CLO_1v1

SLIC (Sequence- and ligation-independent cloning) Protocol

("Novagen protocol", by Jeff Cox Lab, UCSF)

INSERT:
Gel purify PCR product (or clean-up alone if single band)
Spec to quantify
Set up each insert in PCR tubes w/ lids

___µl H20
___ µl PCR Product *(~50 ng)*
2 µl 10X Buffer (T4 DNA Pol)
(Omit all dNTPs)
1 µl 100 mM DTT
0.4 µl T4 DNA Polymerase (LIC-qualified)
20 µl Total

Stir with pipet tip to mix. Incubate at 22°C for 30 min (in rack fine) *(I do 30-35 mins for 30bp homology)*
For 40+bp overlap increase to 40min.
Kill enzyme at 75°C for 20 min

VECTOR:
Cut 1ug vector to linearize (Some people say blunt end may work better, I haven't noticed a difference) – do not CIP
Gel purify
Spec to quantify
T4 as above (may want to scale up by 2x in 40ul w/100ng vector if doing many rxns)

ANNEAL
2ul Plasmid
4ul Insert

In PCR Machine:
Heat to 70°C x 30 sec [Novagen rec]
Cool stepwise to 40-deg (10s/1deg increments), or use the Ramp feature (10s/degree)
Sit on bench 5min ->ice
Transform 10ul to 100ul bugs

I spin down and plate everything after the transformation.