# Blunt ligation

To finish vector creation, we need to close the ends.

## Protocol

1. Amplify the vector
2. Add 1 µl DpnI restriction enzyme directly to 50 µl PCR product.
3. Incubate 37 °C 2 hours
4. Make a gel purification in 50 µl (using the gel purification protocol)
5. Phosphorylate with 0.5 µl PNK4 and 1 µl T4 ligation buffer.
6. Incubate 37 °C 30 minutes.
7. inactivate by placing in 65 °C 20 minutes
8. Add 0.5 µl T4 ligase
9. Store over night in 4 °C or 2 hours in 37 °C.
10. Transform

| **Component** | **10 uL reaction** |
| --- | --- |
| 10x NEB buffer | 1.0 µL |
| Vector | 4.0 µL |
| Restriction enzyme 1 | 0.25 µL |
| Restriction enzyme 2 | 0.25 µL |
| Nuclease-free water | 4.5 µL |

## Incubation

Incubate in PCR machine

1. Incubate: 37 $^{o}$C 30 minutes

Now the vector is ready to insert genes in, by using the USER ligation protocol, or for PCR amplification.