vCAPTURE general protocol and considerations

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1. vCAPTURE uses two AAVs to trap neurons based on the previous activation. The first AAV expresses activity-induced Cre(AAV5-ESARE-ER-Cre-ER-PEST), which drives the Cre-dependent payload of choice (XFP/opsin/DREADDs) from the 2nd AAV (any AAV with a DIO/FLEX).

2. The key for this combination to work is to have sufficient titer after mixing the viruses. Therefore, it is critical to follow **the actual genomic titers** of your virus (by specific lot#), NOT the fold of dilution or mixing ratio. All the following steps are to maximize the final titers of both viruses.

3. Based on our experiences in PFC, hypothalamus, and the Xiphoid, generally, the titer for ESARE should be between 5-9e12 vg/ml. The DIO virus should be >5e12 vg/ml (higher is better), but that is less critical.

4. To achieve #3, we concentrate the ESARE virus to 3-5e13 vg/ml to store as our stock. This can usually be done by concentrating commercial AAV preps (most ESARE prep you get will be in the e12 range) by ~5 fold through a 100kD Amicon ultrafiltration tube.

5. Make the injection viral mix by using your DIO virus to dilute the high-titer ESARE stock. For example, mix 3ul ESARE virus (3e13) and 7ul DIO virus (9e12) will result in final titers: ESARE=9e12 and DIO=6.3e12. This comb could be a good start for a pilot experiment.

6. You can adjust the sensitivity vs. efficiency of CAPTURE by adjusting the final ESARE titer to account for regional/behavior differences. In the example above, you can modify the ratio to 1/9, 4/6 to make the CAPTURE expression weaker or stronger as desired. Ideally, you may want to test a range of titers to see which gives you the largest home cage/behavior difference by histology before finalizing the CAPTURE ratio for the real manipulation experiments. Generally speaking, up and down from 6e12 ESARE titer would be a good start. For manipulation with dreadd or opsin, sometimes you have to compromise between efficiency and background labeling, e.g., to get enough opsin expression to permit optogenetics, you may have to accept some level of expression in the home cage animals as well.

7. CreER PROTEIN expression from ESARE peaks at 2-4 hours AFTER neuronal activity (Kawashima, 2013 Nature Methods) while 4OHT peaks at 30min-1h AFTER IP injection. Therefore, ideally, you want to inject 4OHT 3-4 hours AFTER the behavior or whatever experience you want to capture. Note that any new activity induced during the 3-4h period could also be captured. It is important to design the behavioral experiments to minimize this caveat (e.g, avoid disturbing the animals for at least 4 hours after the initial behavior/stim, not introducing potential new exposures, etc.). However in other cases where the targted neurons are active for prolonged period of time you can chose a middle timepoint. For example Xi neurons during CIEC in Lal et. al 2023, where neurons were active 4 hrs post cold exposure and they continue to show activity thereafterwards, we used a 6 hr timepoint to inject 4OHT and left the mouse in cold for another 2-3 hrs.

For making aqueous 4OHT injectables (easier to make than oil and better PK)

1. Pre-make 25% Tween80 solution by diluting Tween80 in water or PBS. Undiluted Tween80 is hard to pipette. It may take a few hours or overnight to fully dissolve Tween.

2. Dissolve 4TM powder by adding 250ul DMSO into the 10mg bottle (Sigma H6278). You may freeze small vials in DMSO in -20c at this point.
3. Add 400ul 25% Tween80 to 4.35ml of saline, mix well (total volume=4.75ml)
4. Add the 250ul 4TM stock to (3) to make 5ml, mix well by vortexing vigorously. You should get a clear solution after vortexing. Only dilute the 4TM stock into aqueous solutions right before the injection. Please keep it wrapped with foil (to keep it in the dark). Do not let the diluted solution sit for more than a few hours. Do not refrigerate, as the 4TM may precipitate.
5. This is now 2mg/ml 4TM solution in saline w/2% tween-80. Injecting 250ul of this solution to a 25g mouse will deliver 20mg/kg 4TM. You can inject up to 500ul to a 25g mice (ie, 40mg/kg) if needed. You may also dilute it with regular saline for the volume/dose you need.