

Primers:

lzf forward (bwd385): act cgg cgg act ctc gca agc

lzf reverse (bwd386): ggc tct cgt cgg tga tgg cca tga tct tct

product size: 128bp

XbaI fragment sizes: 30bp and 98bp

Method:

1) Fin clip and add 50ul Lysis Buffer and 5ul 10mg/ml Proteinase k per embryo.

2) PCR reaction:

11.8ul ddH<sub>2</sub>O

2ul 10X PCR Buffer

1ul 2.5mM MgCl<sub>2</sub>

1ul 5mM dNTPs

1ul 5um forward primer

1ul 5um reverse primer

2ul DNA

0.2ul Taq Polymerase

PCR Profile:

94C 1 min

94C 20 sec

65C 20 sec

72C 20 sec

Goto step 2 40X

4C soak

3) Restriction Digest:

10ul PCR Product

2ul 10X Buffer

0.5ul XbaI

7.5ul ddH<sub>2</sub>O

incubate at 37C for 3hrs

4) Separate fragments using a 2% Agarose gel