MOLECULAR TECHNOLOGY

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The Molecular Technology Service offers a diverse range of services to individuals and research groups throughout the Institute. The main focus of the facility has centred around the continuing expansion of our Next Generation Sequencing (NGS) services, allied to which, we also offer a range of Single Cell services predominantly focussing on single cell RNAseq. We process samples for a variety of cancer associated projects, in both mouse and human derived materials. We offer a full end-to-end service, from initial study design & planning, through sample QC, full library preparation, sequencing and data return. Allied to these advanced techniques, we continue to offer a range of standard molecular tests covering, plasmid purifications, Sanger sequencing and mycoplasma screening.

The facility's core aim is to provide an efficient and adaptive service to research groups and researchers within the Institute. We have gradually established a core offering centred around NGS, which covers bulk RNAseq, total RNAseq, ChIPseq, RRBS, amplicon-based and whole exome preparations, and are continually striving to add more assays and tests which can be readily utilised by groups across the Institute. We routinely undertake early preparatory meetings with researchers prior to proceeding with NGS based projects and conduct these alongside colleagues from the Bioinformatics & Computational Biology core group. These joint meetings enable us to thoroughly understand the research goals of specific projects and give appropriate advice in order to meet both the experimental and analytic goals of the project.

A major advancement for the service during 2024 was the installation of an Illumina NextSeq 2000 benchtop sequencer. This new instrument was purchased to replace our aging NextSeq 500 instrument which had served well for around 10 years. The introduction of the NextSeq 2000 has given us increased flexibility and enhanced our sequencing capacity significantly. Previously, we were restricted to only a relatively small number of run variants (i.e. only 5 run variants over 2 flow-cell types) which could generate a maximum data output of 120Gbp per run, whereas with our new system we have a much greater number of potential run variants (i.e. 14 in total over 4 flow-cell types) at our disposal, and can now generate up to 600Gbp of data from a single run. Further to this increase in flexibility, the increased capacity has also meant that we can now sequence larger projects more efficiently and economically in-house than previously possible. We have also been able to multiplex projects using the same base assay, which has ultimately had the benefit of increasing turnaround times and reducing sequencing cost liability to the research groups. Up until late September 2024 we had been utilising a locally managed NovaSeq 6000 for larger scale sequencing (principally scRNAseq libraries), this access was removed for reasons outside of our control, and having the NextSeq 2000 in place meant we could now easily cope with sequencing most of these library types in-house, and in most cases, with cost savings associated.

In terms of sequencing content, over the period of 2024 we processed ~110 sequencing runs, covering all three sequencing platforms, with a combined output of ~18Tbp. As an example of our sample throughput, we performed full bulk RNAseq library preparations for more than 1000 samples throughout the year and have also regularly performed a range of other preparations, principally on an ad-hoc basis. We see this throughput increasing steadily year-on-year.

A further development through 2024 is the full integration of the single cell sequencing service into Molecular Technologies, and this is now representing one of our key service areas. The solutions on offer generally cover single cell gene expression analysis (10X Chromium), whereby the generated data can allow researchers to measure gene activity on a single cell basis, and aid in characterising tumour environments through identifying specific cell populations and cell types in diseased versus normal tissues. As with the NGS assays, we are continually striving to add more assays to our service repertoire and have been expanding services this year to include immune profiling capabilities, multiome assays, and gene expression flex assays. The gene expression flex assays represent an exciting area for further establishment, as these assays can potentially open the use of archival and low-quality samples which previously would have been incompatible with the technology (particularly archival FFPE tumour material). In terms of sequencing needs for the Single Cell service, previously we relied on outsourcing this element as the in-house options were insufficient and uneconomical to process such libraries. However, the introduction of our NextSeq 2000 has meant that we can now efficiently process all Single Cell libraries in-house, which has greatly sped up turnaround times and given us control of the complete end-to-end service. Similarly, we work closely with colleagues in the Bioinformatics core group (Y90), who support us with running primary analysis, which forms part of the data deliverables for service users, and is essential to maintain performance thresholds, and allows us to identify any potential sample-sample or run-run variability. Over the course of 2024, we processed and sequenced approximately 200 samples through the single cell service. We are also continuing to explore different technologies in this field, with the aim to offer the most appropriate assay tailored to samples/projects' specific needs and be vendor agnostic. It is likely this will require a degree of benchmarking, which is currently in early planning stages.

Finally, our facility is maintaining the more traditional aspects of our service provisions, which includes plasmid DNA purifications (mini/maxi-prep), collating and administering Sanger sequencing send-outs (including cell-line authentications), and performing Institute-wide mycoplasma testing. These functions still represent a significant portion of the facility's output with several thousand samples processed annually. We have been fortunate to have been able to maintain these services with the assistance of colleagues from the Lab Support team (Abbie McFarlane & Anna Shearer) which has enabled service provision continuation whilst allowing for developments in our core focus areas.

Figure 1. Example NextSeq 2000 primary data analysis, as viewed utilising Sequencing Analysis Viewer (SAV, Illumina). Represents key primary analysis data QC performed by MTS staff prior to full data analysis/data return.