## Colony Screening PCR

**Preparation – the day before**

* White colonies are picked individually into 100ul LB/Carb in sterile 96 well plates (with a lid) and grown, standing, overnight at 37°C.

* The culture is used as template for PCR the next day (or may be held at 4°C prior to use).

* *Be sure to mix thoroughly prior to using the culture for csPCR*. If you don't, you may transfer too many cells and your PCR will not work.

**Reaction Setup**

Sign up for a PCR machine before you start-be considerate here if you are running lots of plates and take them out promptly.

Prepare a cocktail for all PCRs. Usually, n+1 reactions is sufficient, where n is your number of PCRs. However, where you may be setting up >10 reactions, you can use n+2 reactions for your cocktail, >50 reactions n+3, etc.

| Reagent | Stock Concentration | Final Concentration | Vol]/20ul reaction |
|---|---|---|---|
| T7 primer | 20uM | 1uM | 1.0 ul |
| SP6 primer | 20uM | 1uM | 1.0 ul |
| dNTPs | 2.5mM | 200uM | 1.5 ul |
| Buffer | 10x | 1x | 2.0 ul |
| Taq | - | - | 0.1 ul |
| $H_2O$ | - | - | 13.4 ul |

Aliquot 19 ul per well in a 96-well plate. Add1.0 ul of either overnight bacterial culture (feel free to use the 0.1-10 ul multichannel pipets for this) or positive control C2.

**Reaction Conditions**

Record the PCR machine you are using, and the positions in which your tubes are placed.

*PCR Program: Method 14* *Links programs 12, 13, 7, 8*

94°C, 4 minutes……Program 12
**30 cycles:**
94°C, 30 seconds
55°C, 30 seconds
72°C, 1 minute.....…...Program 13
72°C, 3 minutes.....…..Program 7
10°C, forever.....…..Program 8
*The appropriate extension time is 1 minute per 1kb of expected product size. Don't forget that you are adding on about 100bp to your PCR product due to flanking regions of the vector that are between the T7 and SP6 sites.