# Pre-Competition Procedure

Supplies needed:

D ancestor

Dx puromycin +

Mx spectinomycin + tubes

Needles and syringes

Fresh red (CCMA) media

Spectrophotometer

Incubator

1. Label each fresh media tube with the recombined cultures (ex: D0 & M76 or D152 & M76, be sure to include the culture name (ie: HR2, UE3, or US1)
2. Sterilize the tops of all tubes
3. Remove 0.1mL of D ancestor and put in new media tube
4. Remove 0.1mL of Mx and put into designated tube with the D ancestor
5. Remove 0.1mL of Dx and place in different tube then add Mx to designated tube
6. Place in an incubator and measure OD with spectrophotometer daily until reaching 0.300.

An example set up is here:

| D0 & M76 | D152 & M76 |
| --- | --- |
| D0 & M152 | D152 & M152 |
| D0 & M189 | D152 & M189 |
| D0 & M227 | D152 & M227 |

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