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 µl BioLab's buffer 2.1, 0.5 µl T4 DNA polymerase, qsp H2O 10 µl. Incubate 3-4 minutes at room temperature. Stop the reaction on ice.
4. Transform DH5a 50 µl competent cells with the 10 µl 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 µg of each component of the cloning : v, i1, i2, i3... Use 0.1 µl of T4 DNA pol. Incubate 30 min at RT. Stop the reaction with 1 µl 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