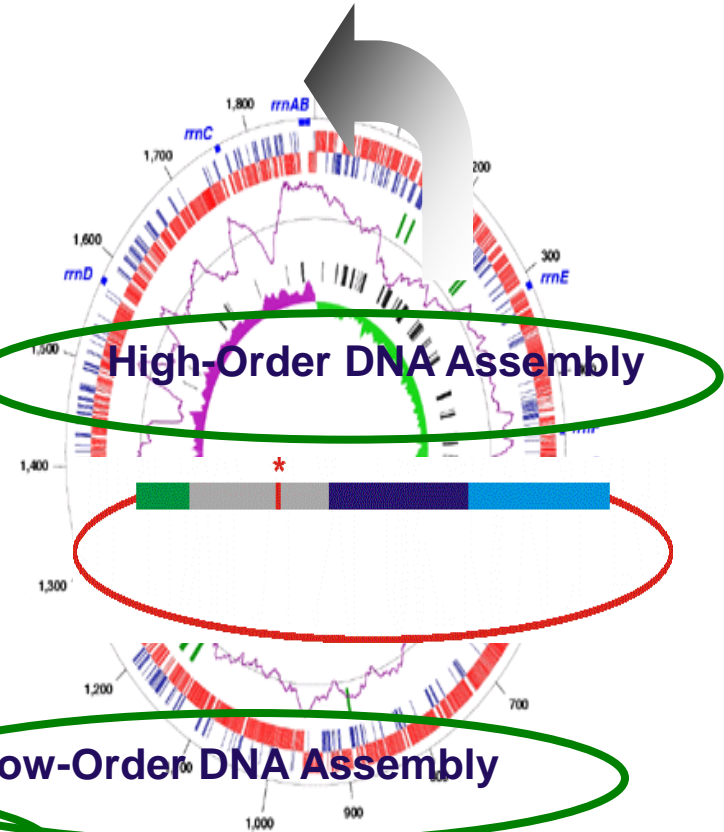
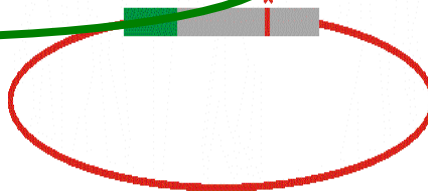


Gateway[®] Recombination Cloning

TOPO[®]

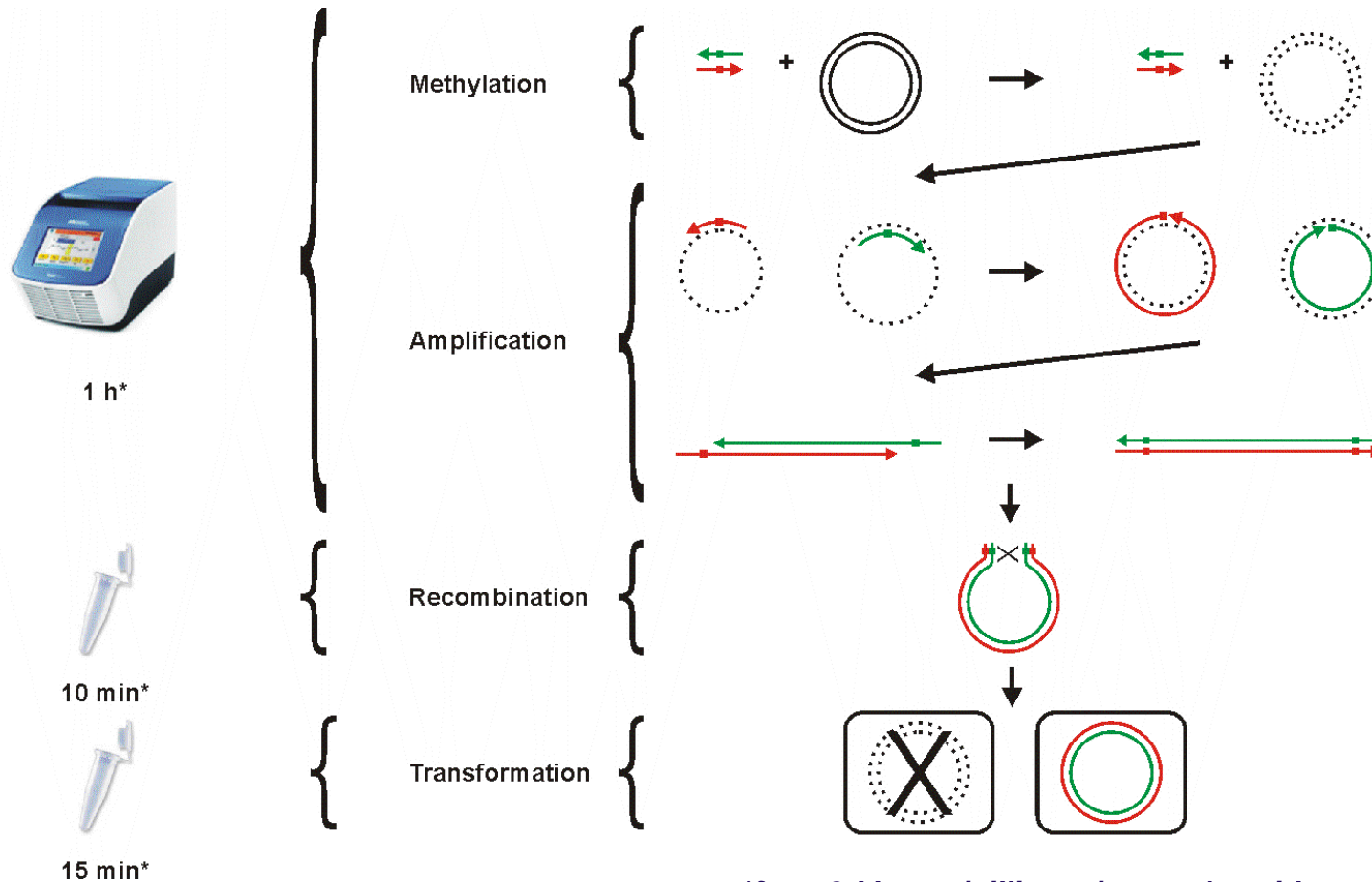


Micro-editing



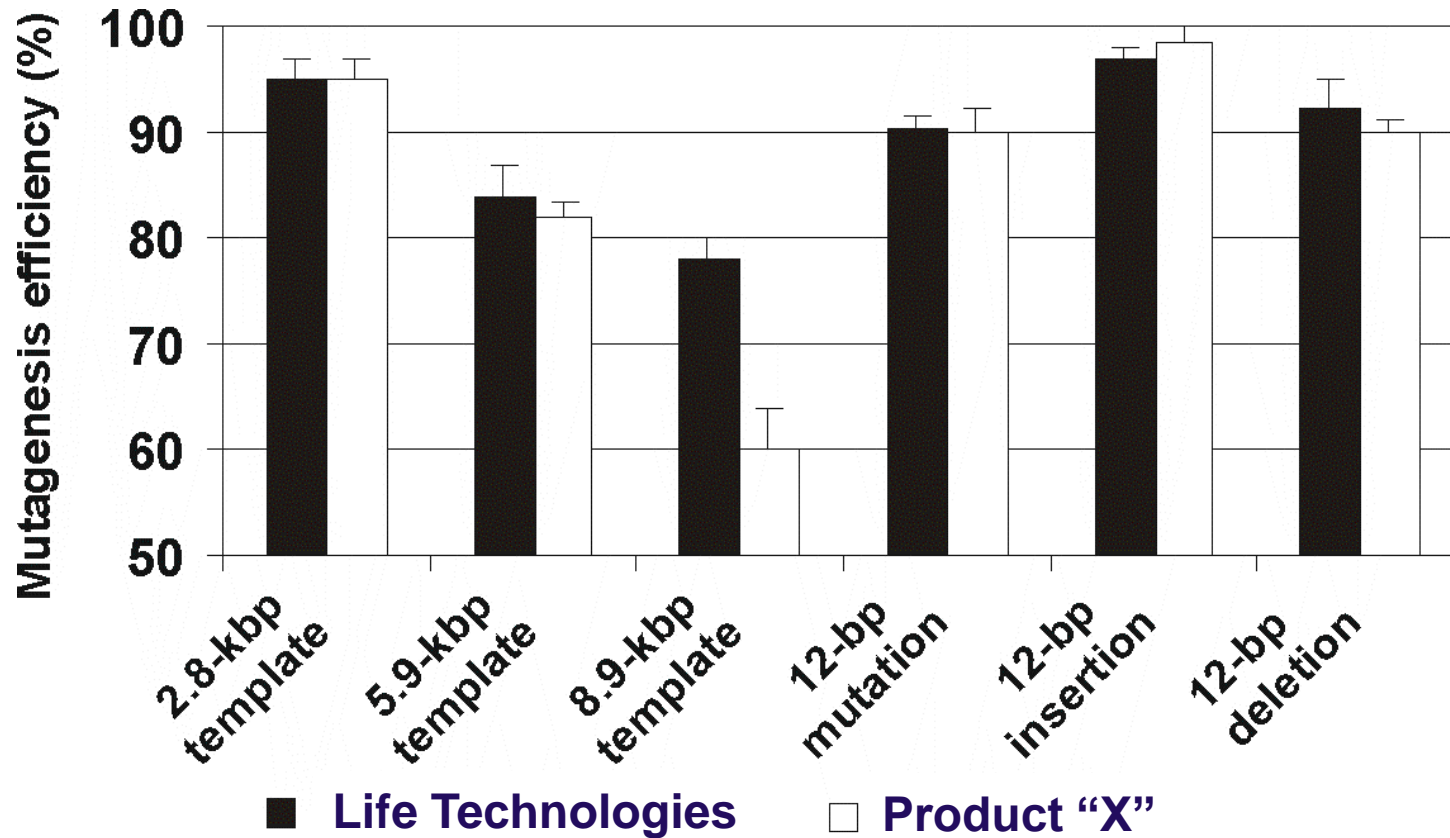
Micro-Editing: Improved Site-Directed Mutagenesis

GENEART[®] Site Directed Mutagenesis - Workflow



**for a 3-kb ampicillin resistant plasmid*

GENEART® Site Directed Mutagenesis: Performance



Assembly/Engineering Technologies for Synthetic Biology

Programming cells by multiplex genome engineering and accelerated evolution

Harris H. Wang^{1,2,3*}

Harnessing homologous recombination to generate recombinant DNA

Mamie Z Li & Stephen

Linker-Mediated Recombination of Large DNA Fragments

DNA assembly and construction of biochemical pathways

Christopher K. Raymond, El...

Zengyi Shao¹, Hua Zhao¹

Complete Chemical Synthesis, Assembly, and Cloning of a Mycoplasma genitalium Genome

One-step inactivation of Escherichia coli K-12 using PCR products

Kirill A. Datsenko and Barry L. Wanner*

Genomic islands and plasmosomal genes in Mycoplasma genitalium

Daniel G. Gibson, Gwynedd A. Benders, Cynthia Andrews-Pfannkoch, Evgeniya A. Denisova, Holly Baden-Tilson, Jayshree Zaveri, Timothy B. Stockwell, Anushka Brownley, David W. Thomas, Mikkel A. Algire, Chuck Merryman, Lei Young, Vladimir N. Noskov, John I. Glass, J. Craig Venter, Clyde A. Hutchison III, Hamilton O. Smith*

In vivo cloning

Jonathan D. Oliner
The Johns Hopkins University

A new logic for DNA engineering using recombination in Escherichia coli

Youming Zhang, Frank Buchholz, Joep P.P. Muyrers & A. Francis Stewart

Chemical synthesis of a mouse mitochondrial genome

Hamilton O Smith², J Craig Venter^{1,2} & J Craig Venter^{1,2}

Assembly of DNA molecules up to several hundred kilobases

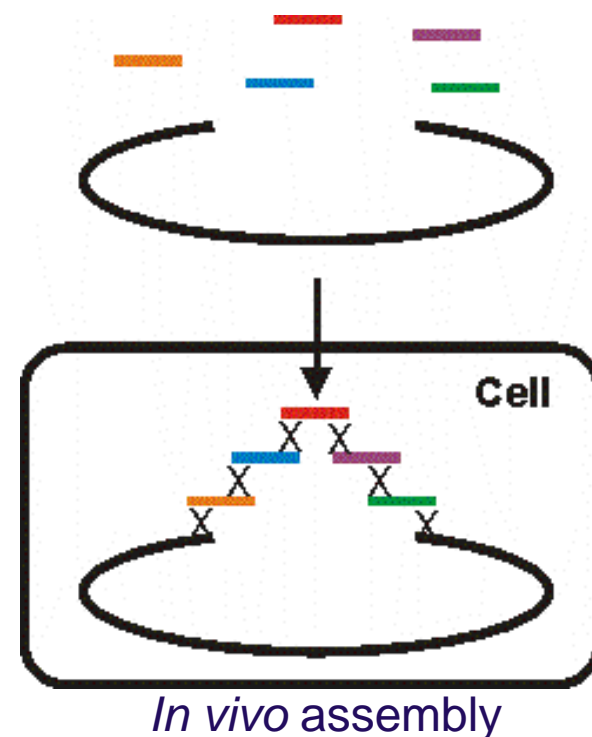
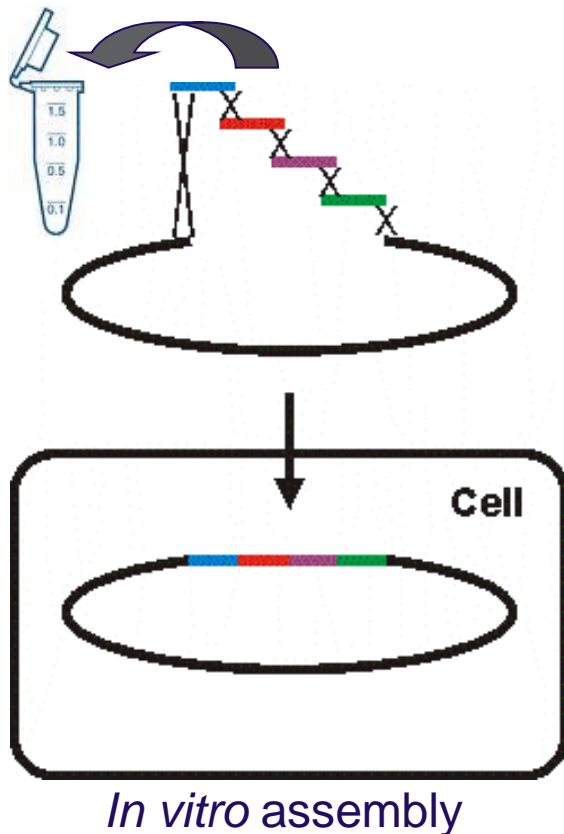
Daniel G. Gibson¹, Lei Young¹, Ray-Yuan Chuang¹, Clyde A Hutchison III² & J. Craig Venter^{1,2}

Transfer of genetic information from one species to another

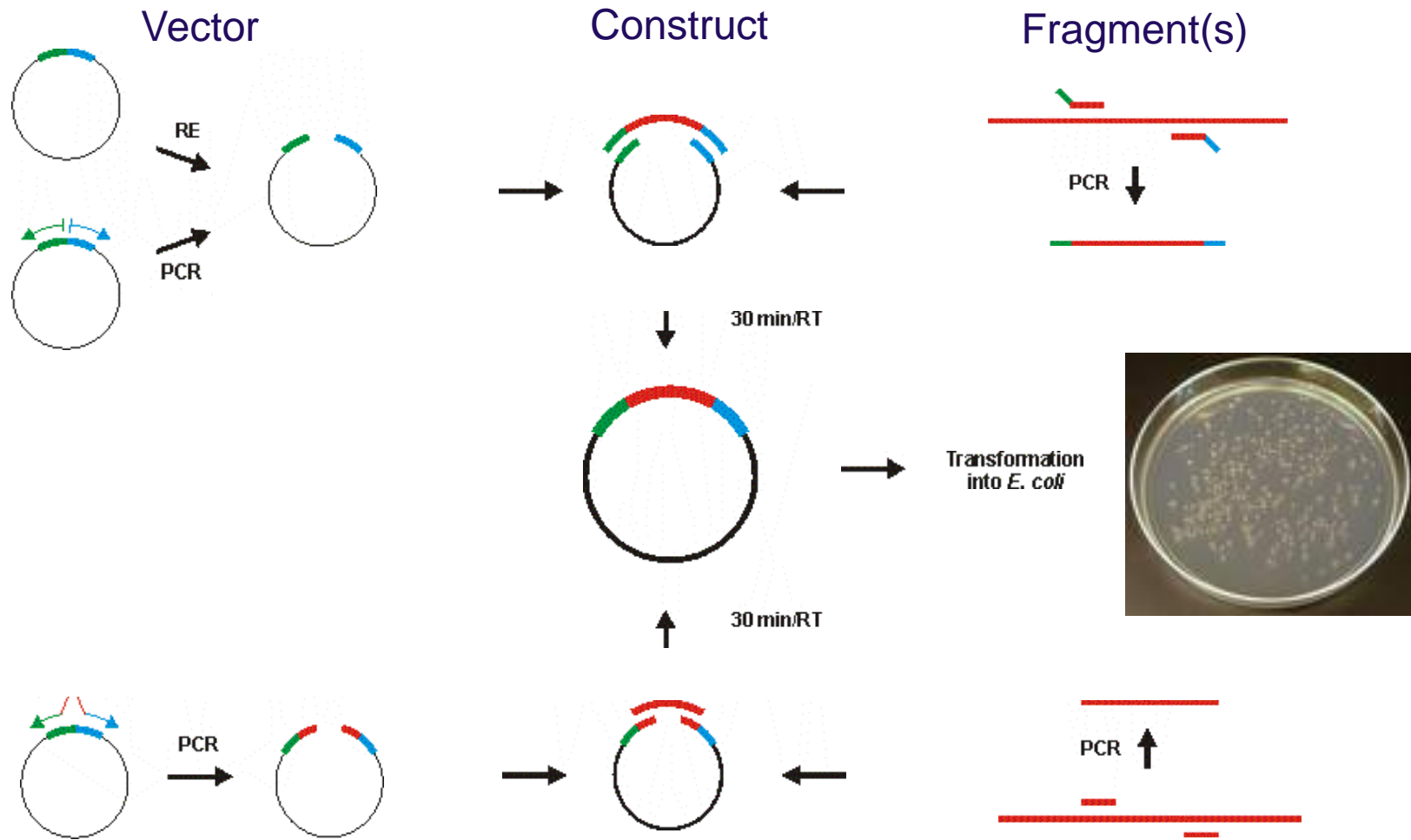
h, Rembert Pieper, Prashanth P. Parmar, Craig Venter

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

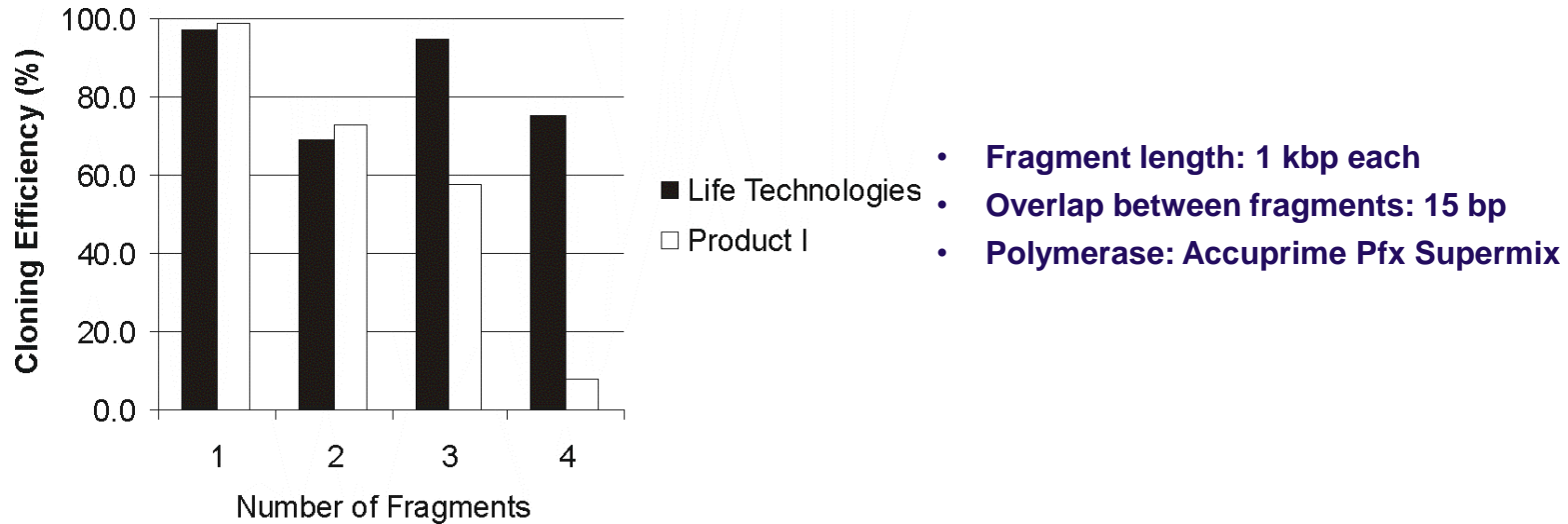
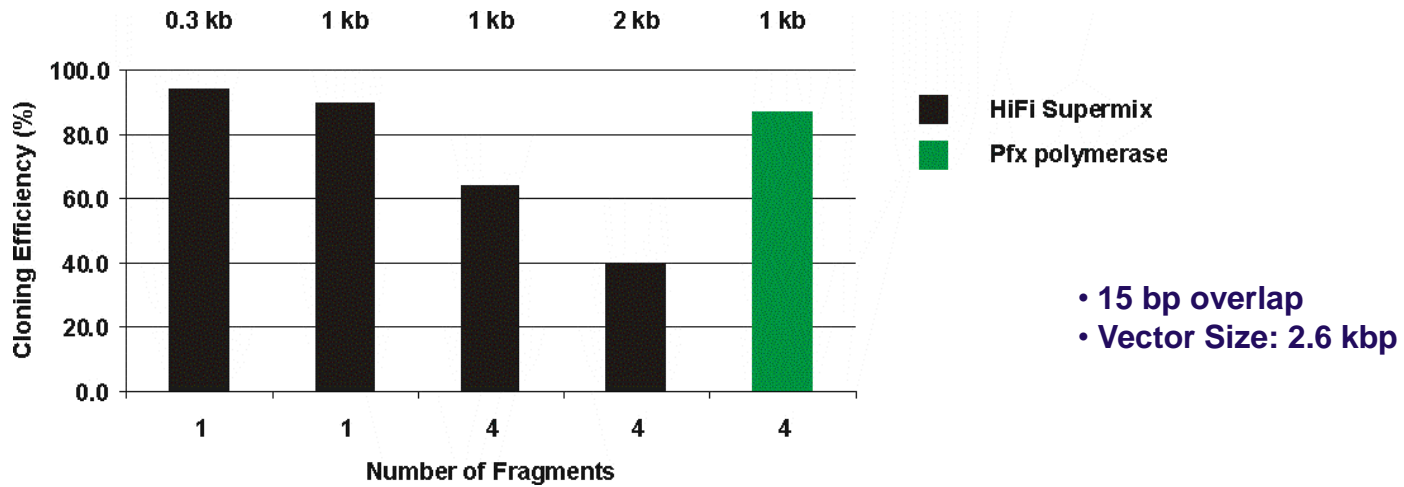


In Vitro Assembly Approach

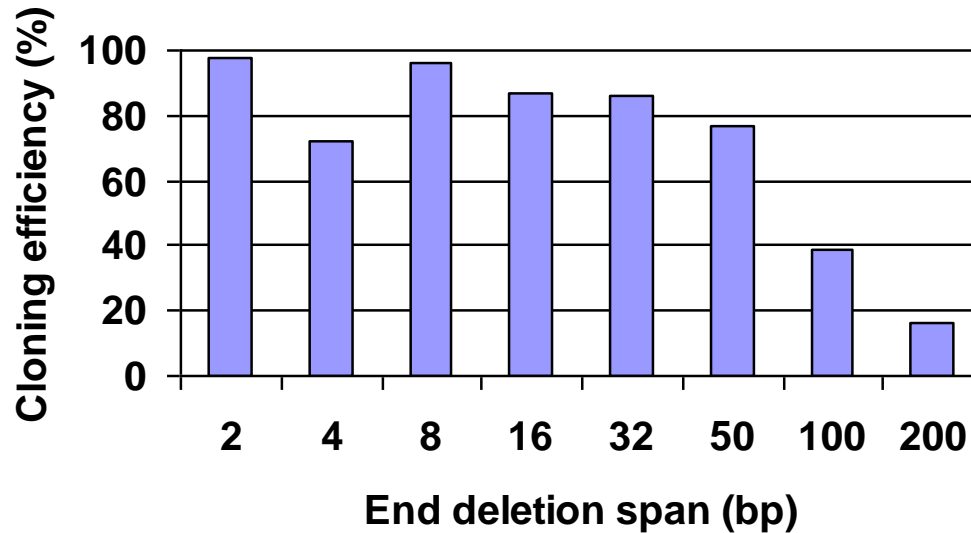
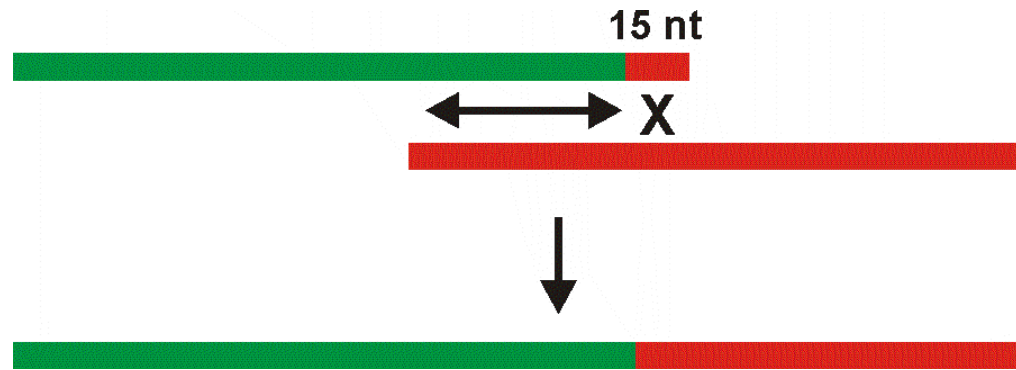


Only 15 bp of terminal homology required

Seamless Cloning: 4 Fragments into a Vector



In Vitro Recombination: End Deletions/Editing

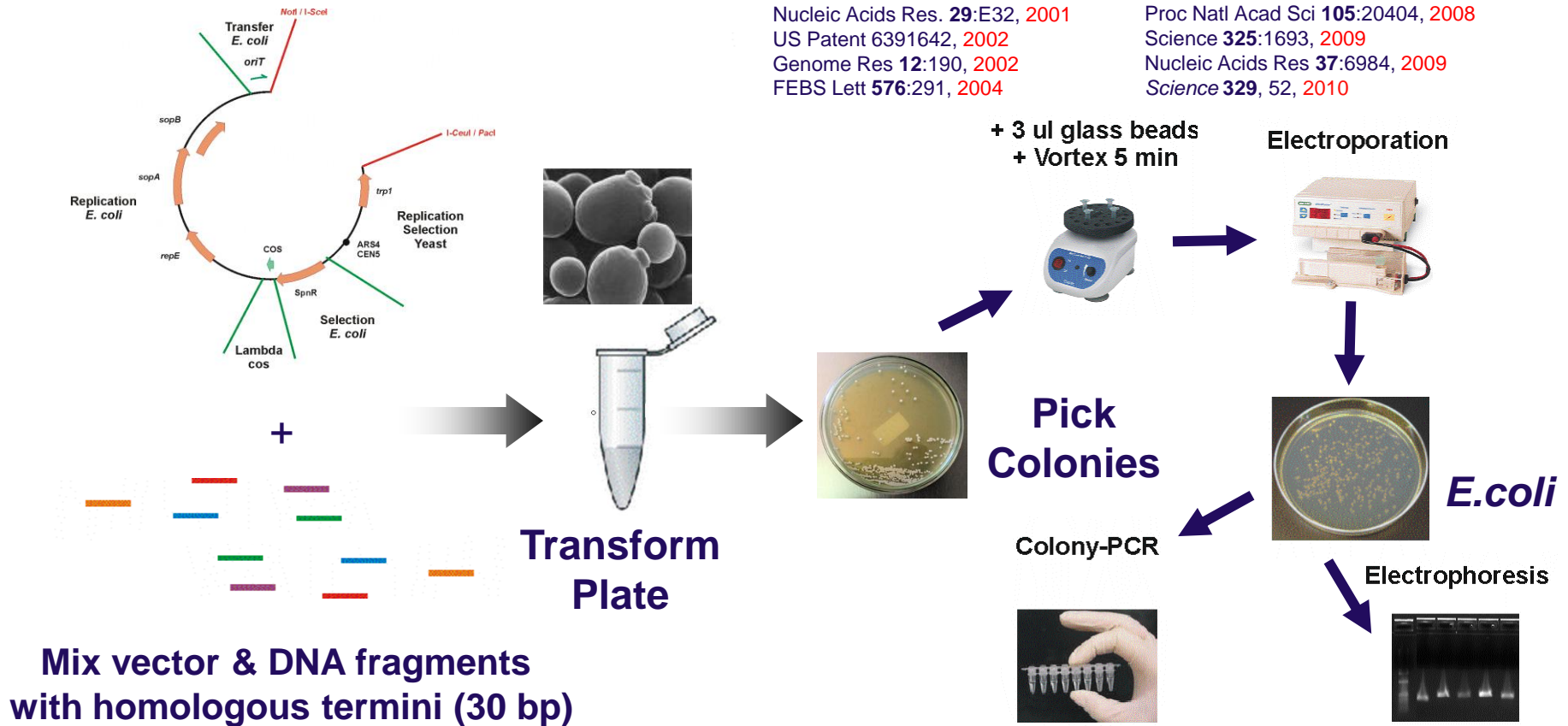


In Vivo Assembly Approach

Transformation-Associated Recombination (TAR) in *S.cerevisiae*

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



Multiple Fragment Assembly in Yeast

No fragments	No/size <u>preexisting</u> fragments	No/size <u>amplified</u> 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%

Bridging Oligos:

Perfect/Imperfect Junction Assembly

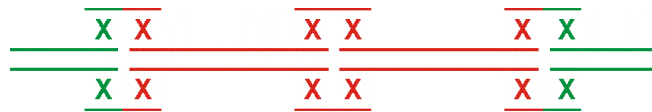
- No homology between adjacent fragments to be joined
- Homology provided in *trans* by designed bridge oligonucleotides
- Allows for reuse of fragments in a new sequence context
- Allows for junction editing

Perfect Junctions



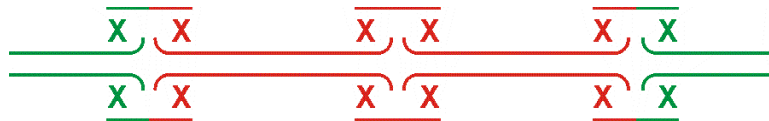
Oligo	Colony #	Cloning Efficiency
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60mer	2685	94%
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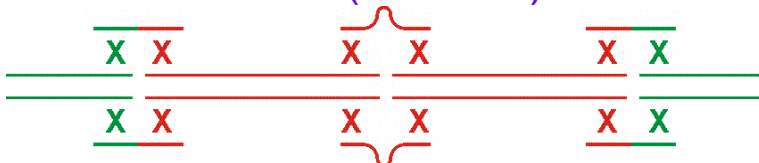
80mer	1240	75%
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Deletion Junctions (12 bases)



60mer	520	25%
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Insertion Junctions (30-X-30)



10 bp	460	63%
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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

Oligo Designer - High and low order DNA assembly

You have chosen invitro assembly type and circular product type. To continue, upload the sequences for your experiment one by one using the upload sequences button below and click the Next button. The first upload sequence will be a vector.

Warning! The following discrepancies were found in the input sequences if you still would like to continue, proceed by clicking the continue button at the bottom of this page:
The pre-existing fragments 2 and 3 do not have enough homology at the terminal ends
The pre-existing fragments 3 and 4 do not have enough homology at the terminal ends
The pre-existing fragments 4 and 5 do not have enough homology at the terminal ends

Fragment #	Fragment Type	Sequence	Upload Sequence	PCR
0	Vector	CCACAC CACACCCACACACCCACACAC CACACCCACACACCCACACACCCACAC	<input type="text"/>	<input type="checkbox"/>
1	AAD44166.2	CCACAC CACACCCACACACCCACACAC CACACCCACACACCCACACACCCACAC	<input type="text"/>	<input type="checkbox"/>
2	AAD44166.3	CCACAC CACACCCACACACCCACACAC CACACCCACACACCCACACACCCACAC	<input type="text"/>	<input type="checkbox"/>
3	AAD44166.4	CCACAC CACACCCACACACCCACACAC CACACCCACACACCCACACACCCACAC	<input type="text"/>	<input type="checkbox"/>
4	AAD44166.5	CCACAC CACACCCACACACCCACACAC CACACCCACACACCCACACACCCACAC	<input type="text"/>	<input type="checkbox"/>
5	AAD44166.5	CCACAC CACACCCACACACCCACACAC CACACCCACACACCCACACACCCACAC	<input type="text"/>	<input type="checkbox"/>

Oligo Designer - High and low order DNA assembly

This Oligo Designer tool facilitates the design of a construct in silico with up to four fragments of interest and a vector for an invitro assembly. The final construct after the input will be displayed if the input fragments sequences comply with the rules.

Designed Construct & Primers

Insert
 Primer
 Vector

Construct 2009 bp

www.invitrogen.com/DNAassembly

Assembly Technologies Summary

In vitro DNA assembly

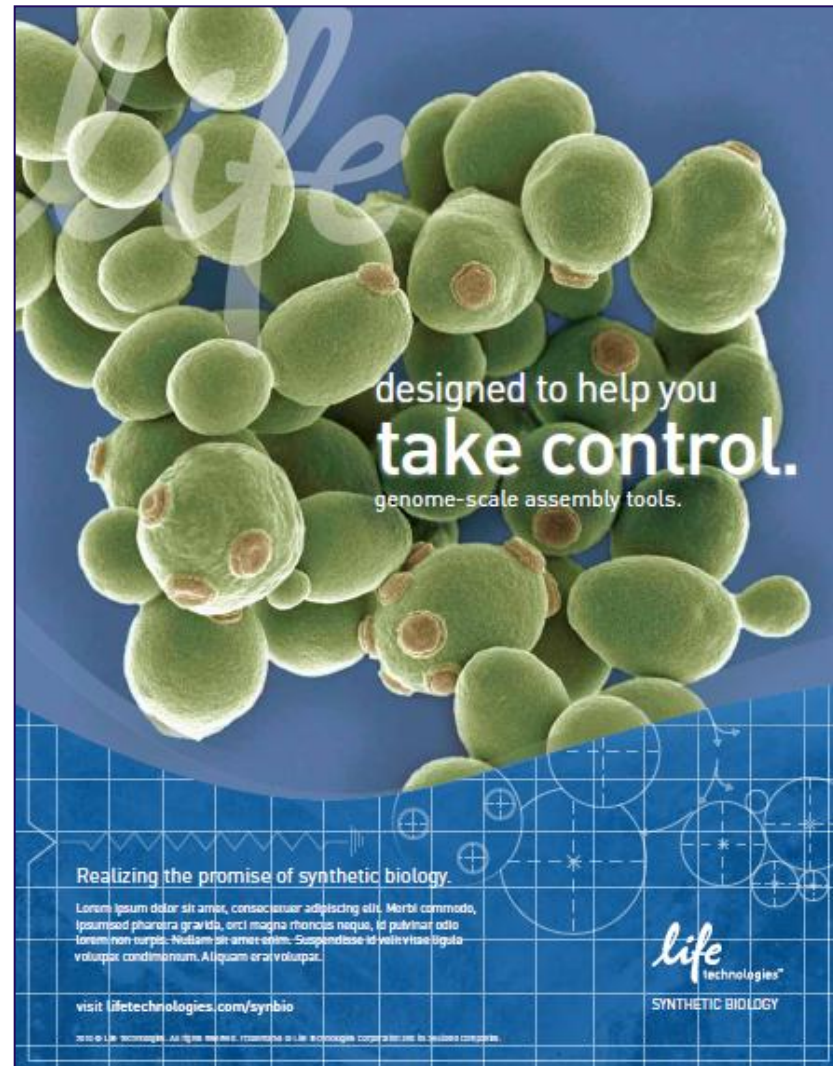
- Up to 4 fragments plus vector
- Seamless (no scars)
- Terminal homology, 15 bp overlaps
- Unmodified oligos/primers
- Constructs up to 15 kb
- Vector-independent
- Isothermal
- 30 minute
- Single tube reaction
- High-fidelity DNA polymerase
- Kitted controls & comp *E. coli* cells
- Web design tools

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

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



The advertisement features a blue background with a cluster of green, textured spheres resembling grapes or berries. A large, faint 'Life' logo is visible in the upper left. The text 'designed to help you take control.' is prominently displayed in white, with 'take control.' in a larger font. Below it, 'genome-scale assembly tools.' is written in a smaller font. At the bottom, there is a grid pattern with various symbols and the text 'Realizing the promise of synthetic biology.' followed by a paragraph of placeholder text. The Life Technologies logo and 'SYNTHETIC BIOLOGY' are in the bottom right corner. A small URL 'visit lifetechnologies.com/synbio' is at the bottom left.

designed to help you
take control.
genome-scale assembly tools.

Realizing the promise of synthetic biology.

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visit lifetechnologies.com/synbio

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SYNTHETIC BIOLOGY