TOPO cloning

PCR cloning

For a 50 mL reaction using Stratagene Pfu Turbo DNA Polymerase (cat# 600250, blunt ends):

1. Combine the following in a PCR tube, addingTaq last.

5 μL	10x reaction buffer
2 μL	dNTPs 5 mM each
1 μL	Forward primer (200 ng/μL)
1 μL	Reverse primer (200 ng/μL)
35.8 μL	dH_2O
0.2	Pfu Turbo
5 μL	template (10ng/μL)

2. Place samples in thermocycler using the following program:

95°C	2 min
95°C	30 sec
Tm*-5°C	30 sec
72°C	1 min/kb
72°C	10 min
4°C	hold

*Tm is calculated as 2x(#A + #T) + 4x(#G + #C)

3. Run samples on an agarose gel to verify amplification. If other bands are present other than the expected it will be necessary to gel purify the correct fragment (QIAGEN cat#28704).

Adding A's

For gel extracted DNA:

1. Combine the following to the gel extracted fragment (for 50 μL eluate).

5 μL	10x Reaction buffer
2 μL	dNTPs 5 mM each
1 μL	Taq DNA Polymerase

2. Incubate at 72°C for 10 min.

For PCR product:

1. Combine the following to the remaining PCR product.

1 μL	Taq DNA Polymerase

2. Incubate at 72°C for 10 min.

TOPO TA cloning (Invitrogen cat#K2500-20)

1. Combine the following:

4 μL	PCR product
1μL	Salt solution
0.5 μL	TOPO vector

2. Incubate at RT for 10 min.
3. Transform 2 μL into 1 vial TOP10 Oneshot competent cells.
4. Incubate transformation on ice for 30 min.
5. Heat shock transformation for 45 sec at 42°C.
6. Incubate on ice for 2 min.
7. Add 250 μL SOC and incubate with shaking 225 rpm, 37°C for 1 hour.
8. Plate 200 μL transformation on LB-Spectinomycin (100 μg/mL Spec) plates.
9. Incubate overnight at 37°C.