ARC-Biotechnology Platform: Service Manual

2018

Version 1.0

1. Background

The Agricultural Research Council Biotechnology Platform (ARC-BTP), situated on the historic Onderstepoort Campus, was established in 2010 as a strategic priority of the ARC. The role of the ARC-BTP is to establish high throughput, high-end technology resources required for applications in advanced genomics, quantitative genetics, marker assisted breeding and bioinformatics, in the areas of plant and animal improvement and health. The ARC-BTP is based on a research and service model, thus providing an environment in which the next generation of highly skilled young researchers can be developed. The technologies established within the platform are accessible as services to the whole of the ARC and outside clients as well as through research collaborations with ARC-BTP research team.

The ARC-BTP has implemented Illumina sequencing and genotyping technologies, with the following instruments currently in operation within the facility: HiSeq2500, two MiSeqs, HiScan and BioNano's Irys Optical mapper. Large sample volumes can be processed with high-throughput liquid handling robotics. High performance computing infrastructure for data analysis is also present within the facility. A detailed description of the infrastructure available at the ARC-BTP can be found on the ARC's website (http://www.arc.agric.za/pages/BTP).

2. Services

The ARC-BTP provides services for next generation sequencing (NGS) of DNA and RNA, as well as for single nucleotide polymorphism (SNP) genotyping on Illumina platforms. You are encouraged to set up a meeting to consult with a member of Genomics Core Facility regarding the experimental design of your research (details bellow). This will help to determine the optimal platform, library preparation protocol, and sample sizes required to answer your research questions.

Broadly, the ARC-BTP services can be divided into the following sections:

1. **Targeted sequencing**: Amplicons including metagenomic samples
2. **Pair-end sequencing**: *De novo* and re-sequencing

3. **Mate pair sequencing**: Enables generation of long-insert *paired-end* DNA libraries for *de novo* sequencing and structural variant detection,
4. **RNA sequencing**: For differential expression analysis, *de novo* transcriptomics, viral metagenomics and other applications. Stranded RNA sequencing with either poly-A capture of mRNA or with ribosomal RNA depletion. Techniques implemented for highly multiplexed pooling of RNA samples to reduce library preparation costs.
5. **Small RNA sequencing**: Discovery of novel miRNAs and other small noncoding RNAs or study differential expression of small RNAs.
6. **Products in development**: methylation sequencing, single cell sequencing, exon sequencing, Chromatin Immunoprecipitation sequencing.
7. **BioNano Irys**: Optical mapping for structural variant detection and *de novo* genome assembly.
8. **Genotyping** using Illumina Infinium BeadChips
9. **High-throughput** Nucleic Acid **Extractions**

3. Submitting your samples

Please ensure that your samples meet the criteria below. Samples that do not meet these criteria will not be processed.

3.1. General guidelines

PLEASE NOTE: Samples that are submitted without a sample sheet and a checklist will not be processed.

3.1.1 Labeling of samples:

- Please label your samples with your name, sample identifier and date. Sample identifiers must be no longer than 6 characters. Simple identifiers, without punctuation characters, are required.
- Make sure that the labels on your tubes are identical to the labels on the sample sheet.

3.1.2. Sample quality check prior shipping/sequencing/genotyping:

- All samples must be intact and not degraded, as assessed by agarose gel or capillary electrophoresis (e.g. a Bioanalyzer).
- All samples (including PCR products) should be clean/purified with 260:280 and 260:230 ratios of between 1.8 and 2.2.

3.1.3 Sample preparation for sequencing and genotyping:

- Samples must be re-suspended in nuclease and PCR inhibitor free water or a Tris-EDTA buffer. Lyophilised samples or samples in ethanol will not be accepted.
- Please refer to Table 1 for detailed guidelines per sample type and library preparation method.
- All samples must conform to the requirements as stipulated in Table 1.

3.2 PLEASE NOTE:

- Samples at ARC-BTP are quantified using fluorometry and results may differ significantly from spectrophotometric results. We strongly recommend clients quantify nucleic acids using more accurate fluorometric methods, such as Qubit or PicoGreen assays.
- If samples are outside of the required specifications, the client will be offered the opportunity to continue with sample preparation and sequencing – BUT with ALL associated COSTS for their account and to be paid in full - even in the event of total failure to produce any data.
- If samples fail sample preparation and the samples complied with minimum sample requirements as outlined in Table 1 the samples will be repeated once (at our cost). If a second failure occurs, the client will be requested to provide a new sample and will be charged for the sample preparation. Any future sample preparations will be seen as additionally sample preparations and charged as such.
- The Genomics Core Facility will verify the integrity and concentration of your samples via intercalating dye and a Bioanalyzer (RNA samples).
- For 16S metagenomic experiments the client must submit purified amplicons. Please contact us for primer requirements. Please provide a gel image of amplicons.

Table 1: Minimum sample requirements for submission to ARC-BTP

Sample Type	Minimum Concentration	Minimum Volume	Library Methods
DNA/Genome Sequencing Prokaryote	50ng/µl	20µl	Nextera/Nextera XT
DNA/Genome Sequencing Eukaryote	50ng/µl	100µl	Illumina TruSeq DNA Nano or PCR-Free
*Bacterial 16S profiling (amplicons)	20ng/µl	20µl	Indexing Nextera XT
PCR product (<550bp)	20ng/µl	20µl	TruSeq DNA Nano
PCR product (>550bp)	20ng/µl	20µl	Nextera/Nextera XT
RNA sequencing	50ng/µl	50µl	TruSeq Stranded mRNA
RNA sequencing with RiboZero Reduction	50ng/µl	50µl	TruSeq Stranded Total RNA
GenoTyping	100ng/µl	100µl	Infinium
Mate Pair Sequencing	100ng/µl	100µl	Nextera Mate Pair
Small RNA sequencing	200ng/µl	20µl	TruSeq Small RNA

4. Packaging for plate submission

Using sticky seals for 96 well plates is typically insufficient to ensure that evaporation does not occur, or ensure no leakage/sample loss occurs. The Core recommends the use of heat or cap mat sealing for sealing of DNA/RNA sample plates. Please do not overfill the plate wells with sample.

5. Shipping guidelines

5.1 Local Shipping within South Africa

- Please inform the Genomic Core Facility, in writing, that your samples have been shipped and confirm that the samples have arrived.
- DNA and RNA must be frozen solid before shipment.
- Please do not send packages that will arrive at our laboratories over a weekend; they will not be received until Monday. Ice or dry ice may thaw during this time and compromise your samples.
- All samples, including templates and primers, should be submitted in the follow formats: 1.5 ml tubes, or 96 well PCR plates.
- Seal the tubes and plates to prevent leakage, sample evaporation and cross-well contamination. The ARC-BTP is not responsible for leakage of samples during shipment.

5.2 International Shipping

- Discuss international shipments with the Genomics Core Facility as early as possible, at least two weeks is preferred.
- Ensure that you as the client understand what is required for each shipment.
- Shipping of some species may require import permits. Processing of permit applications can take a minimum of 4 weeks depending on species and country of origin. It is a client responsibility to ensure that the correct permits are obtained prior to shipment of samples.

6. Pricing

- Pricing is exchange rate dependent and prices are based on current stock availability.

- A formal quotation will be generated for each project.
- Quotes are valid for 30 days or while stocks last.
- Discounted rates are available for academic users, ACGT members and formal collaborations.
- Library preparation, sequencing and genotyping will only commence once a copy of a purchase order/number is received. It is the client's responsibility to ensure that Genomics Core Facility has received it.

7. Processing time

- Turnaround times for sequencing are dependent on the type/amount of requested data and availability of the specific sample preparation kit reactions. The ARC-BTP maintains a diverse range of sample preparation kits but certain kits may need to be purchased prior to the commencement of work. If kits need to be ordered, sample turnaround times will be extended.
- Low and high volumes of requested data may adversely influence turnaround time, however, turnaround times are significantly improved when whole runs are requested.
- Turnaround times for genotyping services are dependent on the number of samples, density of the selected arrays and availability from Illumina.
- Turnaround times and results cannot be guaranteed for samples that do not meet the minimum requirements as stipulated in **Table 1**.

8. Output

8.1. For Next Generation Sequencing:

- Sequence files will be provided in compressed (zipped) FASTQ format.
- The amount of data received might vary, but if more than 90% of the target amount is generated the project will be considered complete. The "target amount" needs to be defined by the client in the sample submission form.
- Any additional data that is generated by the Core will be provided to the user at no additional cost.
- A client must indicate/plan to sequence more data if data yields are critical to their project. Minimum data requirements should be outlined in the sample submission form.

8.2 For Genotyping:

- Genotyping files will be provided in the form of the following reports:
  i. Sample_Map.zip
  ii. SNP_Map.zip
  iii. DNA_Report.zip
  iv. FinalReport.zip
  v. LocusSummary.zip
  vi. LocusXDNA.zip
  vii. Plink output format (sample.map, sample.ped) can be provided on request

9. Physical address:

Agricultural Research Council
Onderstepoort Campus
100 Old Soutpan Road (M35)
Biotechnology Platform (Building 38)

GPS co-ordinates:
25°39'03.10"S
28°11'03.10"E

Working hours: Monday to Friday, 7:30am – 4.00pm

Please ensure that a member of the Genomics Core Facility is available to receive your samples, prior to sending them. Please check this via email or telephonically.

10. Genomics Core Facility Contact Details

Email address:
BTP-Core@arc.agric.za

Telephone numbers:
NGS:

- Jonathan Featherston +27 12 529 9483
- Stephanie Cornellissen +27 12 529 9481

- Nick Mokotoane +27 12 529 9482

Robotics:
- Alister Ngobeni +27 12 549 9483

Genotyping:
- Khulekani Khanyile +27 12 529 9482
- Stephanie Cornellissen +27 12 529 9481
- Nick Mokotoane +27 12 529 9482

Bioinformatics:
- Jonathan Featherston +27 12 529 9483
- Annie Chan +27 12 529 9481

Table 2: Sample submission checklist

Quick Sample Submission Checklist	√
Sample sheet has been e-mailed to the Core staff	
Gel Photo and NanoDrop results including the OD260/280 ratio have been measured	
DNA samples quantified by Qubit or Picogreen assays (optional)	
Agilent 2100 Bioanalyzer data collected (optional)	
All samples must be tightly sealed, spun down and frozen	
Samples must be labeled clearly with the name of the person submitting the sample, the sample name and date of sample submission	
Sample name should be exactly as it appears in the sample sheet	
A hard copy of the sample sheet should accompany the samples in a sample box	
For Genotyping samples need to be normalized to 50ng/µl	