GENOTYPING BY SEQUENCING

Use this guide to help prepare your samples for submission to our **Genotyping By Sequencing** service.

The outcome of your Genotyping by Sequencing project can be affected by the quality and quantity of starting nucleic acid template. Before submitting your samples please ensure that they meet the sample submission criteria listed below.

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1. Sample Labelling and Packaging

- ✓ It is important that your samples are clearly labelled with permanent marker.
- ✓ If submitting in tube format, please use Parafilm to seal the tubes. To prevent crushing during shipping, place the sample tubes in a 50ml tube or a freezer box (with internal rack).
- ✓ If submitting in plate format, please use V-bottom plates and ensure each plate is heat-sealed or strip caps are used to prevent leakage in transit. To prevent crushing during shipping, package plate/s in bubble wrap.

2. DNA Quality Requirements

- ✓ DNA should be free from contaminants and extracted from pathogen- and symbion-free tissues using the same method for all samples.
- ✓ DNA should have an A260/280 ratio of 1.8-2 and an A260/230 ratio of >1.6.
- ✓ We recommend that DNA concentration be assessed by fluorometry.
- ✓ DNA should be a high molecular weight (>20 kb), free of RNA and not form streaks when assessed by gel electrophoresis.
- ✓ DNA should not be concentrated by vacuum to prevent concentration of salts which could have a negative effect on enzymatic reactions during library preparation. Samples with low DNA concentration should be concentrated by *Zymo Research gDNA Clean & Concentrator* or *AMPure XP* beads by adjusting elution volume.
- ✓ DNA should not be viscous during pipetting; such samples may be less accessible to enzymes reducing their contribution to final library preparation.

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3. Standard Establishment Phase Genomic DNA

- ✓ Samples should be submitted in 1.5ml screw cap tubes
- ✓ 3 gDNA samples provided from 3 individuals representative of your species; AGRF will pool these for processing following our QC assessment
- ✓ 500ng DNA (submitted in a minimum volume of 25ul) per sample is required

4. Premium Establishment Phase

- ✓ Samples should be submitted in 1.5ml screw cap tubes
- ✓ 3 gDNA samples are to be provided from 3 individuals
- ✓ 1.5 µg DNA (submitted in a minimum volume of 75ul) per sample is required

5. Batch Submission

- ✓ Samples should be submitted in 96 well plates
- ✓ 200ng of each sample (submitted in a minimum volume of 20ul) per sample is required
- ✓ A single well on one plate should be left empty for a negative control