Genotyping *N. furzeri* embryos or caudal fin tissues

Material

- DirectPCR (Tail) (Viagen #102-T)
- Proteinase K solution, RNA grade (20 mg/ml, Invitrogen)

DNA extraction

1. Collect samples: embryos or caudal fin clippings.
2. Prepare the digestive solution: 2ul (for fin tissue) to 5ul (for embryos) Proteinase K per 1ml DirectPCR (Tail).
3. Incubate each sample with 100 ul digestive solution at 50-55'C for 1 to 2 hours until the sample is completely dissolved. It is also okay to leave it overnight.
4. Inactivate Proteinase K by boiling at 100'C for 10 minutes (leave the cap open for the first minute so the tube does not pop during boiling)
5. Spin at high speed to precipitate debris for clear supernatant.

PCR and restriction enzyme verification

1. Running 20 ul reaction is sufficient for sequencing

10 ul	GoTaq Master Mix
1 ul	DNA extraction
1 ul	10 uM Primer Mix (0.5ul Forward primer + 0.5ul Reverse primer)
8 ul	H2O

2. Running PCR with 40 cycles to obtain more PCR product if purification step will be done by the sequencing company (usually lower yield)

v1.1: Chi-Kuo Hu, 2024/06/21