APR 27, 2018

LVL0 cloning using annealed Oligos

Daniel Stukenberg¹

¹Phillips-Universität Marburg

Daniel Stukenberg

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.pthdnj6

Protocol Citation: Daniel Stukenberg 2018. LVL0 cloning using annealed Oligos. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.pthdnj6>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
We use this protocol and it's working

Created: Apr 27, 2018

Last Modified: Apr 27, 2018

PROTOCOL integer ID:
11849

Annealing of Oligos

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 ₂ O	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

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 ₂ O	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

3 Transform complete reaction mix into competent cells using a chosen protocol