

Catalogue of offering - transcriptome

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Cat No	Bulk Transcriptome	Description and application	Typical applications	Current Capability	Platform	Data	Pre-Project engagement (Optional)	Sample requirements	KeyQC metrics (Data Analysis)	BOM	Analysis Scope	Link to the sample report	Standard Deliverables	Tool versions	Estimated Time
	RNA-seq Eukaryotic poly A model (reference available)	Pros High sensitivity profiling of gene expression changes; sensitive of gene expression with changes gene are in high expression Gene expression over broad dynamic range Suitable for studies focusing on cell-line changes in treatment studies and scale assays is a cost assay to profile differential Can be applied where RNA is limiting and low sample inputs Limitations Profiles the gene expression is gene transcripts captured with a PolyA; non -poly A transcripts not profiled RNA integrity should be high and consistent > RIN 7	Evaluate differential gene expression for treatment Profile genes for perturbations on treatments	Yes	Illumin 150X2	17million* 2 (currently we are asking for 25 millions* 2) is that required?	Sample shipping and preparation SOP Power analysis for RNA-seq Design review analysis	Number of cells - specify range Amount of tissue - amount Sample type - Cells, Tissue frozen; Tissue in RNA-later; FFPE Extracted RNA > conc; Quality	Phred Score >30, % of reads retained after trimming >80 %, % of reads belonging to host >95%, Mapping rate >95%, Exonic rate > 80%	Sample extraction n (12) Sample QC (18) Library prep A (Components) (6 /12) Library QC (14) Sequencing - 6 / 12 *17 million reads Data handling and storage Time for data analysis	Primary processing - FastQC, check for data quality and Pre-processing reads and adapter removal Reference alignment, mapped count file generation Typical comparisons Type 1 - pair of samples controls vs treatment Type 2 - Groups of controls vs treatments Type 3 - Control - treatment vs Modified - treatment (four samples) Data normalization, Differential gene expression	https://workdrive.zohoexternal.com/external/be95ce22e70c96bf19b7ff391625182e3353a2d87272db6dc90b833613a1249	Raw & QC Trimmed data (.fastq files) and QC reports per sample Mapped data (.BAM) files sample and related QC reports Gene expression matrix (.csv/xlsx format) : Raw counts, Normalized counts Sample clustering reports Differential gene expression report Supporting visualization each step (PCA/UMAP plots, Heatmaps, Volcano plots, Functional enrichment gradient plots, KEGG pathview maps*)	1.Fastqc - FastQC v0.11.9 2.multiQC - multiqc, version 1.27.1 3.Kraken - Kraken 1.1.1 4.Cutadapt - 5.0 5.STAR - 2.7.11b 6.Samtools - samtools 1.21 7.Qualimap - Qualimap v.2.3 8.Rseqc - RSeQC-5.0.1 9.FeatureCounts - Version 2.1.1 10.Python - Python 3.12.2 11.R - version 4.4.1 ##Downstream Analysis 12.DESeq2 - 1.46.0 13.edgeR - 4.4.2 14.ClustalPro ler - 4.14.6 15.GSEABase - 1.68.0 16.fgsea - 1.32.4 17.ggplot2 - 4.0.1 18.pheatmap 1.0.13 19.Cytoscape 3.10.3	Hand on-4 days Machine Run-1 week (based on sample count - n=30-40)
	Eukaryotic poly A non-model (reference with denovo) [What is the requirement of reference with denovo?]	Pros Exploratory profiling to create a transcriptome and evaluate gene expression profiles for a fungal/plant/model organism with limited reference genome information in data bases High sensitivity profiling of gene expression changes; sensitive of gene expression with changes gene are in high expression Gene expression	Profile and generate a first reference transcriptome, when a reference is not readily available First line investigation of host-pathogen interactions; where the host OR pathogen reference genome is available Typically suitable for fungal transcriptome where there is considerable strain divergence		Illumin 150X2	25million* 2					Ref alignment, DGE, pathway analysis				

	over broad dynamic range Suitable for studies focusing on cell-line changes in treatment studies and scale assays is a cost assay to profile differential Can be applied where RNA is limiting and low sample inputs Limitations Profiles the gene expression is gene transcripts captured with a PolyA; non -poly A transcripts not profiled RNA integrity should be high and consistent > RIN 7										
Total RNAseq human/mouse/rat	Pros Deeper transcriptome profiling where there is interest in the total transcript complement to include the transcripts lacking poly A; Suitable to studies where total transcript signature is needed, that include circular RNA RNA is lower quality and is degraded partially with RIN > 4 Limitations Requires higher read depth to compensate for ribosomal and structure RNA carry over post ribodepletion - Needs for 20-30% higher read generated to achieve the same coverage as polyA based library for core gene set RNA requirement in quantity needed to support ribodepletion Higher input costs in the overall of read generation and analysis	Total transcript profiling to when interest is in non-polyA transcripts, circular RNA RNA is low RIN and cannot be processed using polyA methods	Yes	Illumin 150X2	30million* 2 [current depth is 25*2 - why the increase in depth?]	Phred Score >30, % of reads retained after trimming >80 %, % of reads belonging to host > 95%, Mapping rate >95%, Exonic rate > 30%	Ref alignment, DGE, pathway analysis, lncRNA, circRNA	https://workdrive.zohoexternal.com/external/a0cf74094cac2ae0575113ccc98987_96c301565c83fac421bc8325b447e347	1) Raw Data and Raw Data counts across all samples. 2) Normalized read count for each samples along with Sample Grouping/Clustering and QC related plots : PCA plot, Heatmap based on top 2000 variant genes, dendrogram and Sample-Sample correlation plot. 3) Differential expression reports for each comparison along with corresponding visualization – Volcano plots. 4) Functional over-representation (enrichment) analysis per comparison, along with corresponding visualization – Gradient Bar plots for top 20 enriched GO: BP, MF, CC and Reactome Pathways. 5) Raw Count and Normalized count of lncRNA for each samples along with Sample Grouping/Clustering and QC related plots : PCA plot, Heatmap on top 2000 variant genes, dendrogram and Sample-Sample correlation plot.	1.Fastqc - FastQC v0.11.9 2.multiQC - multiqc, version 1.27.1 3.Kraken - Kraken 1.1.1 4.Cutadapt - 5.0 5.STAR - 2.7.11b 6.Samtools - samtools - samtools 1.2.1 7.Qualimap - QualiMap v.2.3 8.Rseqc - RseqC-5.0.1 9.FeatureCounts - Version 2.1.1 10.Python - Python 3.12.2 11.R - version 4.4.1 ##Downstream Analysis 12.DESeq2 - 1.46.0 13.edgeR - 4.4.2 14.ClustalPro ler - 4.14.6 15.GSEABase - 1.68.0 16.gfsea - 1.32.4 17.ggplot2 - 4.0.1 18.pheatmap 1.0.13 19.Cytoscape 3.10.3 CircRNA STAR version 2.7.11b circExplorer2 2.3.8 CircRNaprofil r = 1.20.0 Deseq2 = 1.46.0 Clusterprofile = 4.14.6	Hands on time - 1-2 weeks and Machine time - 1 week

- 6) Differential expression reports of known lncRNA for each comparison along with corresponding visualization – Volcano plots.
- 7) Functional over-representation (enrichment) analysis for direct lncRNAs, cis-targets mRNA and mRNA interacting with lncRNA and their supporting plots and tables.
- 8) Identification of co-expressed mRNA and lncRNA followed by visualization Interaction network for lncRNA and mRNA.
- 9) Detection of Known and Novel circRNAs with the supporting plots
- 10) circRNA expression analysis per comparison. Volcano plot and Heatmap of Significant Circular RNAs
- 11) Summary statistics of Circular RNA and their supporting plots
- 12) Analysis - ASE (Alternative Splicing Event) per and Identification of Novel splice variants
- 13) Overall Volcano plot for Differentially Expressed ASE and Individual Volcano plots for each events
- 14) Scatter plots for showing splicing levels (percent-spliced-in, PSI) between two conditions.
- 15) Unsupervised Heatmap for Top 20 Differentially Expressed ASE
- 16) Gene ontology - Biological

										process, Molecular Function and Cellular components (over- representation analysis using enriched genes of top 10 differential alternative splicing events and their supporting plot.	
Bacterial RNAseq (with reference provided)	Pros Sensitivity profiling of gene expression changes; sensitive of gene expression with changes gene are in high expression Gene expression over broad dynamic range Limitations Variable ribosomal RNA removal across bacterial due to ribodepletion process compatibility Attention : Strain purity will be needed to ensure on target mapping; mixed cultures can skew analysis and 16S purity testing is recommended to match reference and analyse purity	Profile gene expression changes in Bacterial cells across treatment or culture conditions	Yes	Illumin 150X2	10million* 2				https://workdrive.zohoexternal.com/extranal/f0397bc957a2b7b4ec98f079d52748552b2863db21994e4646f416c888d994	Raw & QC Trimmed data .fastq files) and QC reports per sample Mapped data .BAM files sample and related QC reports Gene expression matrix .csv/xlsx format) : Raw counts, Normalized counts Sample clustering reports Differential gene expression report Functional enrichment reports (Gene ontology – Biological process, Cellular Component, Molecular Function & KEGG/REACTO ME pathways) Supporting visualization each step (PCA/UMAP/tS NE plots, Heatmaps, Volcano plots, Functional enrichment gradient plots, KEGG pathview maps)	Hands on time - 2 days Machine time -2 days
Blood RNAseq 3'	Pros High efficiency profiling of transcripts - method deplete highly globin genes to improve on- targets reads Limitation Suitable for blood based studies RNA quality with RIN >7 recommended	To explore changes in blood transcript expression profiles during infection, disease or treatment		Illumina 150X2	10million* 2				Ref alignment. DGE	link deliverable tools time	
FFPE RNA human	Pros Transcript profiling in from formalin fixed paraffin embedded samples; with fragmented RNA that is lower quality and degraded partially with RIN >3- 4	To profile transcript from preserved samples - from clinical specimens fixed archival specimens Applied in biomarker discovery involving in transcript	Yes	Illumin 150X2	35million* 2						

	Limitations Requires higher read depth to compensate for ribosomal and structure RNA carry over post ribodepletion - Needs for 30% higher read generated to achieve the same coverage as polyA based library for core gene set RNA requirement in quantity needed to support ribodepletion Higher input costs in the overall of sample extraction, ribodepletion, read generation and analysis	profiles with disease compared to control; or progression of disease						
FFPE Cancer Transcriptome (Discovery)	Pros High depth discovery of total transcript expression, long non-coding RNA, analysis of gene signatures match Supports profiling of RNA-fusions, discovery of differential isoforms, tumour mutation burden, coding mutation - matched to pathogenic variants (limited by depth and coverage) Analysis of match to tumour virome, estimation of immune cell populations Transcript profiling in from formalin fixed paraffin embedded samples; with fragmented RNA that is lower quality and degraded partially with RIN >3- 4 Limitations Requires higher read depth to compensate for ribosomal and structure RNA carry over post ribodepletion - Needs for 50% higher read generated to achieve the same coverage as polyA based library for core gene set RNA requirement in quantity needed to support ribodepletion and dual library preparation to recover gene coverage Higher input costs in the overall of sample extraction, ribodepletion, read generation	Analysis of tumour sample for discovery of biomarker Profiling to guide tumour board in complex investigational tumour cases	Illumin 150X2	50million* 2				

Plant/microbial RNAseq - Host pathogen co expression	Pros Profiling gene expression changes in system with host-fungal/bacterial interaction where both transcripts are profiled - reference alignment for host RNA) followed by analysis of microbial RNA Sensitivity profiling of gene expression changes; sensitive of gene expression with changes gene are in high expression Gene expression over broad dynamic range Limitations Higher depth sequencing needed to cover host and microbial transcripts Variable ribosomal RNA removal across bacterial due to ribodepletion process compatibility; when the microbe is a bacterial Attention : purity will be needed to ensure on target mapping; mixed cultures can skew analysis purity	Applied to interrogate transcript level alterations in and microbe (fungi or during infection when agents are applied to host-microbe system System level cross-talk mapping by matching differential gene expression; experiment with time series/replicates provide power for 'mode of action' investigation	Illumin 150X2	35 million *2			Standard deliverables Dual RNA-Sequencing (Reference based*) – only protein coding genes - Raw & QC Trimmed data (.fastq files) and QC reports per sample Extraction of data for host and pathogen Mapped data (.BAM) files sample and related QC reports, host and pathogen Below analysis performed separately for Host and pathogen : Gene expression matrix (.csv/xlsx format) : Raw counts, Normalized counts Sample clustering reports Differential gene expression report Functional enrichment reports (Gene ontology – Biological process, Cellular Component, Molecular Function & KEGG/REACTOME pathways) Interaction between the pathogen and host transcriptome using WGCNA networks Protein-interaction network analysis Supporting visualization each step (PCA/UMAP plots, Heatmaps, Volcano plots, Functional enrichment gradient plots, KEGG pathview maps* and many more..)

*KEGG Pathview maps for pathways selected according to the interest of the study
 *Reference genome/transcriptome must be known for the organism,

									with annotations made	
Plant RNASeq- Denovo	Pros Generate a reference transcriptome for a plant/microbial strain where there is no suitable reference stain Discovery novel transcripts from ecological isolates, uncharacterized strains Discovery to identify coding potential of novel stains/species for metabolites Rapid identification of variants - SNP and indel to develop molecular markers (when genome is large) Limitations Denovo Transcriptome assembled is usually a complete representation of the coding genome Estimation of splice variants and annotation may be limited by matches to public data set, limiting ability to annotate gene and assign to pathways Splice variants and transcripts may have inaccuracies; limited by assembly algorithms and ploidy	Discovery of gene set and interrogating coding complement First stage to discovery and characterize variations in stains, species create a reference Data sets for metabolites and new transcript discovery	Illumin 150X2							
LongRNAseq (discovery)	Pro Assembly free read out of transcriptomes using long cDNA sequencing Ability to splice variants and isoforms to characterized complex/multiple splice variations Accurately resolve repeats in coding Limitations Limited by depth of reads and ability to high depth sensitive differential gene expression with regard to short read RNA-seq Single read profiles could lead to inaccuracies in variant calling for low depth transcripts	To detect and discover novel transcript, method does not involve graph-assembly therefore can resolve multiple splice forms. Methods can be applied in sensitive of long repeats in transcribed genes Transcriptome from organism with high ploidy - assembly is often inaccurate and that are resolved in long read methods	ONT							
miRNAsq NGS - Discovery	Pro Profile small RNA- match and discovery small RNA High sensitivity differential profiling of	Detection of discovery of RNA [already the novel miRNAs are also targeted in the below assay; change in depth of sequencing	Yes	Illumin 150X2						

		RNA Discover miRNA-gene networks Limitations NGS methods are sampling - some species may not be detected non-target miRNA may also be detected that may increase False discovery	might be required for discovery?)									
miRNaseq NGS - Differential profiling	Pro Profile small RNA - to match small RNA, High sensitivity differential profiling of RNA Discover miRNA-gene networks Limitations non-target miRNA may also be detected that may increase False discovery miRNA - RNA is a computed network	Investigation of small RNA differential gene expression patterns	Illumin 150X2						https://workdrive.zohoexternal.com/external/d/a645d15552cc6eb4ef0a636816530db15503c71a5b38a0529dea8d1a713c3	1)Raw Data QC and Raw Data counts across all samples. 2)Normalized read count for each samples along with Sample Grouping/Clus- tering and QC related plots : PCA plot, Heatmap based on top 500 variant miRNAs. dendrogram and Sample- Sample correlation 3)Known and Novel miRNAs identified by miRDeep2 4)Differential expression reports for each comparison along with corresponding visualization – Volcano plots. 5)Validated targets of Known miRNA identified by miRTarbase 7)Functional over- representation (enrichment) analysis per comparison, along with corresponding visualization – Dot plots for top 20 enriched GO: BP, MF, CC and Reactome Pathways. 8) network for miRNA and mRNA (Validated targets)	FastQC = V0.11.9 MultiQC = V1.17 Cutadapt = Bowtie = 1.0.0 MiRDeep2 = miRDeep2.0.1. 3 cytoscape = 3.10.2 miRBase = 22.1	Hands on time - 4 days Machine time - 1 week (30-40S)
miRNaseq- mRNA integrated analysis human/mou- se	Pro Profile small and RNA expression - measure differential expression profiles Discover miRNA-gene networks and report perturbations Limitations non-target miRNA may also be detected that may increase False discovery miRNA - RNA is a computed network	Discovery and analysis of gene regulatory- networks; combines the analysis of differential RNA analysis and RNA in a sample. Often can be used to interrogate group of controls vs treatments to map perturbations	Yes	Illumin 150X2					https://workdrive.zohoexternal.com/external/d/dddc29f41da1098aadc9993c3857e0a69dc31889c1f83d136dd3e9798b78	"1)Raw Data QC and Raw Data counts across all samples. 2)Normalized read count for each samples along with Sample Grouping/Clus- tering and QC related plots : PCA plot, Heatmap based on top 500 variant miRNAs. dendrogram and Sample- Sample correlation 3)Known and Novel miRNAs identified by miRDeep2 4)Differential	Hands on time- 4 days Machine time - 1 week (20-30S)	

	Specialized libraries for species										
Long non coding rnaseq (Known model species Human/Mouse/Rat)	Profiling the expression of Known and Novel long noncoding RNA in model organisms.	Illumin 150*2							1) Raw Data and Raw Data counts across all samples for known (Gencode). 2) Normalized read count for each samples along with Sample Grouping/Clustering and QC related plots : PCA plot, Heatmap based on top 2000 variant genes, dendrogram and Sample-Sample correlation plot. 3) Differential expression reports of known lncRNA for each comparison along with corresponding visualization – Volcano plots. 4) Identification of mRNA targets for the lncRNA. 5) Functional over-representation (enrichment) analysis for lncRNAs mRNA targets and their supporting plots and tables. 6) Interaction hub network for lncRNA and mRNA. 7) Novel lncRNA identification and Quantification along with annotation	###Known lncRNA data analysis software versions 1.Fastqc - FastQC v0.11.9 2.multiQC - multiqc, version 1.27.1 3.Kraken - Kraken 1.1.1 4.Cutadapt - 5.0 5.STAR - 2.7.11b 6.Samtools - samtools 1.21 7.Qualimap - Qualimap v.2.3 8.Rseqc - RseqC-5.0.1 9.FeatureCounts - Version 2.1.1 10.Python - Python 3.12.2 11.R - version 4.4.1 12.Feelnc - 0.1.1 #####Novel lncRNA 1.StringTie - StringTie v2.2.3 2.Bedtools - v2.27.1 3.cufflinks - cufflinks v2.2.1 4.PLEK - Version 1.2 5.CPAT - 3.0.5 6.BLAST - BLAST 2.5.0+ 7.Diamond - diamond v0.9.30.131 ####Downstream Analysis 12.DESeq2 - 1.46.0 13.edgeR - 4.4.2 14.ClustalPro ler - 4.14.6 15.GSEABase - 1.68.0 16.fgsea - 1.32.4 17.ggplot2 - 4.0.1 18.pheatmap 1.0.13 19.Cytoscape 3.10.3	Hands on time - 2 days Machine time - 4 days (n= 30-40S)
Analysis only											
Publication support standard (RNA 4 sample or group analysis for control and treatments)	Scope Data QC RNA mapping and differential SRA upload of data Custom data extraction - plotting										
Publication support Advanced	Scope										
Raw Data archival - 5 years (RNA seq - Stage/time series data analysis)											
RNA seq miRNA-RNA integration											

RNA seq denovo/gen e annotation, pathway mining and metabolite mapping; Gene ranking and query									
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