

Protocol for genotyping *val* fish

Primer sequences:

Valf: 5'-CCCGCAGACGTTAAGCCTCAC-3'

Valr: 5'-GATCGCGCCGTACTGGTGTT-3'

These primers should amplify a 220 bp fragment surrounding the point mutation in *val*^{b337}. Since the mutation in *val*- DNA destroys a PvuII site, digesting the PCR product with PvuII will cut the wild-type allele down to two fragments of approximately 100 and 120 bp, but will not cut the PCR product amplified from the *val*- allele. Heterozygotes (*val*^{+/-}) will have all three bands (see fig. 2, Moens et al., Development 125: 381-391).

Protocol:

- Place embryos or fin clips into sterile 0.65 ml tubes. It is important to remove as much water as possible from around the embryo or fin clip before adding lysis buffer. For embryos, you can do this with a pulled-out pipette, under the dissecting microscope with epi-illumination.
- Add 50 μ l (for embryos) to 200 μ l (for fin clips) lysis buffer containing 1 mg/ml ProK (ProK should be stored as aliquots at 10 mg/ml in the freezer)
- Incubate at 55°C for 3 hours, either in water bath or in old PCR machine.
- Vortex, spin briefly in microcentrifuge, and inactivate ProK by a 10 minute incubation at 100°C (in old PCR machine).
- Spin briefly before opening tubes. For embryos, use 2-5 μ l of this crude lysate as a PCR template, for tail clips use less (1 μ l is more than enough).
- Check a random few PCR samples to ensure that the PCR worked and to determine how much to digest (see notes below).
- Set up digest with excess enzyme (see notes below) on as little PCR product as will be necessary to visualize on the gel. This will dilute reagents in the PCR reaction that inhibit restriction enzyme digestion, and will encourage the reaction to go to completion.

Notes:

- When genotyping fin clips from adults, you will get two possible genotypes: either homozygous wild-types or heterozygotes. It is important to let the PvuII digest go to completion (use excess enzyme, and avoid using too much PCR product) so that you don't confuse incompletely cut DNA from homozygotes with real heterozygotes.

•To determine how much PCR product to digest, check a few microliters of your PCR product on an agarose gel before doing the digest. Ideally you will be able to dilute 2-5 μ l of your PCR product up to 20 μ l for the digest. This will improve the chances of the digest going to completion.

Reagents:

Lysis buffer:

10 mM Tris pH8

1mM EDTA

0.3% Tween-20

0.3% NP40

Add 1/10th volume of 10 mg/ml ProK