invitrogen

# GeneArt seamless cloning, assembly, and mutagenesis tools

## Engineered for predictability

ThermoFisher SCIENTIFIC

# Cut, edit, create. Break through with GeneArt genetic assembly tools.

Invitrogen™ GeneArt™ products are developed to provide innovative, robust solutions to meet the vast needs of genetic engineers, synthetic biology researchers, and molecular biologists. We've made every effort to provide easy-to-implement workflow solutions to deliver fast, quality results.

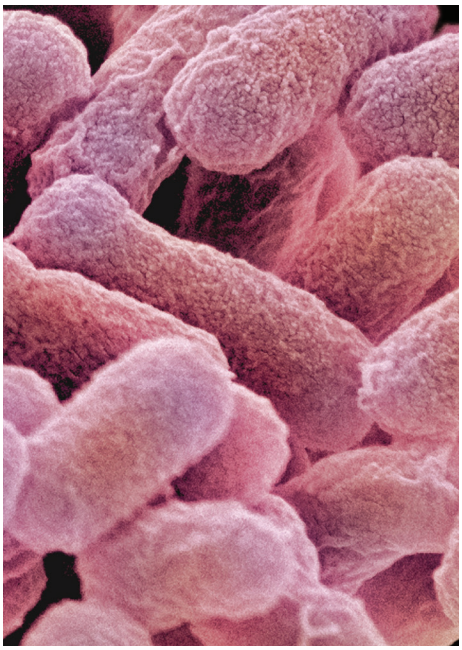

**Free online construct and primer design tool**

Convenient and intuitive, the Invitrogen™ GeneArt™ design tool is accessible to all researchers—free of charge—and is built to ease your workflow and maximize downstream results.

Step by step, the online tool guides users through experimental design and ordering—designing oligos for assembly or mutagenesis of DNA molecules *in silico*.

Explore this one-of-a-kind online tool at **thermofisher.com/GeneArt**

# Contents



# Seamless cloning and assembly

## Flexible workflow solutions to streamline your research

Invitrogen™ GeneArt™ Seamless Cloning and Assembly Kits enable *in vitro* cloning of up to 4 DNA fragments simultaneously into virtually any linearized vector, typically in 30 minutes, without extra DNA sequences, restriction endonucleases, or ligation. With potential construct sizes of up to 40 kb, our kits offer researchers the flexibility and convenience to complete basic, standard, and advanced cloning and assembly protocols.

- **Flexible**—use any vector of your choice
- **Precise**—no scars; clone what you want, where you want it
- **Efficient**—>90% cloning efficiency
- **Convenient**—use our free online web tool to design oligos and assemble DNA molecules *in silico*
- **Fast**—fragment assembly typically in half a day

**Invitrogen™ GeneArt™ Seamless Cloning Enzyme Mix**

- Chemically competent cell transformation
- Linear cloning vector included
- Good for high throughput (HTP)
- Maximum construct size of 13 kb

**GeneArt Seamless Cloning and Assembly Kit**

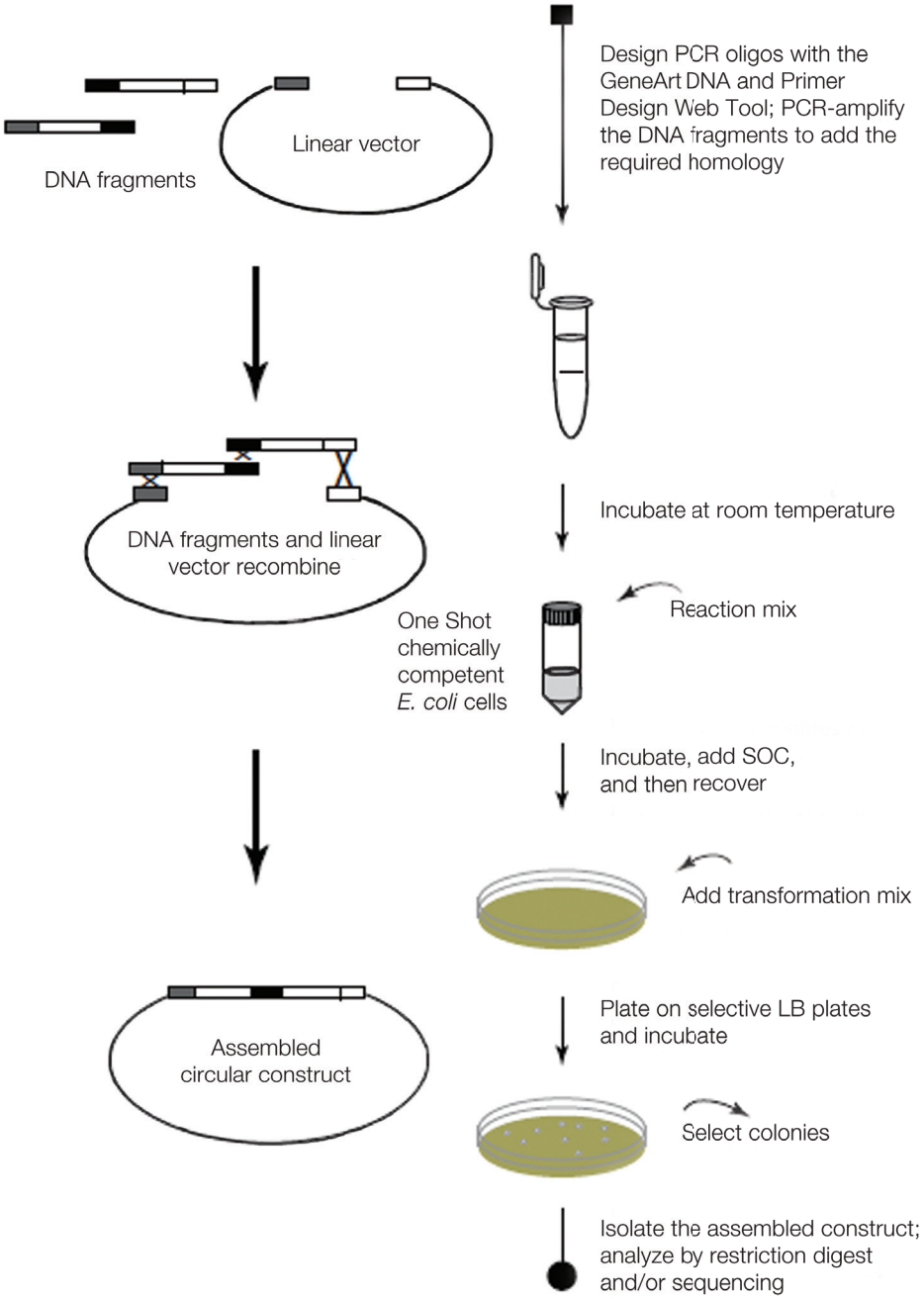
- Good cloning efficiency
- Chemically competent *E. coli* host and linear cloning vector included
- Maximum construct size of 13 kb

**Invitrogen™ GeneArt™ Seamless PLUS Cloning and Assembly Kit**

- Superior cloning efficiency
- Chemically competent *E. coli* host, media, linear cloning vector included
- Maximum construct size of 40 kb
- Good for HTP
- Conjugative gene transfer to most gram-negative bacteria

GeneArt Seamless Cloning and Assembly Kits use a proprietary enzyme mix to recognize and precisely assemble DNA fragments sharing end homology. End homology is created by PCR amplification using primers designed to generate the correct overlap between adjacent DNA fragments to be assembled.

## GeneArt seamless cloning and assembly workflow



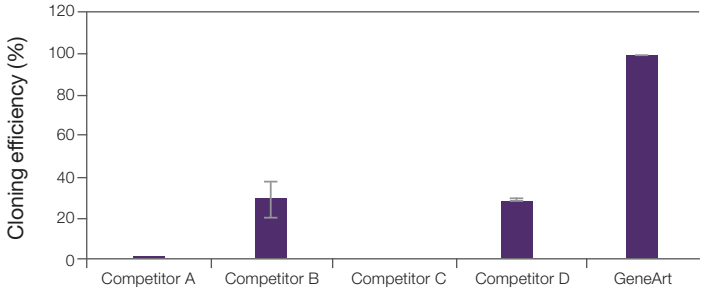




**Figure 1. Cloning efficiency of the GeneArt Seamless PLUS Cloning and Assembly Kit with 4 precloned DNA fragments of 5 kb each, compared to other manufacturer's kits.**

### High-order genetic assembly

In addition to seamless assembly and high efficiency, the vector-independent Invitrogen™ GeneArt™ High-Order Genetic Assembly System for *in vivo* assembly delivers on:

**Speed**—clone up to 10 DNA fragments simultaneously into a single vector (up to 110 kb total)

**Convenience**—assemble existing DNA fragments without restriction digestion or PCR amplification

The GeneArt High-Order Genetic Assembly System relies on yeast's ability to take up and recombine DNA fragments with high efficiency via transformation-associated recombination, greatly reducing *in vitro* handling of DNA and eliminating the need for restriction digestion and ligation.

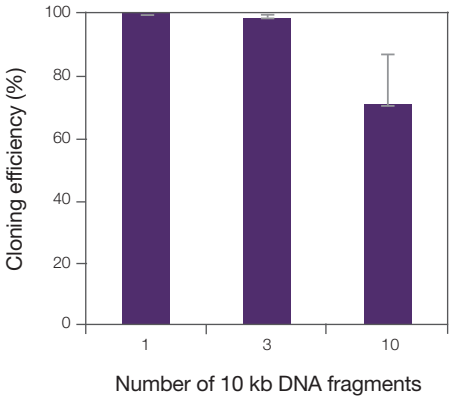



**Figure 2. Cloning efficiencies for 1, 3, and 10 DNA fragments, 10 kb each, amplified using Invitrogen™ Platinum™ PCR SuperMix High Fidelity and cloned into pYES1L using the GeneArt High-Order Genetic Assembly System.**

# Site-directed mutagenesis

## Conveniently mutate 1, 2, or 3 sites from a single plasmid

Invitrogen™ GeneArt™ Site-Directed Mutagenesis Systems provide a simple and highly efficient method for *in vitro* site-directed mutagenesis. These systems can generate base substitutions, deletions, or insertions in plasmids purified from *E. coli*, without specialized vectors, host strains, or restriction sites—an ideal solution for routine or complex mutagenesis.

- **Flexible**—choose from single site or multisite directed mutagenesis products
- **Precise**—alter up to 25 nucleotides when only one site is mutated
- **Efficient**—mutagenesis efficiency >90% (using a 3 kb plasmid)
- **Convenient**—use our free online web tool to design oligos and mutate DNA molecules *in silico*
- **Fast**—time-to-results is typically less than 3 hours (using a 10 kb or smaller plasmid)
- **Flexible**—use any vector of your choice

**GeneArt Site-Directed Mutagenesis System**

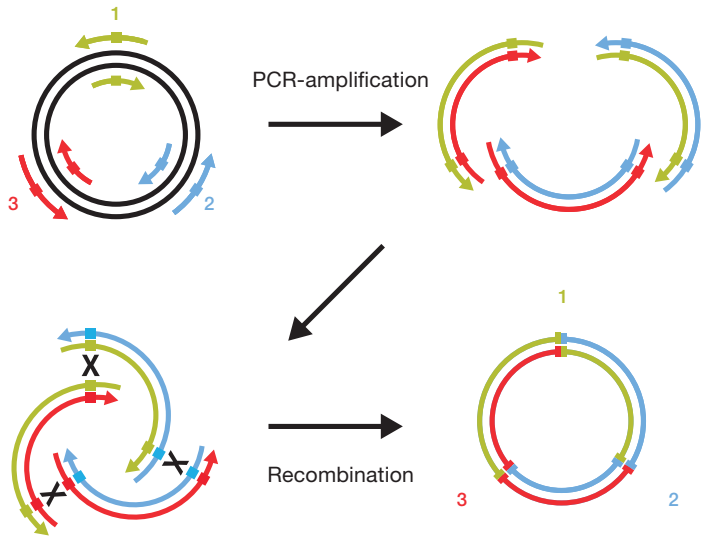
- 1 site/plasmid
- Efficiency >90%

**GeneArt Site-Directed Mutagenesis PLUS System**

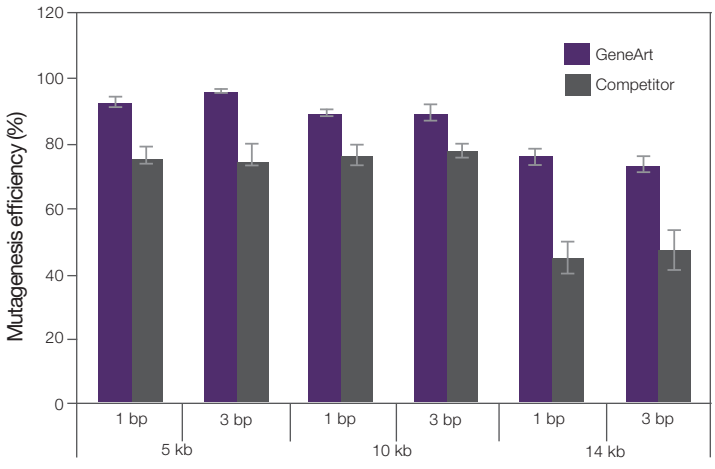
- Perform mutagenesis on 1, 2, or 3 sites—all in the same plasmid
- Efficiency >90%

The GeneArt Site-Directed Mutagenesis Systems utilize mutagenic oligonucleotide primers to generate mutations. The mutagenesis protocol is streamlined by combining DNA methylation and amplification steps into a single reaction, eliminating post-mutagenesis digestion and purification steps.

These systems deliver superior mutagenesis performance with a wide range of plasmid sizes.




**Principle of method for multisite directed mutagenesis.**




GeneArt Site-Directed Mutagenesis PLUS System vs. competitor.

## Ordering information

| Product | Quantity | Cat. No. |
|---|---|---|

### GeneArt Seamless Cloning and Assembly Systems

**GeneArt Seamless PLUS Cloning and Assembly Kit** — 20 reactions — A14603

| Component | Size | Quantity |
|---|---|---|
| 2X GeneArt Enzyme Mix | 100 µL/tube | 1 tube |
| Linear pYES7L Vector (50 ng/µL) | 40 µL/tube | 1 tube |
| One Shot DH10B T1 SA Cells | 21 x 50 µL/tube | 1 tube |
| Control lacZ Insert | 5 µL/tube | 1 tube |
| Linear pUC19L Vector (50 ng/µL) | 40 µL/tube | 1 tube |

| Component | Size | Quantity |
|---|---|---|
| pUC19 Control (10 pg/µL) | 10 µL/tube | 1 tube |
| S.O.C. Medium | 6 mL/bottle | 1 bottle |
| Control Insert (50 ng/µL) | 5 µL/tube | 1 tube |
| Stbl3/pRK2013 Glycerol Stock* | 1 mL/tube | 1 tube |

**GeneArt Seamless Cloning Enzyme Mix** — 20 reactions — A14606

| Component | Size | Quantity |
|---|---|---|
| 2X GeneArt Enzyme Mix | 100 µL/tube | 1 tube |
| Linear pUC19L Vector (50 ng/µL) | 40 µL/tube | 1 tube |
| Control Insert (50 ng/µL) | 5 µL/tube | 1 tube |

**GeneArt Seamless Cloning and Assembly Kit** — 20 reactions — A13288

| Component | Size | Quantity |
|---|---|---|
| 10X Enzyme Mix | 45 µL/tube | 1 tube |
| 5X Enzyme Buffer | 90 µL/tube | 1 tube |
| Linear pUC19L Vector (50 ng/µL), 4 control reactions | 8 µL/tube | 1 tube |
| Control Insert (50 ng/µL) | 5 µL/tube | 1 tube |

| Component | Size | Quantity |
|---|---|---|
| One Shot TOP10 Chemically Competent *E. coli* | 50 µL/tube | 21 tubes |
| pUC19 Control (10 pg/µL) | 10 µL/tube | 1 tube |
| S.O.C. Medium | 6 mL/bottle | 1 bottle |

**GeneArt Linear pUC19L Vector for Seamless Cloning** — 20 reactions — A13289

### GeneArt High-Order Genetic Assembly Systems

**GeneArt High-Order Genetic Assembly System** — 10 reactions — A13285

**GeneArt High-Order Genetic Assembly System (with Yeast Growth Medium)** — 10 reactions + 2 L medium — A13286

**GeneArt High-Order Linear pYES1L Vector** — 10 reactions — A13287

**CSM Medium for Mav203 Yeast Cells** — 2 L — A13292

**GeneArt High-Order Vector Conversion Cassette** — 10 reactions — A13291

### GeneArt Site-Directed Mutagenesis Systems

**GeneArt Site-Directed Mutagenesis PLUS System** — 10 reactions — A14604

| Component | Size |
|---|---|
| One Shot MAX Efficiency DH5α T1 | 1 box |
| DNA Methylase (4 units/µL) | 12 µL |
| 200X SAM (S-adenosine methionine) | 10 µL |
| 2X GeneArt Enzyme Mix | 100 µL |
| 10X PCR Enhancer | 100 µL |

| Component | Size |
|---|---|
| PCR Water | 1.8 mL |
| pMSDM-White Vector (20 ng/µL) | 5 µL |
| 0.5 M EDTA | 500 µL |

| Component | Size |
|---|---|
| Control Primer Mix 1 (10 µM) | 25 µL |
| Control Primer Mix 2 (10 µM) | 25 µL |
| Control Primer Mix 3 (10 µM) | 25 µL |

**GeneArt Site-Directed Mutagenesis System** — 16 reactions — A13282

| Component | Size |
|---|---|
| DNA Methylase (4 units/µL) | 20 µL |
| 200X SAM (S-adenosine methionine) | 10 µL |
| 10X Enhancer | 100 µL |
| 0.5 MEDTA | 500 µL |

| Component | Size |
|---|---|
| pUC19WHITE Control Plasmid (20 ng/µL) | 100 ng |
| Control Primer Mix (10 µM) | 25 µL |
| PCR Water | 1.8 mL |
| 5X Reaction Buffer | 90 µL |

| Component | Size |
|---|---|
| 10X Enzyme Mix | 45 µL |
| One Shot MAX Efficiency DH5α T1 | 1 box |

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Find out more at **thermofisher.com/geneart**

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