

INTRSECT SOP

Updated 3/28/19

I. Working with plasmids

The plasmids supplied by the Deisseroth lab are from produced using the Qiagen endotoxin-free maxi prep kit. Plasmids are sequenced prior to release. The annotation of each plasmid describes the backbone (eg AAV), promoter (eg Ef1a), expression logic (eg Con/Fon means requires both Cre AND FIp for expression), and payload (eg Chr2-YFP). The '2.0' annotation on some constructs indicates that they have been modified for improved FIp activity.

Specific considerations in working with plasmids:

1. **Tube labeling.** The INTRSECT toolset nomenclature requires that investigators be careful and consistent in labeling tubes; we recommend you include the backbone, promoter, logic of expression, payload, date produced, and concentration.
2. **Sequencing.** We sequence the plasmids from the end of the promoter to the 3' functional elements (eg WPRE). It is important to sequence the plasmid after any manipulation, in order to exclude mutations, as single base mutations may affect any of the complex functional elements.
3. **Amplification.** We use RecA- bacteria for transformation, such as Invitrogen's OneShot Stbl3 cells (C737303) to avoid unwanted recombination and Qiagen endotoxin-free maxi prep kit (12362) for preparations of distribution plasmids. If you use a cartridge-based plasmid preparation kit, please exercise extreme caution in testing plasmids by sequencing and culture expression prior to experimental use or viral production, as we have previously observed contamination across preparations due to failure of bottoms of the elution tubes. As exceedingly small amounts of recombinase are sufficient to catalyze recombination, contamination may result in aberrant expression in downstream applications.
4. ***In vitro* expression.** During design and validation, we test our plasmids in cultured HEK293 cells and primary neuronal cultures (derived from p0 rat pups). Plasmids are delivered using either lipofectamine (ThermoFisher) or CaPO4 transfection. We co-transfect them with expression plasmids for recombinases, as appropriate, in all combinations (eg no recombinase, co-transfection with Cre, co-transfection with FIp, co-transfection with Cre and FIp) and assay the expression pattern based on fluorophore expression (or using a proxy, such as a fluorescently-labeled antibody), both using a microscope and by flow cytometry. When you test constructs, be aware of different iterations of FIp recombinase; we use the 'optimized' variant FIpO; it is possible that different iterations of FIp will have variable efficacy in your specific experimental design.
5. **Working with recombinases.** Recombinases are highly functional catalysts and should be handled with caution. We employ a number of engineering controls to minimize the chance of contamination, including preparing stocks of recombinases and recombinase-dependent constructs on different days, physically separating tubes containing these tools from one another in storage containers, changing gloves between constructs when

there is a chance of contamination, employing master mixes to minimize potential droplet contamination, and frequently testing constructs.

II. Working with viruses

INTRSECT viruses are produced in collaboration with a number of separate core facilities. **We do not test every lot of virus produced by these independent groups.** When collaborators have notified us that they have had difficulty integrating INTRSECT viruses into their experimental design or have noted aberrant expression, we have reached out to virus cores and work together to independently test the viruses. On occasion, we have found viruses that have been mislabeled or contaminated. Although we work with the core facilities to optimize their processes, we do not have control over the production pipeline for viruses and recommend that downstream users test each batch of virus, regardless of whether these are INTRSECT or other viruses, prior to experimental use. Following are some guidelines we use for INTRSECT experiments. These apply to AAV; additional safety and handling considerations apply to other viruses.

1. **Receiving new viruses.** When receiving a new lot of virus, thaw it on ice and aliquot into individual tubes to minimize freeze-thaw cycles, then store at -80C. **Ensure that every tube is labeled.** If possible, label tubes with viral payload information, titer, and lot.
2. ***In vitro* testing.** We test all of our INTRSECT viruses in cultured neurons using co-infection with all appropriate combinations of viruses (eg no recombinase, Cre, Flp, Cre and Flp) to ensure that a) the virus is what we ordered, b) that there is no spurious expression, and c) that the virus expresses in the expected pattern.
3. **Note about titers:** Different viral cores use different approaches to describe the concentration (titer) of a virus. Even within a single core facility, the approach used to titer a virus may change over time. If you use more than a single lot of virus, we recommend discussing the titer with the viral core facility and independently testing every lot of virus.
4. **Note about serotypes:** Adeno Associated Virus (AAV) has exploded in popularity due to its stability, infectivity, relative safety, and post-infection tolerability. There are a number of serotypes available, which infect cells based on the expression of multiple 'viral receptors'. This 'tropism' is a critical component of experimental design, as the expression of these receptors does differ by cell type (neuron subtype).

III. Use of INTRSECT tools in animals

This toolset has been designed using a murine platform, due to the increasing array of molecular tools available in this species. It is likely to be useful in other species (rats, NHPs), although we have not assayed approaches outside of mice. We use a high level of caution when injecting these tools in mice, extending similar considerations as detailed above.

1. **Animals.** When using transgenic animals, it is critical to be cognizant of the limitations of a transgenic animals line. We recommend assaying transgene expression specificity (how true is the expression of the transgene in the cell type of interest?) and sensitivity (how many of the cell type of interest express the transgene?) prior to using a

transgenic animal for any experiment, but especially for INTRSECT experiments that may rely on crossing two transgenic mouse lines, which can have the effect of compounding poor sensitivity and specificity. Also, please note that these numbers may vary by brain region.

2. **Viral mixes.** We sometimes use a mix of viruses, such as INTRSECT virus plus recombinase virus, in a single injection site in a single animals. To decrease the possibility of cross-contamination, we do not open multiple stock tubes at the same time and try to minimize their physical proximity to each other. We also briefly spin viral aliquot tubes (after thawing on ice), to decrease the possibility of viral contamination of common surfaces, such as gloves.
3. **Syringes.** To decrease the possibility of cross-contamination, we utilize a dedicated syringe for each virus combination. For instance, we use different syringes to inject AAV-EF1a-Con/Fon-YFP by itself and AAV-EF1a-Con/Fon-YFP + AAV-EF1a-Cre. We, again, thaw the virus on ice and briefly spin aliquot tubes prior to opening them.
4. **Controls: part 1.** To maximize internal validity, it is critical to integrate control transgenic animals and injections into your experimental scheme. For instance, if using a double transgenic animal with a Cre AND Flp (Con/Fon) INTRSECT virus, also inject the virus into wild-type animals, Flp-only animals, and Cre-only animals. We recommend doing this before any behavior or circuit tracing to ensure that you are using a virus that expresses in the expected pattern and that your transgenic platform is suitable for use with the INTRSECT toolset.
5. **Controls: part 2.** An additional consideration when using double-transgenic animals is to ensure that Cre and Flp are co-expressed. For instance, in an animal cross where both parental lines express recombinases with 90% sensitivity, an intersecting subpopulation comprising even 20% of the total may not have recombinase expression (in the extreme). We recommend injecting control animals with single-recombinase-dependent viruses (e.g. Cre-dependent cDIO-YFP and Flp-dependent fDIO-mCherry) to ensure that the overlapping population you expect to exist based on *in situ* or IHC data of the driver gene is reflected by expression of the recombinase transgene.

IV. Further resources

If you have additional questions, please email charur@stanford.edu. Below are examples of experimental use of INTRSECT

Marcinkiewicz, Catherine A., et al. "Serotonin engages an anxiety and fear-promoting circuit in the extended amygdala." *Nature* 537.7618 (2016): 97.

Tovote, Philip, et al. "Midbrain circuits for defensive behaviour." *Nature* 534.7606 (2016): 206.

Clemente-Perez, Alexandra, et al. "Distinct thalamic reticular cell types differentially modulate normal and pathological cortical rhythms." *Cell reports* 19.10 (2017): 2130-2142.

Yu, Kai, et al. "The central amygdala controls learning in the lateral amygdala." *Nature neuroscience* 20.12 (2017): 1680-1685.

Fadok, Jonathan P., et al. "A competitive inhibitory circuit for selection of active and passive fear responses." *Nature* 542.7639 (2017): 96.

Capelli, Paolo, et al. "Locomotor speed control circuits in the caudal brainstem." *Nature* 551.7680 (2017): 373.

Poulin, Jean-Francois, et al. "Mapping projections of molecularly defined dopamine neuron subtypes using intersectional genetic approaches." *Nature neuroscience* 21.9 (2018): 1260.

Chuhma, Nao, et al. "Dopamine neuron glutamate cotransmission evokes a delayed excitation in lateral dorsal striatal cholinergic interneurons." *eLife* 7 (2018): e39786.

Gao, Zheng-Run, et al. "Tac1-Expressing Neurons in the Periaqueductal Gray Facilitate the Itch-Scratching Cycle via Descending Regulation." *Neuron* 101.1 (2019): 45-59.

Lazaridis, Iakovos, et al. "A hypothalamus-habenula circuit controls aversion." *Molecular psychiatry* (2019): 1.