Design, Synthesis, Assembly Technologies for Synthetic Biology Engineering

Metabolic Design Summit 2011

Del Mar, California
May 25, 2011

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VP, Synthetic Biology R&D

Presentation Overview

- Synthetic Biology
  - Definition
  - Integrated technologies
- Bioinformatics and Design
  - "Omics" and NGS → Models
  - Engineering tools and workflows
- Gene synthesis
  - Process, design/optimization, industrial scale
- 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

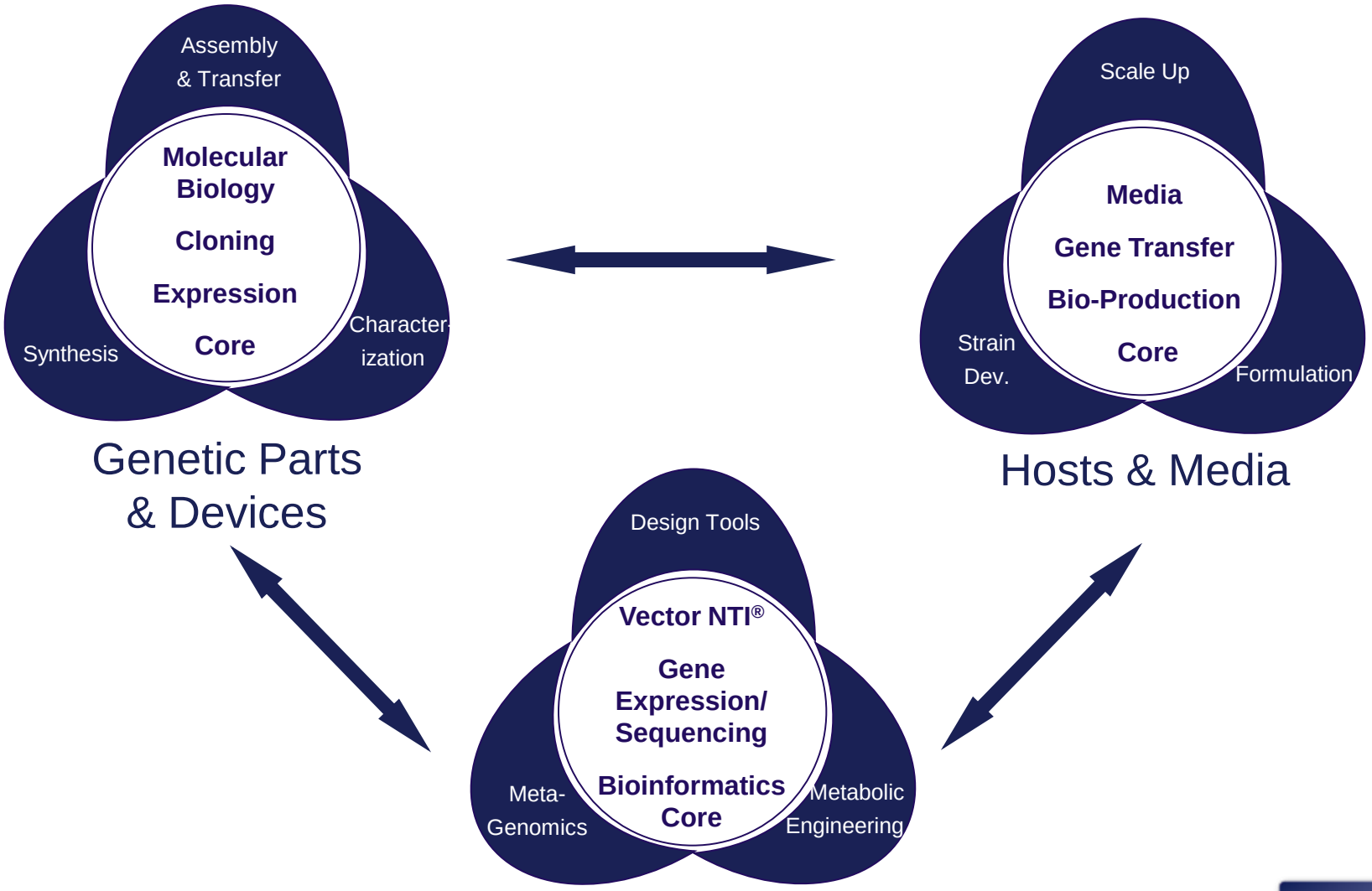
Energy

Chemicals

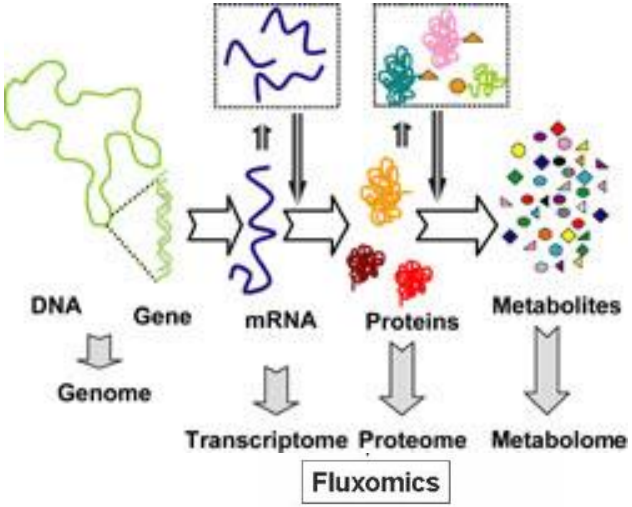
Agriculture

Bio-Remediation

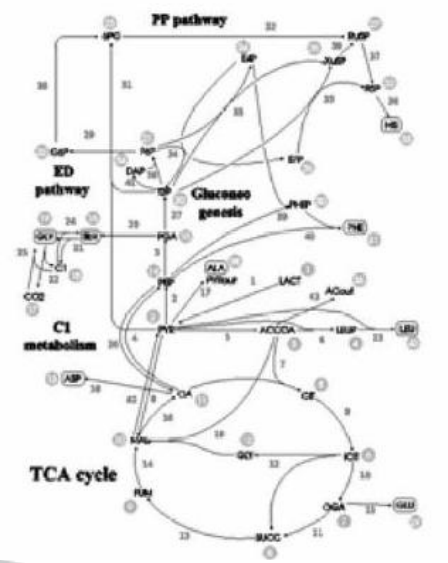
Integrating Synthetic Biology Elements



Synthetic Biology Systems Characterization



functional output of combined omics



- System “omics” characterization
- Anticipate production conditions
- Model/understand the system
- Prioritize candidates for modification
- Identify best methods for modification
- Manage design & revision cycles
- Analytics to verify/iterate designs
- Scale/optimize
- Validate

NGS Analysis Engine

Modified PGM™ Sequencer

5500 SOLiD™ System or
5500xl SOLiD™ System

.XSQ File

1 file/lane

LifeScope™ Genomic Analysis Software
Mapping/Pairing Engine

SAET

Improved .XSQ File

Mapping

Pairing

Mapped reads

maToBAM

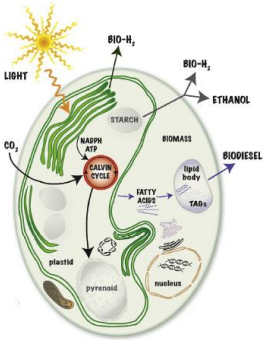
.BAM File

LifeScope™ Genomic Analysis Software
Analysis, Viewing, Reporting

SNP	Coverage
Indel	Exon Counting
CNV	Junction Finder
Inversion	Fusion Transcripts

Export results for additional viewing and analysis

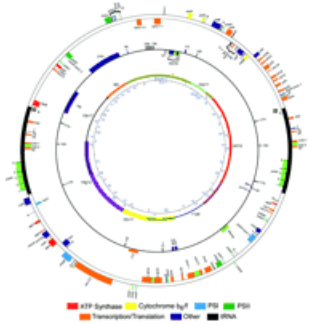
Primary Analysis	Secondary Analysis	Tertiary Analysis
Imaging & Conversion to Color or Base Space (on-instrument)	Mapping Reads to References (off-instrument)	Finding Biological Meaning (off-instrument)



Genomic DNA

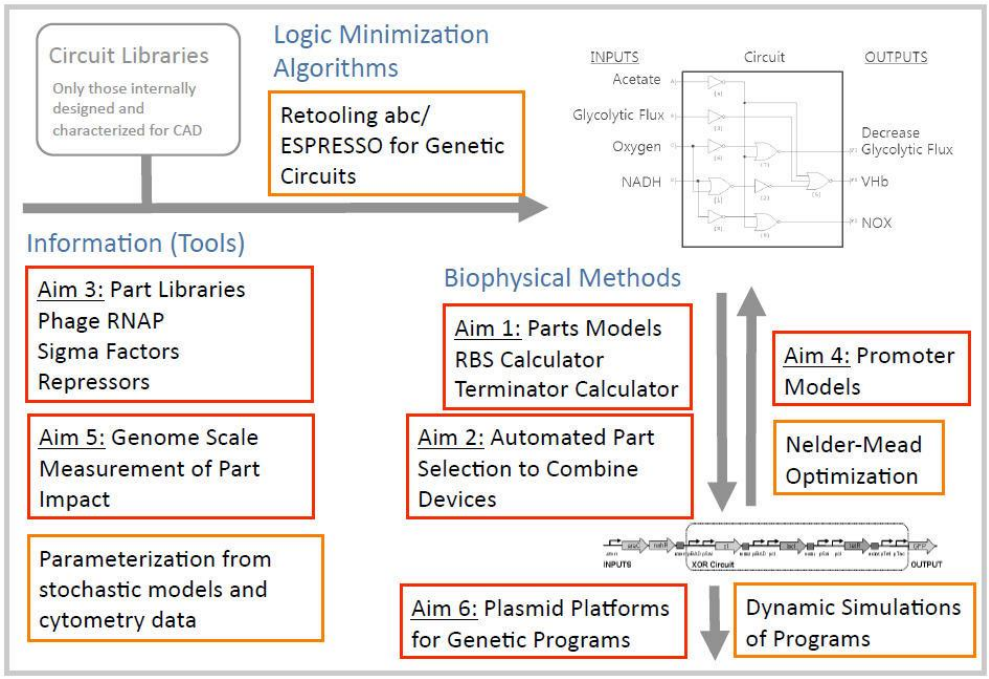
Transcript RNA

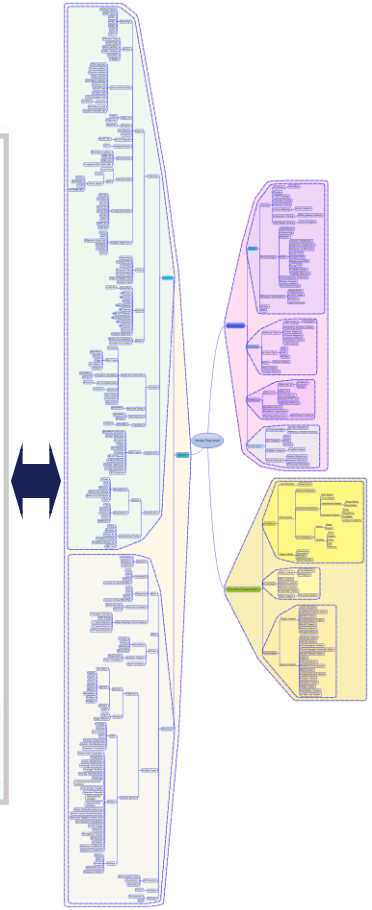
Hybrid Assembly



Synthetic Biology Design and Simulation Tools
SRA with the Voigt Lab

- Genetic design algorithms
- Development of design SW tools
- Emphasis on design automation/scale
- Circuit simulation
- Characterized libraries of parts
- Design SW modules interfaced with VNTI

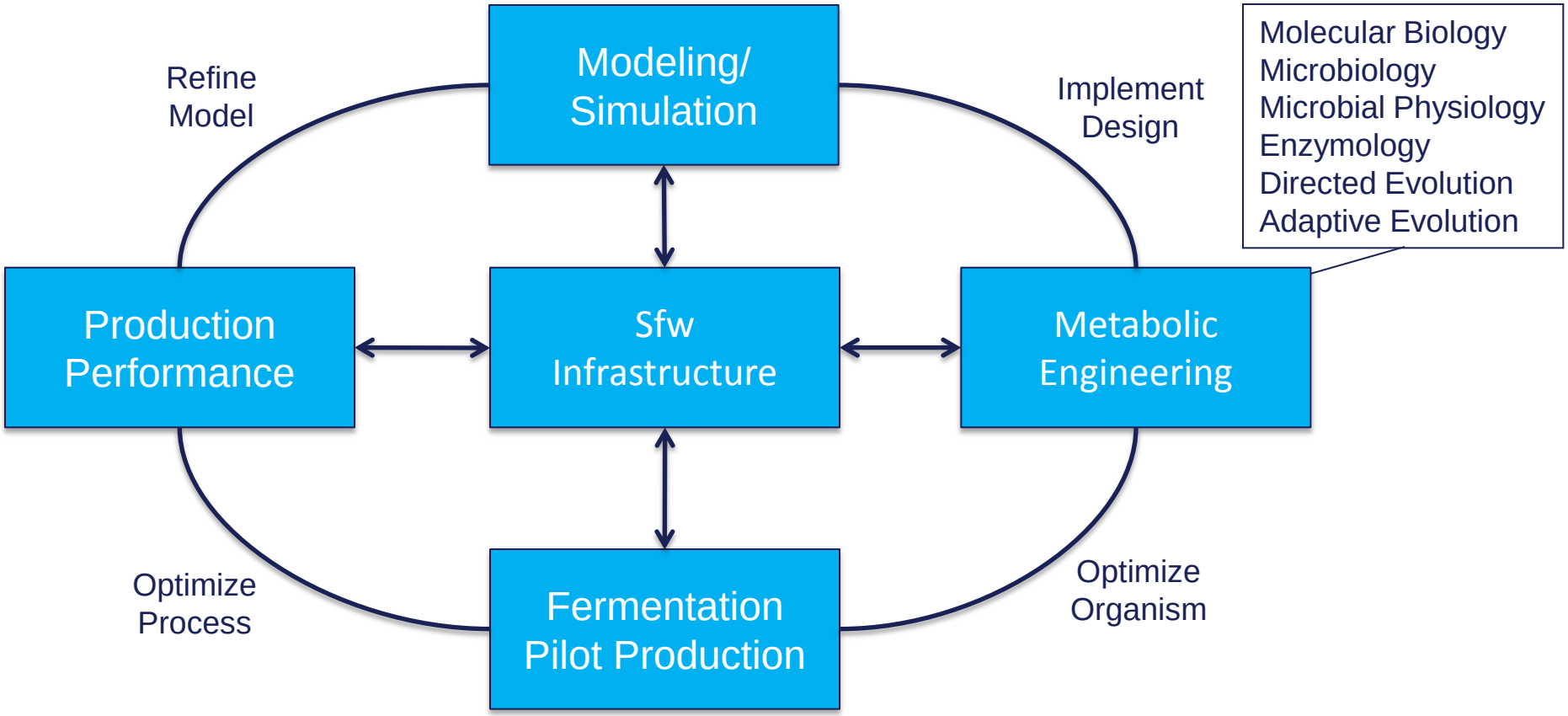




UCSF

Integrating Design Engineering Workflows

From Models to Engineered Strains



Modelers, Molecular Biologists, Fermentation Engineers Together Early and Throughout Project is Key to Success

Gene Synthesis:
Foundational Technology and Capability for Synthetic Biology

Key Considerations

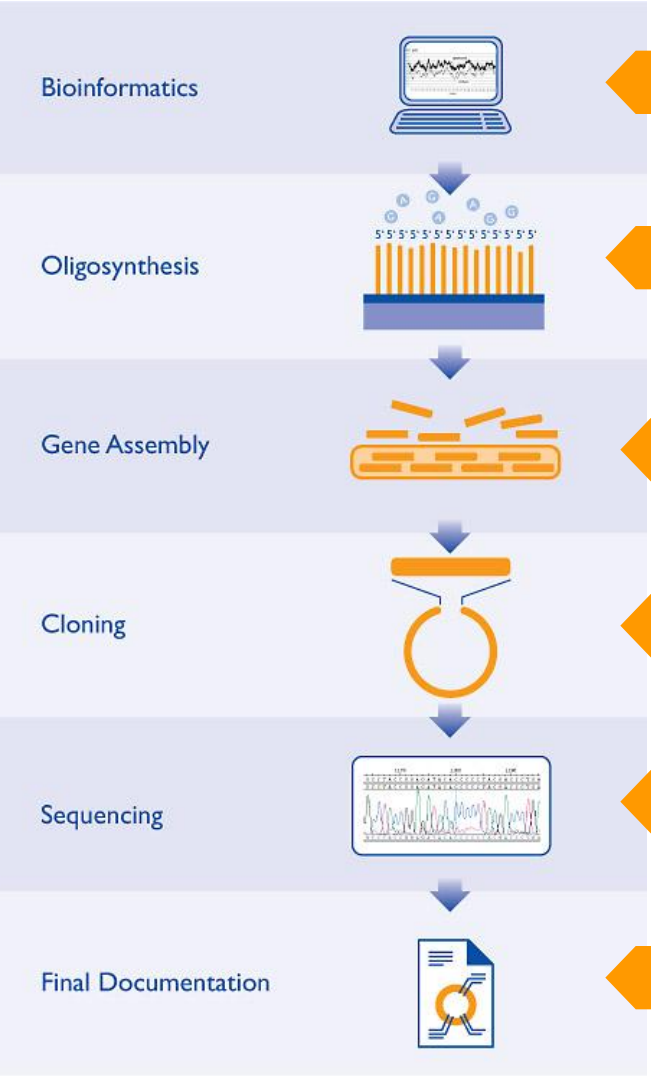
- Highest quality
- Ability to synthesize difficult sequences
- State-of-the-art design/optimization
- Cost effective
- Delivery time
- Scale and capacity
- Market and brand leadership
- Thought leadership
- Synthetic biology vision

life technologies™

GENEART
THE GENE OF YOUR CHOICE

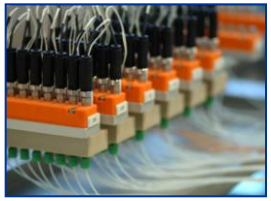
Majority stake obtained April 2010
Tender period closed May 2010
Acquisition closed December 2010

Gene Synthesis: Technology and Process Overview

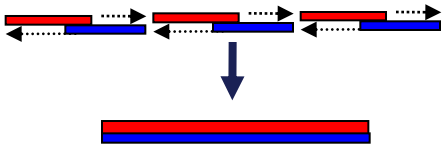


- Optimization/CAD
- Design fragments and oligos
- Alignment and simulation *in silico*

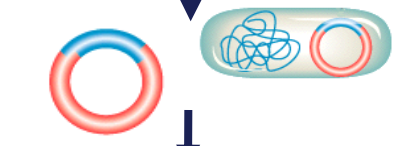
- Nano scale synthesis
- High throughput/quality



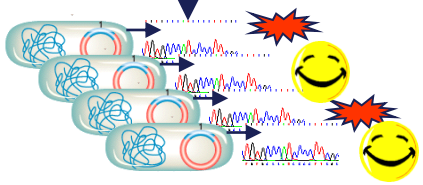
- Several approaches
- High throughput
- Up to 2.0 kb/fragment



- High throughput
- Semi-automated



- automated CE sequencing
- automated alignment



- Product report
- Ship overnight
- ISO 9001

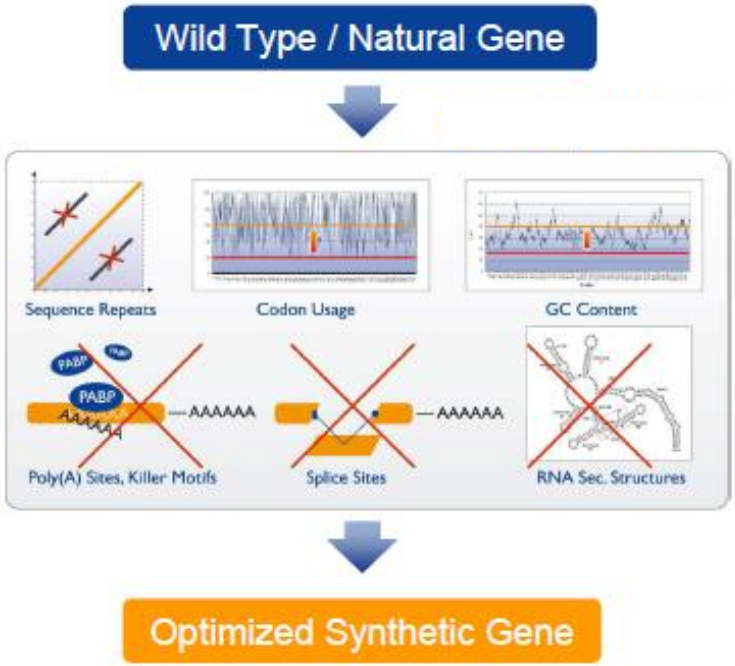
LIMS

GENEART
THE GENE OF YOUR CHOICE

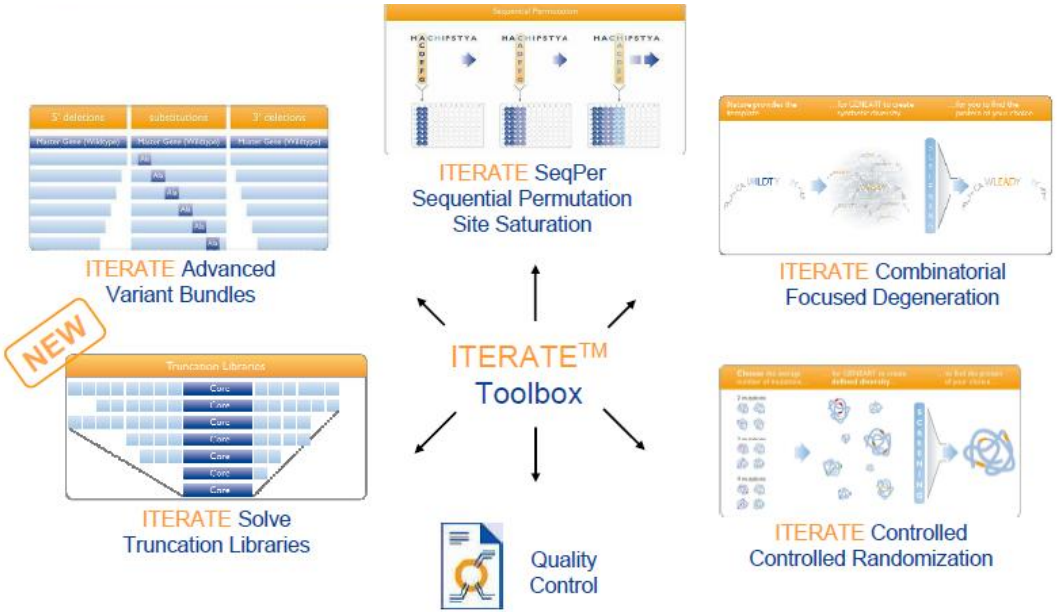
life

Gene Design: Expression and Activity Optimization

GeneOptimizer®

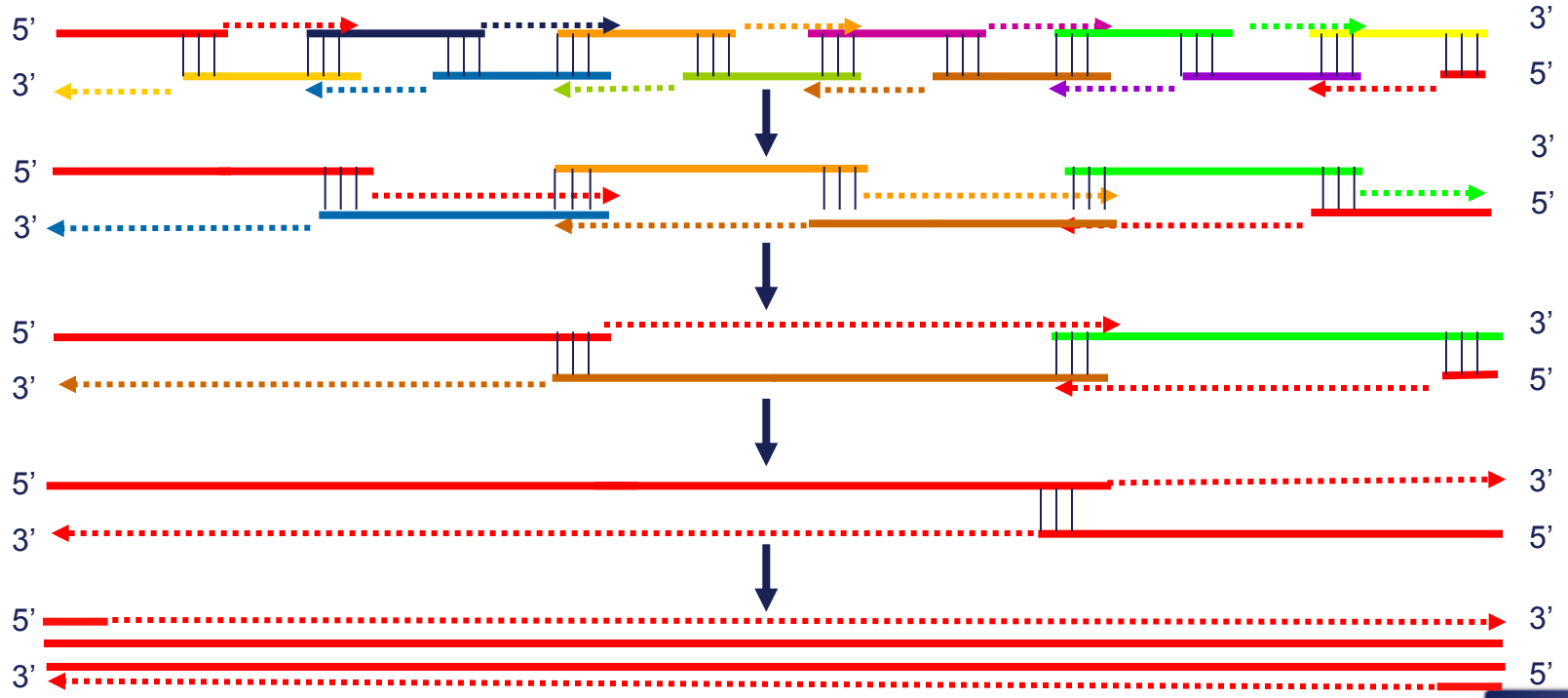


ITERATE™ Toolbox Libraries



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:
  - 1: All oligonucleotide tiles; 2: Terminal Primers
- Assembled fragment is cloned and sequenced



Double Stranded DNA Fragment up to 2.0 kb

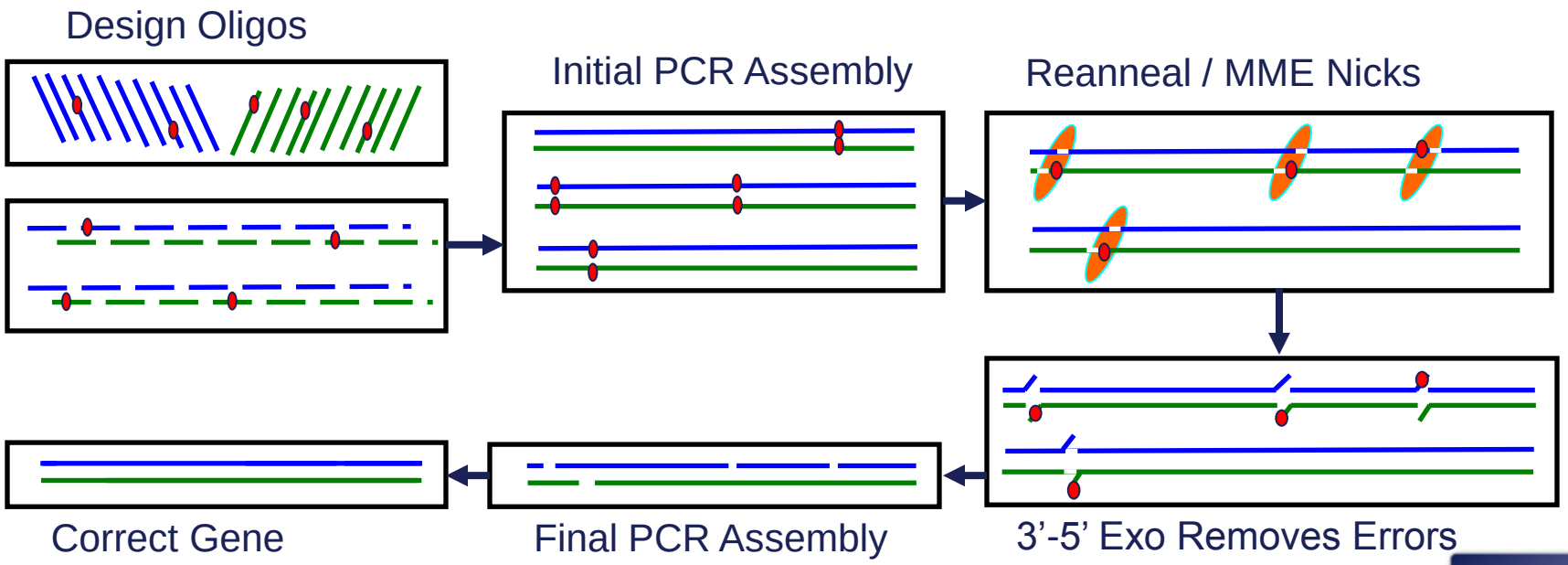
Gene (and Larger) Synthesis Considerations

- 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, chemicals, 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

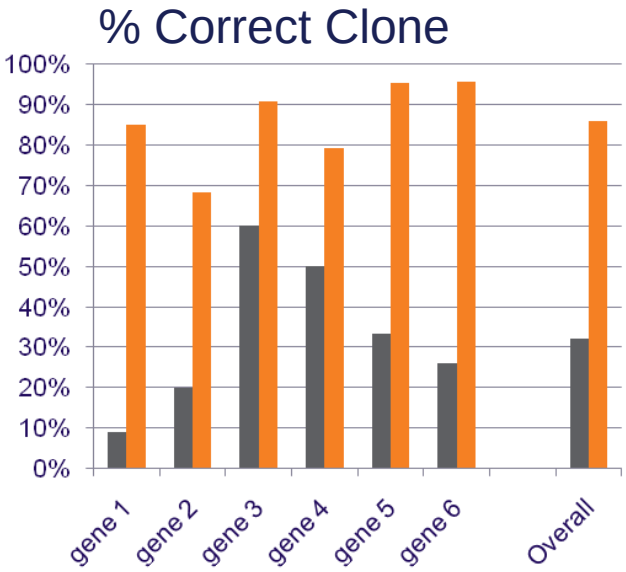
Life Technologies Signs Exclusive License Agreement for DNA Error-Correction Technology from Novici Biotech LLC
October, 2010

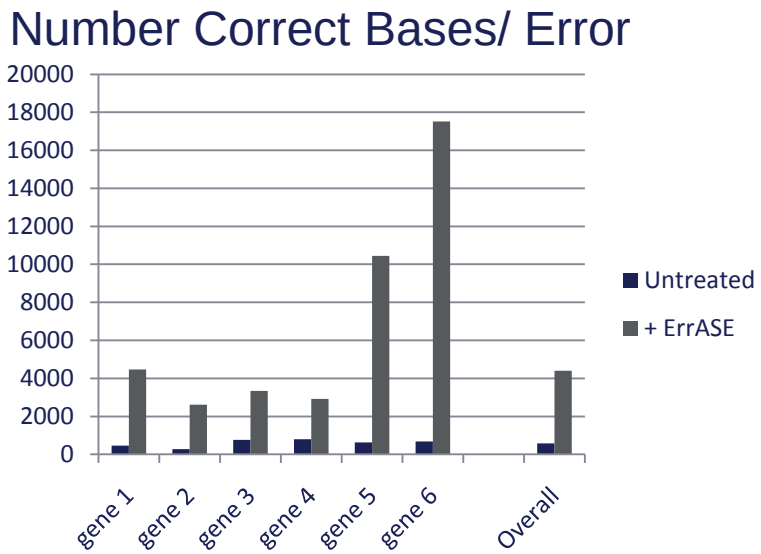
Error Correction

- Oligonucleotides have error rate (~1/300-500 bases) due to synthesis
- Synthetic genes must be cloned and sequenced for mutations
  - >80% of errors are single frameshift deletions/insertions
  - <2% from Polymerase in high fidelity PCR amplification
- Mismatch endonuclease (MME) correction using fixed protein:DNA ratio



Error Correction Results for Test Genes

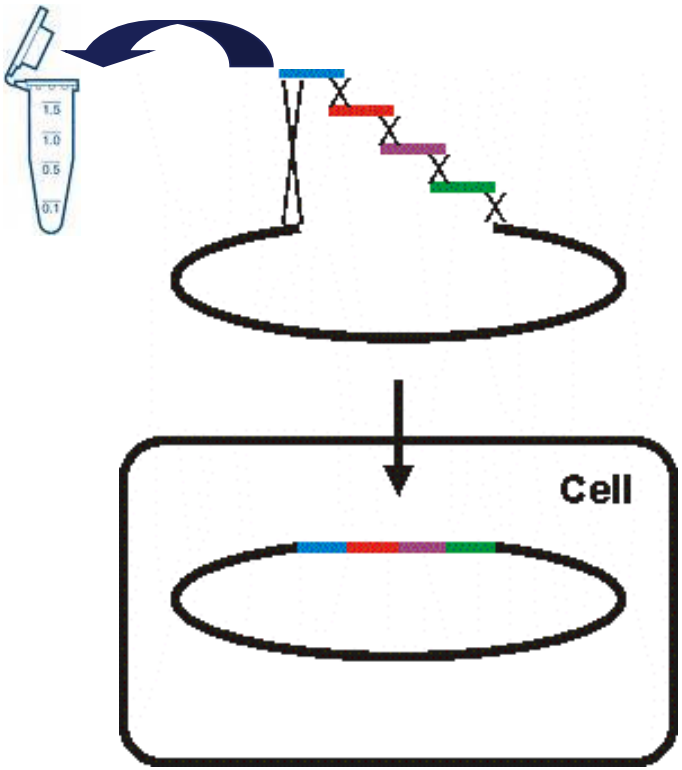




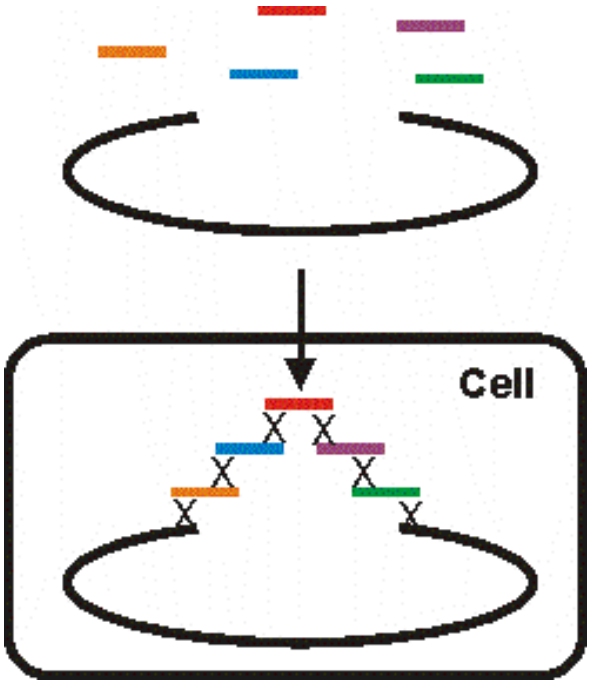
- Integrated 2-step PCR assembly protocol
- Genes from 220 bp to 830 bp in length including low to high GC content
- Average Error Rate improved from 1/570 to 1/4400 after ErrASE Treatment
- Average Frequency of getting correct full length clone improved from 32% to 86%
- For a 1kb gene ~95% chance that at least 1 of 2 clones picked will be correct

Simple Version of Assembly Solutions

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



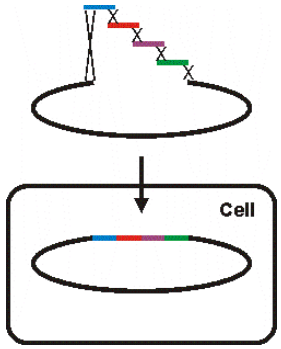
In vitro assembly

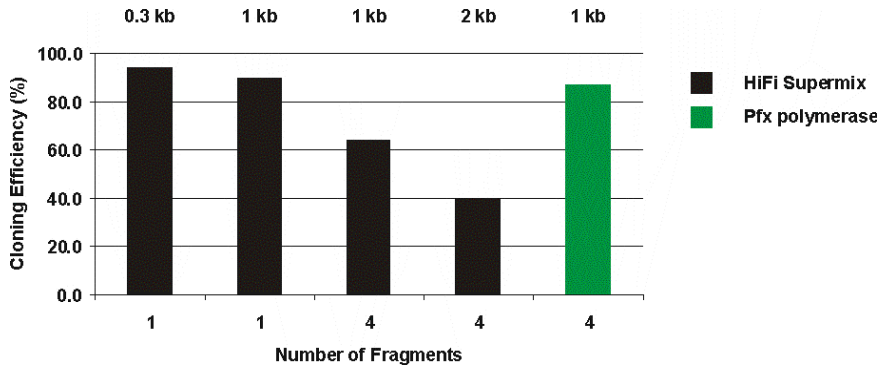


In vivo assembly

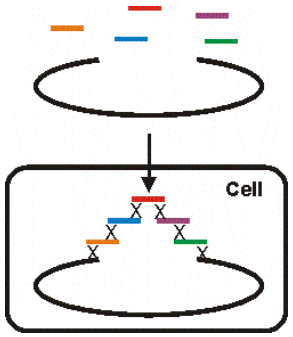
Initial Products Launched Fall, 2010

In vitro **Recombination (*E. coli*)**



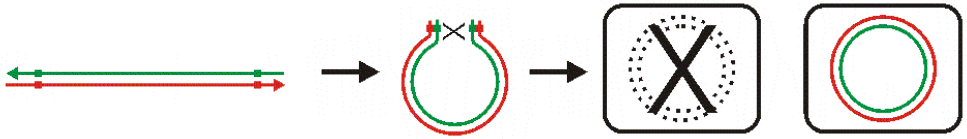


In vivo **Recombination (yeast)**

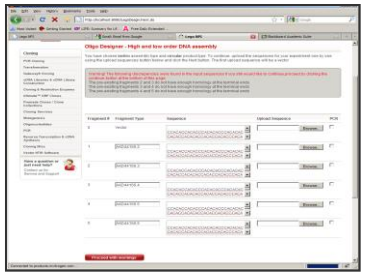


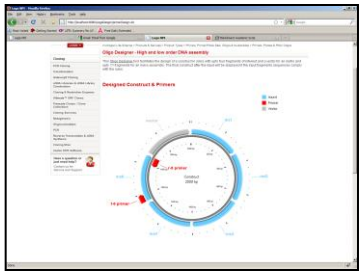
# fragm	#/size preexisting fragm	Total Size (kb)	#/size amplif fragments	Overlap	Colony output	Cloning effic
3	3 x 10 kb	38	0	80 bp	4000	100%
5	5 x 10 kb	58	0	80 bp	1400	100%
10	10 x 10 kb	108	0	80 bp	670	50%
20	8 x 10 kb	108	12 x 0.5-2.5 kb	80 bp	770	83%

Micro-Editing/ Site-Directed Mutagenesis



Web Design Tools

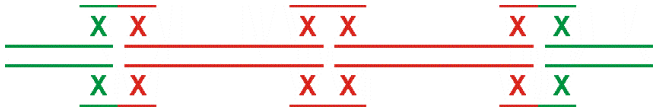
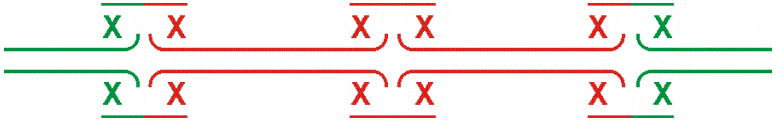
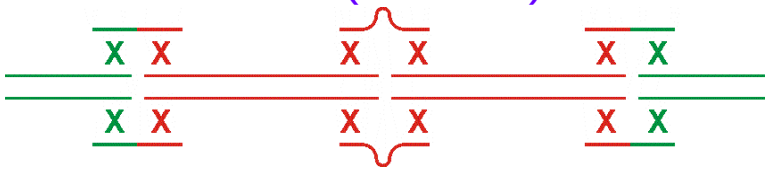




Bridging Oligonucleotides for *in Vivo* Assembly:

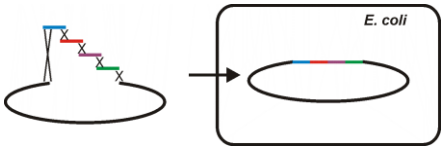
Perfect/Imperfect Junction Assembly and Editing

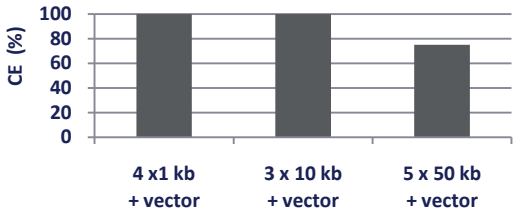
- Homology provided in *trans* by designed "bridge" oligonucleotides
- Allows for reuse of fragments in a new sequence context
- Allows for junction editing

	Oligo	Colony #	Cloning Efficiency
Perfect Junctions			
	60mer	2685	94%
	80mer	1240	75%
Deletion Junctions (12 bases)			
	60mer	520	25%
Insertion Junctions (30-X-30)			
	10 bp	460	63%
	20 bp	430	50%

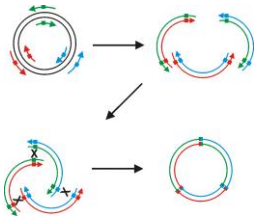
Tools In Development

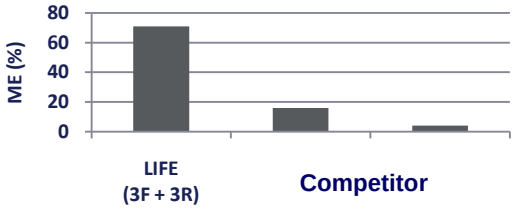
In vitro **High Order DNA Assembly**



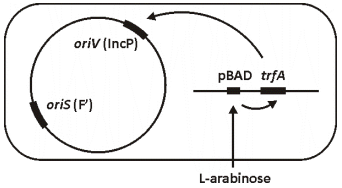


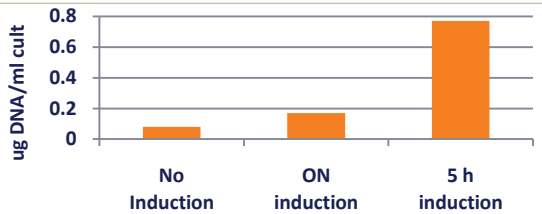
Multi-site directed mutagenesis



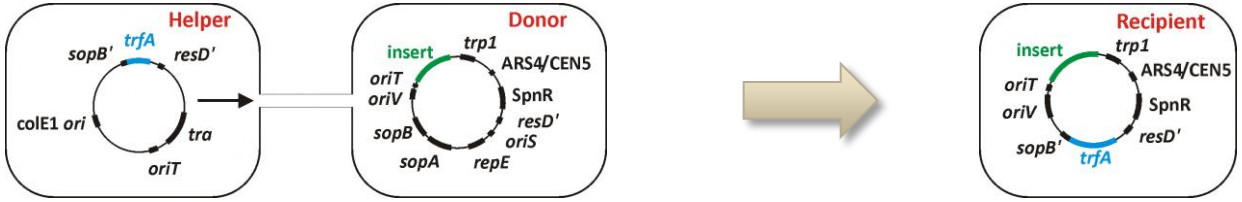


Plasmid copy number control



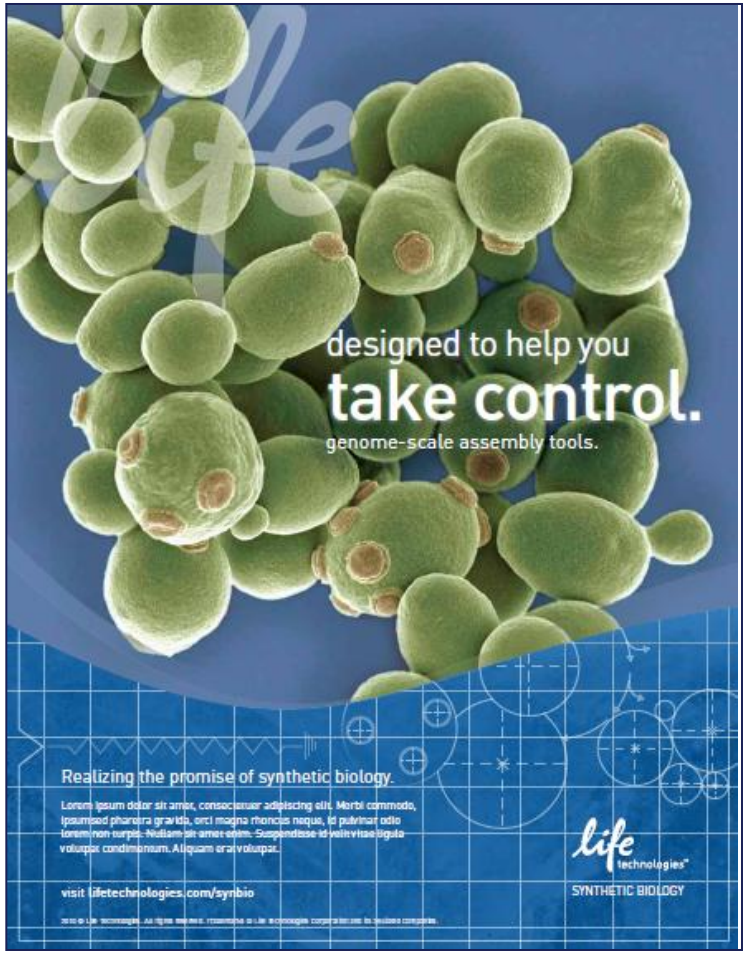


Interspecies conjugative transfer



Acknowledgements

- Informatics/Genetic Design
  - Kevin Clancy
  - NGS Sequencing Groups
  - Voigt Lab, UCSF
- Gene Synthesis
  - Ralf Wagner et al/GENEART
- Error Correction
  - Jason Potter, Sihong Chen
  - Hal Padgett, Novici
- *In Vitro* Assembly
  - Billyanna Tsvetanova
  - Federico Katzen
- *In Vivo* Assembly
  - Lansha Peng
  - Ke Li



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