Tailing and Genotyping Fish

1. Add 100 ul of Tail Lysis buffer to PCR strips or 96 well PCR plate
2. Anesthetize juvenile or adult fish in tricaine
3. Place fish on a petri dish with a little liquid and cut off 3-5 mm away from fin end with a razor blade
4. Transfer fish into individually numbered tanks and transfer cut fin with forceps into appropriate and labelled tube
5. Put at 98C for 10 min in thermocycler
6. Add 10 ul of 1:10 dilution of ProtK (~1-10 mg/ml ProtK) (for 50 fish: 5 ul of protk, 500 ul of lysis buffer) into each tube
7. Digest at 55C for 2-10 hours
8. Denature ProtK enzyme at 98C for 10 min
9. Set up 10 ul PCR reactions with KAPA HIFI Hotstart polymerase (KK2602)
    - 5 ul 2x KAPA Master Mix
    - 3.5 ul of water
    - 0.5 ul of 10 uM primers
    - 1 ul of DNA from prep

PCR program:

1. 95C	3:00	
2. 98C	0:20	
3. 60C	0:15	
4. 72C	0:30	<- go up to 1 min for longer than 1 kb
5. Go to step 2	34x	
6. 72C	1:00	
7. 12C	forever	

* I use a 60C mt as my starting point for genotyping the first time and the product should ideally be less than 1 kb but can be anywhere from 200 bp-1.5 kb

* if there's a lot of noise in the product, touchdown PCR starting at 72C and working down to 60C has been great

Lysis Buffer Solution (500 mls):

- 5 mls 1M Tris (pH 8)
- 1 ml 0.5M EDTA
- 1.5 ml Tween
- 1.5 ml Glycerol
- 491 ml diH2O