Genotyping for *stl445* at *inppl1a* locus

The predicted *stl445* causative mutation disrupts an Hpy188III site within the *inppl1a* locus.

Wildtype sequence: 5′–AAATCATATCCAGAG–3′
Mutant sequence: 5′–AAATCATAACCAGAG–3′

gDNA isolation

Lyse tissue samples in 50 mM NaOH (fin clip samples in 50 µL, <30 dpf fish in 100 µL). Expose to high heat (95 C) for 20 minutes, then neutralize with 1 M Tris-HCl (pH 8) at a 1:4 ratio. For example, if 100 µL of NaOH was used, neutralize by adding 25 µL Tris.

Amplify *inppl1a* locus using PCR:

Reagent	**Volume**	**Notes**
2X GoTaq Green	12.5 µL	Promega #M7123
[10 µM] Forward Primer	1.5 µL	5′–CTTCCTGTCATGTGACCTTCAG–3′
[10 µM] Reverse Primer	1.5 µL	5′–GTTTGCATGTGTGTGTGTGTTC–3′
Nuclease Free Water	4.5 µL	
gDNA	5 µL	
Total	**25 µL**	

Run PCR using the standard **55 ANNEAL** thermocycler program. Make sure to let the chamber come to temperature before adding samples.

1. Pre-denaturation: 95 C for 3 minutes
2. Denature: 95 C for 30 seconds
3. Anneal: 55 C for 30 seconds
4. Extend: 72 C for 30 seconds
5. Go to step 2, 34X
6. Final extension: 72 C for 5 minutes
7. Infinite hold: 12 C

Hpy188III digestion on *inppl1a* PCR product:

Reagent	**Volume**	**Notes**
PCR Product	13 µL	Save remaining 12 µL to run on the gel, can be stored at 4 C overnight
10X CutSmart Buffer	1.5 µL	NEB #B7204S
Hpy188III Restriction Enzyme	0.5 µL	NEB #R0622S
Total	**15 µL**	

Leave at 37 C for 4 hours, or overnight. Kill the reaction at 65 C for 20 minutes.

Separation of PCR and digestion products with gel electrophoresis:

Pour a 3% agarose gel (or 2% metaphor + 2% agarose) in TAE Buffer. Add 5 µL EtBr per 100 µL of gel. Load ~10 µL of the PCR and digest products along with a 100 bp ladder. Apply voltage for 30 minutes to 1 hour. Detect bands with UV light.

NOTE: Although bands can be distinguished after 30 minutes of electrophoresis, dye front often obscures the bands when imaging at this time point.

Analysis of results:

Uncut PCR product migrates with the 200 bp marker in the 100 bp ladder.

Hpy188III digestion of wildtype amplicon generates two cut fragments that migrate further in the gel. Homozygous mutant amplicon remains uncut. Digestion of heterozygous amplicon results in 3 bands, with a bright uncut band at ~200 bp, and two faint cut bands.