You can find this and other more detailed tutorials at <http://the-odin.com/tutorials/>

This kit contains everything you need to genotype samples from Fish.

Background Reading

<http://www.nature.com/articles/srep15894>

**Protocol**

1. Rinse the fish with water
2. Take fish and place in 300uL 50mM NaOH in 1.5mL microcentrifuge tube
3. Heat tube in almost boiling water for 10 minutes (95C)
4. Add 300uL of 50mM Tris
5. For a 50uL PCR reaction
   1. 1uL of fish solution for PCR reaction template
   2. 1uL of Fish primer
   3. 10uL of 5x Master Mix(or 25uL of 2x Master Mix)
   4. 38uL water with 5x Master Mix(or 23uL with 2x Master Mix)
6. See PCR temperatures and times below

You can find how to cast and run a gel here: <http://the-odin.com/tutorials/>

1. First, make TAE if you don’t have it by adding 5g of TAE mix to 1L of distilled water or 50 to 1L to make a 10x stock and then dilute that down to 1x(i.e. 100mL 10x and 900mL water)
2. Next, add 0.5g of agarose to 50mL of TAE and microwave it on high for 1 minute or until all agarose is dissolved(the solution should be clear)
3. Add 5uL of Gel Green to the still hot molten agarose solution and swish around to mix
4. Dump the molten agarose into your gel mold and let set (this usually takes around 20-30 minutes but can be sped up by placing it in the fridge)
5. Add 3.5uL of 6x loading dye to your digest and add each of your digest.loading dye solutions to the individual wells on the gel (one well for one reaction)
6. Add 4uL of 100bp ladder to the first or last lane of the gel

**PCR Reaction**

**1 x** 95C 10 Minutes

**35 x**

95C 30s

52C 1 minute

70C 1 minute

**1x** 70C 10 minutes