**Viral Transfer Protocol**

*9/25/2018 by Sean Buskirk*

**Transformation of Donor Strains with Indicator Plasmid**

1. Transform your donor strains with pGIL154 (to utilize URA3 as indicator of cytoplasmic mixing during kar matings).
   1. Select on SC-Ura
   2. Stock strains

**Kar Mating Round 1 (into kar mutant)**

1. Pass pGIL154-containing donor strains (into SC-Ura) and resurrect recipient strain yGIL1353 (into YPD).
   1. yGIL1353 (M10, LA0, KanMX, *MATα*, *kar1Δ15*)
2. Mix saturated cultures 5:1 in favor of the donor
   1. Once factoring in carrying capacity of media: 150 uL of donor and 10 uL of recipient.
3. Spot 20 uL of mixture onto (dry) YPD agar. Let soak into agar. Incubate for 6 hr at 30°C.
4. Using a sterile loop, collect cells from the agar surface and resuspend in 500 uL sterile water. Pellet cells, aspirate, and resuspend in ~20 uL sterile water.
5. Streak cells onto SD+MSG+G418 agar and incubate at 30°C O/N.
6. Once colonies have formed, select 8-16 colonies per kar mating to seed YPD+G418 in 96-well plate. Incubate at 30°C O/N.
7. Spot cultures onto SC-Ura and YPD+Nat (to identify haploid cytoductants). Perform killer assay using yGIL432 (to test for immunity) and/or yGIL1097 (to test for killing ability).

**Kar Mating Round 2 (out of kar mutant)**

1. Pass identified cytoductants (into SC-Ura) and resurrect recipient strain (into YPD).
   1. Stock cytoductants.
2. Mix saturated cultures 5:1 in favor of the donor
   1. Once factoring in carrying capacity of media: 150 uL of donor and 10 uL of recipient.
3. Spot 20 uL of mixture onto (dry) YPD agar. Let soak into agar. Incubate for 6 hr at 30°C.
4. Using a sterile loop, collect cells from the agar surface and resuspend in 500 uL sterile water. Pellet cells, aspirate, and resuspend in ~20 uL sterile water.
5. Streak cells onto SD+CSM-Ura+Nat agar and incubate at 30°C O/N.
6. Once colonies have formed, select 8-16 colonies per kar mating to seed YPD+Nat in 96-well plate. Incubate at 30°C O/N.
7. Spot cultures onto SC-Ura and YPD+G418 (to identify haploid cytoductants). Perform killer assay using yGIL432 (to test for immunity) and/or yGIL1097 (to test for killing ability).

**Curing Cytoductants of Indicator Plasmid**

1. Streak identified cytoductants onto SC-Arg+5FOA (to cure strains of pGIL154).
2. Select 4 colonies per cytoductant to seed into YPD in 96-well plate.
3. Spot cultures onto SC-Ura (ensure loss of plasmid). Perform killer assay using yGIL432 (to test for immunity) and/or yGIL1097 (to test for killing ability).
   1. Also perform growth curve if so inclined.
4. Pass identified cytoductants into YPD to stock.