Next-Generation Sequencing: Sample Preparation Guide

Use this guide to help prepare your samples for submission to our Next Generation sequencing services.

The outcome of your next generation 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 pen or adhesive labels (please ensure the labels will withstand any freezing)
- Sample names should be simple with up to 8 alpha-numeric characters (avoid using spaces and punctuation characters)
- We recommend 1.5ml or 2 ml screw-cap DNase- and RNase-free microcentrifuge tubes
- Please use Parafilm to seal each tube before package.
- To prevent crushing during shipping, place the sample tubes a 50 ml tube (Falcon tube) or a freezer box (with internal rack). Samples can be further secured with padding such as paper towel or tissue.

2 Amplicons

- DNA must have an A260/280 ratio of 1.8-2
- We recommend that DNA concentration be assessed by fluorometry (Picogreen, Qubit QuantIt or similar assay)
- Amplicons for Nextera XT sample prep should be >300 bp

3 Genomic DNA

- DNA must be high molecular weight (>40Kb) and free of RNA as assessed by gel electrophoresis
- DNA must have an A260/280 ratio of 1.8-2
- We recommend that DNA concentration be assessed by fluorometry (Picogreen, Qubit QuantIt or similar assay)
- Gel electrophoresis results should be included when submitting sample(s)

Library	Sample Type	DNA Quantity	DNA Concentration	Recommended Buffer
Shotgun, bead size selection	gDNA	1 µg	≥20 ng/µL	Nuclease free water, TE Buffer
Shotgun, Pippin Prep size selection	gDNA	5 µg	≥100ng/µL	Nuclease free water, TE Buffer
Nextera	gDNA	500 ng	≥20 ng/µL	Nuclease free water $TE_{0.1}$ pH 7.5-8.0
Nextera XT	gDNA	200 ng	≥10 ng/µL	Nuclease free water $TE_{0.1}$ pH 7.5-8.0
Mate Pair	gDNA	> 20 µg	≥100ng/µL	Nuclease free water $TE_{0.1}$ pH 7.5-8.0

4 ChIP-Seq

- 20 ng of ChIP DNA
- ChIP DNA should be between 200-600 bp
- We recommend that DNA concentration be assessed by fluorometry (Picogreen, Qubit Quantit or similar assay)

5 RNA

- AGRF strongly advises DNase treatment (on-column)
- Quality of total RNA should be assessed by bioanlayzer, with a RIN (RNA Integrity Number) ≥ 8
- RNA should have an A260/A280 ratio of 1.8 – 2
- We recommend that RNA concentration be assessed by fluorometry (RiboGreen)
- Samples should be resuspended in nuclease-free water
- If you are submitting for Small RNA sample preparation, please ensure your samples have been isolated with a protocol that will retain small RNAs such as Qiagen's miRNeasy Mini Kit or Ambion's mirVana™ miRNA Isolation Kit.

Library	Sample Type	RNA Quantity	RNA Concentration	Recommended Buffer
mRNA-seq	Total RNA	3 µg	≥100 ng/µL	Nuclease free water
	Purified mRNA	200 ng	≥10 ng/µL	Nuclease free water
Whole Transcriptome	Total RNA	3 µg	≥100ng/µL	Nuclease free water
Small RNA	Total RNA	3 µg	≥100ng/µL	Nuclease free water
	enriched small RNA	500 ng	≥20 ng/µL	Nuclease free water

6 Sequencing Libraries

- AGRF will accept sequencing libraries prepared with standard Illumina protocols
- Details of the library preparation protocol must be provided
- AGRF requests 25 µl of library at a concentration of 10 nM or greater
- Recommend buffer for storage and dilution of libraries is 10 mM Tris-HCl pH 8.5, 0.1% Tween 20
- AGRF will perform a quality check of each library via automated electrophoresis (Agilent Tape Screen or Bioanalyzer) and qPCR.
- Custom sequencing libraries may be accepted on consultation (custom sequencing primer must be provided at 100 µM)

If you have any questions regarding your sample submission please email nextgenseq@agrf.org.au or call us on 03 9345 2683 for assistance.