Transcriptomics at the Genetics Core, Edinburgh Clinical Research Facility

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The **Genetics Core** provides support for transcriptomic projects through **RNA extraction**, **Library Preparation** and **Sequencing**. We can support many different protocols depending on your experimental needs. Below are some of our more popular workflows.

RNA Extraction

Blood – Paxgene (preferred) or Tempus
Tissue or Cells – Qiagen RNeasy Mini Kit
FFPE – Covaris truXTRAC FFPE Total NA Plus Kit
miRNA - Qiagen miRNeasy Mini Kit

Sample QC by Qubit & Agilent Fragment Analyzer, or Agilent Bioanalyzer, to determine RNA yield, RIN & DNA yield (to identify possible DNA contamination)

Library Preparation

Total RNA-seq (by ribodepletion)
Good – Information on coding & non-coding RNA; Can work with poor quality samples, including FFPE
Bad – Most expensive as requires a lot of reads
Can require 50-100M reads/sample

Input: 5ng-1µg & RIN: Any
NEB NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (rRNA depletion workflow)

or

Input: 250pg-10ng & RIN: Any
Takara SMARTer Stranded Total RNA-Seq Kit v2 - Pico Input Mammalian

mRNA-seq (by polyA pull-down)
Good – Focus on coding regions
Bad – Requires good quality RNA
Can require 30-50M reads/sample

Input: 10ng-1µg & RIN: ≥7
NEB NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (polyA mRNA workflow)

or

Input: 2pg-200g & RIN: ≥7
NEB NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina

Gene Expression Profiling
Good – Very affordable; works on poor quality samples; simple analysis
Bad – Information on level of transcripts only; no sequence data across the transcripts; less useful for isoforms.
Can require 5-10M reads/sample

Input: 0.5ng-500ng & RIN: Any
Lexogen QuantSeq 3' mRNA-Seq Library Prep

miRNA-seq
A specific kit and sequencing run is required; miRNA data is not present in a standard RNA-seq or mRNA-seq run.
Can require 5-10M reads/sample

Input: ≥200ng & RIN: Any
Perkin Elmer NEXTFLEX Small RNA-Seq Kit v3 for Illumina

or

Input: ≥1ng & RIN: Any
Qiagen QIAseq miRNA Library Kit

Sequencing Platforms

Selecting the best platform for your experiment
2x NextSeq2000 & 1x iSeq for fast & flexible sequencing
Choose the read length and number of reads you need per sample and we will do the rest.

Coming Soon – direct RNA sequencing on the ONT PromethION

Cycles (actual bp)	iSeq 4M Reads	P1 100M Reads	P2 400M Reads	P3 1200M Reads	P4 1800M Reads
50 (88bp)				60Gb	90Gb
100 (138bp)		10Gb	40Gb	120Gb	180Gb
200 (238bp)			80Gb	240Gb	360Gb
300 (327bp)	1.2Gb	30Gb	120Gb	360Gb	540Gb
600 (638bp)		60Gb	180Gb		

(iSeq 100 | NextSeq 2000)