The background of the slide is a close-up photograph of several green, unripe tomatoes hanging from a vine. The tomatoes are round and have a slightly bumpy texture. The vine and leaves are also visible, creating a natural, organic feel. The lighting is soft, highlighting the green color of the tomatoes.

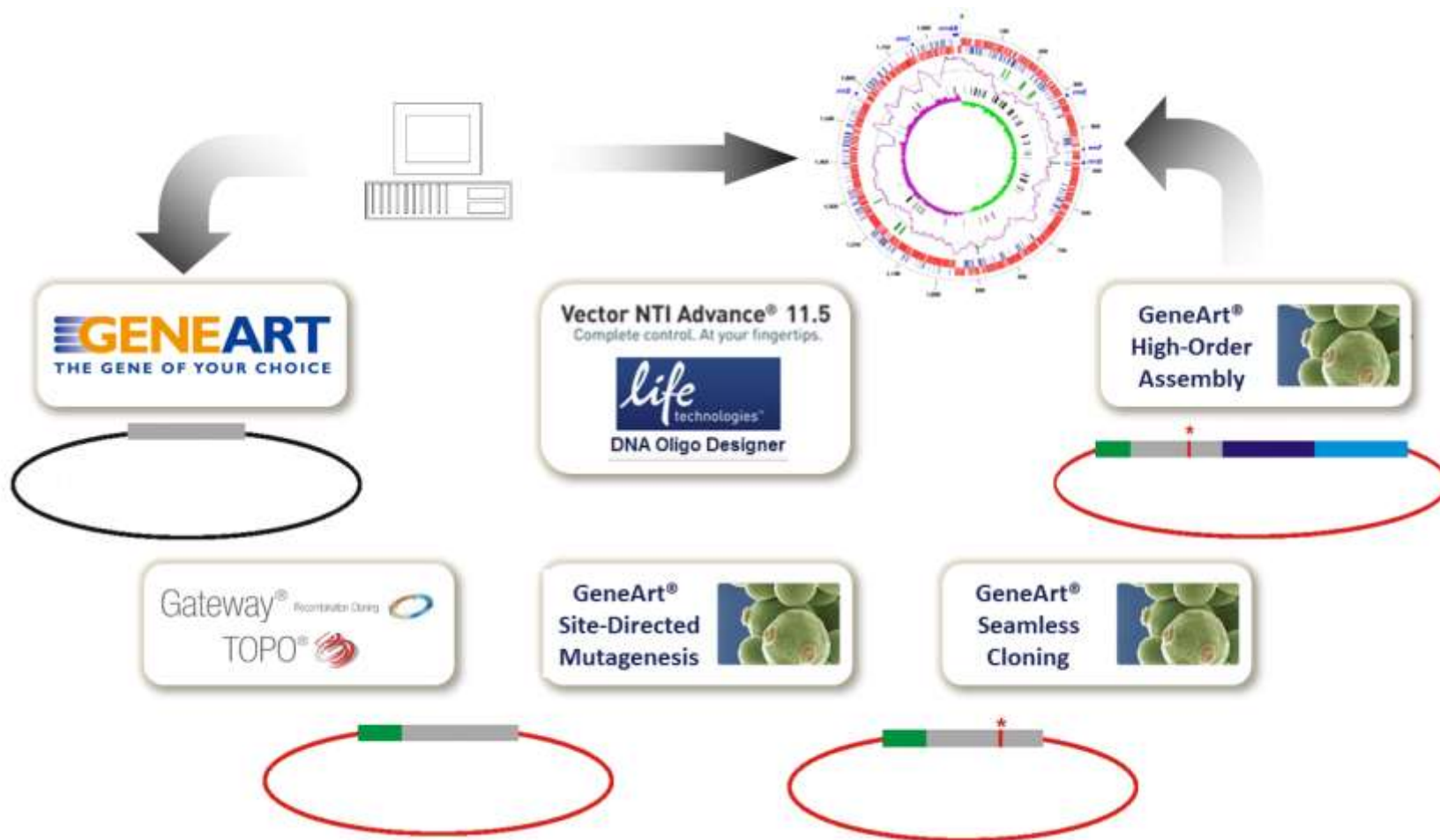
# **GENEART® Genetic Assembly Tools for Synthetic Biology Engineering**

**Federico Katzen, Ph.D.  
Senior Staff Scientist  
Synthetic Biology R&D**

# *life*



# Our Current Gene Synthesis and Assembly Solutions





# Our Current Gene Synthesis and Assembly Solutions

## In Development

Error correction  
for Gene Synthesis

Interspecies  
Horizontal Transfer

High-Order  
DNA Assembly  
*in vitro*

Multi site-Directed  
Mutagenesis

## Related Products

GeneArt®  
*Synechococcus*  
Engineering kit

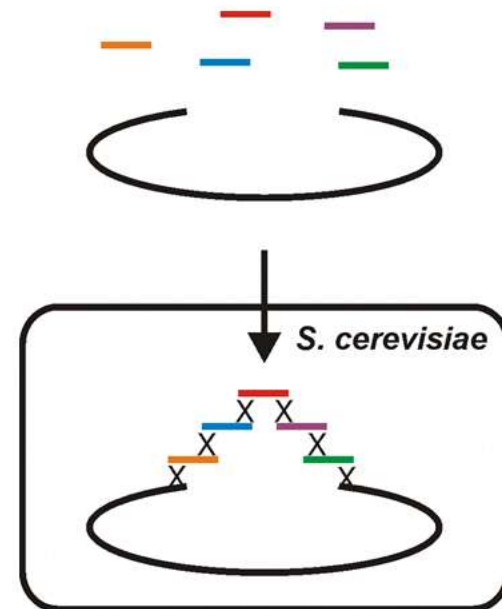
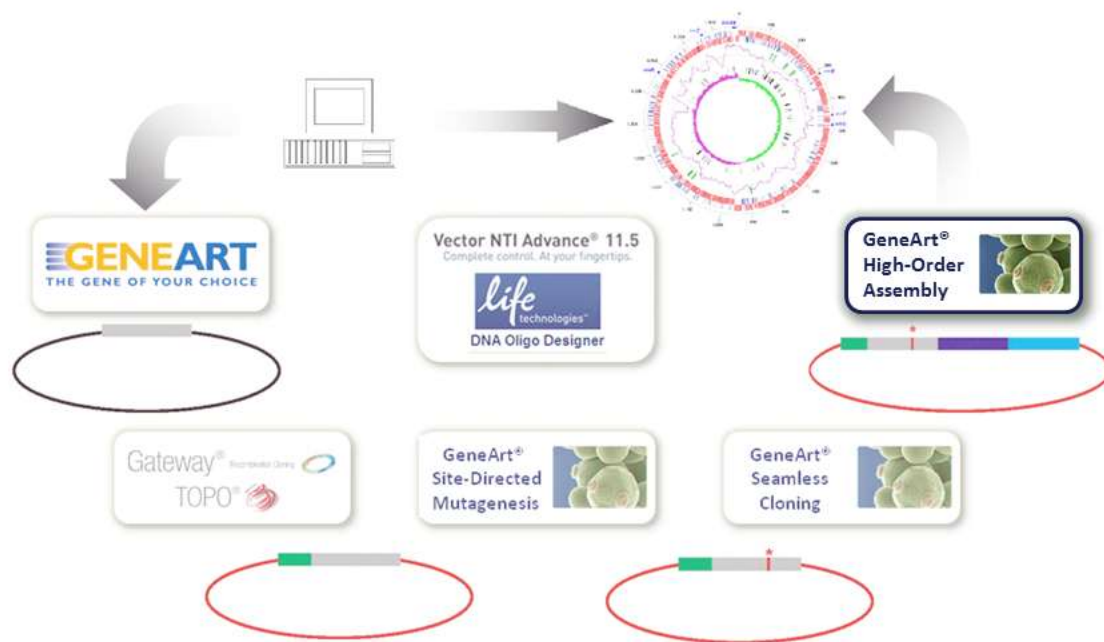


GeneArt®  
*Chlamydomonas*  
Engineering kit





# GeneArt® High-Order Assembly





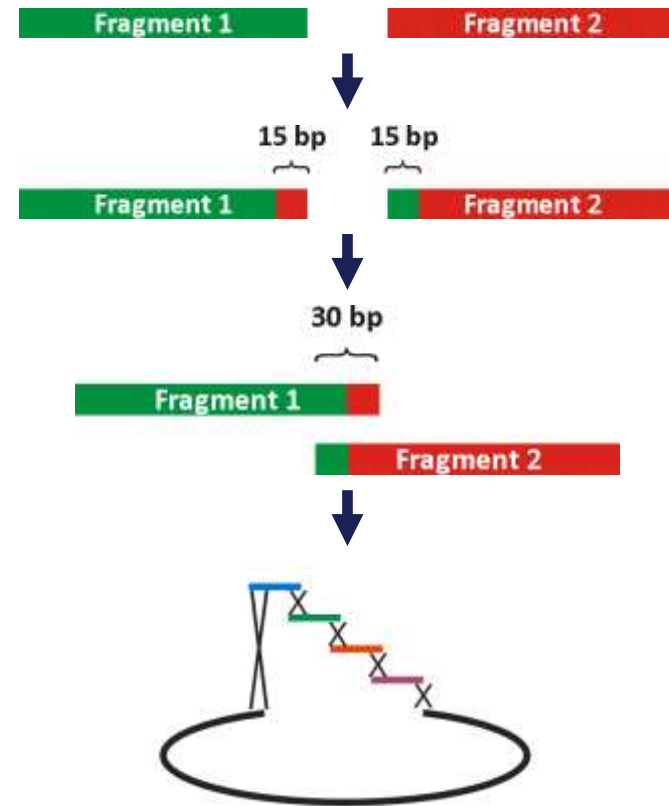
# Homologous Recombination using GeneArt® High-Order Assembly

Fragments to be cloned

Corresponding fragment sequence added by PCR

Fragments align by terminal homologies

Assembly of final construct

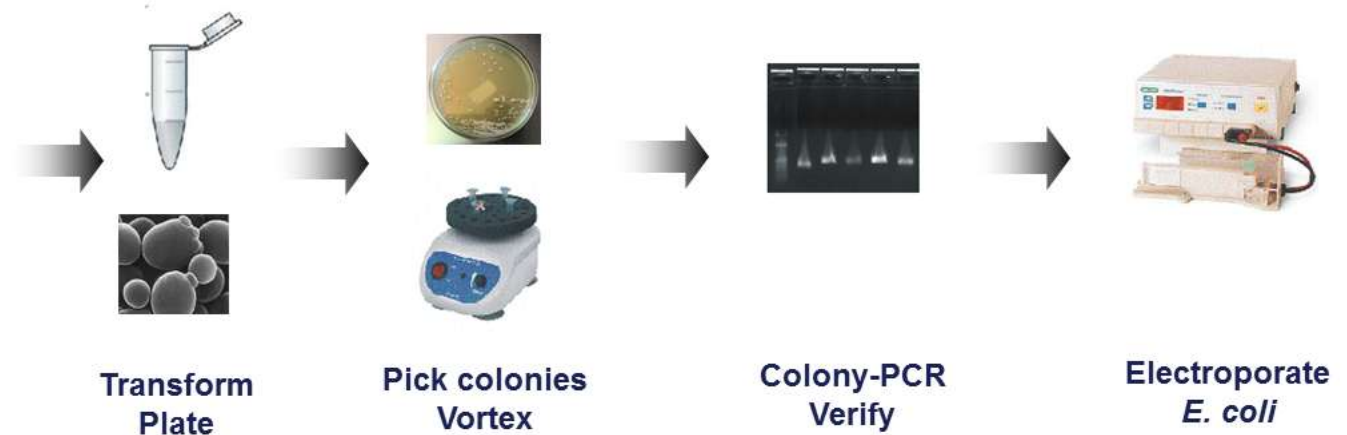
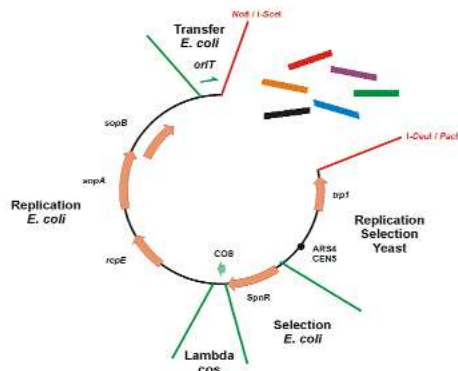


Terminal homology enables simultaneous and directional cloning of fragments





# GeneArt® High-Order Assembly Workflow



- Overall time: 4 days
- Hands-on time < 30 min
- No yeast liquid culture required
- Yeast assembly/*E. coli* propagation
- Free webtool design





## *In vivo* High Order Assembly – Typical Results

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 10 kb	0	80 bp	100	4000	100%
5	5 x 10 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	200	770	83%

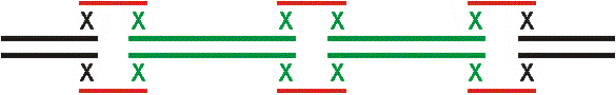
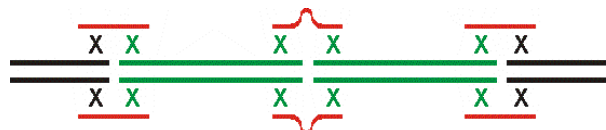
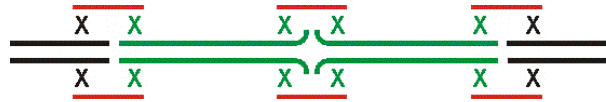
Assembly Type	Fragment Overlap	Colony Output	Cloning Efficiency
1 x 10 kb	80 bp	1140	100%
1 x 10 kb	30 bp	1160	100%
10 x 5 kb	30 bp	1850	92%





# *In vivo* High Order Assembly - Bridging Oligonucleotides

1. Homology provided in *trans* by designed bridge oligonucleotides
2. Allows for reuse of fragments in a new sequence context
3. Allows for junction editing

	Fragment #/Size	InDel Size (nt)	Colony #	Cloning Efficiency
<b>Perfect Junctions</b>				
	2x5 kb	N/A	1240	75%
<b>Insertions</b>				
	2x5 kb	10	460	63%
<b>Deletions</b>				
	2x5 kb	12	520	87%





# Webtool for DNA Assembly Experimental Design

## DNA Oligo Designer - GENEART® Seamless Cloning and High-Order Genetic Assembly Kits

You have chosen **High-Order Genetic Assembly Kits** and Assembly kit and a **circular** graphical representation. To continue:

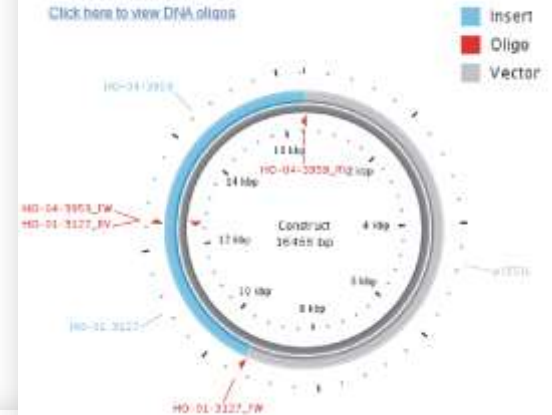
- 1) Upload or paste the sequence of each DNA fragment in order of assembly, starting with the vector. Sequences can be uploaded in FASTA file format.
- 2) Enter a name for each fragment.
- 3) Select the PCR checkbox next to each fragment to automatically design PCR primers for amplifying the insert. If you are using the GENEART® High-Order System, synthetic linkers for stitching can be designed by leaving the PCR button unselected.

[Refresh & Start Over](#)

#	Upload/Copy & Paste Sequence <a href="#">info</a>	Sequence <a href="#">info</a>	Fragment Name <a href="#">info</a>	PCR <a href="#">info</a>	
Vector	<input type="text"/> <a href="#">Browse...</a>	<div>CCTCGCCGCGAGTTAA</div> <div>TTAAAGTCAGTGAGC</div> <div>GAGGAAGCGCGTAA</div> <div>CTATAACGGTCCTAA</div>	pYES1L	<input type="checkbox"/>	<a href="#">delete</a>
Fragment 1	<input type="text"/> <a href="#">Browse...</a>	<div>ggccgccaatttttagtttaggtt</div> <div>cccggtaaatgacattaa</div> <div>ggaaaacgtgctgaatcctc</div> <div>aataatc</div>	HO-01-3127	<input checked="" type="checkbox"/>	<a href="#">delete</a>

## Final Construct & Oligos

[Click here to view DNA oligos](#)



Oligo Name	Oligo Sequence	Purity	Tm	GC
HO-01-3127_FW	GCGCAGCGGCGGCCG	25N.DSL	96.0	67.0
HO-01-3127_RV	TAAACGCCCAACAATA	25N.DSL	79.0	47.0
HO-04-3959_FW	TACATCGTGCTGCTAT	25N.DSL	77.0	44.0
<input type="checkbox"/> HO-04-3959_RV	GCTCACTGACTTTAATT	25N.DSL	83.0	43.0

[Add To Cart](#)

[Download assembled molecule and DNA oligos](#)

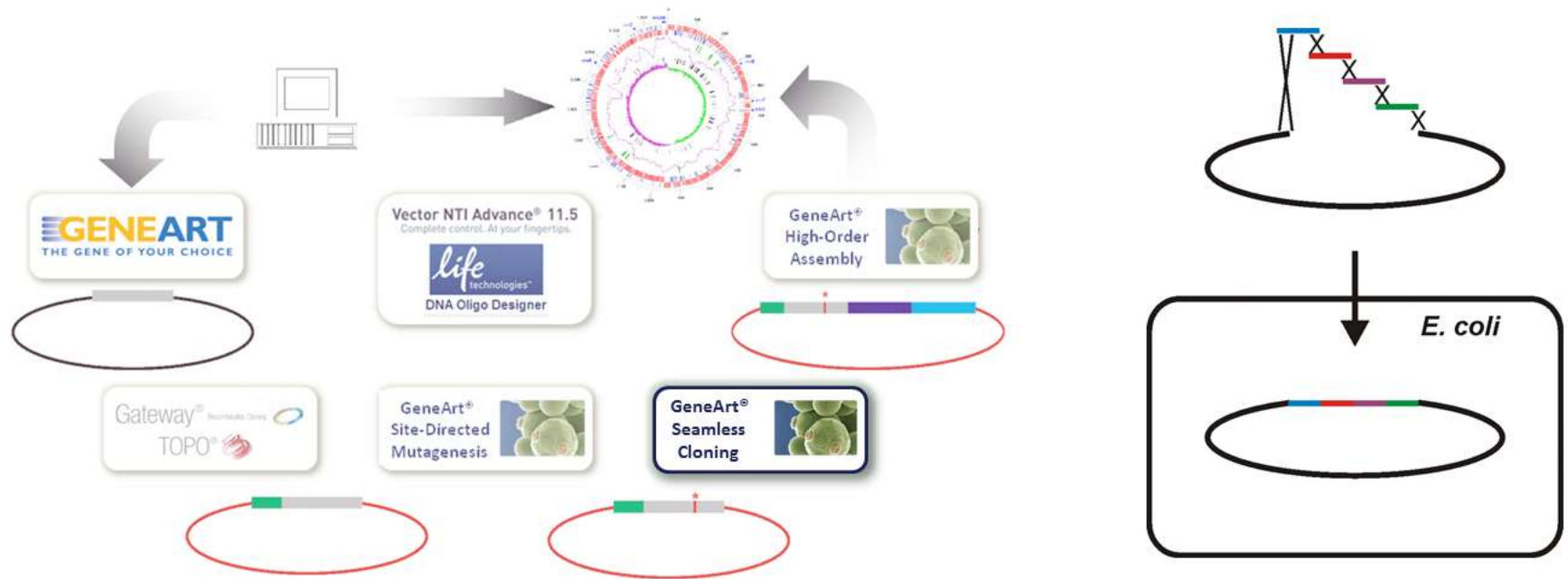
[www.invitrogen.com/designdnaassembly](http://www.invitrogen.com/designdnaassembly)



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# GeneArt® Seamless Cloning





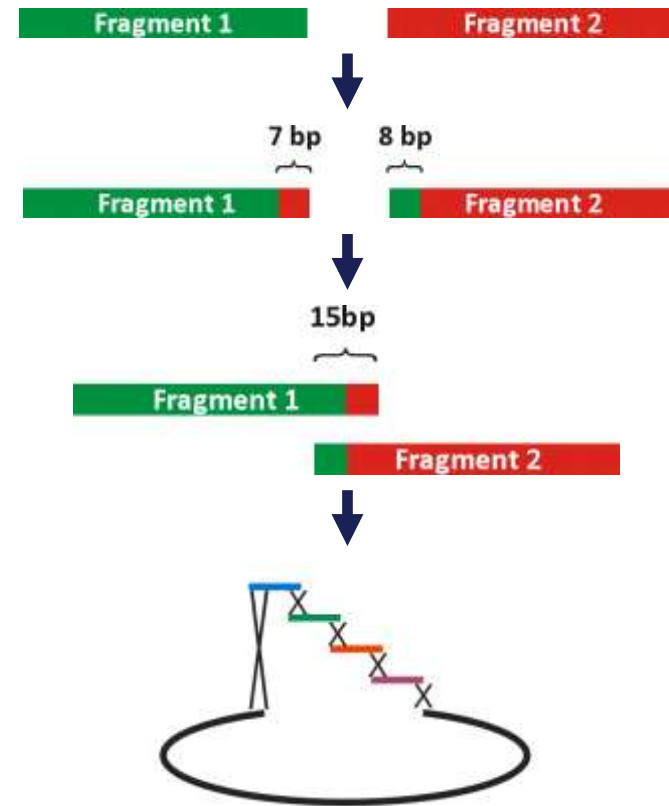
# Homologous Recombination using GeneArt® Seamless Assembly

Fragments to be cloned

Corresponding fragment sequence added by PCR

Fragments align by terminal homologies

Assembly of final construct

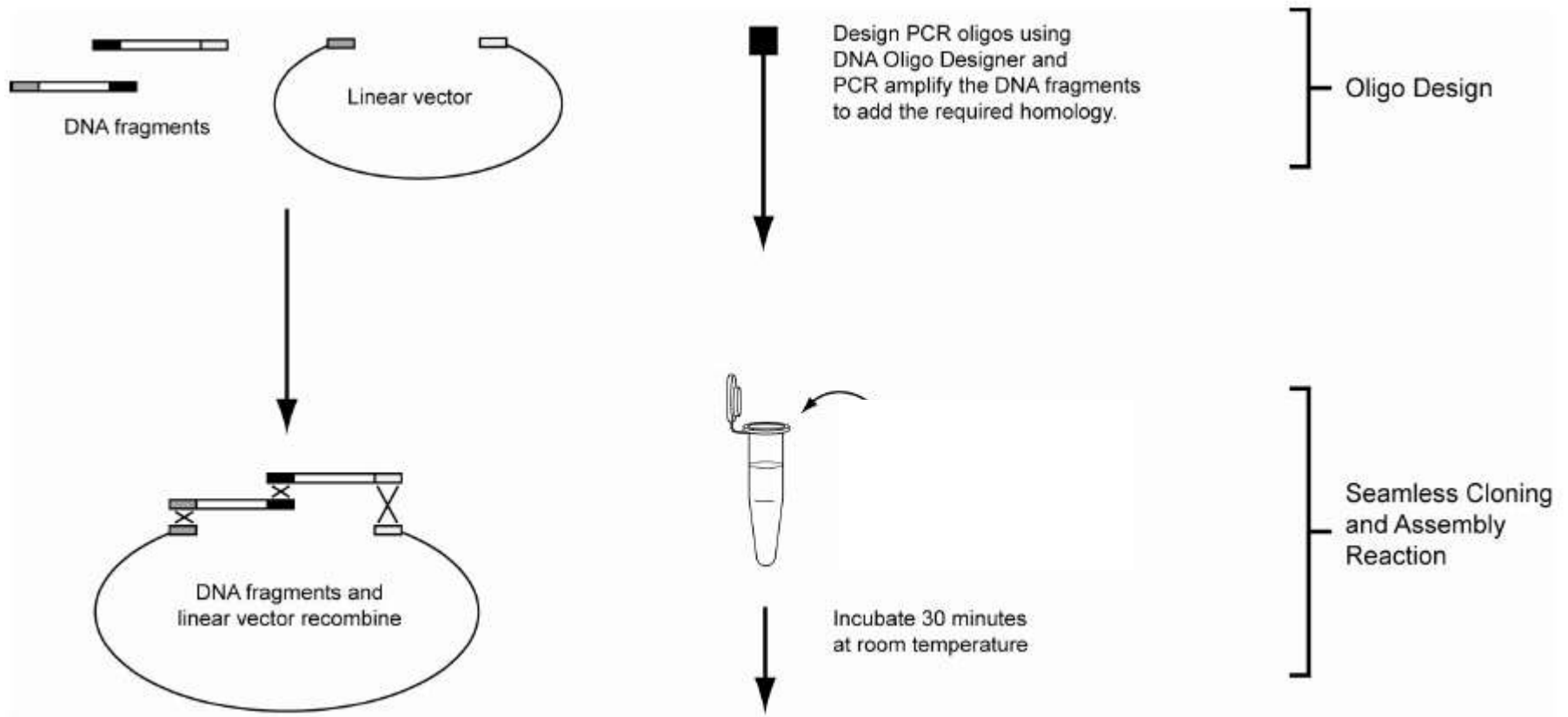


Terminal homology enables simultaneous and directional cloning of fragments





# Seamless Cloning and Assembly Workflow

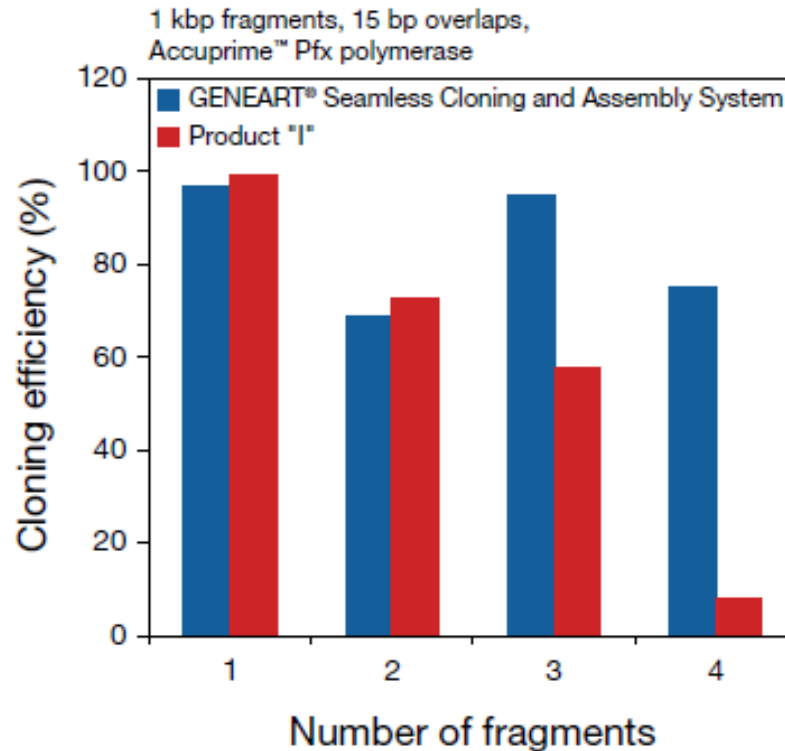


Transform into One Shot® TOP10 chemically competent cells





# Cloning Efficiency vs. Competitive Product



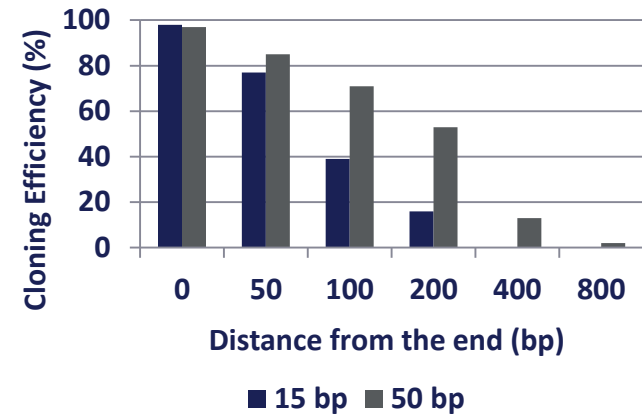
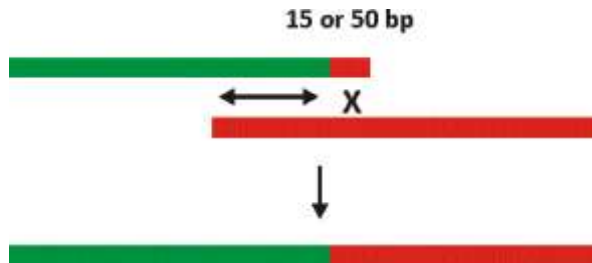
**GeneArt® Seamless Cloning and Assembly System maintains a high cloning efficiency as the number of fragments cloned increases.**



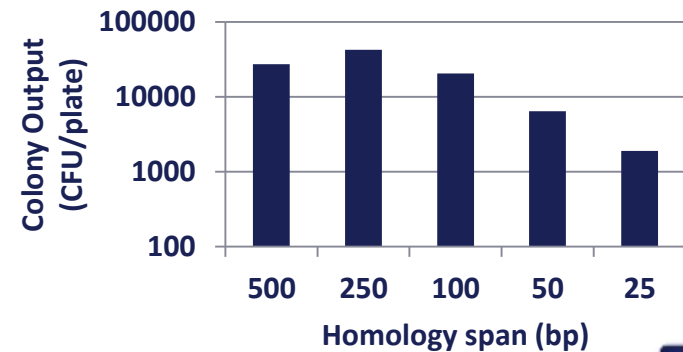
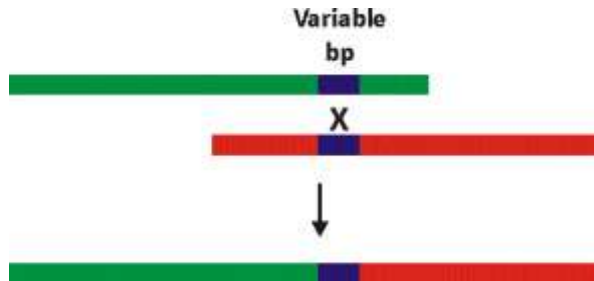


# Recombination away from the ends (end editing)

## *In vitro* - end vs internal



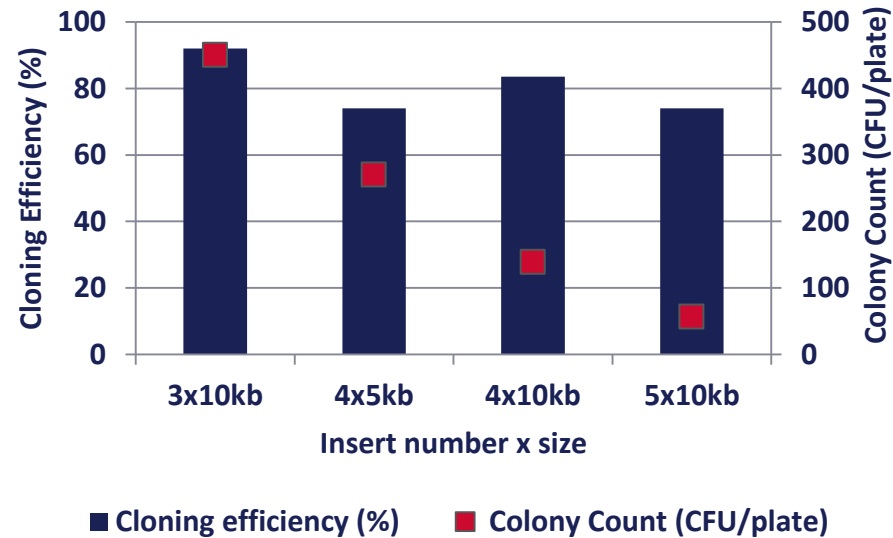
## *In vivo* - internal vs internal





# Improved *in vitro* Assembly

In development



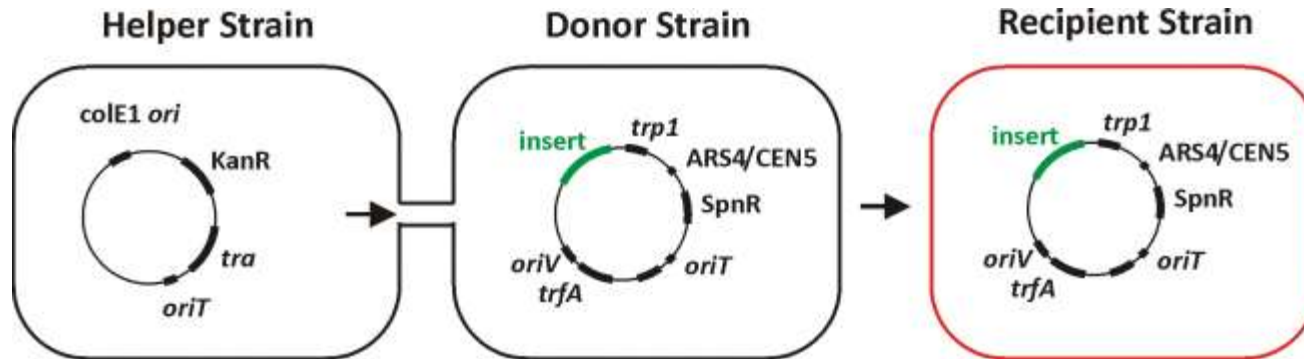
1. New formulation
2. Improved reaction conditions
3. Alternate transformation conditions





# Interspecies Horizontal Transfer

In development

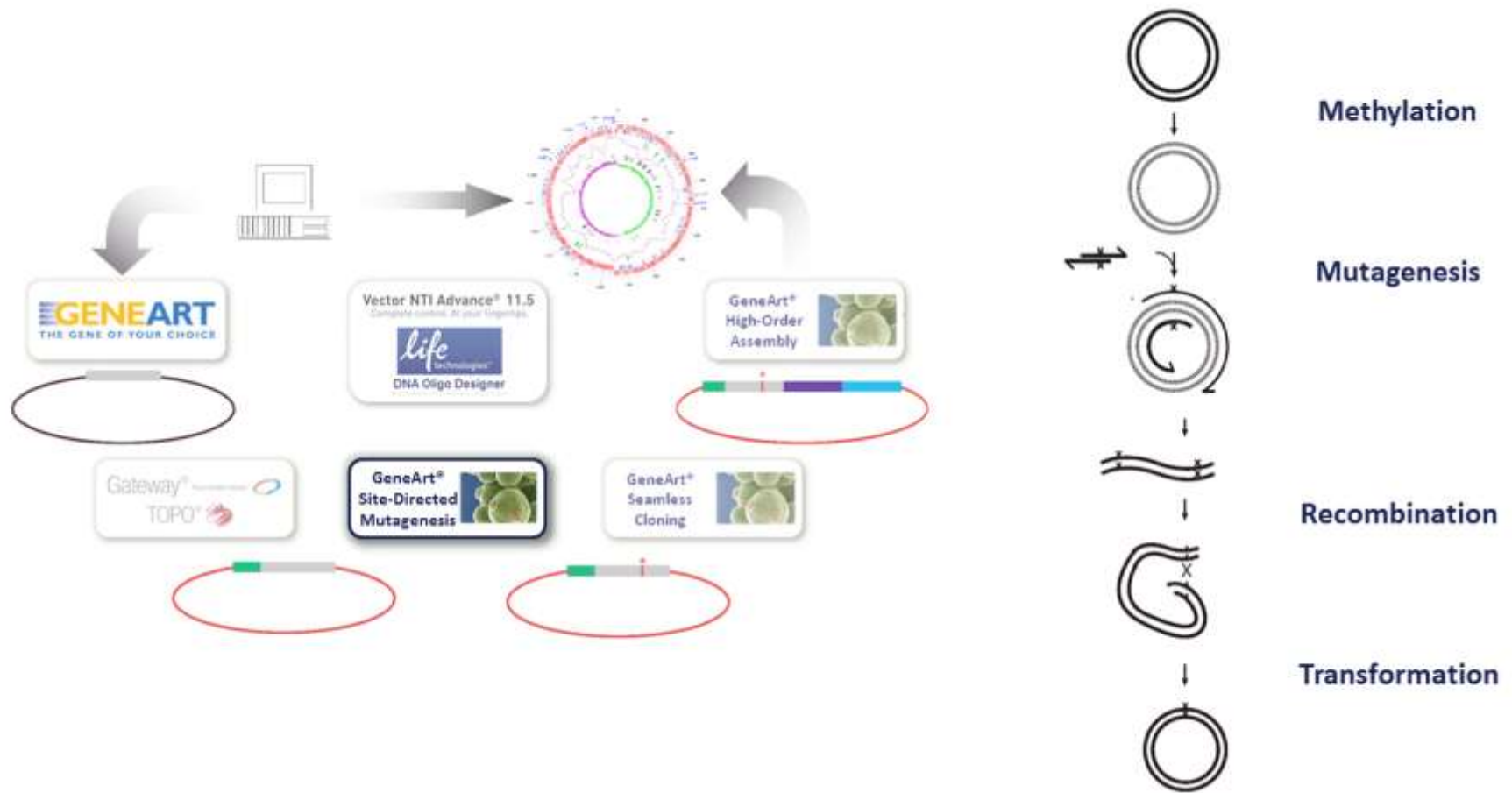


Recipient	Donor + Helper	Recipient	Donor + Helper + Recipient
<i>A. tumefaciens</i>	0	0	>50,000
<i>R. meliloti</i>	60	0	360,000
<i>S. cerevisiae</i>	0	0	5



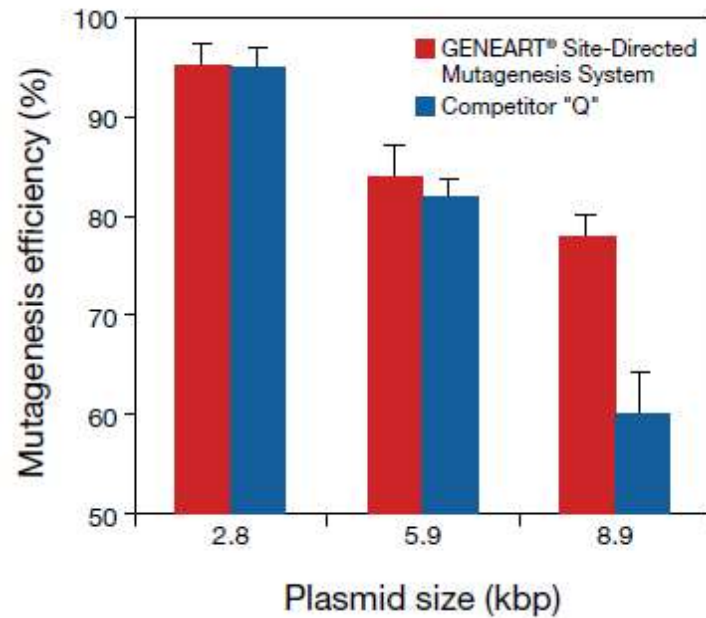


# GeneArt® Site-Directed Mutagenesis





# Mutagenesis Efficiency vs. Competitor



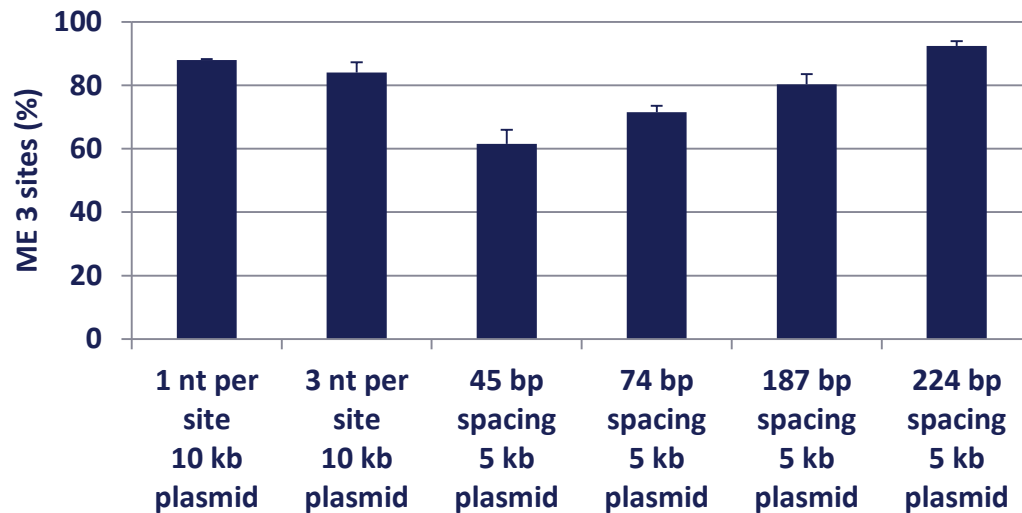
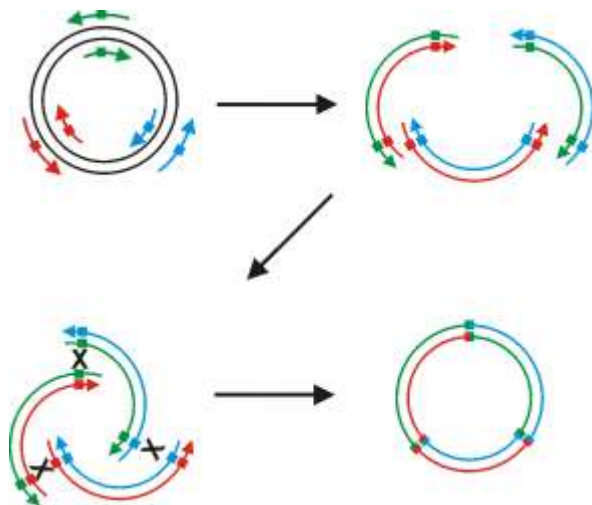
**GeneArt® System has a higher mutagenesis efficiency than a major competitor's kit as the size of the construct increases**





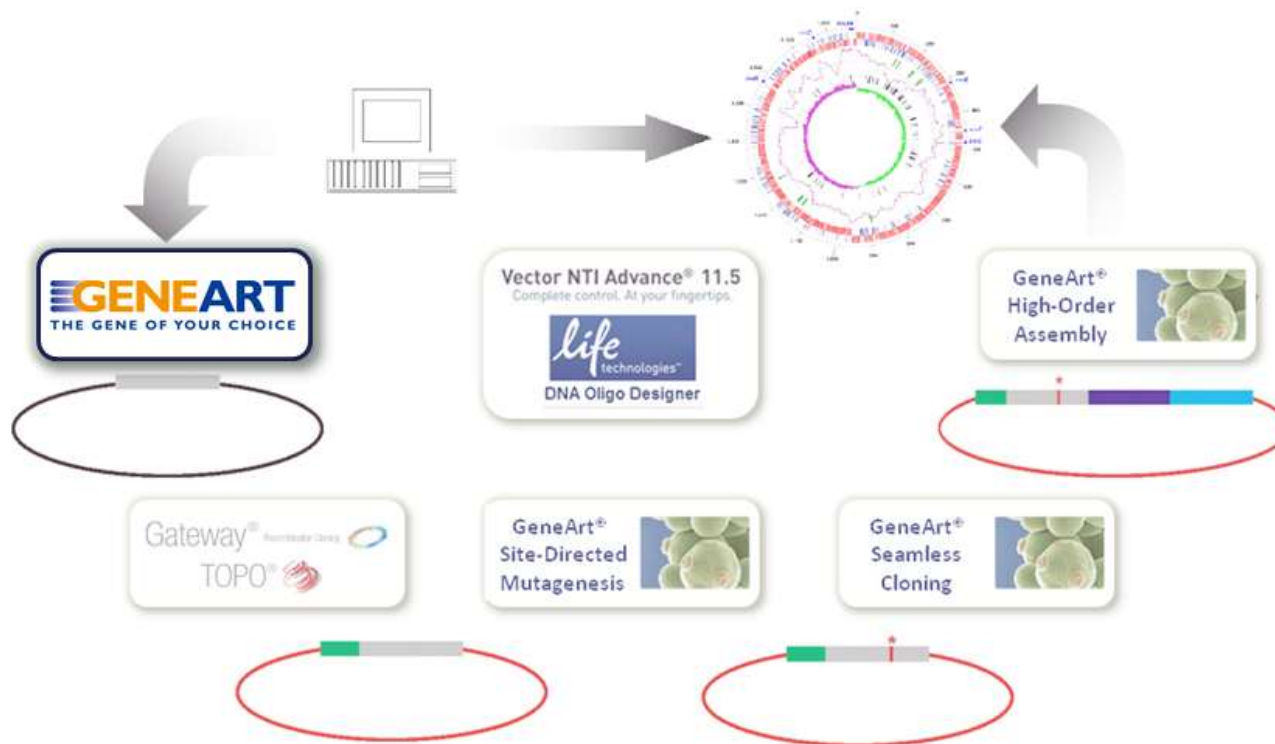
# Multisite-directed mutagenesis

*In development*



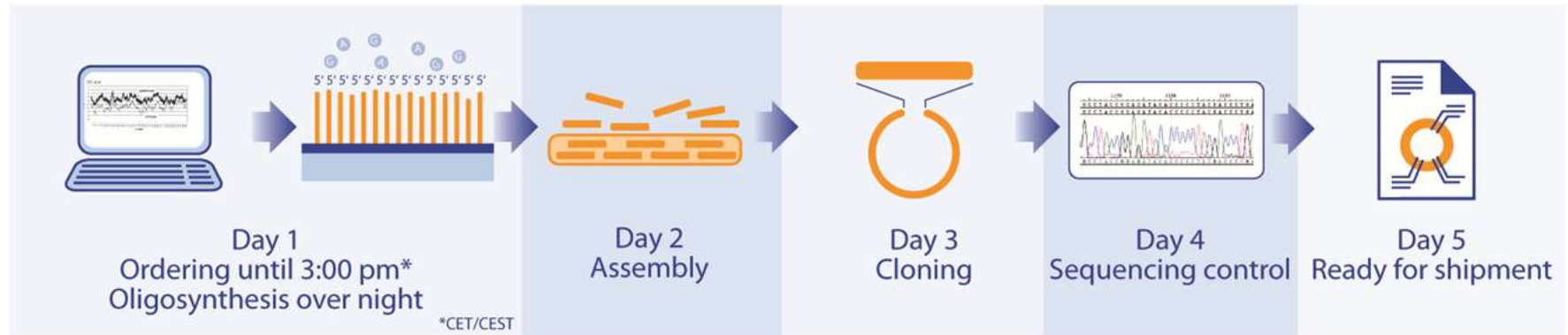


# Gene Synthesis by GeneArt®





# Gene Synthesis by GeneArt® - Fast Access to any Sequence



## ***SuperSPEED***

### **De novo gene synthesis of sequences**

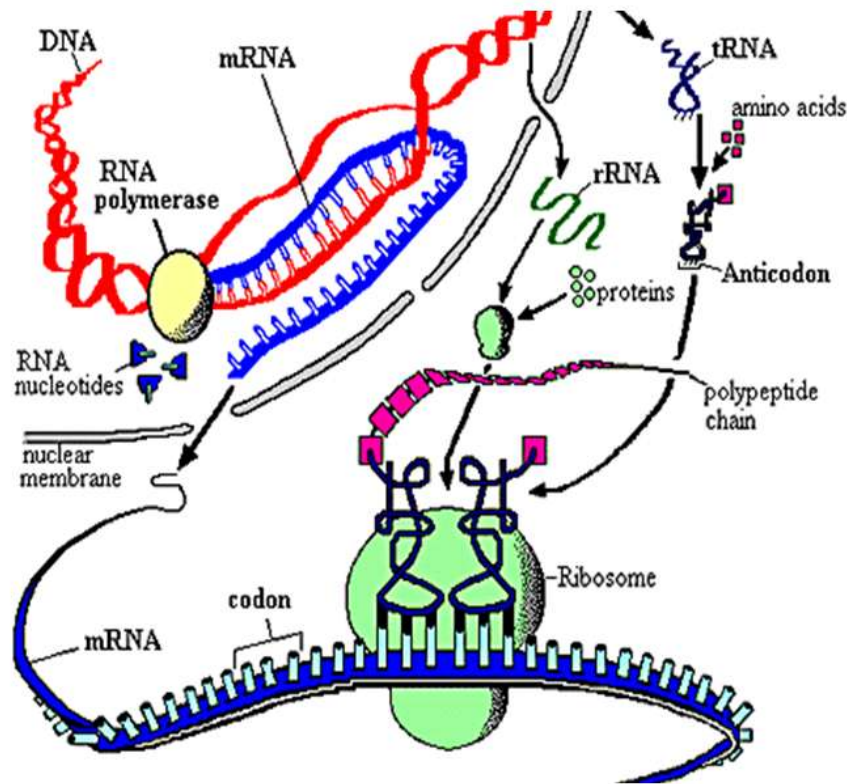
- up to 1,2 kb in 5 business days
- up to 1,8 kb in 7 business days

***Ongoing expansion***





# Multi-Parameter Gene Optimization Challenge Solved by GeneArt®



## Coding Region

- codon usage
- GC content
- cryptic splice sites
- direct repeats
- RNA sec. structures
- instability sequences

GeneOptimizer®





# How to solve the multi-parameter optimization problem?

Iterative



Test all possible



Monte Carlo



GeneOptimizer®  
sliding window

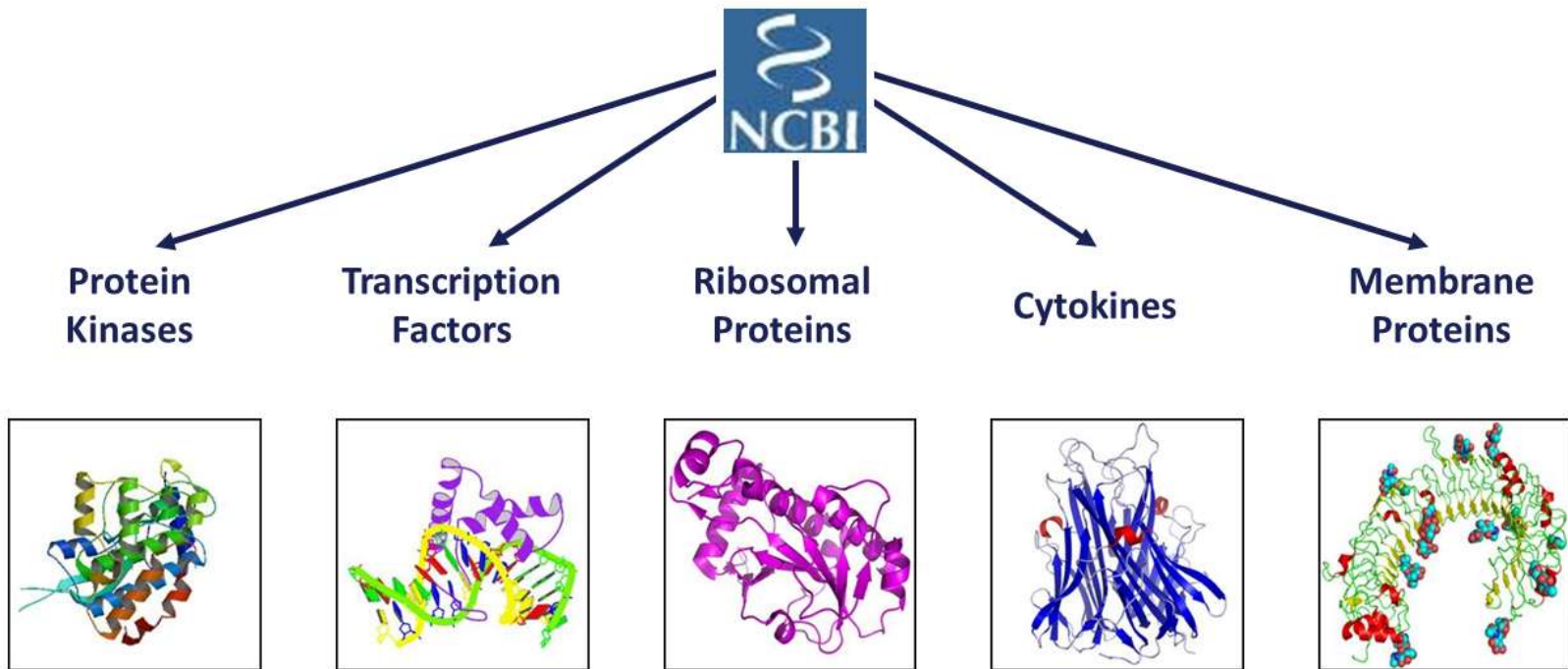




# Multigene study in mammalian HEK293T cells

Gene optimization as a general strategy to improve autologous expression of human genes

50 standard human genes representing the most interesting protein classes were selected from the NCBI data bank



*PLoS One.* 6:e17596, 2011

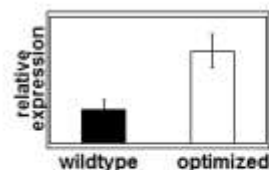
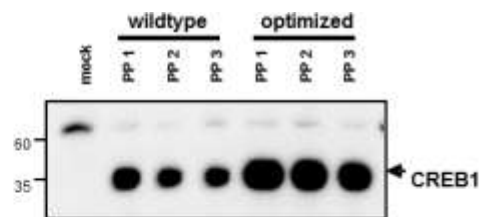
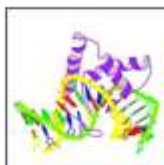


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# Examples and Expression statistics

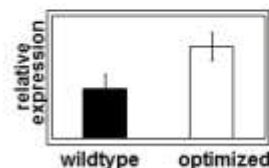
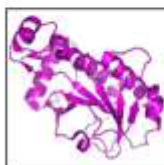
## Transcription Factors



**CREB1**  
▲  
x 2.8

opt > wt	opt = wt	opt < wt	only opt
4	none	none	none

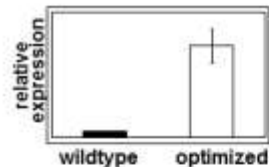
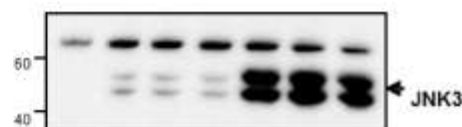
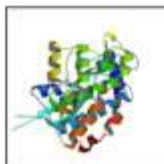
## Ribosomal & other Proteins



**SMARCD1**  
▲  
x 1.8

opt > wt	opt = wt	opt < wt	only opt
4	none	none	none

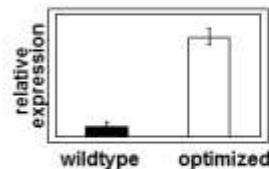
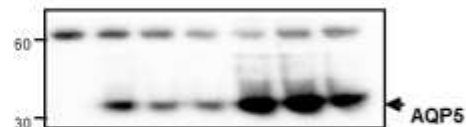
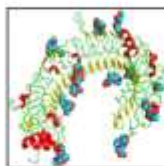
## Protein Kinases



**JNK3**  
▲  
x 14

opt > wt	opt = wt	opt < wt	only opt
13	3	none	none

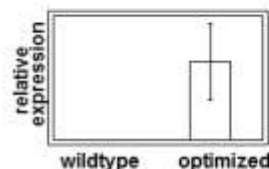
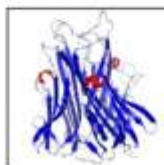
## Membrane Proteins



**AQP5**  
▲  
x 9

opt > wt	opt = wt	opt < wt	only opt
15	1	2	4

## Cytokines



**IL-2**  
▲  
only opt

opt > wt	opt = wt	opt < wt	only opt
6	2	none	2

*PLoS One.* 6:e17596, 2011





# Summary

1. Yeast-based high order assembly method efficiently assembles up to 110 kb.
2. Oligo stitching method: no homology between fragments required.
3. Seamless cloning capable of assembling multiple fragments with high efficiency.
4. Highly-efficient site-directed mutagenesis based on homologous recombination.
5. Gene optimization as a general strategy to improve gene expression.





# Selected Publications

**PLoS One. 6:e17596, 2011**

OPEN ACCESS Freely available online



## Multiparameter RNA and Codon Optimization: A Standardized Tool to Assess and Enhance Autologous Mammalian Gene Expression

Stephan Fath<sup>1</sup>, Asli Petra Bauer<sup>2</sup>, Michael Liss<sup>1</sup>, Anne Spriestersbach<sup>3</sup>, Barbara Maertens<sup>3</sup>, Peter Hahn<sup>3</sup>, Christine Ludwig<sup>1</sup>, Frank Schäfer<sup>3</sup>, Marcus Graf<sup>1</sup>, Ralf Wagner<sup>1,2\*</sup>

<sup>1</sup> Geneart AG, BioPark, Regensburg, Germany; <sup>2</sup> Molecular Microbiology and Gene Therapy Unit, Institute of Medical Microbiology and Hygiene, University of Regensburg, Regensburg, Germany; <sup>3</sup> QIAGEN GmbH, Hilden, Germany

**Syst Synth Biol. 4:215, 2010**

RESEARCH ARTICLE

## The GeneOptimizer Algorithm: using a sliding window approach to cope with the vast sequence space in multiparameter DNA sequence optimization

David Raab · Marcus Graf · Frank Notka ·  
Thomas Schödl · Ralf Wagner

**Methods Enzymol. 498:327, 2011**

## GENETIC ASSEMBLY TOOLS FOR SYNTHETIC BIOLOGY

Billyana Tsvetanova, Lansha Peng, Xiquan Liang, Ke Li, Jian-Ping Yang, Tony Ho, Josh Shirley, Liewei Xu, Jason Potter, Wieslaw Kudlicki, Todd Peterson, and Federico Katzen

**Methods Mol Biol. 834:93, 2012**

## Recombination-Based DNA Assembly and Mutagenesis Methods for Metabolic Engineering

Xiquan Liang, Lansha Peng, Billyana Tsvetanova, Ke Li, Jian-Ping Yang, Tony Ho, Josh Shirley, Liewei Xu, Jason Potter, Wieslaw Kudlicki, Todd Peterson, and Federico Katzen



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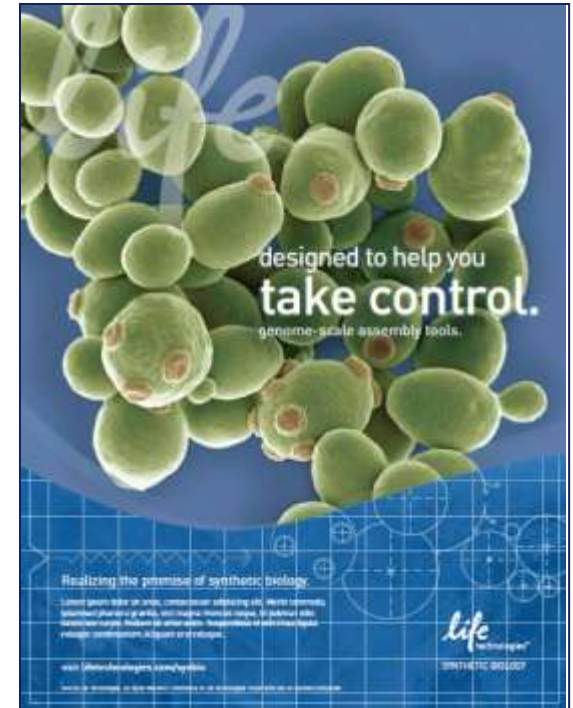
# Acknowledgements

## Gene Assembly

- Lansha Peng
- Billyana Tsvetanova
- Ke Li
- Xiquan Liang
- Linda Hammond
- Todd Peterson

## Gene Synthesis

- Stephan Fath
- Michael Liss
- David Raab
- Marcus Graf
- Frank Notka
- Thomas Schödl
- Christine Ludwig
- Ralf Wagner



[www.lifetechnologies.com/syntheticbiology](http://www.lifetechnologies.com/syntheticbiology)



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