life technologies™

synthetic biology
by life technologies™

Bio Pacific Rim
Summit on Industrial Biotechnology and Bioenergy

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Genetic Design, Assembly and Editing Tools for Synthetic Biology Engineering

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Overview:

1. Synthetic biology technology tools platforms
2. Workflows for rational design and engineering
3. Building an informatics platform powering rational design
4. Tools toward scaling engineering standards
    - Error correction
    - Assembly and editing
5. Targeted regulation and genome engineering tools

Leading the Way in Synthetic Biology

- Building a comprehensive synthetic biology tool box
- Collaborative deployment with leading industry players & researchers

Our Synthetic Biology Toolkit

DNA Parts & Devices

NGS Analysis

Gene Synthesis

Error Correction

Seamless Assembly & Mutagenesis

Synthetic Libraries

Combinatorial Assembly/ Libraries

Gene Evolution

Jump-In™ Integration Systems

TAL Effectors

Genome/Pathway Engineering

BioDesign Software

Bio-CAD™

Vector NTI Express

Gene Optimizer

Metabolic Modeling

Lab Kits & Custom Services

Advanced Analytics and Engineering Tools

Industrial Hosts with genetic tools

Standardized Hosts

Host Systems

Enabling Solutions across Industries

Energy

Healthcare

Chemicals

Invitrogen™ Applied Biosystems® Gibco® Molecular Probes® Novex® TaqMan® Ambion® Ion Torrent™

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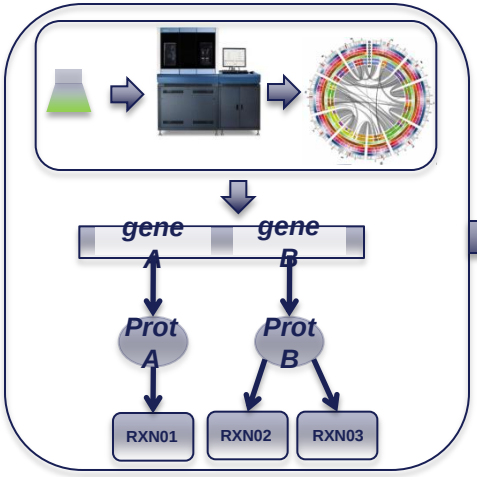
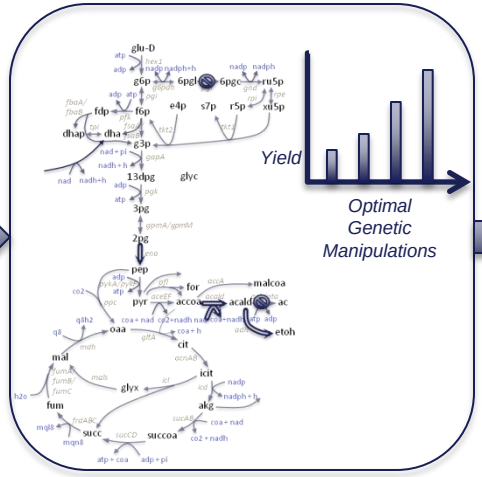
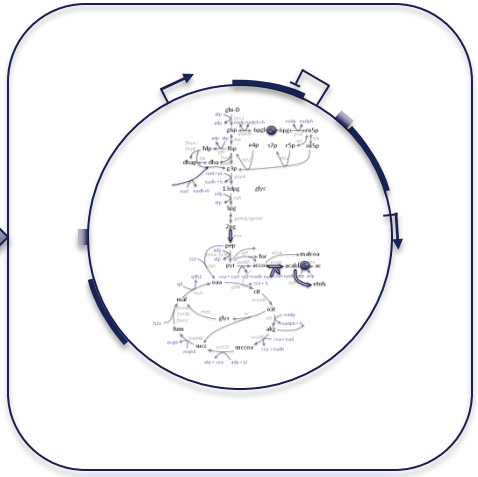
Metabolic Engineering Workflow



Characterize/Understand



Design



Optimize/Build

- Sequence (genome/transcriptome)
- Annotate
- Gene-Protein-Reaction (GPR) maps
- Construct “Draft Metabolic Model”
- Biomass synthesis & regulation
- Gap Filling

- Characterized base strain
  - Medium-specific data
  - Gene expression data
  - Flux Analysis
- Mine for optimal targets

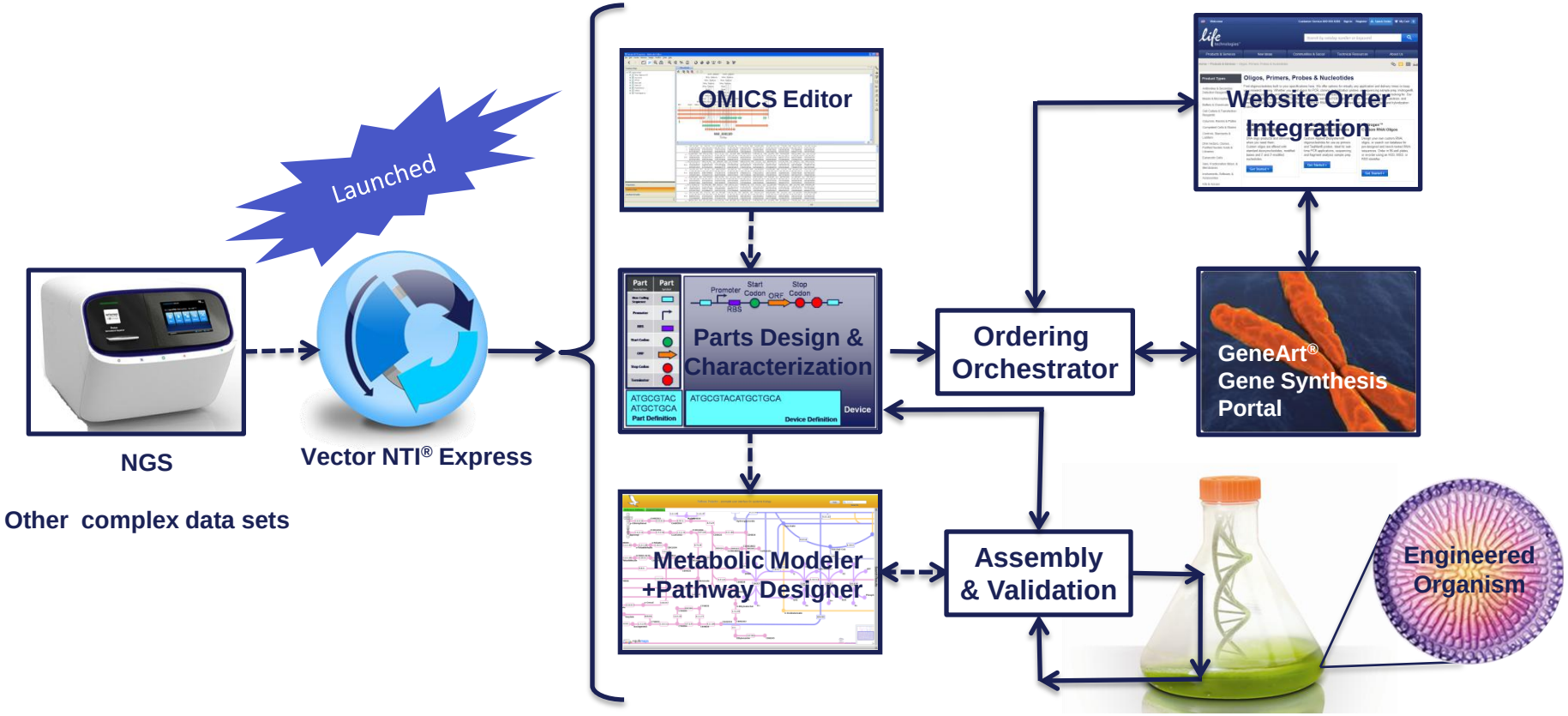
- Synthetic Biology Engineering
  - Characterized parts
  - BioCAD™ software for design
  - Apply toolbox, kits & services
- Engineer hosts
- Monitor Changes

Traditional Strain Improvement

NGS

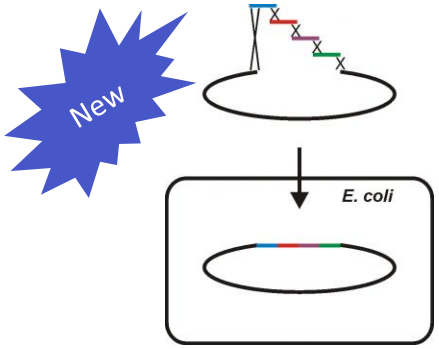
BioCAD™
From gene to system based analysis

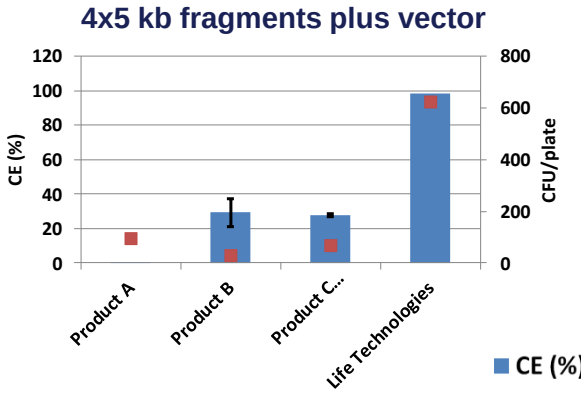
- An information fusion software platform
- Composed of various data preprocessing and functional modules
- Will serve as an interface between wet lab and computational engineering

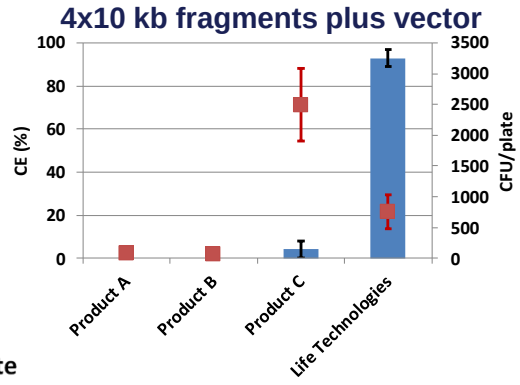


GeneArt® Assembly and Mutagenesis Technologies

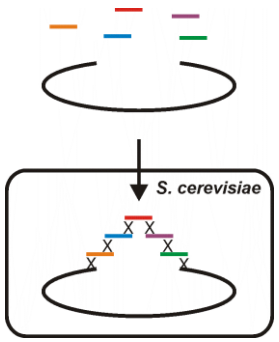
Seamless Assembly PLUS



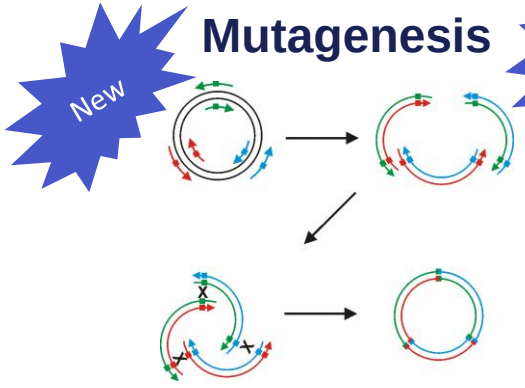




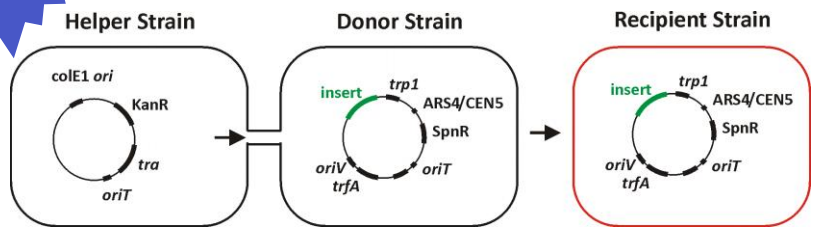
High Order Genetic Assembly



Multi-site directed Mutagenesis



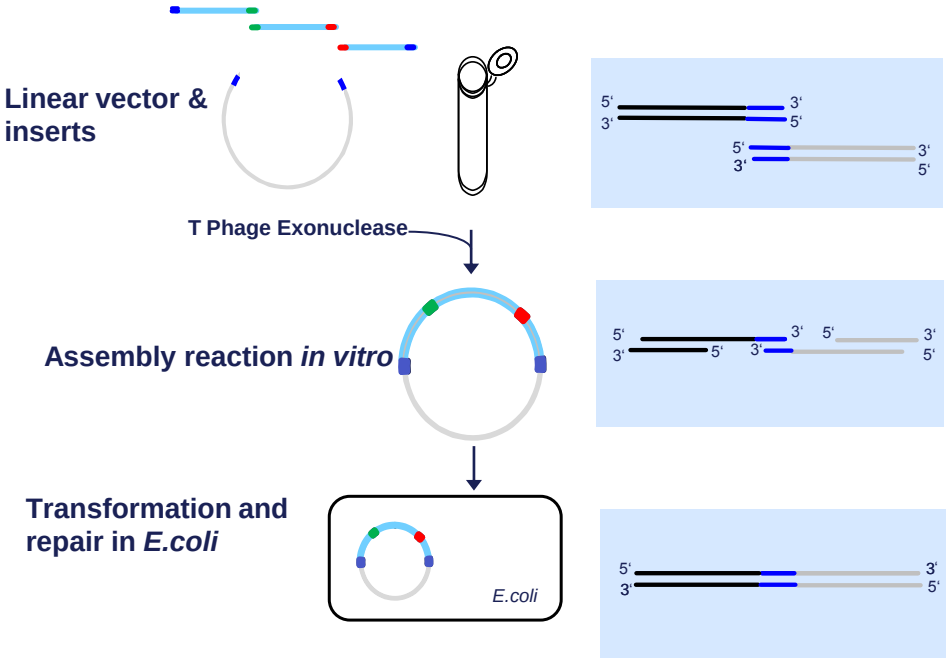
Seamless Assembly PLUS



Methods Enzymol. 498:327, 2011
Methods Mol Biol. 834:93, 2012

HTP Homologous Assembly of DNA Fragments in a Production Environment

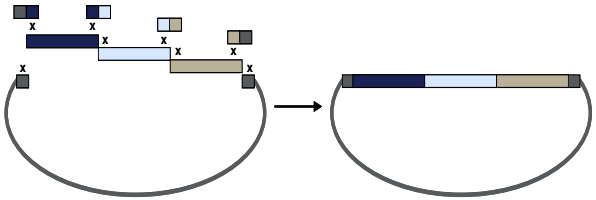
Sequence-independent, ligation free *in vitro* assembly



- Assemble gene synthesis intermediates (1-5 fragments)
- Generate large DNA fragments up to 50 kb
- Vector construction
- Subclone gene synthesis products in customer's vector

In vivo assembly using homology bridging fragments or oligos

- Fragments lacking homologous termini can be joined using DNA bridges
- Bridges prepared as gene synthesis fragments or as oligonucleotides
- Assembly of multiple fragments plus vector up to 150 kb final size



Verified ds DNA linker vs long ss Oligos

- Increased transformation efficiency
- 100% sequence correctness of dsDNA linker
- Length of linker is individually adjustable
- Flexibility for integrating various motifs
- HTP-compatible

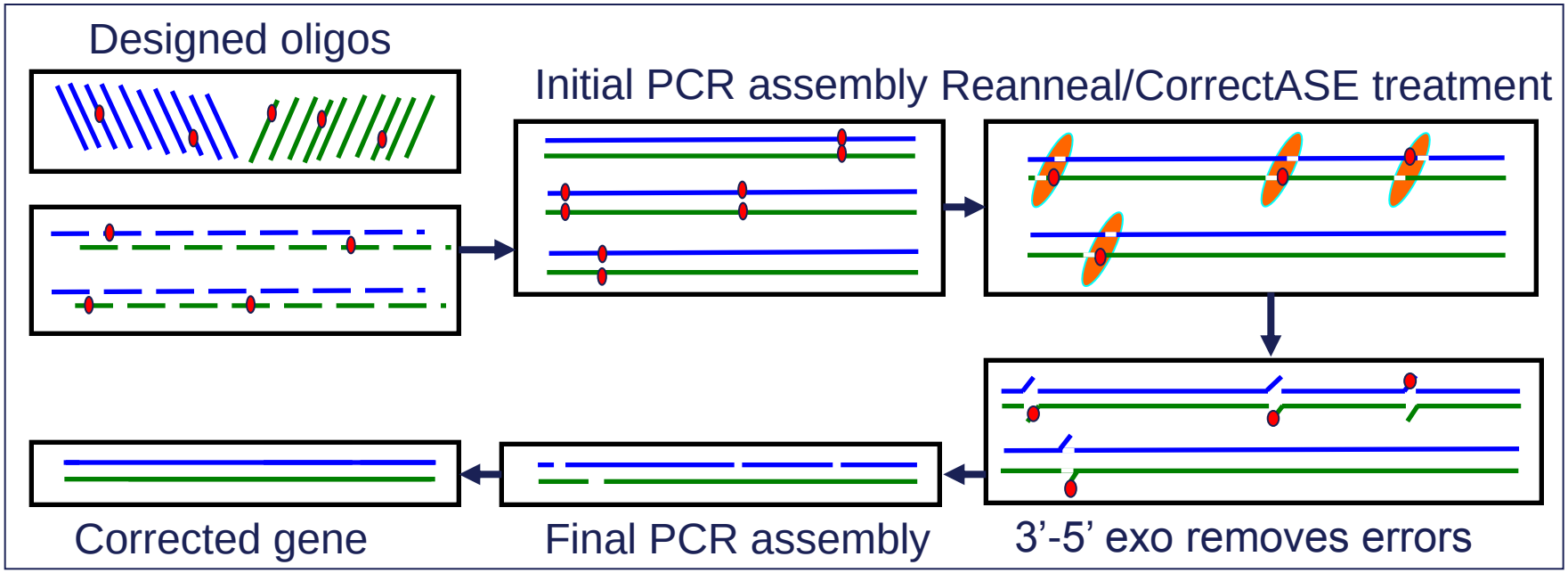
Gene Synthesis Error Correction: CorrectASE™

Source DNA oligonucleotides contribute to gene error due to synthesis errors (n-1)

Oligonucleotide quality is variable among suppliers and lots

Synthetic genes must be sequenced for mutations.

- ~90% of errors are single frameshift deletions in oligonucleotides
- <2% of errors from DNA polymerase in PCR amplification



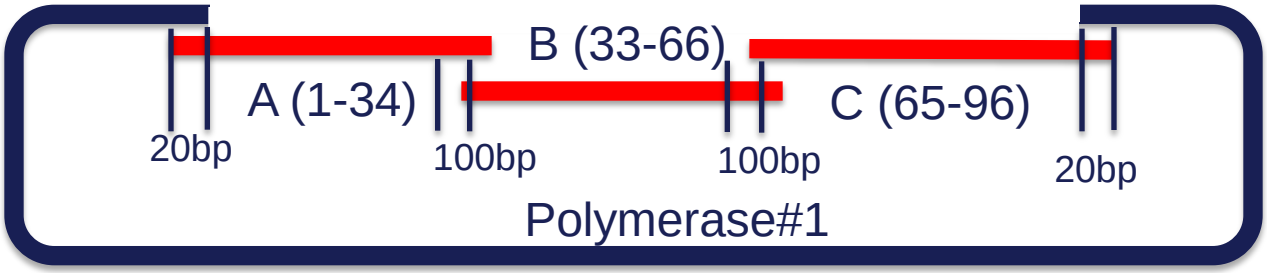
~6 hours from oligos to transformation

CorrectASE™/Seamless Multi-Fragment Assembly

Use deslated oligos (55-60mers) of moderate quality for 2 phage polymerases

Polymerase#1	2850bp	polA family	3 fragments	96 oligos
Polymerase#2	1690bp	polB family	2 fragments	46 oligos

Designed so the end fragments overlapped vector by 20bp and internal overlap by 2 oligos (~100bp).



1 day
- Set up normal 1st & 2nd PCR to get full length fragments ~1kb each
- Did CorrectASE reaction and final PCR for each fragment
- GeneArt Seamless assembly of error-corrected PCR fragments & linear vector

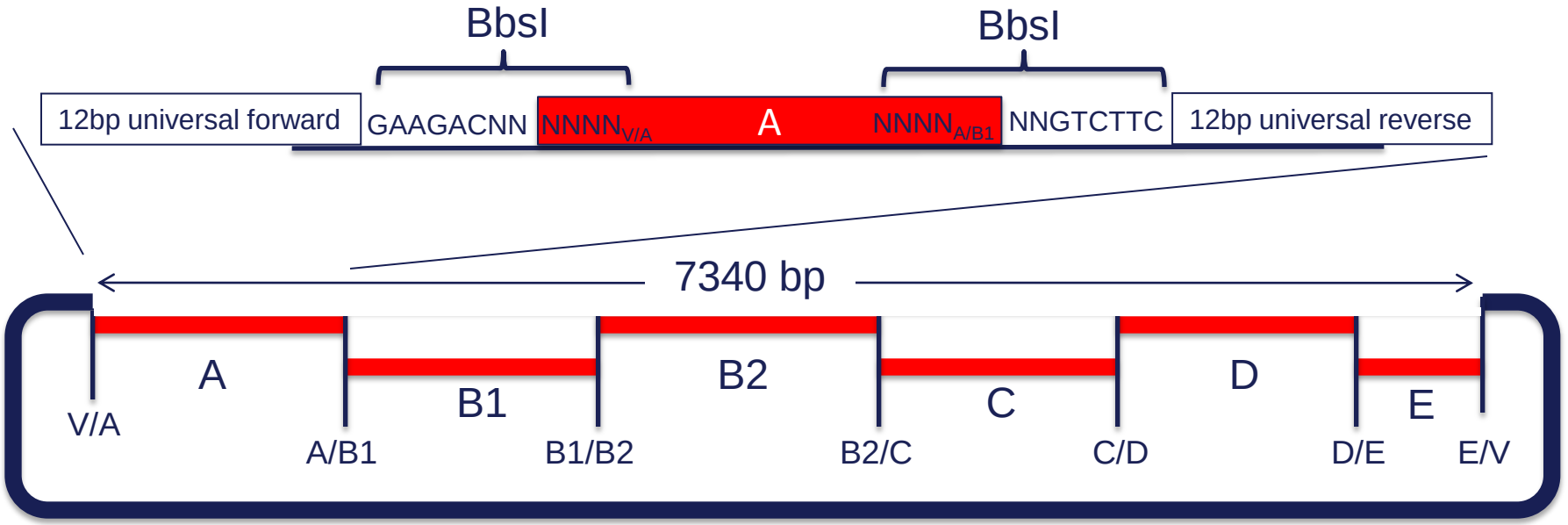
Pick 4 CFU/polymerase gene:

2/4 had correct size insert by miniprep 2/2 had correct sequence

CorrectASE™/Golden Gate Multi-Fragment Assembly

~7.3kb violacein pigment pathway assay is 5 genes (A,B,C,D,E)
All genes need to be in frame and functional to get purple CFUs

Moderate quality desalted oligonucleotides
Design for Golden Gate assembly due to complexity and repeats
Split into 6 fragments from 640bp to 1600bp in size with unique BbsI sites



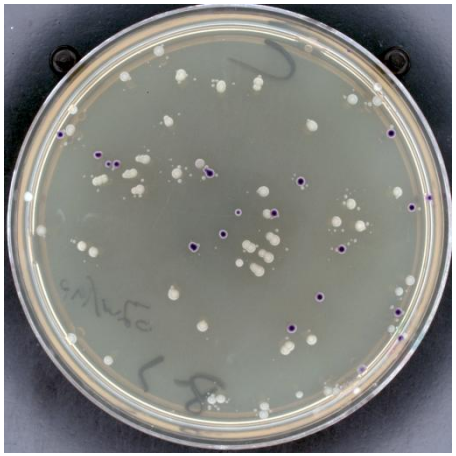
- Design oligos for each fragment (296 oligos, 40-60 base in length)
- PCR assemble 6 fragments in parallel and error correct with CorrectASE
- Use error corrected PCR fragments in Golden Gate assembly reaction with vector
- Transform Top10 cells and plate

CorrectASE™/Golden Gate Multi-Fragment Assembly

- CorrectASE™

1 purple/ 20 white, 5%

+ CorrectASE™



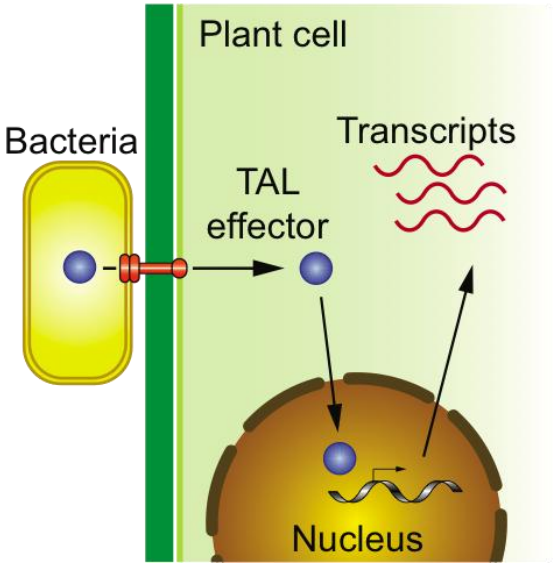
19 purple/ 52 white, 27%

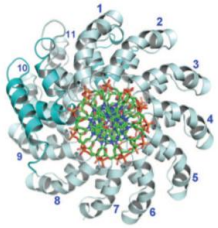
~1 in 4 correct clones
for 7.3kb pathway

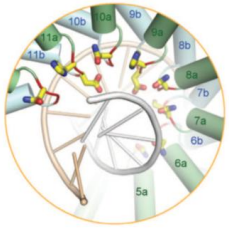
- PCR gene assembly, error correction, sub-fragment assembly, screening
- 7.3kb multi-gene pathway
- Moderate quality, desalted oligonucleotides
- One day from designed oligonucleotides to functional pathway

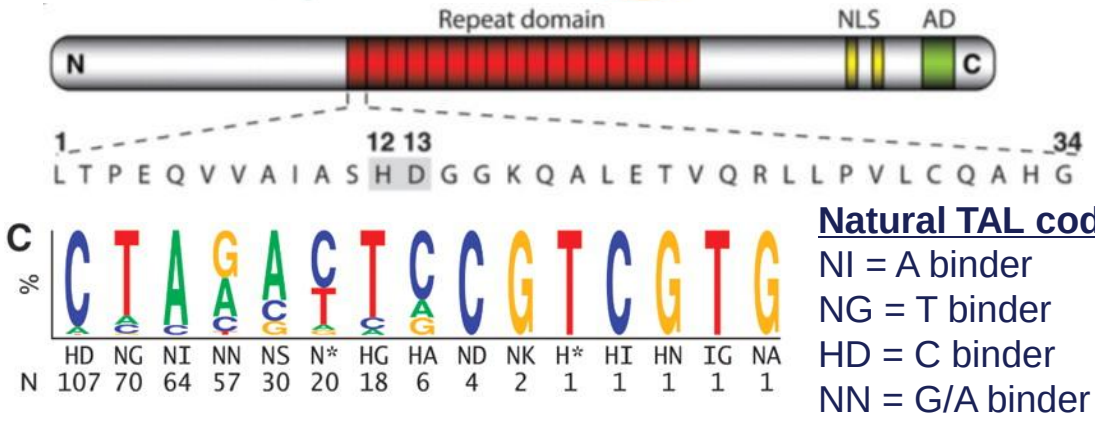
Regulation and Genome Editing: Custom TALs Effectors

TAL (transcription activator-like) Effectors









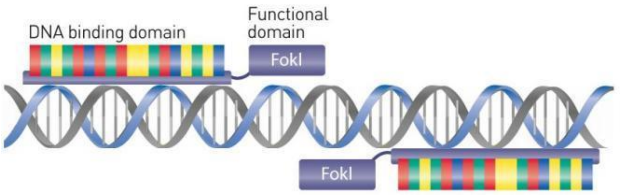
Natural TAL code
NI = A binder
NG = T binder
HD = C binder
NN = G/A binder

- Bacterial TAL effectors bind to DNA sequences based on amino acid/nucleotide relation defined by a specific code
- DNA base binding specificity is defined by di-variable amino acids 12 and 13 in each repeat
- Tune binding specificity & affinity by number of repeats & sequence
- Function in wide variety of species

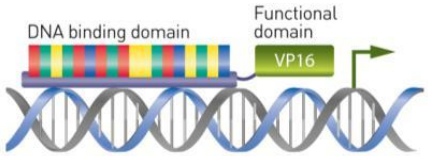
GeneArt® Precision TALs:

Custom DNA binding proteins for precision DNA targeting

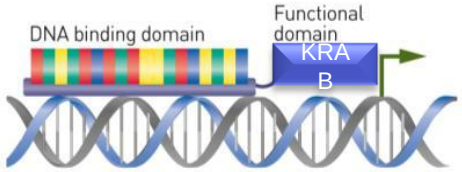
- Gene targeting (Fok1 Nuclease Pair)
  - Silencing/Stable Knock-Outs
  - Incorporation of exogenous DNA/Knock-Ins



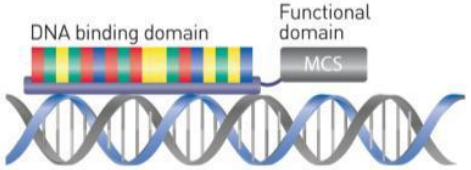
- Activation of transcription (Activator vp16 or vp64)
  - Increasing expression level of endogenous genes



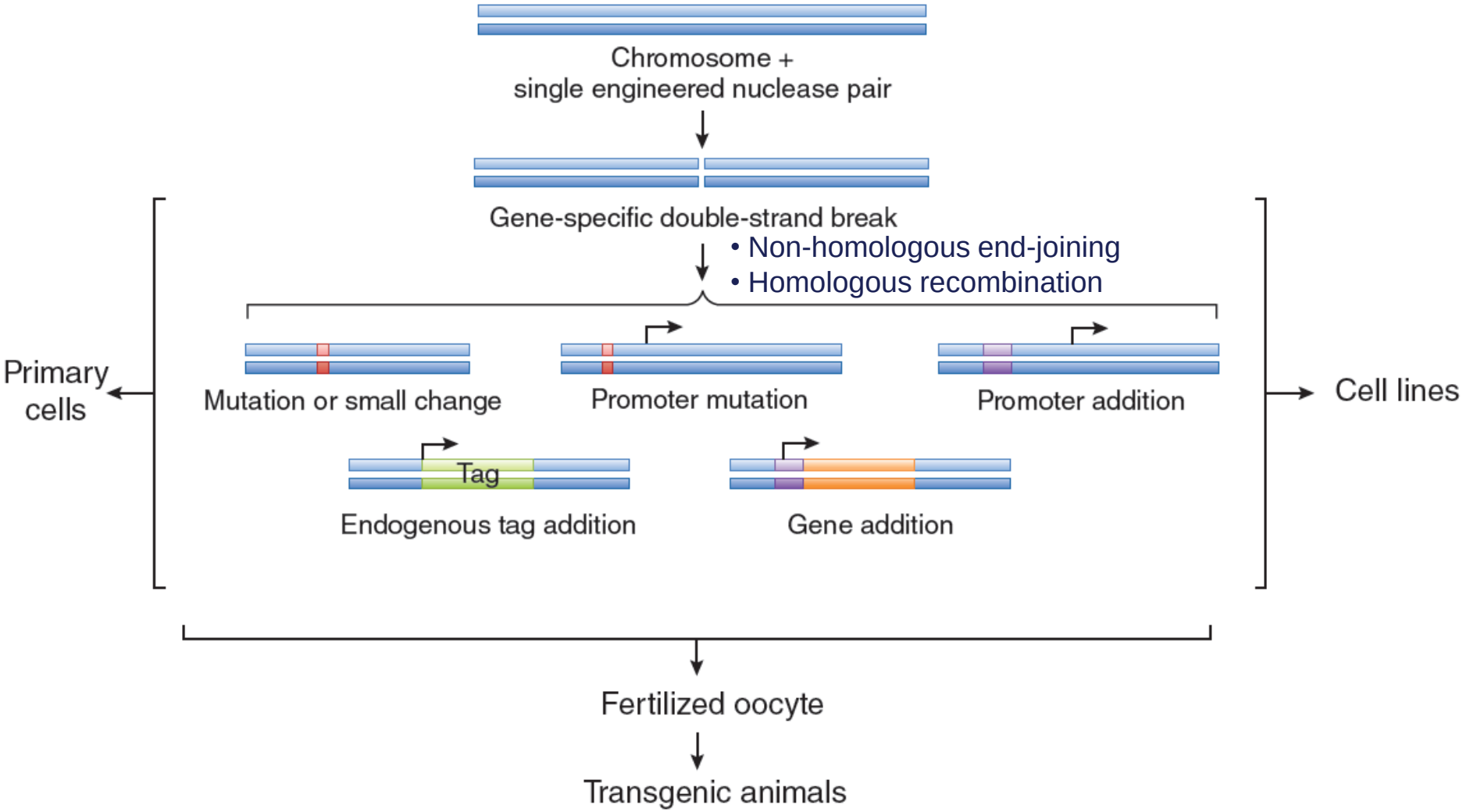
- Repression of transcription (Krab)
  - Targeted repression of gene expression



- Steric repression & custom design (MCS)
  - Transient knock-down of gene expression
  - Target any locus in the genome with the effectors domain of your choice



TALE Nuclease -Mediated Genome Editing



McMahon et al. 2012, Nature Methods

Thoughts Looking Forward

Current Status

- Can engineer biosynthetic route for most fuels/chemicals
- Advanced synthesis and assembly decreasingly rate limiting (cost)
- Increasingly complex workflows
- Design tools & standards fragmented
- Limited standards for measurement
- Limited circuit complexity and predictable function portability

Gaps

- Known synthesis and process paths to economic viability
- Decrease cost of synthesis at scale
- Project and FAB level workflow management
- Compatibility and organization of complex data sets
- Understanding and harnessing genetic context at part, device & system levels

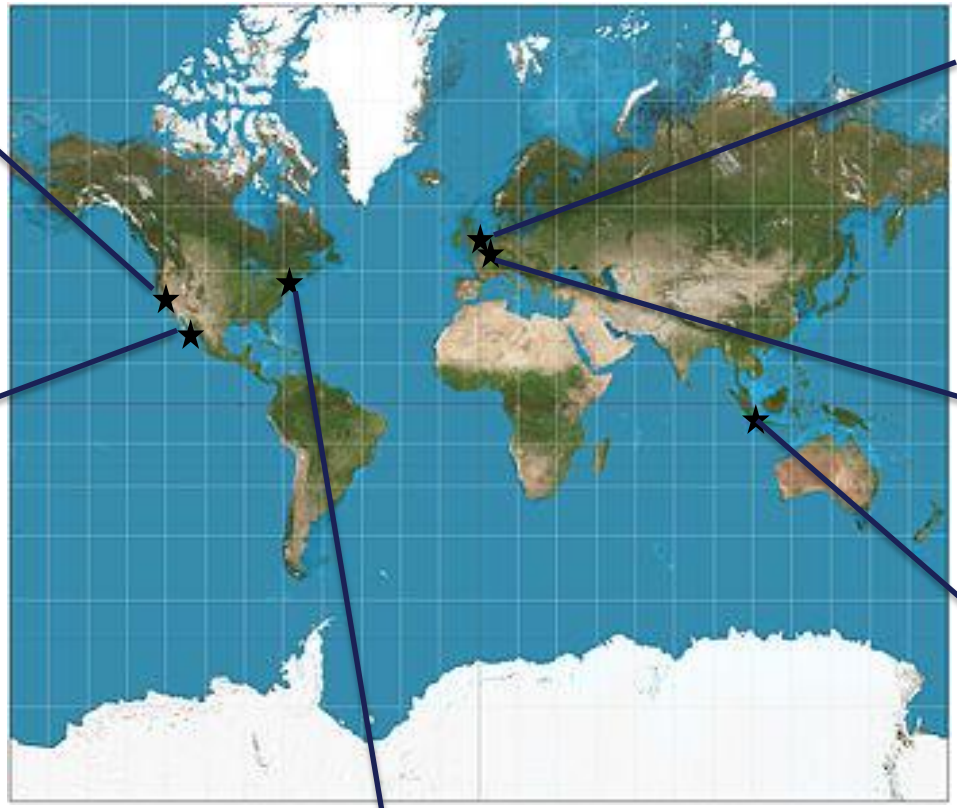
Opportunities

- Integrated intelligent genetic design and process optimization tools
- Industrializing next leap in synthesis cost reduction
- Standardize across measurement, assays & data formats
- Intentionally integrate synthetic biology & traditional strain development methods
- Systematic capture & application of engineering successes & failures

Impact

- Enhance portability and predictability of engineering outcomes
- Accelerate transition from top down to bottom up engineering
- Engineer for a process outcome, not within a system's capabilities
- Move from engineered pathways in a living system to living systems in an engineered (overall) process

Acknowledgements



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