Zebrafish Core Facility User's Manual

Services and Pricing

Rack Rental Rates

1 Rack = $175/month

2 Racks = $275/month

Each rack is capable of holding up to 1,350 fish at maximum densities.

The 2-rack discount is only available on every second rack. If you have three racks, the charge will be $450 ($175 + $275) per month, whereas at four racks the charge will be $550 ($275 + $275) per month and so on.

Rack space is determined by the number of tanks, not the number of fish. Twice per month the tanks for each user are counted and rack space is calculated by adding the tanks as fractions of a rack where the 1.5L, 3L, and 10L tanks are equal to $1/80^{th}$, $1/60^{th}$, and $1/30^{th}$ of a rack respectively. The lesser of the two counts for a given month is used for the assigning a user their rental fees for that month. As a user is growing or expanding their colony, the charge will increase by 1 rack per month for that month once they have moved beyond the limitations of the previous rack (*e.g.* increasing from 0.9 racks to 1.1 racks will result in being charged for 2 racks). If you have budget restrictions for the number of racks you would like maintained, communicate this with the manager and you will be notified if you ever surpass your limit and you will be allowed a grace period in which you can reduce your numbers to drop you back into the previous number of racks.

All fish related maintenance (feeding, health checks, breeding, system tests) is conducted by CORES staff.

Procurement

As an alternative to renting racking space, users can purchase fish from the facility on an as needed basis. The standard rate for zebrafish is $10 per fish for both wildtype and transgenic lines. Requests can be made directly by e-mail to the facility manager the day before indicating the strain, number of fish, and when the fish should be ready. Embryos are also available for purchase at $15 per breeding tank for internal academics and $25 for external academics. These requests should be made using the breeding request form (see Breeding Requests section for more information).

Shipments are available for external academics and will be charged a $50 administrative/processing fee as well as shipping fees on top of the cost for the fish and/or embryos.

CFIA Imports

All imports into Canada will need to enter through the CFIA import quarantine lab. The process will take 8-12 weeks from time of import to having fish released for research use. The cost breakdown is as follows:

	Faculty of Medicine ($)	External ($)
Administrative fees	150	150
Permit and Inspection fees	300	300
Shipping costs	TBD	TBD
Husbandry fees (Importing Embryos)	50/week (minimum 12 weeks*)	140/week (minimum 12 weeks*)
Husbandry fees (Importing Adults)	50/week (minimum 8 weeks)	140/week (minimum 8 weeks)
Shipping costs to final destination	TBD	TBD
Total (Embryos):	1,050 + shipping costs + 50/additional week	2,130 + shipping costs +140/additional week
Total (Adults):	850 + shipping costs + 50/additional week	1570 + shipping costs +140/additional week

*A minimum of 12 weeks is based on an expected maturation rate. This value may increase for certain transgenic lines that have reduced growth, maturation and/or breeding rates.

Molecular and Genotyping Services

Genotyping is available for all transgenic and mutant lines. Associated fees include costs of reagents as well as the expected labor cost.

Fin Clipping and gDNA extraction ($1 / fish)

Trained staff will perform fin clips and gDNA extraction using the HotSHOT protocol that yields 55 µl of 100 – 300 ng/µl gDNA per fin clip.

Primer Design

You may have a primer that you have designed yourself or taken from a paper that can be ordered from IDT. If not, as long as you can provide information on the sequence of the transgene to be targeted then we can design a primer that will amplify a region within the target sequence.

Note: Since PCR is a presence/absence test, positive results do not ensure functionality of the transgene. When a functional integration site is not known, a combination of genotyping and functional assays is advised. When a functional integration site is known, primers should be designed to hybridize within the transgene and one of the flanking arms to only yield amplicons for integrations at the functional locus.

PCR ($3.50 / sample)

PCR can be performed either using a PCR kit from your lab or we can perform the test using the Hot Start KAPA Taq polymerase PCR kit owned by the CORES facility and charged to the user at reagent cost. This kit will be sufficient for most PCR's with the exception of very large amplicons (>1,500 bp) in which a long-amp PCR kit would be more suitable. Thermocycler programs are run in the CORES facility using a touch-Down thermocycler protocol modified for the specific needs of the amplicon (annealing temperatures and fragment length).

Gel Electrophoresis ($20 / gel)

Gel electrophoresis and gel imaging can be done at the CORES facility and the images will be processed and delivered to the user for their records. Each gel can hold up to 36 samples.

User Resources

Spreadsheets for Rack Rentals

All users renting rack space are provided with a shared google sheet that lists all of their tanks as well as various information about that tank including: tank ID, age of fish, filial generation, parental Tank ID, tank size, approximate number of fish, screening information, and additional notes as required. These sheets are updated regularly by CORES staff and are intended to allow users easy access to information about their colonies and can be shared with all lab members.

Breeding Requests

A breeding request form is e-mailed out to all users on Mondays for the following week and is due by end of day Thursday. On Fridays the completed breeding schedule is circulated for users to verify that their requests have been listed accurately for the following week which goes from Saturday to Friday. Fish are set up for breeding the day before and will be listed on the final breeding schedule by the day they are set up for breeding the following day.

When completing the breeding request form, fill out all columns that apply for each line that you want bred. Here is a breakdown of how the breeding requests are interpreted and some commonly used jargon/abbreviations that are used to ensure clarity of the request.

Date fish are set up / bred: We set up the fish in the afternoon (date set up) to allow for the exchange of pheromones overnight to stimulate ovulation in the female the following morning (date bred). The date bred reflects an age of 0 – 3 hpf by 11 AM with the following day being considered 1 dpf.

Creating a new line: This column specifically refers to microinjected embryos / larvae which you are using to create a new line. If you are simply crossing two already established lines (outcrosses) then you don't need to fill this column out as we know that the new line is a combination of the two lines that were used to create it.

Embryo quantity requested: This is an important column – here is how it is interpreted:

- **Production of embryos for experimental purposes and line propagation**
- "500": 1x 10 L tank containing 9 males and 9 females is set up to produce at least 500 fertilized viable embryos.
- "1000": 2x 10 L tanks
- "1500": 3x 10 L tanks, etc.
- "MAX": Use this when you want to breed all fish of that line – generally when you only have 1 or 2 tanks of that line.
- For all breedings conducted for line propagation and generating experimental embryos, breeders will be placed back in their original tanks after breeding unless otherwise specified by the user.

- **Screening Adults for Transmission and/or Breeding Performance**
- If you are trying to figure out which adults will transmit the gene of interest (establishing F0's and F1's) or wish to assess the breeding performance of individual fish then use this format:
- "5 INX's": 5 males will be incrossed with 5 females of the same line / tank in pair-wise breedings (useful for F1 INX's to generate F2's).
- "10 INX's": 10 males x 10 females, etc.
- "MAX INX's": All fish from this line will be incrossed in a pair-wise manner.
- Parents will be kept separate from their original tanks after breeding and placed in individual tanks containing the pair (male and female together) with a number corresponding to the plate of embryos they produced.
- Use the same format for outcrosses (OX's):
- "5 OX's"
- "10 OX's"
- "15 OX's" etc.

- This works the same as the INX's; however, how the parents will be separated can vary:
- Transgenic OX'd to wildtype or Casper: transgenic individuals will be isolated and numbered while wildtypes will be returned to original tanks.
- Transgenic OX'd to transgenic: Indviduals from the line that is considered stable will be placed back into their original tanks while individuals from the line you are screening will be isolated and numbered.

Always feel free to contact the facility manager if you are unsure about how to accurately list your request to ensure that the breeding serves the purpose it was intended for.

Embryo Care and Maintenance

Embryo maintenance is conducted by researchers/lab members and will need to be done for the first 5 days post fertilization. Lab members can find their fertilized embryos in the incubator located in the embryo cleaning station of the zebrafish core lab in their respective shelf area. Booking of the embryo cleaning station is done via the "ZCF Embryo Cleaning Station" Google calendar. Embryo maintenance should be conducted as follows:

- **Day 0 (afternoon of breeding day):** Embryos should be split into new petri of fresh E3 medium at 50 – 100 embryos/plate to allow for appropriate oxygenation and minimize contamination from non-viable embryos. Remove any scales, debris and non-viable embryos (discernable by white/opaque coloration) from the plates. Expect that ~20% of day 0 embryos will be non-viable; you should account for this when collecting the number of embryos you wish to keep.
- **Days 1-2:** Decant E3 from petri plates and replace with fresh E3 from your designated wash bottle. On day 2 you will likely notice that some larvae have already hatched from their chorions, the broken chorion should be removed from petri plates and discarded in the waste container along with any newly found non-viable embryos.
- **Day 3:** By day 3 the vast majority of larvae should have hatched from their chorions, so it is particularly important to clean on this day to remove the broken chorions from the plates. This is also an appropriate time for screening fluorescent zebrafish as the vast majority of structures are developed and the larvae being out of the chorion allows for easier visualization.
- **Days 4-6**: If you have removed all of the broken chorions from the plates on day 3 and were only left with hatched larvae at 50/plate, then cleaning may not be necessary during these days. It is advised that larvae be set up in the clean room for exogenous feeding during this time period. To do this, simply place your plates of larvae on the bottom shelf labelled for plates to be put online. All of the following procedures for larval maintenance are adapted for 50 larvae/tank. By providing your plates at 50 larvae/plate or less it will maximize the growth and developmental potential of the larvae in the nursery.
- **Day 7**: This is generally considered the latest that larvae should be placed online, withholding exogenous feed past this point has been shown to result in permanent growth/developmental retardation.

Euthanizing larvae: Live embryos and larvae should be placed in a waste container of E3 medium with bleach. Allow larvae to sit in 20% bleach solution for 10-minutes and discard down the sink.

System and Husbandry Parameters

Water Quality Parameters

Temperature:	28.0 – 28.5 °C
Conductivity:	700 – 800 µS/cm
pH:	7.2 – 7.6
dissolved O_2 (tanks):	6.59 - 6.90 ppm (85 – 89% saturation)
Hardness:	70 – 90 mg/L $CaCO_3$
Ammonia:	0 mg/L
Nitrite:	0 mg/L
Nitrate:	0 – 20 mg/L

Housing Densities

10L tank:	30 – 50 fish
3L tank:	10 – 20 fish
1L tank:	1 fish (long term isolation can be detrimental to fish health)

Note: Zebrafish tend to become aggressive at low densities with both the aggressor and their target showing high stress hormone levels. Low densities (<3 fish/L) are avoided as much as possible.

Feeding

5 – 14 dpf (larvae):	Rotifer polyculture (continuous feed)
15 – 29 dpf (larvae):	150 µm Gemma Micro (twice daily)
30 – 89 dpf (juvenile):	300 µm Gemma Micro (twice daily)
90+ dpf (adult):	300 µm Gemma Micro (once daily)

Health Status

The health status of individual zebrafish is monitored daily during feeding. Sick and/or distressed fish are isolated and/or culled. The facility health status is monitored yearly by sending sentinel fish and recent culls for PCR testing. The clean room is positive for *pseudoloma neurophilia* and the zebrafish picornavirus. Both of these pathogens are subclinical and should not interfere with most research.

Additional Information

Additional information on the CORES facility including procurement pricing and equipment can be found on our website: medicine.dal.ca/zebrafish

For any specific questions related to the zebrafish CORES facility, the facility manager can be reached at zebrafishcore@dal.ca