Triple Immuno: RFP, GFP, cFos (Rebecca) - BLA hM4Di

1. **Day 1 - Primary**
   1. Wash 3 x 10 minutes PBS to remove sodium azide
   2. Block 2 hours @ RT in 5% BSA
   3. Primary antibody incubation - 48 hours @ 4C in 1% BSA
      * 1. 1:1000 rabbit polyclonal anti-cfos (SySy; -20C Freezer; Rb label)
        2. 1:1000 chicken anti-GFP (4C Fridge; #1 label)
        3. 1:1000 guinea pig anti-RFP (-20C Freezer; #8 label)
2. **Day 2 - Secondary** 
   1. Wash 3 x 10 minutes PBS
   2. Secondary Ab Incubation - 2 hours @ RT in 1% BSA
      * 1. 1:200 Alexa 488 goat anti-chicken (4C Fridge) - #3
        2. 1:500 Alexa 555 goat anti-guinea pig (4C Fridge) - #4
        3. 1:200 Alexa 647 goat anti-rabbit (4C Fridge) - #22 (new)
   3. Wash 3 x 10 minutes PBS
   4. Mount slides w DAPI

GFP, cFos (Rebecca) - Engram tagging/cFos overlaps

1. **Primary antibodies - 1mL/well**
   1. 1:1000 rabbit polyclonal anti-cfos (SySy; -20C Freezer; Rb label)
   2. 1:1000 chicken anti-GFP (4C Fridge; #1 label)
2. **Secondary antibodies - 1mL/well** 
   1. 1:200 Alexa 488 goat anti-chicken (4C Fridge; #3 label)
   2. 1:200 Alexa 555 goat anti-rabbit (4C Fridge; #2 label)
3. **Day 1 - Primary**
   1. Wash 3 x 15 minutes PBS to remove sodium azide
   2. Block 2 hours @ RT in 5% BSA
   3. Primary antibody incubation - 48 hours @ 4C in 1% BSA
4. **Day 2 - Secondary** 
   1. Wash 3 x 10 minutes PBS
   2. 1% BSA Secondary Ab Solution - incubate 2 hours @ RT
   3. Wash 3 x 10 minutes PBS
   4. Mount slides w DAPI