Seamless Cloning and Assembly

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) (200ng each)	x ul
Linear cloning vector (50ng)*	1 ul
Deionized water	to 5 ul

   * You can use 50 ng of pYES7L, linear pUC19L, or your own linearized cloning vector. The volume of your own linearized cloning vector depends on its concentration.

2. Quickly thaw the GeneArt 2x Enzyme Mix on ice and pipette up and down to mix thoroughly. Add 5ul of the thawed GeneArt 2x Enzyme Mix to the reaction mix, and immediately return it back to -20˚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 (<500rpm for <5 seconds) to collect the reaction components to the bottom of the microcentrifuge tube.
5. For small assemblies (<13 kb) containing inserts with 15-bp end-terminal homology, incubate the reaction mix at room temperature for **15-30 minutes**. Do **not** incubate more than 30 minutes.

   For large assemblies (>13 kb) containing inserts with larger end-terminal homology, incubate the reaction mix at room temperature for **60 minutes**. Do **not** incubate more than 60 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 samples stay on ice for more than 5 minutes before transformation.