University of Southampton iGEM 2009 Protocol:
TA cloning

Ligation of insert with pGEM® easy vector

Reaction component	Standard Reaction/ µL	Positive control/ µL	Background control/ µL
2x rapid ligation buffer	5	5	5
pGEM® Easy vector	1	1	1
PCR product	X	-	-
Control insert DNA	-	2	-
T4 DNA ligase	1	1	1
Nuclease free water	X	1	3

X is dependent on the ratio between the vector and insert (usually 1:3), which can be calculated using the mass and the concentration of the insert.

Transformation of Ligated TA cloned vector

1. Add 5µl of the DNA solution to DH5α competent cells and incubate on ice for 30 minutes.

2. Heat-shock the cells by placing them in a thermostatically controlled water bath for 45 seconds **only** at 42°C.

3. Place the heat-shocked cells on ice for 2 minutes.

4. Add 1 mL of SOC growth media and incubate using a shaking incubator at 37 °C for 1.5 hours.

5. Plate the cells in two dilutions onto LB agar plates (Amp resistant/IPTG/X-Gal). First, plate 100 µL of cells then prepare a second plate with 10 µL of cells diluted with 90 µL of SOC.

6. Incubate the LB agar plates at 37 °C overnight. **Important!** Do not grow the plates for longer as colonies will multiply and could become indistinct. Colonies should appear blue for negative ligations and white for complete ligations.

7. The plates can be stored at 4°C to increase the appearance of the blue colonies. White colonies are then taken and grown up.