Quick Ligase ligation

Materials

2x Quick ligase buffer (in 40µl aliquots; these are 1-time use since freeze-thaw cycles degrade the ATP in the buffer).

Quick Ligase from NEB

ddH2O

Purified, linearized vector (likely in H_2O or EB)

Purified, linearized insert (likely in H_2O or EB)*Italic text*

Procedure

For 10µl reaction

Larger volumes can be scaled up if needed

5 µL 2X Quick ligase buffer

0.5 µl *Quick* ligase

6:1 Molar ratio of insert to vector (~10ng vector). Try to keep total DNA concentration <100ng/rxn for optimal efficiency.

Add (4.5 - vector and insert volume)µl ddH_2O

Method

1. Add appropriate amount of deionized H_2O to sterile PCR tube
2. Add in appropriate amounts of vector and insert. Heat the mixture to 42*C for 2min to free up sticky ends (can set up a thermocycler for this).
3. Add 5 µL of 2X ligation buffer to the tube.
   Pipette buffer up and down before pipetting to ensure that it is well-mixed.
4. Add 0.5 µL of *Quick* ligase. PIPETTE half the volume of the mixture UP AND DOWN to ENSURE MIXING OF THE ENZYME.
   Also, the ligase, like most enzymes, is in some percentage of glycerol which tends to stick to the sides of your tip. Just touch your tip to the surface of the liquid when pipetting to ensure accurate volume transfer.
5. Let the 10 µL solution incubate at room temp for 5-10min.
6. Denature the ligase at 65°C for 10min.
7. Store at -20°C

T4 ligase ligation

Materials

T4 DNA Ligase

10x T4 DNA Ligase Buffer -> make sure it smells bad (like "wet dog"); if it doesn't smell, it might be bad.

Deionized, sterile H_2O

Purified, linearized vector (likely in H_2O or EB)

Purified, linearized insert (likely in H_2O or EB)*Italic text*

Procedure

10µl Ligation Mix

Larger ligation mixes are also commonly used

1.0 µL 10X T4 ligase buffer (use 10µl aliquots in -20 freezer; repeated freeze-thaw cycles can degrade the ATP in the buffer that's critical for the ligation rxn)

6:1 Molar ratio of insert to vector (~10ng vector)

Add (8.5 - vector and insert volume)µl ddH_2O

0.5 µL T4 Ligase

Method

1. Add appropriate amount of deionized H_2O to sterile PCR tube
2. Add in appropriate amounts of vector and insert. Heat the mixture to 42*C for 2min to free up sticky ends (can set up a thermocycler for this).
3. Add 1 µL ligation buffer to the tube.
   Pipette buffer up and down before pipetting to ensure that it is well-mixed.
4. Add 0.5 µL T4 ligase. PIPETTE half the volume of the mixture UP AND DOWN to ENSURE MIXING OF THE ENZYME.
   Also, the ligase, like most enzymes, is in some percentage of glycerol which tends to stick to the sides of your tip. Just touch your tip to the surface of the liquid when pipetting to ensure accurate volume transfer.
5. Let the 10 µL solution incubate at 16*C for 1hr.
6. Denature the ligase at 65°C for 10min.
7. Store at -20°C