

NEBuilder® HiFi DNA Assembly Chemical Transformation Protocol (E2621, E5520, E2623)

Materials Required but not Supplied

NEBuilder® HiFi DNA Assembly Master Mix

- Nuclease-free Water (NEB #B1500)

NEBuilder® HiFi DNA Assembly Bundle for Large Fragments

- Nuclease-free Water (NEB #B1500)

NEBuilder® HiFi DNA Assembly Cloning Kit

- Nuclease-free Water (NEB #B1500)

Overview

For use with NEBuilder® HiFi DNA Assembly Cloning Kit (NEB #E5520), NEBuilder HiFi DNA Assembly Bundle for Large Fragments (NEB #E2623), and NEBuilder HiFi DNA Assembly Master Mix (NEB #E2621)

Protocols.io also provides an [interactive version of this protocol](#) where you can discover and share optimizations with the research community.

1. Thaw competent cells on ice.
2. Transfer 50 µl of competent cells to a 1.5 ml microcentrifuge tube (if necessary).
3. If the chemically competent cells are from New England Biolabs, add 2 µl of assembled product to NEB competent cells and go to step 4 directly. If competent cells are purchased from another manufacturer, dilute assembled products 4-fold with H₂O prior transformation. This can be achieved by mixing 5 µl of assembled products with 15 µl of H₂O. Add 2 µl of the diluted assembled product to competent cells.
4. Mix gently by pipetting up and down or flicking the tube 4–5 times. Do not vortex.
5. Place the mixture on ice for 30 minutes. Do not mix.
6. Heat shock at 42°C for 30 seconds*. Do not mix.
7. Transfer the tubes to ice for 2 minutes.
8. Add 950 µl of room temperature SOC* medium to the tube. When using NEB 10-beta or NEB Stable E. coli competent cells, add 950 µl of the NEB 10-beta/Stable Outgrowth Medium.
9. Incubate the tube at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate.
10. Warm selection plates to 37°C. Spread 100 µl of the cells onto the selection plates. Use Amp plates for the NEBuilder Positive Control sample. Both the transformation control pUC19 and the NEBuilder Positive Control are plated on Amp

plates.

11. Incubate overnight at 37°C.

* Please note: Follow the manufacturer's protocols for the duration and temperature of the heat shock step, as well as the optimal medium for recovery. Typically, transformation of our positive control assembly product will yield more than 100 colonies on an Amp plate with greater than 80% colonies containing inserts.

NEB recommends NEB 5-alpha Competent *E. coli* (NEB #C2987) for transformation of NEBuilder HiFi DNA Assembly products. NEB recommends NEB 10-beta Competent *E. coli*, High Efficiency (NEB #C3019) for assemblies larger than 15 kb. NEBuilder HiFi DNA Assembly Bundle for Large Fragments (NEB #E2623) offers a discount for purchasing these competent cells with the NEBuilder HiFi DNA Assembly Master Mix.