Mitfa$^{w2/w2}$; *roy*$^{a9/a9}$ (AB) (CZRC catalog ID: CZ73)

Nature of mutation

Casper is a *w2*, *a9* double mutant with combinatorial pigmentation mutants, yielding an almost transparent zebrafish. Mitfa$^{w2/w2}$ is a mutation (Q113X, creating a premature stop codon, truncated protein) in a Mitf gene, completely lacking melanophores. Roy$^{a9/a9}$ is a spontaneously occurred genetic mutation, which has no iridophores, only uniformly pigmented eyes, sparse melanocytes, and a translucency of the skin (White, Sessa et al. 2008).

Genotyping assay

1. Genotyping of the *Casper* allele is based on the microscope.

Figure. The *Casper* line exhibits almost transparent phenotype.
The upper figure shows the lateral view of *Casper* embryos and wild-type embryos at 5 dpf. The lower figure shows the lateral view of *Casper* line at adult.

2. Genotyping of the *Casper* line can also be performed via allele-specific PCR using *w2*-specific primers.

Primers:

W2 _forward: 5’ GTGCAGGTTAATACAGATATAGGT 3’

W2 _reverse: 5’ CCAATGGTAAACAAGACAATG 3’

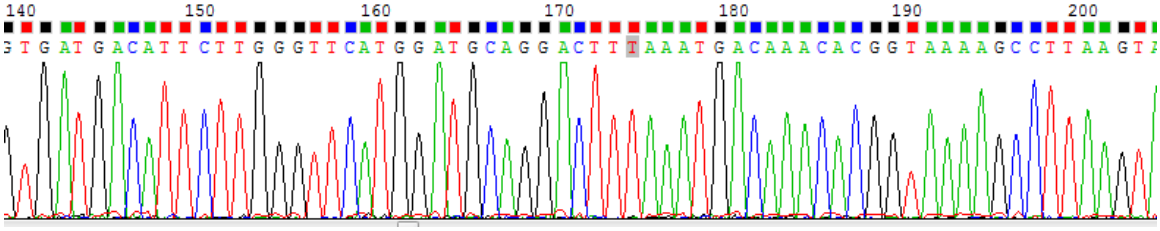
PCR program:

94℃	4min	
94℃	30 sec	30 Cycles
55℃	30 sec	
72℃	30 sec	
72℃	5min	
4℃	hold	

Product size: 463 bp:

The sequencing results of the parents:

CZ73 (-/-)　　TGGATGCAGGACTTCAAATGACAAACACG　　(wild-type)



Reference

White, R. M., A. Sessa, et al. (2008). "Transparent adult zebrafish as a tool for in vivo transplantation analysis." Cell Stem Cell **2**(2): 183-189.