University of Glasgow | College of Medical, Veterinary & Life Sciences

Glasgow Polyomics
www.glasgow.ac.uk/polyomics

Polyomics NGS Project Form

P/N:

Contact details

Name		Email		Date	
PI		GUID		IP Address	

Project details

Project title	
Services required	
Project summary	☐ Yes ☐ No
Funding	
Number of samples	
Number of replicates	

Samples

Organism		Reference available	☐ Yes ☐ No

Sample type	

DNA	RNA
☐ WGS	☐ Poly A selection
☐ Exome	☐ Ribosomal depletion
☐ Targeted capture	☐ Ribosomal and globin depletion
☐ Meta genome	☐ miRNA
☐ 16s rRNA	

Sequence	☐ PE ☐ SE ☐ Mate Pair	Read length	
Number of reads			
Sample due date			
Sample preparation method			
Delivery method	☐ Internal mail	☐ Post/Courier	☐ Personal
Estimated sequence date			

Analysis

Deliverables	
Analysis time	

Additional information

Quote	
Budget code	
Authorship	☐ Yes ☐ No

Notes

Information
We generally recommend PolyA selection, and Paired-end sequenced reads. Read depth depends on the sample type, though we would recommend you err on the high side.

Amount of DNA/RNA
Genomic DNA $1\mu g$
Total RNA $1\mu g$
If these amounts are difficult to get we have various protocols for low input DNA/RNA.

RNA library prep options:
1. PolyA selection (PS) - this is a good option if you are interested in eukaryotic mRNA and not too concerned about the lack of non-coding species. Here we will use 'TruSeq stranded mRNA sample prep kit'.
2. Ribosomal RNA depletion (RD) - this is a good option if you work with prokaryotic RNA or are interested in sequencing both coding and long non-coding species. Here we will use 'TruSeq stranded totalRNA with Ribo-Zero' kit. It is important to realise that the downstream sequencing will result in a high proportion of reads covering the non-coding species which will reduce the number of reads covering mRNA. To this end we would suggest that the total number of reads is increased by 50% compared to sequencing with polyA selection.
3. small RNA selection (SR) - this is a good option if you are interested in microRNAs. Here we will use 'TruSeq small RNA sample prep kit.

The general recommendation is that you only request the RD library prep if non-coding species are really important to the project. If not, then it is better to stick with PS.

Sequencing mode
Single end (SE) - RNA fragments are sequenced from one end only. This is a cost-effective option which could be recommended for transcriptome interrogation at a gene level. By default we sequence with 75bp long reads (SE 1x75bp). Illumina recommends a low number of reads 10-12M. You will not be able to confidently differentiate between expression of different transcripts and given low read count may not be able to see weakly expressed genes. We are in the process of investigating the performance of the above. SE is also used for analysing smallRNAs where 10M reads per sample would be recommended.

Paired end (PE) - RNA fragments are sequenced from both ends. This provides more reliable expression estimations at the transcript level and therefore can identify alternative splicing with more confidence. This is recommended for transcriptome interrogation at the transcript level. By default we sequence with paired end 75bp long reads (PE 2x75bp, 150bp per fragment)

Sequencing depth recommendations for RNAseq of PS libraries:
1. Bacterial transcriptomes: SE 5-10M reads
2. Small eukaryotic genomes e.g. yeast: PE 10-15M reads
3. Medium eukaryotic genomes: C. elegans, D. melanogaster, A. thaliana: PE
4. 15-25M reads
5. Large eukaryotic genomes: PE 25-40M reads

Data analysis (optional)
The RNAseq analysis (costed separately) will provide:
1. Raw data in form of fastq files containing unaligned reads for each sample

2. Aligned reads in form of bam files for each file
3. Normalised and raw expression estimates for gene/transcripts for each sample
4. Gene/transcript lists showing statistical measures of differential expression for each requested comparison.