Molecular cloning and genetic engineering – Colony PCR

Aim

Use colony PCR to determine whether the colonies contain target plasmids.

Materials

rTaq

Template DNA

ddH_2O

Primer mix

Procedure

1. Prepare one of the following reaction mixes on ice:

25ul rTaq

5ul primer mix

10ul ddH_2O

10ul bacterial fluid

2. Run PCR with the following program:

STEP	TEMP	TIME
Initial Denaturation	95℃	3min
30 Cycles	98℃	10sec
	55℃	30sec
	72℃	1min
Final Extension	72℃	5min

Note

Initial Denaturation: Complete denaturation of template DNA and complete activation of PCR enzyme are very important to the success of PCR. Generally, the activation time of unmodified Taq enzyme is two minutes.

Denaturation: Hydrogen bonds of double-stranded DNA templates break down under thermal action to form single-stranded DNA.

Anneal：When the temperature decreases, primers bind to DNA templates to form local double strands.

Extension：Under the action of Taq enzyme (about 72℃, with the best activity), dNTP was used as raw material to synthesize DNA chains complementary to the templates, starting from the 3'-end of the primer and extending in the direction of 5'-end to 3'-end.

Final Extension：After the last cycle, the reaction lasted for 5 minutes at 72 ℃. The primers were fully extended and the single chain products were annealed into double chains.