

Anealing the oligos

Oligo annealing buffer: **50mM Tris, 5mM MgCl₂, 200mM NaCl**

2ug each oligo, 36ul annealing buffer,

Thermal condition:

98C, 2min;

65C, 10min;

42C, 30min;

25C, 30min,

End

Column purify annealed oligos, concentration, 83ng/ul

Setup ligation:

Molar rati (vector/oligo): 1:10, 1:5; 1:2, for total of 3 ligations

Use the rapid ligation kit from Roche (www.roche-applied-science.com,

Cat# 11635379001).

Then transformation of bacteria using the ligation products.