# Plasmid Colony PCR

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1. Set up PCR strips (12) or microtiter plates (96) with 20 ul 2xYT+ appropriate antibiotic (e.g. 2xYT + ampicillin for pBluescript and pGEM-T, 2xYT + kanamycin for kanamycin resistant plasmids, etc.)
2. Pick a single colony into each tube/well. Swirl tip in medium to get bacteria into suspension. Cover with parafilm and place on rotary shaker at room temperature while you prepare PCR master mix.
3. For each colony, you will need 20 ul of PCR master mix. Use oligonucleotides primers present in the polylinker of your plasmid, or use custom oligos that will anneal to the insert:

| | Primer 1 | Primer 2 |
|---|---|---|
| pGEM-T/pGEM-T easy | SP6 | T7 |
| pBluescript | T3 | T7 |

| | |
|---|---|
| 10X BRL PCR Buffer (without Magnesium) | 2 ul |
| BRL MgCl2, 50mM | 0.8 ul |
| dNTP, 2.5 mM | 1.6 ul |
| Primer 1, 75 ng/ul | 0.5 ul |
| Primer 2, 75 ng/ul | 0.5 ul |
| BRL Taq polymerase | 0.05 ul |
| Clontech Taq Start Antibody | 0.05 ul |
| H2O, PCR grade | 13.7 ul |

Make enough for each clone to be amplified, plus a bit extra to compensate for pipetting error.
==MIX MASTER MIX WELL==

4. Aliquot 20 ul mix into clean PCR tubes
5. Transfer 2 ul of resuspended colony into PCR tube. Make sure colony is evenly resuspended. Do not transfer clumps of cells as these will inhibit PCR reaction! Seal PCR tubes and run PCR machine using conventional cycling program:

1-94oC 3'
2-94oC 30"
3-50oC 30"
4-72oC (1 minute/kilobase of insert)
5-Return to Step 2, 34 times
6-72oC 5'
7-4oC 10'
8-END

6. Carefully cover tubes/wells containing live bacteria in LB+ ampicillin and store in refrigerator until results of PCR are known.
7. At conclusion of PCR reaction, run entire PCR volume into a checking gel. Those clones that have inserts of interest, inoculate minipreps or maxipreps by pipetting 10 ul of colony into culture medium.