Colony PCR

Purpose:

In order to determine the presence and absence of insert DNA in plasmid constructs.

Materials:

- Primers (including prefix, suffix, kpnI, overlap primers and adding one)
- Colonies
- ddH2O
- 2X Taq DNA polymerase Mastermix-Red
- LB agar plate

Procedures:

1. Premix the reaction mixture as following:

	µl
DNA or colony	(1pg~10ng)
2X mastermix	5
Primer-F	0.2
Primer-R	0.2
ddH2O	4.6
	10.0

2. Pick single colony, inoculate on the agar plate first, and then stain it into the PCR microtube.
3. Run PCR program as following: 98°C 1 min, 98°C 10 sec, 56°C 15 sec, 72°C 40 sec, 72°C 7 min for 35 cycles.
4. Run the PCR product on the agarose electrophoresis, and determine which sample to be restriction enzyme analyzed according to the presence of band.