Screening protocol for the identification of spontaneous mutations in Ciona intestinalis

1. Acclimate individuals of wild *Ciona intestinalis* in 35-50 l tanks for 2-3 days (feeding regime as in Cirino et al. 2002): check daily animal conditions and discard dead/unhealthy individuals; if gonoducts contain few gametes, expose animals to continuous light for 2-3 days.
2. Dissect gonoducts and collect gametes separately in 2 ml vials:
   - open the animal at the base of the atrial siphon with a sterile blade;
   - collect eggs from the oviduct with a Pasteur pipette and transfer them in a 15 cm Petri dish filled with 0.22 µM MFSW (millipore filtered natural sea-water);
   - collect sperm from the spermiduct with a Pasteur pipette and transfer it into a 2 ml vial in ice.
3. Perform i*n vitro* self-fertilization by washing the pipette used for sperm collection into the egg-containing Petri dish (at 2-3 PM in order to have tadpole larvae ready for morphological screening the morning after) and store in an incubator at 17-18°.
4. Cryopreserve *Ciona* semen - see **Cryopreservation of *Ciona intestinalis* sperm**.
5. The morning after (9:30-10 AM), analyze carefully live progeny at the stereomicroscope for obvious phenotypes that affect gross anatomy. Stereotyped abnormal phenotypes must be around 20-25%. In case, add few menthol crystals for light anesthesia of tadpole larvae
6. If no obvious phenotypes are detected, fix about 100 larvae for ISH analysis - see **Automated whole-mount in situ hybridization on developmental stages of *Ciona intestinalis* for the identification of recessive mutations with subtle phenotype** -.
   - fix in 2 ml vials 1-1.5 hr in 1:1 8% PFA in 0.1 M MOPS pH 7.4:MFSW;
   - wash 3 x 10 min in 1 ml 1X PBT in DEPC H_2O;
   - dehydrate in EtOH series (3 x 10' in 1 ml 30%, 50% and 70% EtOH in DEPC H_2O);
   - change last 70% EtOH in DEPC H_2O and store at -20°C.

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Reagents: 0.22 µM Millipore filtered natural sea water (MFSW), DEPC - H2O, paraformaldehyde, NaCl, MOPS, 10x PBS in DEPC-H2O, EtOH, TWEEN-20

Equipment: blades (flame sterilized with alcohol), Pasteur pipettes, 15 cm Petri dishes, 2 ml vials, 50 ml Falcon tubes, 65 C water bath, Parafilm, 0.22 µM Filters, Bunsen burner, incubator, 35-50 l tanks, stereomicroscope.

Additional information:
Cirino, P., A. Toscano, D. Caramiello, A. Macina, V. Miraglia, and A. Monte. Nov. 27, 2002. Laboratory culture of the ascidian Ciona intestinalis (L.): a model system for molecular developmental biology research. Mar. Mod. Elec. Rec. [serial online]. http://www.mbl.edu/html/BB/MMER/CIR/CirTit.html

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SOLUTIONS

8% PFA in 0.1 M MOPS pH 7.4

Reagent	Quantity (for 50 ml)	Final concentration
Paraformaldehyde	4 grams of Paraformaldehyde	
NaCl	5 ml of 5 M NaCl stock	0.5 M
MOPS	5 ml of 1 M MOPS pH 7.4 stock	0.1 M
DEPC H_2O	up to 50 ml	

Seal the 50 ml Falcon with Parafilm and mix well by shaking it manually.
Incubate in 65° water bath for 1 hr (shake manually each 10 minutes until paraformaldehyde is dissolved).
Filter the solution at 0.22 µM.
Aliquot in 2 ml vials and store at -20°.

1X PBT in DEPC H_2O

Reagent	Quantity (for 50 ml)	Final concentration
PBS	5 ml of 10X PBS stock	1X
100% TWEEN-20	50 µl	0.1%
DEPC H_2O	up to 50 ml	

Mix gently and store at room temperature.

30% ETOH in DEPC H_2O

Reagent	Quantity (for 50 ml)	Final concentration
100% EtOH	15 ml	30%
DEPC H_2O	up to 50 ml	

Mix gently and store at room temperature.

50% ETOH in DEPC H_2O

Reagent	Quantity (for 50 ml)	Final concentration
100% EtOH	25 ml	50%
DEPC H_2O	up to 50 ml	

Mix gently and store at room temperature.

70% ETOH in DEPC H_2O

Reagent	Quantity (for 50 ml)	Final concentration
100% EtOH	35 ml	70%
DEPC H_2O	up to 50 ml	

Mix gently and store at room temperature.