

TITLE: Zebrafish Import and Quarantine Procedures

SOP Category: Aquatic

RUAC SOP #: 5.11

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Effective Date: 6/17/2025

Approval:

David C. Rein DVM

Revisions:

SCOPE:

This document describes the procedures to be followed when importing zebrafish from external sources to the Research Tower Zebrafish Core Facility. This SOP applies to all Animal Care Staff (ACS), ACS Supervisors (ACSS), Veterinary Staff (VS), and Research Staff members (RS) at the Rutgers University facilities.

OBJECTIVE:

The objective is to describe the process of importing zebrafish into the Research Tower Zebrafish Core. Quarantine procedures aim to reduce the risk of pathogen dissemination to established stock when new lines are introduced. Imports must be coordinated with the VS and initiated by the requestor first placing an iLab importation request under Aquatic. Only disinfected (bleach and/or iodine-dipped) eggs are allowed from outside sources. Health reports are requested from the sending/source institution and must be reviewed by a RUAC Veterinarian for approval to import. Only disinfected embryos from healthy parents can leave the quarantine and be introduced into the main facility. Adult fish from an external provider are prohibited from direct entry into the main facility.

PROCEDURES:

The quarantine rack system is physically separated and functionally independent from the main colony room and centralized life support system. The quarantine room utilizes dedicated equipment (tanks, nets). Only authorized personnel are permitted to enter the room.

Import Request Processing

1. The receiving lab places import requests into iLab.
2. Health reports are obtained from the sending institution.
 - a. A CMR Veterinarian will review the health documentation to determine if any excluded agents exist.



Stand-alone Tecniplast rack used in the quarantine room.

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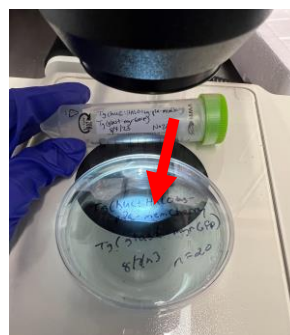
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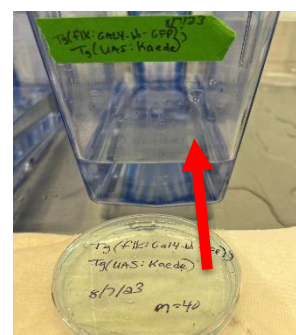
- b. Excluded agents: *Aeromonas hydrophila*, *Pseudocapillaria tomentosa*, *Pseudoloma neurophila*, *Mycobacterium marinum*, *Edwardsiella ictaluri*, *Flavobacterium columnare*, *Ichthyophthirius multifiliis*, *Piscinoodinium pillulare*, *Pleistophora hyphessobryconis*, *Saprolegnia brachydanis*, Infectious pancreatic necrosis virus (IPNV), Infectious spleen and kidney necrosis virus (ISKNV), and Zebrafish Picornavirus.
 - c. If health reports are unavailable within one year or testing reports are limited in scope, only bleached eggs will be accepted into Quarantine.
3. The final location of imported eggs (the Main or Quarantine room) is determined on a case-to-case basis. Bleached eggs may be approved for direct entry onto the main system under extremely rare circumstances of extensive current and historical testing that demonstrates negative results for all excluded agents.

Receiving the Shipment

1. Zebrafish eggs are shipped overnight by commercial carriers (FedEx or UPS) in sealed tubes (or flasks) at a density of no more than one egg per 2 mL. The tubes should be packed tightly into insulated shipping containers. During winter months, a heat pack should be placed in the box.
2. Obtain the shipment's tracking number from the sender to monitor the package's arrival time.
3. On receiving, embryos are visually inspected under a dissecting microscope. Only live viable embryos are transferred to a clean Petri dish. Any mortalities or non-viable embryos are discarded.
4. Label each Petri dish with the lab name, strain, and date of fertilization and place it within the 28C incubator located in RB-20.
5. Four to six days after fertilization, transfer healthy larvae into 3.5-liter tanks fitted with the 300-micron mesh baffle to the rear of the tank and place them onto the designated rack "nursery" location.
6. Record the total number of fish on the lab's accounting log and the new tank total on the lab's per diem census sheet.



Import tube content is transferred to a petri dish.



5dpf larvae are transferred to a tank.

How the Strain Exits Quarantine

1. All imported animals directed to the quarantine facility will stay under quarantine containment. Only surface-disinfected embryos from excluded agent-free and healthy parents are permitted exit from quarantine for introduction into the main facility.
 - a. After a period of growth (2-3 months), a sub-sample of five imported fish is sacrificed for infectious disease testing with the Zebrafish Surveillance Plus PCR Panel.

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- b. The pathogen report is obtained and reviewed.
 - c. When the strain's health report is negative for excluded pathogens, the strain is propagated through breeding while on the quarantine rack to generate embryos that are surface-sanitized with dilute bleach or iodine before being transferred to tanks inside the main facility. (See Iodine and Bleach Dip for Zebrafish Embryos SOP).
 - d. If a strain's health report returns positive for an excluded agent, steps a and b are repeated.
2. Once the line has been successfully established in the main facility, all fish of the imported line maintained in quarantine are culled.

REFERENCES

Zebrafish: Housing and husbandry recommendations. *Lab Animals* 2020, Vol 54 pg. 215-217

Laboratory Animal Medicine-Chapter 20: The Biology and Management of the Zebrafish, pg.1032-1033

<https://zebrafish.org/wiki/protocols/shipping>