

A fertilized mouse oocyte micro-injection experiment involving nucleases or transgenes requires a **minimum of 8-9 weeks** from the time of experiment initiation to the time when you will receive the first set of biopsies for screening, and approximately **12-14 weeks to complete the entire project** (for a full session of 35-40 live pups).

**Why does it take so long?** In a word - **“biology”**

The following is a typical timeline of events in generating modified C57BL/6 mice:

The TAMC will order the requisite numbers of C57BL/6 female embryo donors once we receive the constructs and paperwork from the Investigator. The mice usually take **one week to arrive**, and then are unpacked and set up in cages in the UMMS vivarium where they are allowed to **settle for one week** (or oocyte yields will be too low). The TAMC will super-ovulate some 20 WT females 2 days before the injection, and mate these females with stud C57BL/6 males the afternoon before the injection. The next morning, we check the females for coitus plugs, recover the ovaries and uterine tissue from the plugged mice, and flush oocytes with a fine needle. We then load the micro-injection needle(s) with the Investigator's guide RNA-nuclease mix or transgene, and micro-inject those oocytes (usually 100-120 oocytes) that exhibit signs of successful fertilization (i.e. obvious pronuclei). The embryo harvests and micro-injection process to inject 100+ oocytes requires 4-5 hours minimum. The injected oocytes are then placed into an incubator to see which will survive, while we check on the foster females we will need (these are ICR or SW female mice that were set up 1-2 days before the experiment with vasectomized male mice). The surviving oocytes are then surgically transferred that late afternoon (depending on the embryo recovery and nuclei fusion rate) or the next morning (at the 2-cell stage) into the oviducts of 3-5 pseudo-pregnant females. Once the foster females recover from the surgery and anesthesia, they are placed into the mouse colony for care and observation, and will usually **deliver in 18 days**. Not all of these moms will maintain the pregnancy or deliver pups, but we usually obtain 2 or 3 pregnancies from this process (about 10-15 live pups). The timeline to this point: **1 week to order, plus 1 week to settle, plus 3 weeks to perform the injection and have the foster moms deliver pups = 5 weeks**.

When using C57BL/6 host oocytes for injections, many of the resulting litters must be fostered perinatally onto other surrogate moms. This is because C57BL/6 pups tend to grow larger *in utero*, which induces stress and/or dystocia in the recipient foster mom and places the valuable litter at great risk. To avoid losing litters derived from injected oocytes, the TAMC sets up additional wild type mating cages in parallel with the surgical transfer foster dam cages, thus assuring that we have additional surrogate moms on hand. We can then remove the surrogates' natural litters and c-section the experimental litters over to the surrogate moms. Regardless of whether the foster moms deliver or surrogate moms are used, the resulting pups remain with the mother for 3-4 weeks (strain depending) of age prior to weaning and tail biopsy. This adds another 3-4 weeks to the timeline. **Thus, the absolute shortest time in which we can provide biopsy samples to the Investigator for screening is 8 weeks (for hybrid) or 9 weeks (for inbred) from the starting date** (the date when the TAMC has the construct and paperwork in hand).

Note- the above procedure describes the generation of 10-15 pups. But the TAMC guarantees an Investigator will receive 35-40 live pups for screening. This is a MUCH

more exacting standard than set by most Transgenic facilities (which instead usually guarantee numbers of transfers performed, or numbers of injected oocytes transferred, or even just number of scope-hours or sessions attempted). **The very high standard of the UMMS TAMC means that we REPEAT the entire process described above 3 or more times for a full-session using C57BL/6 mice**, or once or twice more if a half session of 20 pups was ordered. Please note this large number of live pups simply cannot be generated in a single day or in a single week. The TAMC usually completes a full-session project over 4-5 weeks. Since the TAMC provides the biopsy samples ASAP to allow the Investigator to identify founder mice, the samples will come to the Investigator in 3-4 sets of 10-15 samples over the course of 4-5 weeks (unless the mice are a little small and we delay weaning and biopsy for a week to ensure their safety).

Unless previously arranged, the TAMC does not wait for results from one injection experiment to begin the next injection experiment for a given project. Repeat injection experiments are ongoing and usually a week apart. An exception to this would be if the TAMC notes something amiss, like needles clogging, oocytes lysing, or no two-cell embryos being generated. If such were the case, we would immediately contact the Investigator and place a hold on the rest of the injection series. **Otherwise, the entire project will be completed in 12-14 weeks** from the time the TAMC receives the construct/paperwork to the time the Investigator obtains the last set of biopsies for analysis.

Once putative founder mice are available, the TAMC can easily arrange with UMMS Animal Medicine to transport the cages of founders (that you identified) to your vivarium space. Other arrangements are also possible (ex. all pups are transported to your space).