LVL0 cloning using annealed Oligos

Daniel Stukenberg

Abstract

Citation: Daniel Stukenberg LVL0 cloning using annealed Oligos. **protocols.io**
dx.doi.org/10.17504/protocols.io.pthdnj6
Published: 27 Apr 2018

Protocol

Annealing of Oligos

Step 1.

Set up Annealing reaction in 1,5 mL microcentrifuge tube

fwd Oligo	1,5 µL (10 µM)
rev Oligo	1,5 µL (10 µM)
T4 ligase buffer	5 µL (10x)
ddH_20	42 µL

Incubate in heatblock for 10 min at 85°C

Turn off heatblock and allow samples to remain in the heatblock for slow cooling to room temperature.

Proceed with next step or freeze annealed oligos for long term storage.

Golden Gate Reaction

Step 2.

Set up Golden Gate Reaction

Entry Vector	50 - 70 ng
T7-Ligase (NEB)	1 µL
BsmBI (NEB)	1 µL
T4-Ligas Buffer	1 µL
ddH_20	Ad 10 µL

Start Golden Gate Reaction in Thermocycler

Digest	42°C	2 min
Ligation	16°C	5 min
Final Digest	60°C	30 min
Inactivation	80°C	19 min

Transformation

Step 3.

Transform complete reaction mix into competent cells using a chosen protocol