GENOTYPING TADPOLES

Initials SR

Reference

Recipes used in protocol	Amount Used
genotyping buffer, tads	
Taq Buffer, 10X	

Protocol

1) Tail snip collection:
[If anesthesia desired (OPTIONAL):
Tadpoles are anaesthetized by transferring to dishes containing 0.025% ethyl 3-aminobenzoate methanesulfonate salt (tricaine/MS222; SIGMA) in distilled water. Anaesthesia usually takes 1 to 2 minutes, depending on the size of the tadpole. Clean scissors are then used to cut away the posterior 5 mm of the tail. Tadpoles are returned to tanks containing 1 to 2 inches of water to recover. A teaspoon is a useful means of transferring the anaesthetized tadpoles. Recovery usually takes 5-10 minutes, after which the tadpoles should be able to swim and feed normally.]

Pick up the tadpole in a glove free hand, snip off 0.5 cm, and transfer the tail to a sterile eppendorf tube containing 0.1 ml genotyping buffer, and one scoop of 0.5 mm zirconium oxide beads. It helps if two people work together on this: one to hold and snip, the other to pick up the tail with forceps and put it in buffer (dip the forceps in 70% ethanol and wipe with a Kimwipe between tails). Place tadpoles individually in numbered plastic cups while you do the PCR reactions.

2) DNA extraction using the Bullet Blender.
1. Place samples in every other spot in the machine (using empty tubes if necessary to make 12 total). Set controls for Speed: 10, Time: 3 min. Press start.
2. After blending visually inspect samples. If there are still chunks then blend again.
3. Pellet debris 2 minutes, 14,000 rpm. Make sure to balance your tubes!
4. Pipette 40 ul of supe into a clean tube and add an equal volume of isopropanol.
5. Spin 10 minutes, 4C.
6. Wash pellet with 70% ethanol.
7. Air dry and dissolve in 20 ul $T_{10}E$.

3) PCR:
Use 1 ul of the above DNA in a standard 25 ul reaction, (94°C for 5 minutes, followed by 30 cycles of 94°Cx30s denaturation, 55°Cx30s annealing, and 72°Cx30s extension) with

primers SR494, 495, 496, 497. Females will give two bands (DM-W 260bp and DMRT 206bp), males one (DMRT, 206 bp only). Once we have a good DNA prep, we will always run one of each as a control. If you get no product, dilute your samples further and try again.

Primers:
Dm-W_for: CCACACCCAGCTCATGTAAAG
Dm-W_rev: GGGCAGAGTCACATATACTG
Dmrt_for: AACAGGAGCCCAATTCTGAG
Dmrt1-rev: AACTGCTTGACCTCTAATGC

Master mix

10x Taq buffer	100 ul	50 ul
H20	735 ul	368 ul
dNTPs(10 mM)	25 ul	12.5 ul
SR464	25 ul	12.5 ul
SR465	25 ul	12.5 ul
SR466	25 ul	12.5 ul
SR467	25 ul	12.5 ul
Taq	5 ul	2.5 ul
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