D. PCR SAMPLE PREPARATION

1) To obtain samples for genotyping, anesthetize fish in Tricaine/Mesab (MS-222; http://zfin.org/zf_info/zfbook/chapt10.html - wptohtml63) as described in the following sections of *The Zebrafish Book*:
Chapter 2.8, 2.9; http://zfin.org/zf_info/zfbook/chapt2/2.8.html#4,
10.63; http://zfin.org/zf_info/zfbook/chapt10.html#wptohtml63. Clip tail fins and transfer them into 50 μl aliquots of lysis buffer in a 96-well plate. The figure below shows a position within the tail fin where the fin clipping typically takes place (marked by a dotted line).

Lysis Buffer:

- 10 mM Tris-HCl (pH 8.0)
- 50 mM KCl,
- 0.3% Tween 20
- 0.3% NP40
- 1 mM EDTA

2) While the fins are being clipped, keep the plate on ice.

3) After clipping, cover the samples with 20 μl mineral oil and incubate at 98ºC for 10 minutes.

4) Add 5 μl Proteinase K solution (20 mg/ml) and incubate overnight at 55ºC. This incubation ends with 10 minutes at 98ºC to denature the Proteinase K.

5) Store the plate at –20ºC.

6) For PCR, dilute the samples in water (1:20) and use 2.5 μl of this dilution in the reaction.

7) Occasionally, samples are not prepared from fin clips but from embryos or juvenile fish. In these cases, an identical protocol is used to lyse the samples, except that the lysis buffer contains 4 mM EDTA. For PCR, 5 μl of 1:10 dilution (in water) is used.

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Last Updated May 20, 2008