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GeneArt® Seamless Cloning and Assembly Enzyme Mix

Cat. No.	Size	Storage
A14606	20 reactions	See below

Part no. 100009437 **Pub. no.** MAN0006910 **Rev.** 2.0

Component	Cap Color	Amount	Storage*
GeneArt® 2X Enzyme Mix	Pink	100 µL	–80°C
Linear pUC19L Vector (50 ng/µL)	Clear	40 µL	–20°C
Control insert (50 ng/µL)	Green	5 µL	

*For convenience, you may store all components at –80°C

Description

- GeneArt® 2X Enzyme Mix is used for the simultaneous and seamless assembly of up to 4 DNA inserts between 100 bp and 10 kb and virtually any linearized *E. coli* vector, totaling up to 13 kb in length. The DNA fragments must share an end-terminal homology of 15 bp.
- Linear pUC19L Vector, a 2,659-bp linearized *E. coli* plasmid containing the ampicillin resistance gene, is provided as a cloning control vector for the assembly of DNA inserts. It can also be used as a depository vector for generating pre-cloned fragments or as a final cloning vector, if desired.
- Control insert, a 1.3 kb DNA insert sharing a 15-bp homology with the linear pUC19L vector at both ends, is used as a control in the assembly reaction.

A short protocol describing the GeneArt® Seamless Cloning and Assembly reaction is provided below. For detailed protocols and additional information, refer to the GeneArt® Seamless PLUS Cloning and Assembly Kit manual available at **www.lifetechnologies.com/geneart**.

The GeneArt® Seamless Cloning and Assembly Enzyme Mix is used for the simultaneous and seamless assembly of up to 4 DNA fragments and a vector, totaling up to 13 kb in length. To assemble larger constructs of up to 40 kb in length, use the GeneArt® Seamless PLUS Cloning and Assembly Kit (Cat. no. A14603), which includes the One Shot® DH10B™ T1R SA Chemically Competent *E. coli* optimized for the assembly of small, medium, and large constructs.

Homology Requirements for Adjacent DNA Fragments

The GeneArt® Seamless Cloning and Assembly technology relies on homologous recombination to assemble adjacent DNA inserts sharing end-terminal homology. Therefore, each DNA insert must have a 15-bp overlap with the adjacent insert (including the cloning vector). This homology may be split between adjacent fragments in any combination (e.g., 7+8, 6+9, 5+10 etc.).

Guidelines for Seamless Cloning and Assembly

- Devise your DNA assembly strategy and verify it by performing *in silico* cloning using the web-based GeneArt® Primer and Construct Design Tool, available at **http://rnaidesigner.invitrogen.com/oligoDesigner**.
- Follow the guidelines for small constructs (i.e., total construct size < 13kb) provided in the GeneArt® Seamless PLUS Cloning and Assembly Kit manual and the GeneArt® Design Tool to generate your linear cloning vector and to prepare your DNA inserts.
- Pre-cloning is not necessary for small constructs.
- The GeneArt® 2X Enzyme Mix might appear slightly turbid; this is normal and does not affect the enzyme activity. Do **not** centrifuge the enzyme mix to produce a clarified supernatant.
- Add the GeneArt® 2X Enzyme Mix to the assembly and cloning reaction **separately at the end** after you have mixed all the other reaction components.
- After adding the GeneArt® 2X Enzyme Mix to the reaction mixture, promptly return it to –80°C.

Seamless Cloning and Assembly Reaction

1. In a microcentrifuge tube, set up the seamless cloning and assembly reaction. **It is crucial that you add the GeneArt® 2X Enzyme Mix as the last component** (see Step 2).

Insert(s) (200 ng each)	x µL
Linear cloning vector (50 ng)	1 µL
Deionized water	to 5 µL

   If desired, set up a positive control reaction with 1 µL of the linear pUC19L vector, 2 µL of the Control insert, and 2 µL of deionized water in a separate tube.
2. Quickly thaw the GeneArt® 2X Enzyme Mix on ice and pipette up and down to mix thoroughly. Add 5 µL of the thawed GeneArt® 2X Enzyme Mix to each reaction mixture, and immediately return it back to –80°C.
3. Mix the reaction components completely by pipetting them up and down 3 times and then gently tapping the sides of the tube 3–5 times.
4. Briefly centrifuge (<500 rpm for <5 seconds) to collect the reaction components to the bottom of the microcentrifuge tube.
5. Incubate the reaction mix at room temperature for **15–30 minutes**. Do **not** incubate longer than 30 minutes.
6. After incubation is complete, place the reaction mix on ice for 2–5 minutes before proceeding to the transformation step. Do **not** let the samples stay on ice for more than 5 minutes before transformation.

Transformation

1. Use 3 µL of the seamless cloning and assembly reaction to transform One Shot® TOP10 Chemically Competent *E. coli* or other chemically competent cells of your choice, following the instructions provided by the manufacturer. **Do not use electrocompetent cells.**
2. Plate the transformations on appropriate pre-warmed selective LB plates. Plate the positive control on LB plates containing 50–100 µg/mL of ampicillin and 30–100 µg/mL X-Gal for blue-white screening. Incubate the plates overnight at 37°C.
3. The next day, pick individual colonies (pick white colonies if you have used the linear pUC19L vector) and isolate the plasmid DNA, or screen for the presence of the insert(s) by colony PCR.

Technical Support

For additional product and technical information, such as Safety Data Sheets (SDS), Certificates of Analysis, etc., visit our website at **www.lifetechnologies.com**. For further assistance, email our Technical Support team at **techsupport@lifetech.com**.

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