

AQUATIC ZEBRAFISH CORE

Core User Advanced Training Checklist

Name of Core User/:	PI:	IACUC Protocol # (non ZFC 1154 only requires Initial introduction)
		1154

Date of training	Technique	Protocol and/or hands-on training	Description or focus of training	Trainer initials (1)	Core User initials (2)
	Initial introduction to animal and fish facility and IACUC regulations	Training and signed sheet	Explain LAF access regulation, IACUC regulation and protocol compliance, Fish: 2 independent water systems, fry system and maintenance		
	Euthanasia fry (from 1- 15dpf)	Protocol and training	Follow protocol, euthanize with ice water bath, add bleach as 2 nd step		
	Euthanasia adults	Protocol and training	Follow protocol, use mating tank insert on top of ice water bath, dispose in biohazard bag in freezer		
	Dead/sick/injured fish	Training	Notify Core staff, record in appropriate log, if dropped and injured must euthanize, if found dead place in labeled biohazard bag, if sick place in individual tank with label		
	Mating pair set up	Training	Check last/record current set up date, Remove/replace tank, how to handle fish with net, determine sex		
	Embryo collection	Training	Collect embryos using strainer, rinse debris, collect into petri dish, bring up to 11 th floor incubator		
	Return fish to tank	Training	Sort fish if necessary (outcross), remove tank from rack to add fish, replace tank and resume water flow		
	Sort embryos	Training	Remove dead or unfertilized embryos from plates, keep no more than 60 embryos per dish		
	Microinjection of embryos	Training	Injector set up, needle calibration, injection technique		
	Fin clipping for genotyping	Protocol and training	Use MS222 (tricane) to anesthetize fish, use of razor blade and forceps, keep tissue samples on ice, put fish in genotyping tank		

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	Bleach/ pronase embryos	Protocol and training	At about 24 hpf sort embryos before bleaching to remove any dead and check their stage of development, follow bleaching protocol		
	Remove pronase from embryos	Training	Should be removed 24 hrs after bleaching, if embryos are not hatched may use gently pipetting to dechorionate,		
	Extract DNA from fry/ embryos	Protocol and training	Use MS222 (Tricane) to anesthetize, place fry in per tube on ice, remove excess water, use ice-cold extraction kit and thermocycler		
	Freeze fry/embryos using dry ice for tissue, RNA, DNA extraction	Protocol and training	Use MS222(Tricane) to anesthetize, place fry in tube and remove excess water, dip tube in ethanol bath with dry ice until frozen, keep tube on dry ice		
	Fix fry embryos for tissue prep	Protocol and training	Use MS222(Tricane) to anesthetize, place fry in tube and remove excess water, put tube on ice and add ice-cold fixative (e.g. PFA, GA)		
	Put fry on system	Training	Sort 5-7dpf fry not more than 30 per dish, set up tank with screen baffle, fill out paperwork, keep all fry on rack 3, adjust water flow, feed Gemma 75, activate cage card		
	Use of dissecting scope	Training	Use of dissecting scope		
	Use of fluorescent dissecting scope	Training	Use of fluorescent dissecting scope		
	Use of fluorescent microscope	Training	Use of fluorescent microscope		
	Mounting of fish on methyl cellulose	Protocol and training	Use MS222(Tricane) to anesthetize, mount fish on small drop of methyl cellulose in embryo medium		

- (1) By initialing this box the trainer acknowledges they have provided the required documents and hands on training, and that the user is authorized to perform the task on their own and train others.
- (2) By initialing this box the user acknowledges they have read the appropriate protocols and completed the required training