

## Generating Keio Knockout

1. Make a P1 lysate on the Keio strain that contains the Keio (FRT)kan deletion of choice.
2. Move the Keio (FRT)kan deletion of choice into the Kan(S) strain of choice by P1 transduction and select for Kan(R).
3. Make competent cells and transform the strain with the Keio (FRT)kan deletion with pCP20, which is Amp(R) at 30°C.
4. Restreak two colonies at 30°C and if most of the resulting colonies have similar morphologies, restreak one colony at 42°C.
5. Pick 10 of the 42°C single colonies and patch onto a Rich, a Rich Amp, and a Rich Kan plate at 37°C.
6. Choose an Amp(S) Kan(S) colony. This is a colony that has undergone a FRT recombination that displaced Kan and lost the pCP20 plasmid which was temperature sensitive.