# DNA cassettes

**Material**

Oligonucleotides

Restriction enzymes from New English biolabs

T4 Ligase from New English biolabs

Competence cell

dd$H_2O$

**Procedure**

1. The DNA cassettes encoding stemloop structures are synthesized (Beijing Genomics Institute, Beijing, China) as two complementary DNA oligonucleotides.
2. These oligonucleotides are annealed at high concentration by heating from 95℃ to above their annealing temperature gradually by 0.1 ℃ / s. And then we makes it to 12℃ by 4℃ / s.

1. The DNA cassettes are inserted into the plasmid using unique restriction sites at the ends of the cassettes and within the plasmid itself.
2. The plasmid and the cassettes were digested and ligated together.
3. The ligation products are transformed into E. coli. The transformed cells containing the plasmid with the cassette insert were screened by sequencing.

**Attention**

When designing complementary oligonucleotides , we need to compare the annealing temperature of the primers to the temperature of stemloop dimmers. If the annealing temperature of primers is lower, we design the pair of primers based on required sequence with sticky ends at 5' end. Otherwise, we add continuous guanines at the both ends of one primer while cytosines to the other one.