



# Genetic Synthesis and Assembly Tools for Synthetic Biology

PacRim BioEnergy Conference  
Engineering Biological Systems Workshop  
Renewable Chemicals and Metabolic Engineering Track

Honolulu, HI  
December 13, 2010

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VP  
Genomics Technologies R&D

# Presentation Overview

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

# 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**

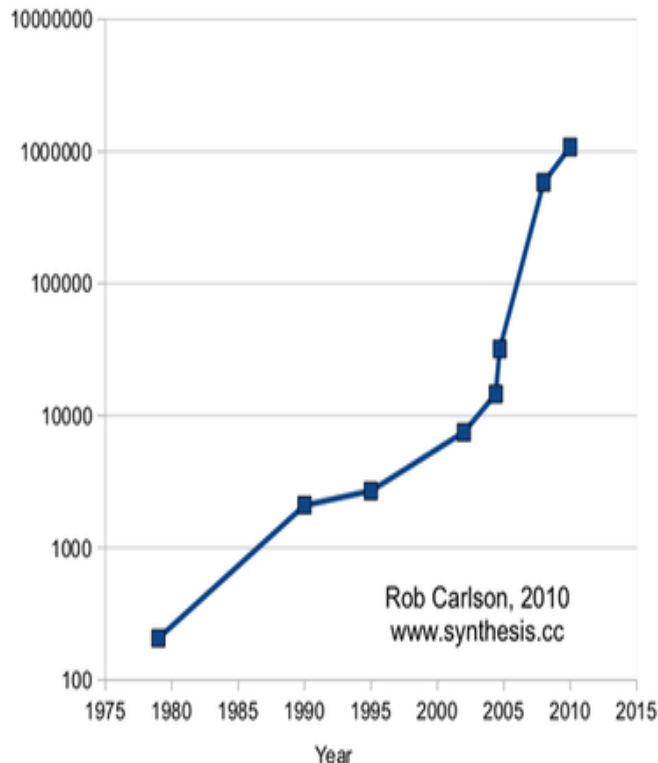


# Technology Drivers & Synthetic Biology

## 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

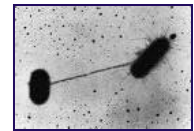
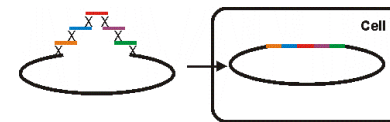
Bioinformatics  
Systems and  
Computational Biology

Industrial Microbiology  
Chemical Engineering  
Fermentation Science

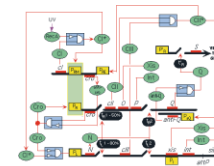
## Synthetic Biology

Standardized Genetic  
Parts/Devices

Engineered Hosts



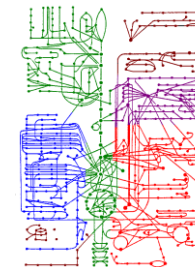
Gene/Chromosome Assembly/Transfer Tools



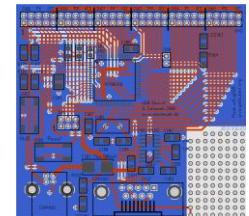
Engineered  
Genetic Circuits



Predictive  
BioCAD/FAB  
Optimized Applications

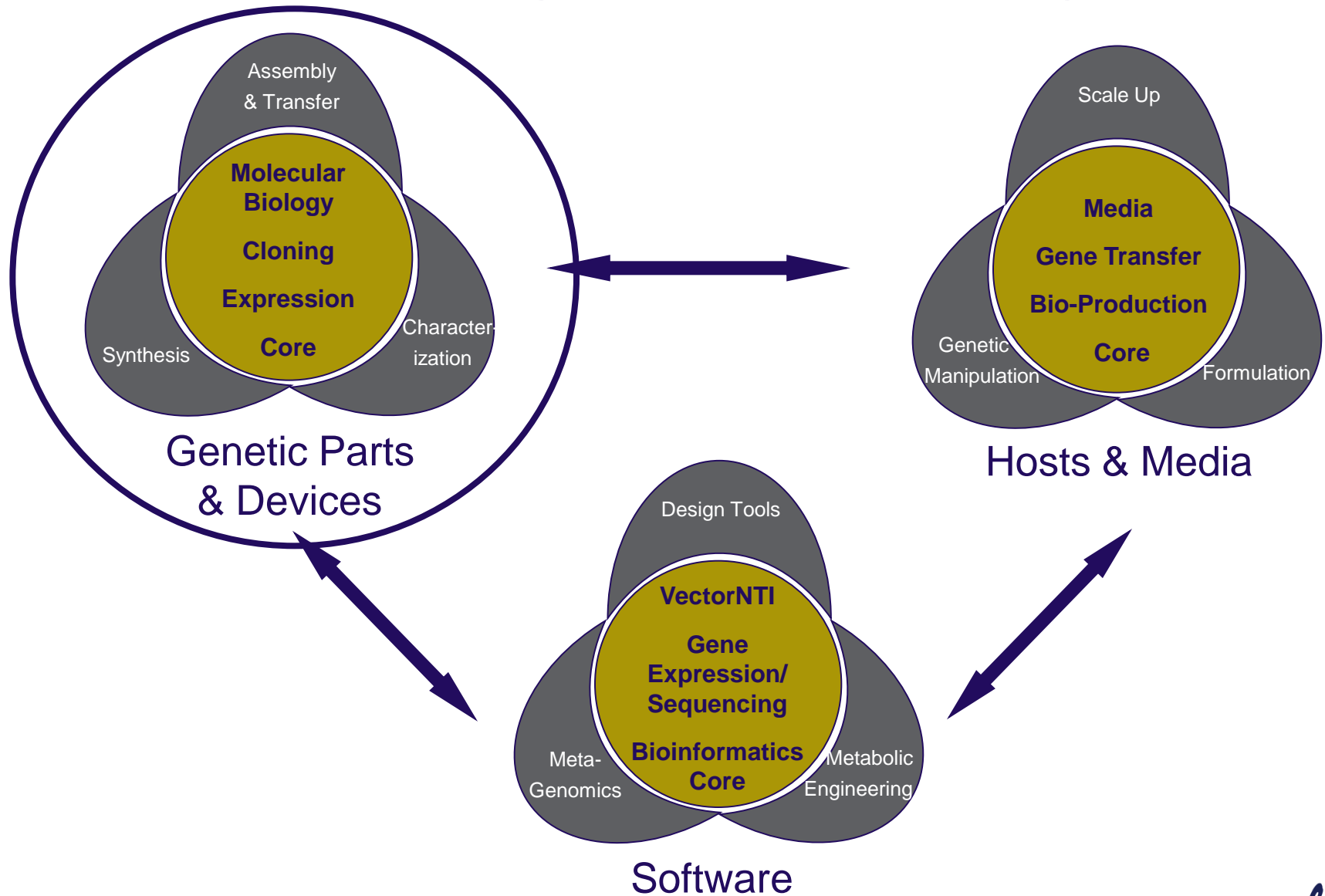


Genetic/Biochemical  
Pathways



Design Engineering

# 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



## 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

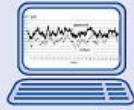
Anticipate acquisition will be completed early Q1 2011



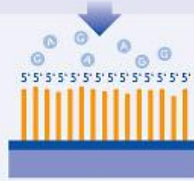


# Technology and Process Overview

Bioinformatics



Oligosynthesis



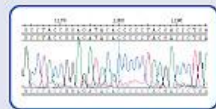
Gene Assembly



Cloning



Sequencing



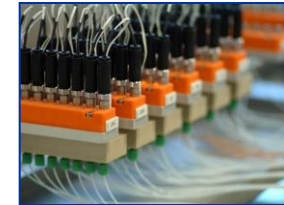
Final Documentation



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



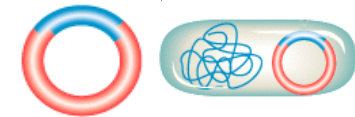
- Nano scale synthesis
- High throughput/quality



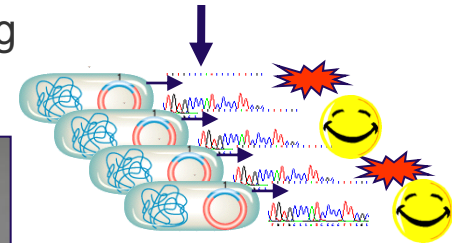
- Several approaches
- High throughput
- Up to 2.0 kb/fragment



- High throughput
- Semi-automated



- automated CE sequencing
- automated alignment



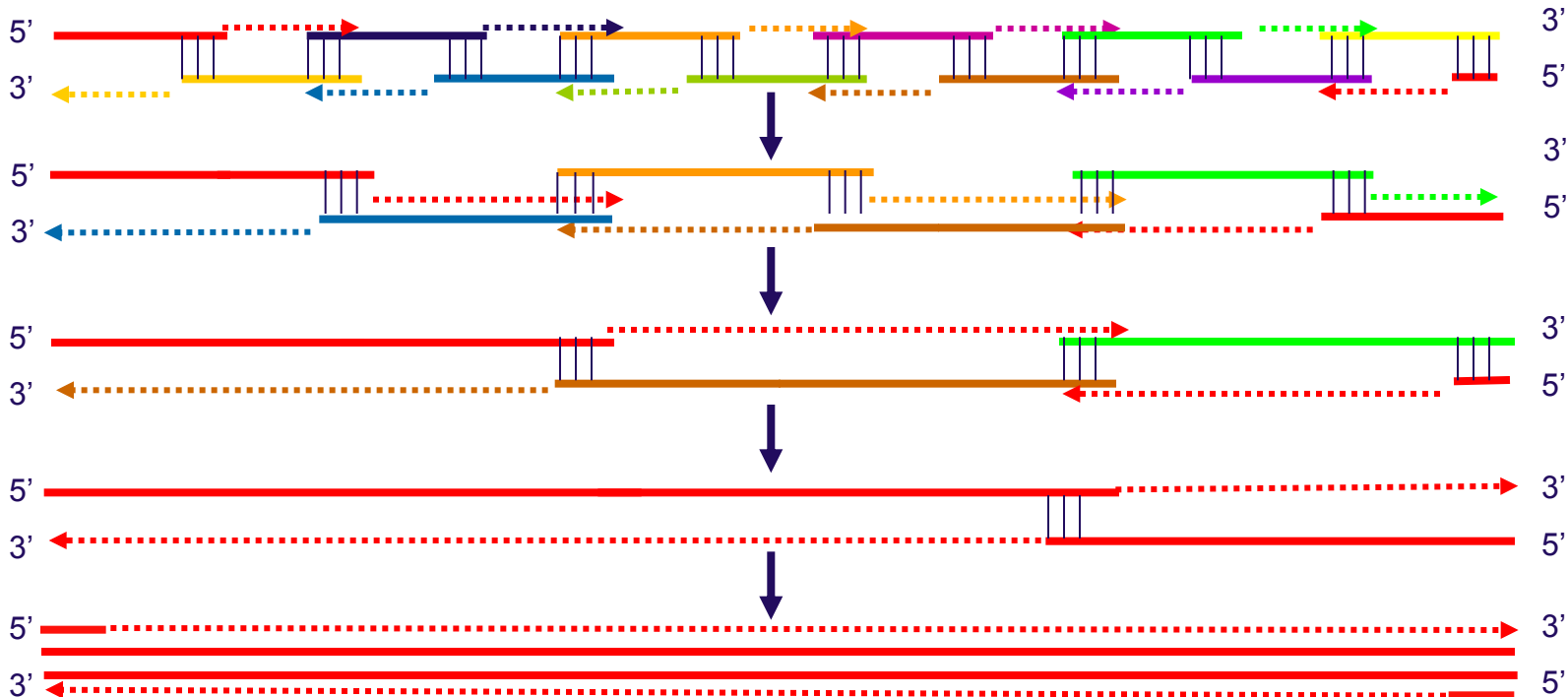
- Product report
- Ship overnight
- ISO 9001



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



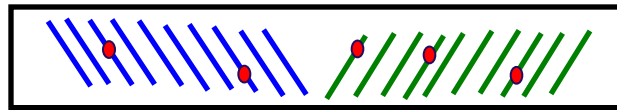
# Gene (and Larger) Synthesis Considerations

- 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

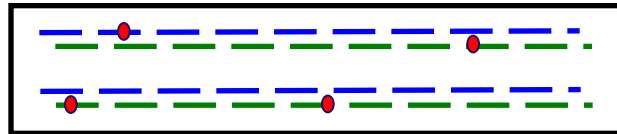
**Life Technologies Signs Exclusive License Agreement for DNA Error-Correction Technology from Novici Biotech LLC**  
October 11, 2010



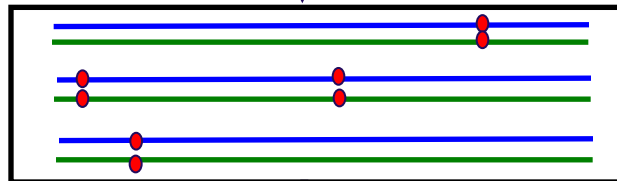
# ErrASE Synthetic Gene Assembly Method



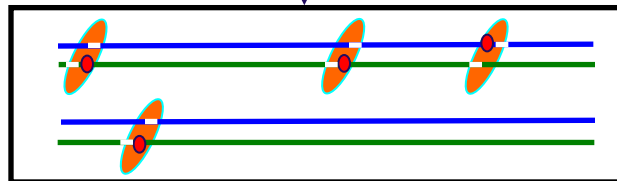
Oligos designed & mixed.  
Some oligos have errors.



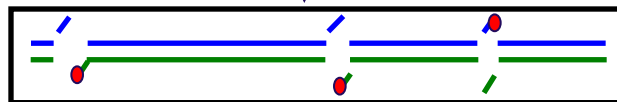
PCR assembled 1st round



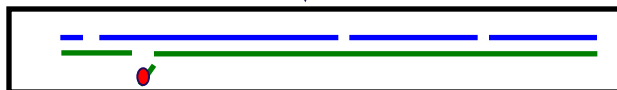
PCR assembled 2nd round  
(Can also use assembly by ligation)



DNA denatured & reannealed  
Errase nicks at errors



HiFi Polymerase exonuclease  
blunts ends, removing errors



Long "corrected oligos" are PCR re-  
assembled in 3<sup>rd</sup> 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

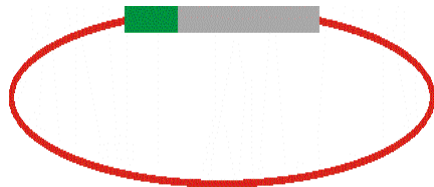
# Genetic Assembly & Editing



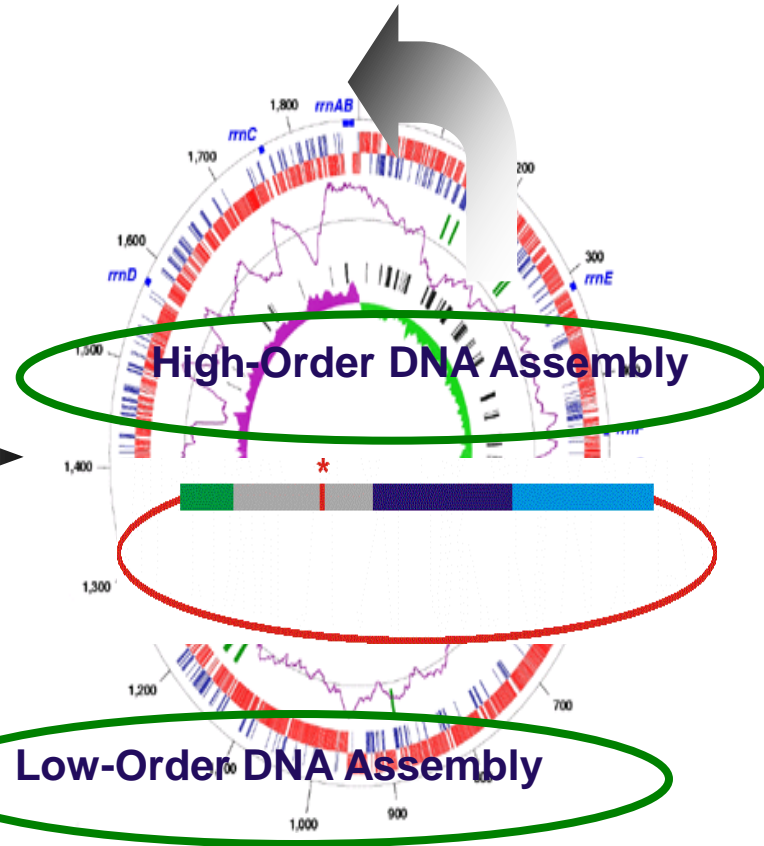
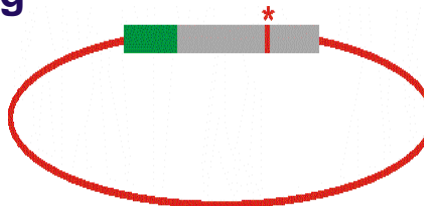
**GENEART**  
THE GENE OF YOUR CHOICE

Gateway<sup>®</sup> Recombination Cloning

TOPO<sup>®</sup>



Micro-editing



# Assembly/Engineering Technologies for Synthetic Biology

Programming cells by multiplex genome engineering and accelerated evolution

Harris H. Wang<sup>1,2,3\*</sup>

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

Zengyi Shao<sup>1</sup>, Hua Zhao<sup>1</sup>

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\*

A new logic for DNA engineering using recombination in *Escherichia coli*

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

In vivo cloning

Jonathan D. Oliner  
The Johns Hopkins University

Chemical synthesis of mouse mitochondrial genome

Hamilton O Smith<sup>2</sup>, J Craig Venter<sup>1,2</sup> &

Assembly of molecules up to several hundred kilobases

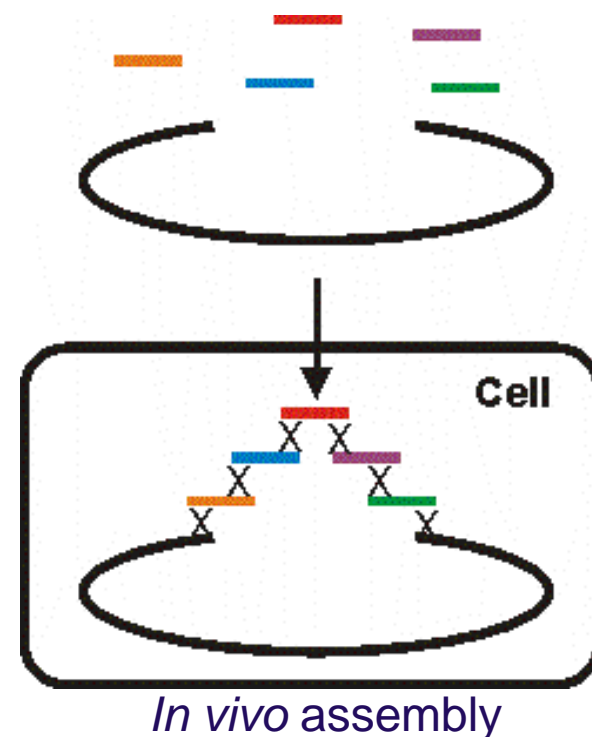
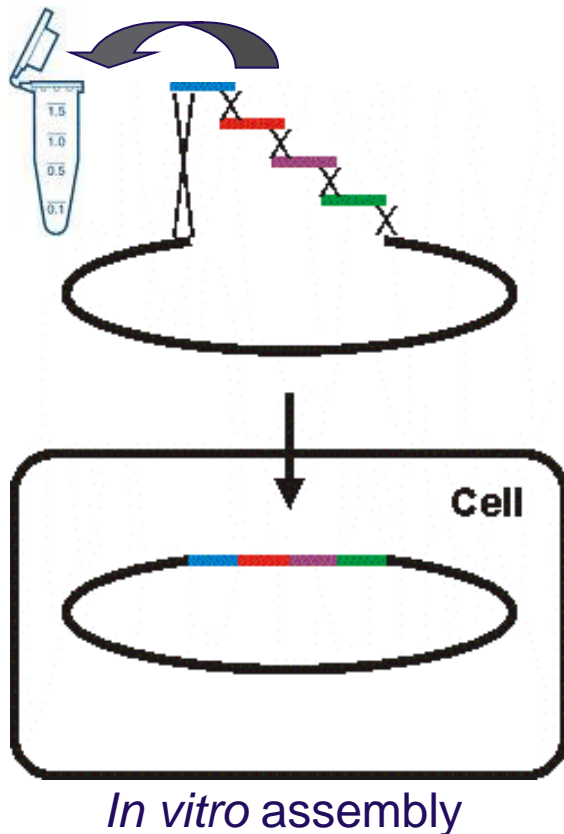
Daniel G. Gibson<sup>1</sup>, Lei Young<sup>1</sup>, Ray-Yuan Chuang<sup>1</sup>, Clyde A. Hutchison III<sup>2</sup> &

Transfer of genetic information from one bacterium to another

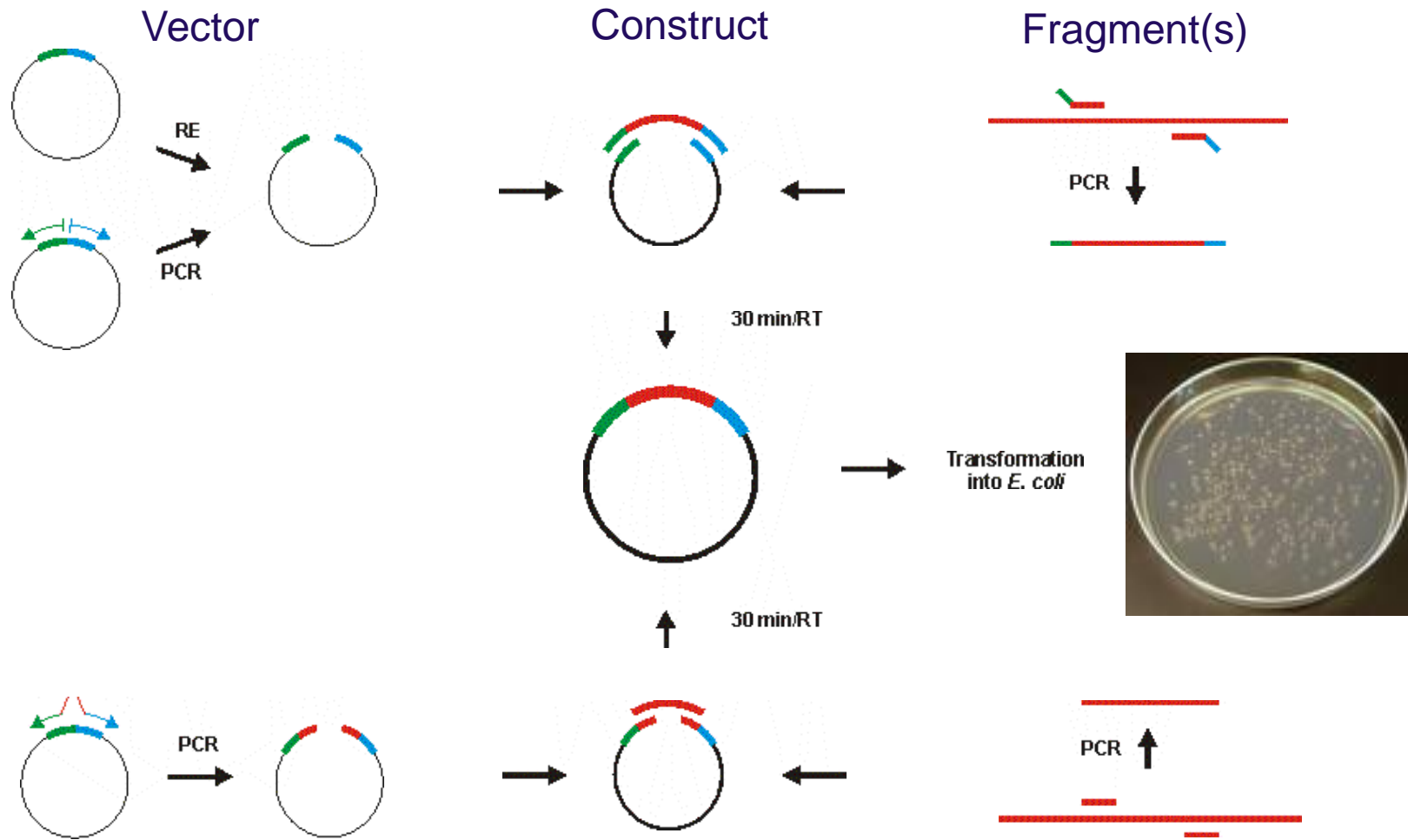
h, Rembert Pieper, Prashanth P. Parmar, Craig Venter

# 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



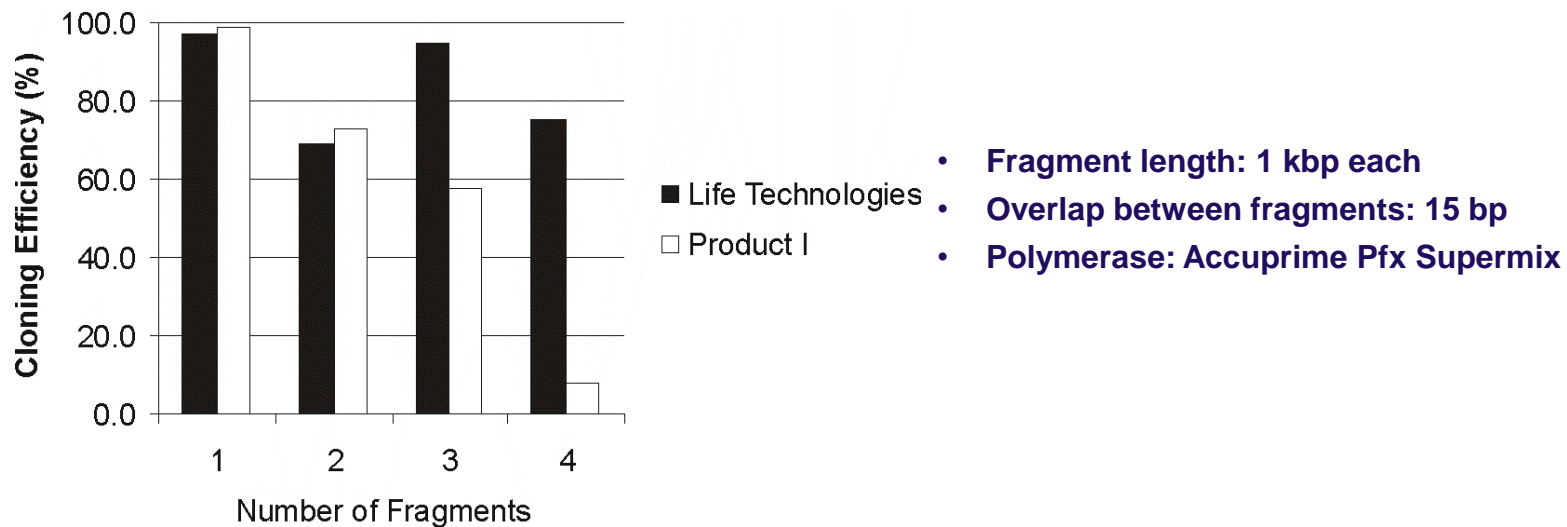
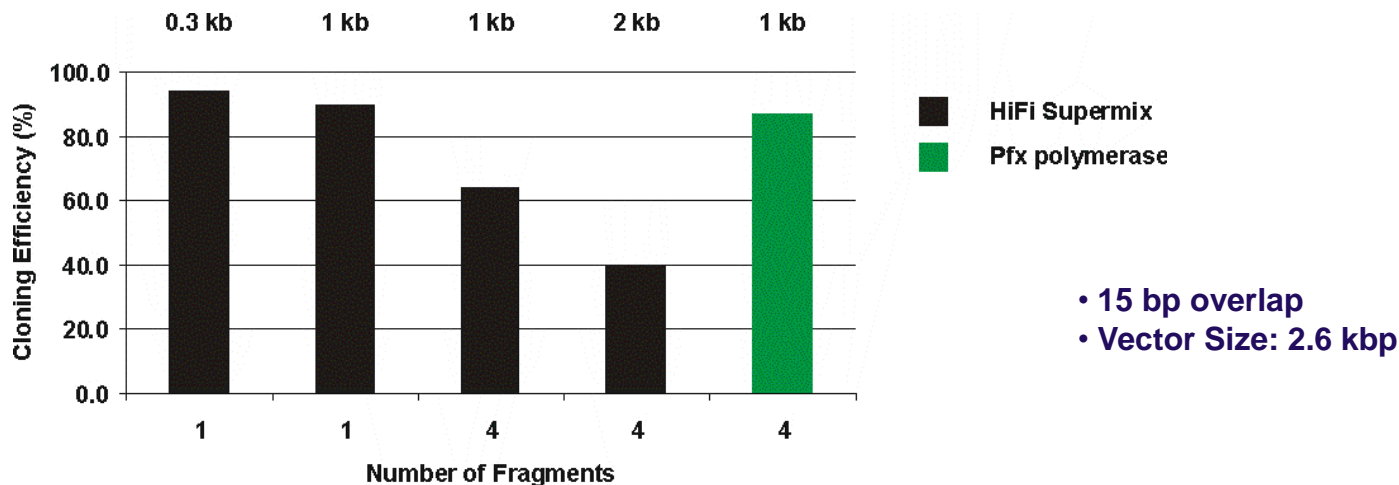
# *In Vitro* Assembly Approach



Only 15 bp of terminal homology required



# Seamless Cloning: 4 Fragments into a Vector

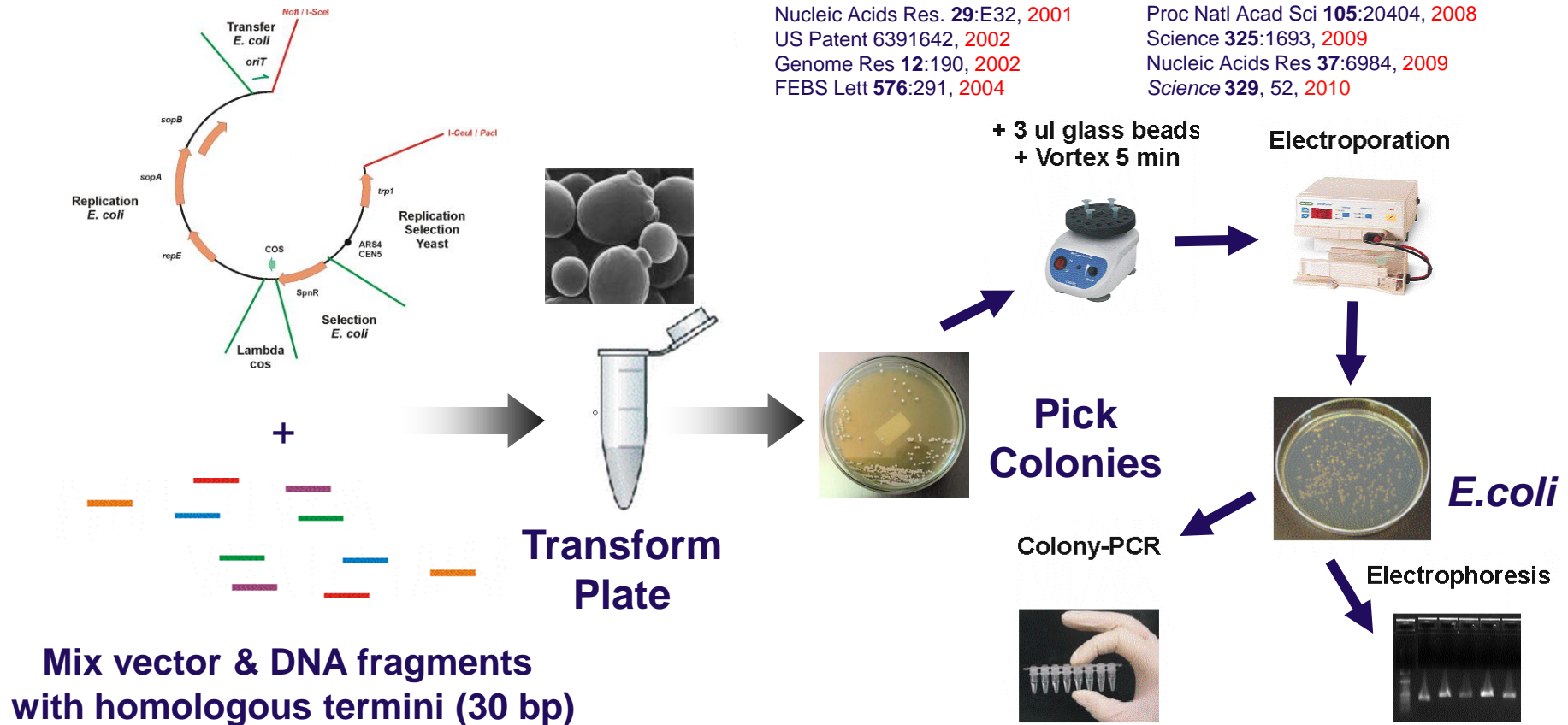


# In Vivo Assembly Approach

## 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



# 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

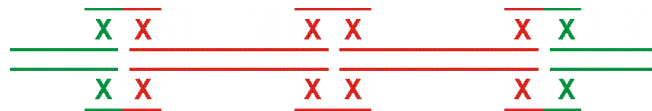
- No homology between adjacent fragments to be joined
- Homology provided in *trans* by designed bridge oligonucleotides
- Allows for reuse of fragments in a new sequence context
- Allows for junction editing

### Perfect Junctions



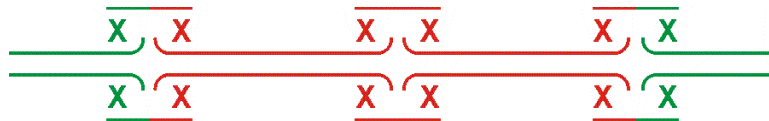
Oligo	Colony #	Cloning Efficiency
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60mer	2685	94%
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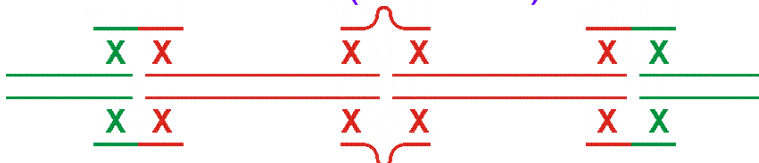
80mer	1240	75%
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### Deletion Junctions (12 bases)



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

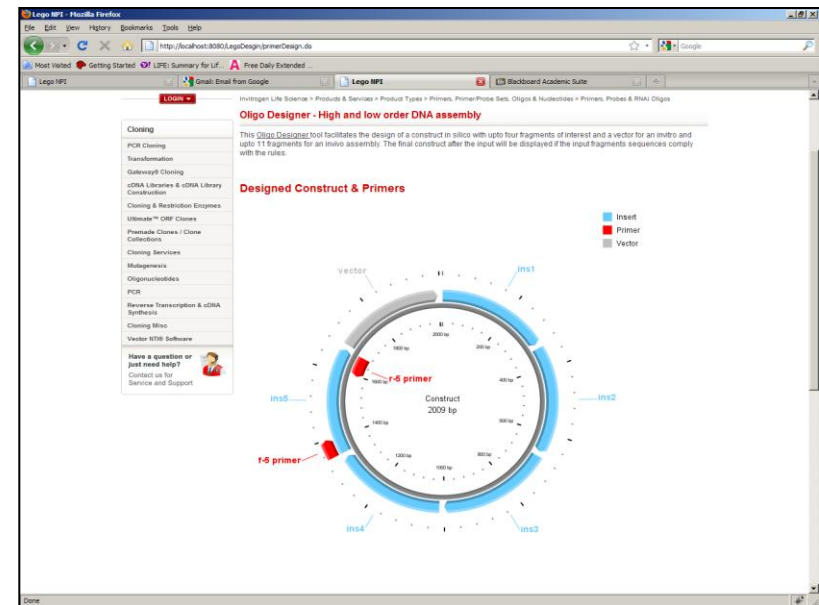
**Oligo Designer - High and low order DNA assembly**

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	CCACACACACCCACACACCCACACAC CAGACACACACACACACACACACGACA	<input type="text"/> <input type="button" value="Browse"/>	<input type="checkbox"/>
1	[A]44166.2	CCACACACACCCACACACCCACACAC CAGACACACACACACACACACACGACA	<input type="text"/> <input type="button" value="Browse"/>	<input type="checkbox"/>
2	[A]44166.3	CCACACACACCCACACACCCACACAC CAGACACACACACACACACACACGACA	<input type="text"/> <input type="button" value="Browse"/>	<input type="checkbox"/>
3	[A]44166.4	CCACACACACCCACACACCCACACAC CAGACACACACACACACACACACGACA	<input type="text"/> <input type="button" value="Browse"/>	<input type="checkbox"/>
4	[A]44166.5	CCACACACACCCACACACCCACACAC CAGACACACACACACACACACACGACA	<input type="text"/> <input type="button" value="Browse"/>	<input type="checkbox"/>
5	[A]44166.5	CCACACACACCCACACACCCACACAC CAGACACACACACACACACACACGACA	<input type="text"/> <input type="button" value="Browse"/>	<input type="checkbox"/>



[www.invitrogen.com/DNAassembly](http://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
- **Micro-Editing/Site Directed Mutagenesis**
  - Xiquan Liang
- ***In Vitro* Assembly**
  - Billyanna Tsvetanova
  - Federico Katzen
- ***In Vivo* Assembly**
  - Lansha Peng
  - Ke Li

