Ligation Reactions:
20ul total volume
3:1 to 10:1 insert to vector ratio
Sticky End Ligation: 30 min to 2hrs @ RT
Blunt End Ligation: at least o/n @ RT

Ligation of Sample	Ligation Control
1ul T4 DNA Ligase	0ul T4 DNA ligase (no ligase control)
2ul 10x T4 DNA ligase buffer	2ul 10x T4 DNA ligase buffer
(X)ul insert DNA	(X)ul insert DNA
(Y)ul vector DNA	(Y)ul vector DNA
(17-X-Y)ul dH_2O	(18-X-Y)ul dH_2O

Transformation of Competent E. coli cells:
Thaw aliquot from –80C
Combine 100ul competent E. coli for P3 plasmids and 10ul ligation sample DNA
Incubate on ice 2min
Heat shock @42C for 45 sec to 1 min
Incubate on ice 2min
Add 500ul SOB to sample
Incubate 37C for 40 min to 1 hr w/ shaking
Spin cells @6000rpm, 6min
Pour off supernatant, resuspend cells in residual liquid (~50 to 100 µl)
Plate cells on selective media, incubate 37C for 12-16hrs
Compare colony number on the ligation control plate to the sample plate(s)

Diagnostic of Transformed Cells:
Select several (i.e.8-12) isolatable colonies from the sample plate and inoculate in 5ml LB media with antibiotic selection (per 5ml: 5µl (100mg/ml) ampicillin, 5 µl (50mg/ml) kanamycin)
Incubate 6-8hrs (optimally) with shaking @37C
Conduct Miniprep protocol, conduct diagnostic digest, run on 1% TAE agarose gel to determine if appropriate band sizes are seen

Preparation of a Glycerol Stock:
900ul from a 5ml o/n bacterial culture
100ul 100% glycerol
Store @-80C