

## SLIC Cloning

1. Digest and gel-purify the vector.
2. PCR amplify the insert with 20-30 pb sequence overlap with the vector sequence (use Phusion polymerase) and purify the PCR product (after agarose gel or DpnI digestion)
3. Mix 100 ng vector with 200 ng insert, add 1  $\mu$ l BioLab's buffer 2.1, 0.5  $\mu$ l T4 DNA polymerase, qsp H<sub>2</sub>O 10  $\mu$ l. Incubate 3-4 minutes at room temperature. Stop the reaction on ice.
4. Transform DH5a 50  $\mu$ l competent cells with the 10  $\mu$ l reaction mix. Spread on a LB + AB plate.

### For cloning multiple inserts :

1. and
2. are the same
3. Make a separate T4 DNA pol reaction with as much as 1  $\mu$ g of each component of the cloning : v, i1, i2, i3... Use 0.1  $\mu$ l of T4 DNA pol. Incubate 30 min at RT. Stop the reaction with 1  $\mu$ l of 10 mM dCTP. Keep on ice.
4. Mix each component of the cloning reaction with buffer (2.1 or ligase buffer). Incubate 30 mn at 37°C.
5. Transform

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