COLONY PCR

1. Prepare PCR master mix. Typical reaction:

   2µl 10X Taq buffer
   2µl $MgCl_2$
   1µl dNTP's (2.5mM)
   0.5µl Primer A
   0.5µl Primer B
   0.2µl Taq
   Up to 20µl H_2O

2. Aliquot master mix into 20µl reactions. Keep on ice.

3. Take a fresh LB + selection plate. Draw a grid with appropriate number of squares.

4. Using a 20µl pipette pick a colony from the ligation plate with the tip.

5. Streak the tip onto the grid.

6. Then draw up and expel the PCR mix in the PCR tube a few times with
   the innoculated tip.

7. Repeat for all samples.

8. Once all samples ready, run PCR. Suggested program:
   94°C - 5mins
   94°C - 30 sec
   55°C - 30 sec
   72°C - 30 sec
   72°C - 10 mins
   4°C - Forever….

Repeat steps 2-4 25 times.
Obviously annealing temp and extension time dependent on reaction.

9. Put gridded plate in 37 °C incubator

10. Run samples on gel.

11. Set up O/N cultures of +ve clones.

HINTS: Use a +ve and a –ve control in PCR if possible

If cloning non-directionally, try and use primer pairs which indicate presence of insert AND direction of insert.