

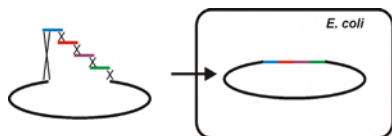


life

**GeneArt® Seamless PLUS and Site-Directed Mutagenesis PLUS**  
**GeneArt® Construct and Primer Design Webtool**

# New Products Key Benefits

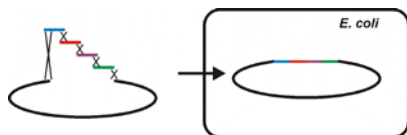
## GeneArt® Seamless PLUS Cloning and Assembly



### Complete Kit for Seamless Cloning & Assembly

- *in vitro* assembly of 4 fragments for constructs  $\leq 40$ kb
- Ability to clone fragments up to 10kb
- Enough pUC19L for 20 reactions
- All-in-One enzyme/buffer mix with 1 hr RT stability
- One Shot® DH10B™ T1<sup>R</sup> SA Competent Cells
- Interspecies conjugative transfer

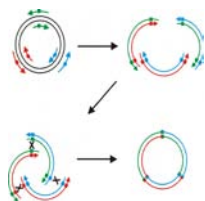
## GeneArt® Seamless Cloning and Assembly Enzyme Mix



### Economic option for Seamless Cloning & Assembly

- *in vitro* assembly of up to 4 fragments for constructs  $\leq 13$ kb
- Enough pUC19L for 20 reactions
- All-in-One enzyme/buffer mix with 1 hr RT stability

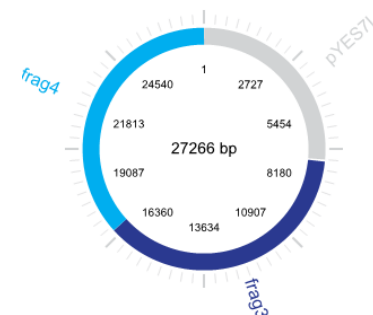
## GeneArt® Site-Directed Mutagenesis PLUS



### Single AND multi-site mutagenesis in one kit

- Up to 3 nucleotides may be inserted, deleted or changed in 1, 2, or 3 separate sites
- All-in-One enzyme/buffer mix with 1 hr RT stability
- All single-site functionality of the previous GeneArt® Site-Directed Mutagenesis

**Updated Free Webtool**  
now works for  
All Seamless Assembly  
& Mutagenesis  
Products



Oligo Name	Oligo Sequence	Purity	Tm	GC	Clonetrans
fragment1_FW	AAATTGAGCTGCTGCTAC	25kDLS	63.0	56.0	
fragment1_RV	TATGTAGGGGCTATGCT	25kDLS	74.0	51.0	
fragment2_FW	GCCATGCCCCCTACATA	25kDLS	76.0	57.0	
fragment2_RV	GCCAGGCTTCATGCT	25kDLS	67.0	58.0	

[Add To Cart](#)

[Download assembled molecule and DNA oligos](#)

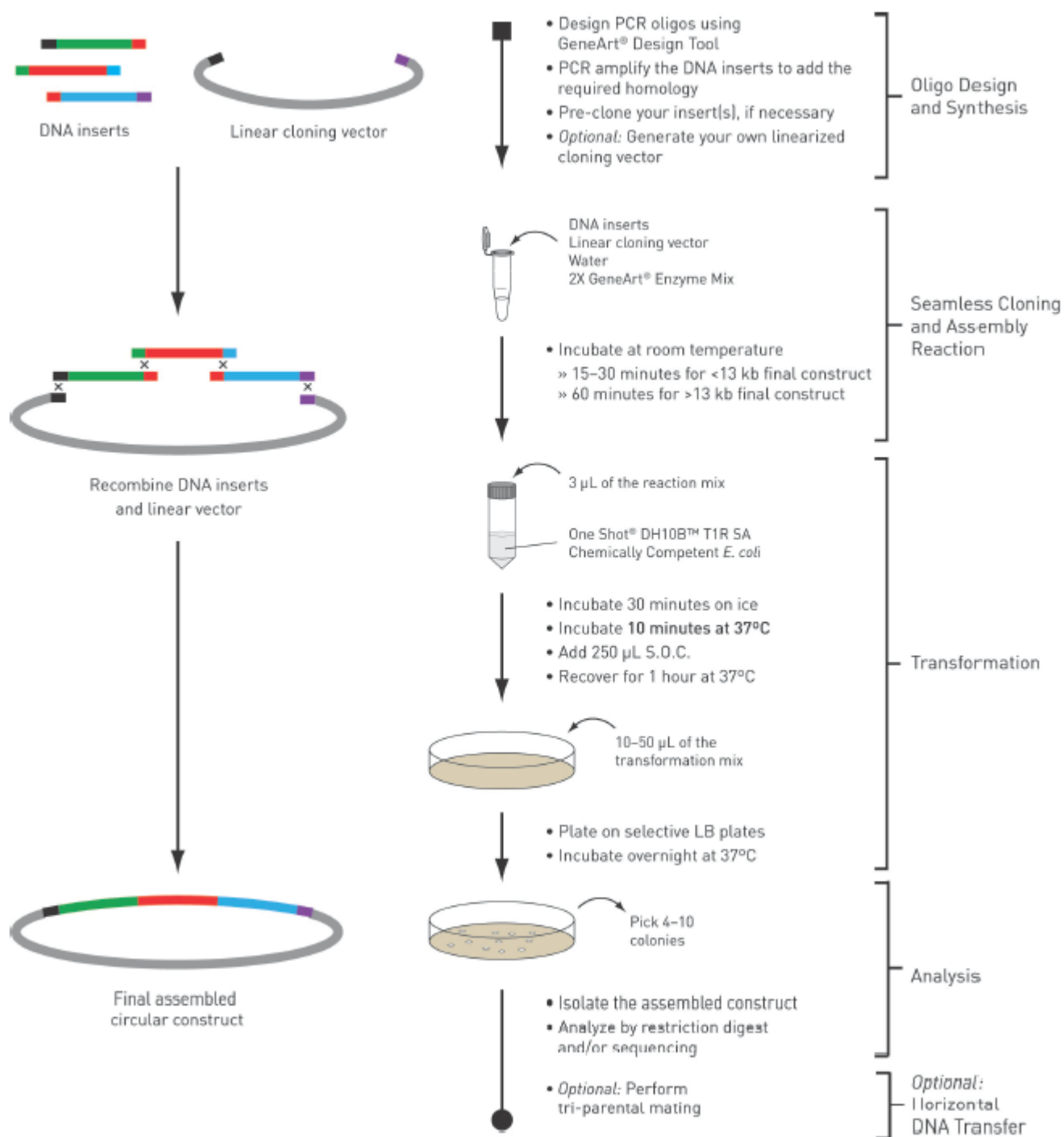
# GeneArt® Seamless PLUS Cloning and Assembly

- *in vitro* assembly of up to 4 fragments and constructs  $\leq$  40kb
- 30 minute RT reaction for most constructs
- Ability to clone fragments up to 10kb
  - Precloning option minimizes laborious PCR optimization
  - New webtool guides user through precloning process, designs primers, provides one-click purchase of oligos
- Use any linearized vector or pUC19L included in the kit
- All-in-one enzyme/buffer mix
  - Less pipetting
  - Store at -20°C and room temperature stable for 1 hour
  - Good for high through-put workflows
- One Shot® DH10B T1R SA competent cells
  - Transform 10 minutes @ 37°C
- Free, easy-to-use GeneArt® webtool assists with construct and primer design
- Interspecies conjugative transfer

# GeneArt® Seamless Cloning and Assembly Enzyme Mix

- *in vitro* assembly of up to 4 fragments
  - Max construct size = 13kb
  - Max fragment size = 10kb
- Economical choice for high-throughput workflows with smaller construct sizes
- Use any linearized vector or pUC19L included in the kit
- Free, easy-to-use GeneArt® webtool assists with construct and primer design
- Contents:
  - All-in-One enzyme/buffer mix
  - pUC19L
  - Control insert

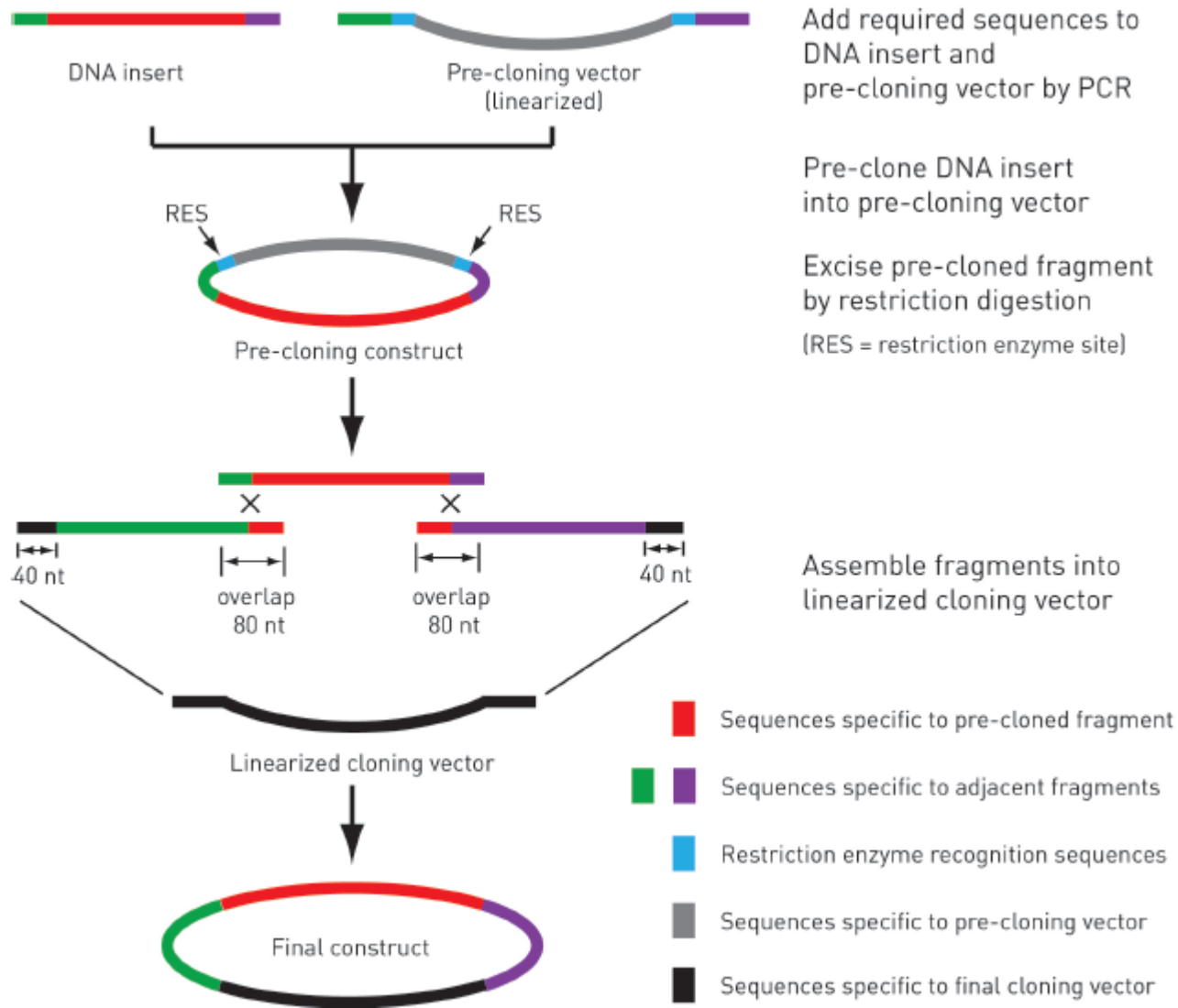
# GeneArt® Seamless Cloning and Assembly Workflow



# Pre-cloning

- Cloning a DNA insert generated by PCR amplification into a small intermediate vector, the pre-cloning vector, and then excising by restriction digestion prior to final assembly
  - Inserts >2.5 kb and belonging to large assemblies (>13 kb) should be pre-cloned
  - Reduces PCR optimization for large fragments
  - Minimizes the size of the primers required for generating the DNA insert to be pre-cloned
  - Increase the cloning efficiency and colony output for the final assembly
- Process
  - PCR amplify the insert(s) to incorporate the sequences required for pre-cloning and final assembly
  - Transform to propagate clone
  - Excised fragment has “cleaner” ends and no contamination from other bands
  - Detailed protocols in manual and GeneArt® Construct and Primer Design webtool

# Precloning Workflow

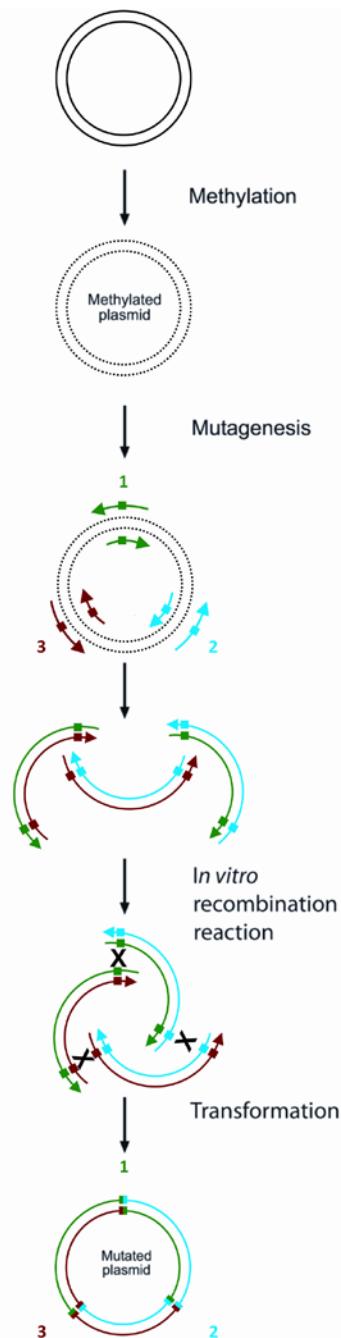


# GeneArt® Site-Directed Mutagenesis PLUS

- Single AND multi-site mutagenesis in one kit
  - Up to 3 nucleotides, including degenerated, may be inserted, deleted or changed in 1, 2, or 3 separate sites
  - Up to 25 nucleotide, or 12 degenerated nucleotide, mutation in single site
  - Plasmids up to 14kb
  - >90% efficiency for 1, 2, or 3 sites
  - All single site functionality of current mutagenesis kit
  - DNA methylation and amplification in a single step, no *in vitro* digestion or purification steps required after mutagenesis reaction
- All-in-one enzyme/buffer recombination mix
  - Less pipetting
  - Store at -20°C and room temperature stable for 1 hour
  - Good for high through-put workflows
- Detailed rules for primer design included in the manual
- Both mutagenesis kits supported by free GeneArt® construct and primer design webtool



# GeneArt® Site-Directed Mutagenesis Workflow



Plasmid template  
PCR Reagents  
Methyl Transferase Reagents



1. Methylate plasmid DNA and amplify the plasmid in a mutagenesis reaction with up to three overlapping primers containing the target mutations

PCR Sample  
2X Enzyme Mix



2. Perform the *in vitro* recombination reaction.

Add 1  $\mu$ L 0.5 M EDTA to stop the reaction  
Use 2  $\mu$ L of recombination reaction sample for transformation



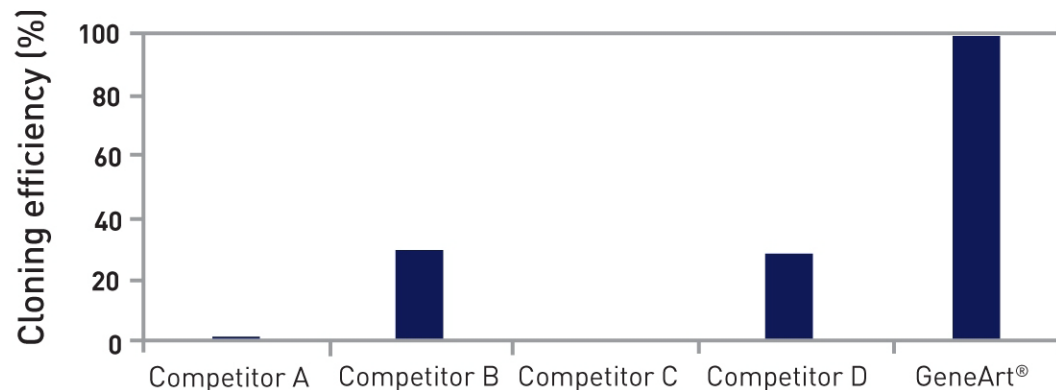
3. Transform the sample into DH5 $\alpha$ ™-T1<sup>R</sup> competent *E. coli*. The host cell circularizes the linear mutated DNA, and *McrBC* endonuclease in the host cell digests the methylated template DNA, leaving only unmethylated, mutated product

Incubate on ice for 15 minutes  
Heat shock for 30 seconds  
Add 250  $\mu$ L SOC  
Recover for 1 hour at 37°C



# GeneArt® Outperforms the Competition

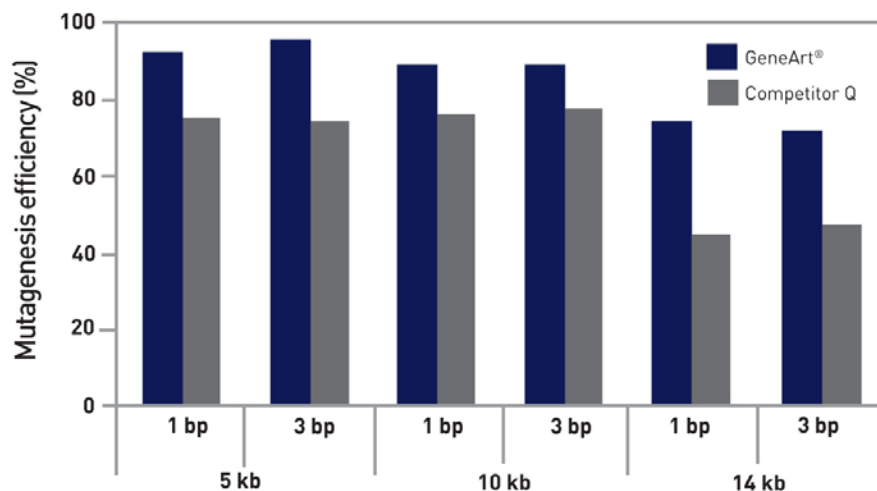
## GeneArt® Seamless PLUS Cloning and Assembly vs the Competition (%CE w/4 inserts, 5 kb each)



GeneArt® Seamless PLUS Cloning and Assembly outperforms the competition. In this experiment, 4 DNA fragments, 5 kb each, were pre-cloned\* and then assembled into linear vector pYES7L (Life Technologies, Inc) using manufacturer's recommended protocols.

Cloning efficiency (%CE) = % clones with correct inserts and construct design.

pre-cloning\* avoids problems of PCR amplification of large fragments



## GeneArt® Site-Directed Mutagenesis PLUS Kit vs Competitor Q

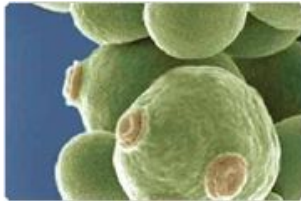
GeneArt® Site-Directed Mutagenesis PLUS (blue columns) outperforms Competitor Q (grey columns). In this experiment, 3 sites of 1bp or 3bp each were changed (including 1 insertion, 1 deletion, and 1 substitution) in 5, 10, and 14kb plasmids.

Mutagenesis efficiency (%ME) = % clones with correct changes.

# GeneArt® Primer and Construct Design Tool for Seamless or High-Order Assembly and Mutagenesis

<http://rna.qa2.invitrogen.com/oligoDesigner/>

## GeneArt® Primer and Construct Design Tool for Seamless or High-Order Assembly and Mutagenesis



This tool facilitates the in silico design, assembly, or mutagenesis of a DNA molecule using GeneArt® technology. Use the GeneArt® Design Tool in conjunction with GeneArt® Seamless Cloning and Assembly Kits (e.g., [Seamless PLUS](#), [High-Order](#), [Seamless Cloning and Assembly Kit](#), and [Seamless Enzyme Mix](#)) and GeneArt® Mutagenesis Kits (e.g., [Site-Directed Mutagenesis PLUS](#) and [Site-Directed Mutagenesis](#)) to simplify construct and primer design.

Starting with the sequence information for your DNA fragments, the GeneArt® Design Tool:

- Guides you through construct design, including fragment import, order, and orientation
- Checks for areas of homology and potential design issues
- Creates primers for pre-cloning or incorporating end-homology between fragments (if needed) or primers for mutagenesis, depending your workflow
- Shows updated amino acid sequence for mutagenized constructs
- Allows one-click online ordering for custom primers (not available in all countries)
- Generates final construct sequence, in GenBank format, and map files for easy download or import into [Vector NTI® software](#)

For additional information regarding this tool or the products it supports, visit our [DNA Assembly page](#) or contact Technical Support.

To begin, choose your kit from the list below:

### Select Product Type

GeneArt® Seamless Cloning and Assembly ▼

GeneArt® Site-Directed Mutagenesis ▼

Continue

# GeneArt® Primer and Construct Design Tool

## Select Product Type

### GeneArt® Seamless Cloning and Assembly

- ☐ **GeneArt® Seamless PLUS Cloning and Assembly Kit (A14603):** **NEW**  
Highest-efficiency *in vitro* system for assembly of up to 4 DNA fragments and any vector totaling up to 40 kb in length, with the added convenience of increased enzyme stability, "all-in-one" enzyme/buffer mix, pre-cloning option for large fragments for increased cloning efficiency, and optional horizontal gene transfer.
- ☐ **GeneArt® High-Order Genetic Assembly (A13285):**  
Highest efficiency *in vivo* system for simultaneous and seamless assembly of up to 10 fragments and any vector totaling up to 110 kb in length. This system relies on yeast's ability to take up and recombine DNA fragments with high efficiency.
- ☐ **GeneArt® Seamless Cloning and Assembly Kit (A13288):**  
For *in vitro* assembly of up to 4 DNA fragments and any vector totaling up to 13 kb in length.
- ☐ **GeneArt® Seamless Cloning and Assembly Enzyme Mix (A14606):** **NEW**  
The economic solution for *in vitro* assembly of DNA fragments and any vector totaling up to 13 kb in length.

### GeneArt® Site-Directed Mutagenesis

- ☐ **GeneArt® Site-Directed Mutagenesis PLUS Kit (A14604):** **NEW**  
Generate base substitutions, deletions, and insertions of up to 3 nucleotides in 1, 2, or 3 separate sites in DNA plasmids of up to 14 kb
- ☐ **GeneArt® Site-Directed Mutagenesis Kit (A13282):**  
Generate base substitutions, deletions, and insertions of up to 25 nucleotides in a single site in DNA plasmids of up to 14 kb.

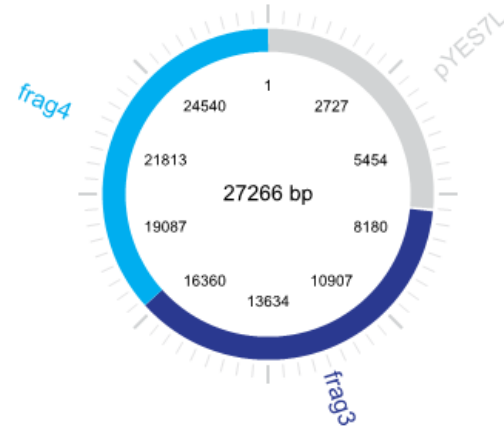
Continue

# New and Improved GeneArt® Web-Design Tool








Facilitates the *in silico* construct and primer design

- **Now includes *in silico* design for mutagenesis!**
- Interface for product selection and sequence entry
- Designs DNA oligos for PCR amplification prior to assembly, mutagenesis, or oligo stitching
- Checks for potential issues during assembly or mutagenesis, ie terminal or internal homology regions
- Provides option to download final construct and primer sequences in GenBank format
- Option to purchase oligos directly from the tool
- Detailed cloning and assembly instructions

## Final Construct



 [Download GenBank File](#)

<input type="checkbox"/>	Oligo	Oligo Sequence	Oligo Size	Purity	Tm (°C) (Template Specific)
<input type="checkbox"/>	 pYES7L_FW	AAGTACGACGTGCTGC	35 nt	<input type="text" value="25 nanomoles, desalted"/>	53.2
<input type="checkbox"/>	 pYES7L_RV	AATACCCAGCGTAATG	35 nt	<input type="text" value="25 nanomoles, desalted"/>	53.2
<input type="checkbox"/>	 frag3_FW	CGGCCGCGCTGATACC	41 nt	<input type="text" value="25 nanomoles, desalted"/>	51.0
<input type="checkbox"/>	 frag3_RV	TTCGATAACTACACTG	46 nt	<input type="text" value="25 nanomoles, desalted"/>	51.0
<input type="checkbox"/>	 frag3_pUC19L_RV	GGTATCAGCGCGGCC	43 nt	<input type="text" value="25 nanomoles, desalted"/>	60.3
<input type="checkbox"/>	 frag3_pUC19L_FW	CAGTGTAGTTATCGAA	48 nt	<input type="text" value="25 nanomoles, desalted"/>	51.0
<input type="checkbox"/>	 frag4_FW	CCAGCCCGGAGGCGAA	48 nt	<input type="text" value="25 nanomoles, desalted"/>	51.2

# Which cloning method is right for you?

<http://www.invitrogen.com/site/us/en/home/References/an-introduction-to-cloning-a-researchers-guide-to-cloning-dna/choose-a-cloning-method.html>

## When to Choose a Particular Cloning Technology

	Restriction Enzyme Cloning	TA-Cloning	TOPO® (TA, blunt, directional)	Gateway®	GeneArt® Seamless Cloning	GeneArt® High-Order Genetic Assembly
Fragments Cloned Simultaneously	1	1	1	Up to 4	Up to 4	Up to 10
Max Fragment (s) Size	Variable	1-3 Kbp	<5 kbp (<10 Kbp for XL-TOPO®)	Variable	Up to 40 Kbp max total size of 13 Kbp	Up to 100 Kbp, max total size of 110 Kbp
Gene Shuttling Between Vectors w/o PCR or Restriction Enzymes	NO	NO	NO	YES	NO	NO
Seamless (No Extra Sequences)	NO	NO	NO	NO	YES	YES
Use Your Own Vector w/o Modification	YES	NO	NO	YES	YES	YES
Use Pre-existing Fragments w/o Modifications	NO	NO	NO	NO	YES 4 Fragments	YES 10 Fragments
Time to Clone Multiple Fragments	Days to Weeks	Not Possible	Not Possible	>4 Days	1 Day	3 Days
4 Fragment Cloning Efficiency	NA	NA	NA	30% - 85%	75%	>90%
Web-Based Vector Design Tool	NO	NO	NO	NO	YES	YES
List of Available Vectors	GO	GO	GO	GO	GO	GO

© 2012 Life Technologies Corporation. All rights reserved.  
The trademarks mentioned herein are the property of Life Technologies Corporation and/or its affiliate(s) or their respective owners.