

Work order for NGS services at ILS

PI name: _____

√ Tick whichever is applicable

1. Application for which samples / libraries given for NGS:
 - a. ChIP-seq
 - b. RNA-seq / Transcriptome
 - c. 16S metagenome
 - d. Shotgun metagenomics for de novo or re-sequencing
 - e. Exome sequencing
 - f. Small RNA
 - g. Others (discuss with the Genomics core facility)
2. Sample type given for NGS: (Attach annexure with the work order)
 - a. DNA (Provide quality file 260/280 and Qubit concentration with sample)
 - b. RNA (Provide quality file i.e. Qubit concentration and RIN score with sample)
 - c. Prepared NGS library for ChIP-seq / RNA-seq / 16S / Metagenome / exome / others (Provide Qubit concentration with fragmentation results from Tapestation or Bioanalyzer)
 - d. Tissue / bacterial pellet etc
 - e. Organism (Human, Mouse, Rat, Bacteria, others please specify)
3. Coverage required for your experiment
 - a. RNA-seq / Transcriptome for PE run (10mi, 20mi, 30mi, 50mi, 100mi)
 - b. RNA-seq / Transcriptome for SE run (10mi, 20mi, 30mi, 50mi, 100mi)
 - c. ChIP-seq SE run (10m, 20mi, 30mi, 50mi)
 - d. For 16S, Exome and Shotgun sequencing (need to be discussed with facility)
4. Analysis Required (a) Yes, (b) No
5. Fund to be used for NGS service:
 - a. ILS Core fund
 - b. Project fund (Please specify the project name and no)
 - c. In case of external sample (PO is required from institute)

Date:

PI Signature:

Tips for cost-calculation for NGS using Nextseq and general recommendations:

- a. Total output from a high output Nextseq run is 350-400million single end reads and $350 \times 2 - 400 \times 2$ for paired end reads. Therefore you can estimate how many samples you can pool per sequencing run.
- b. For RNAseq / transcriptome analysis, if you have to only check global expression of genes, 75 bp SE run is fine. For exon skipping and new gene identification using RNAseq, 75bp PE run should be used with high coverage (100mi / sample). The cost goes higher as total output is 350 to 400mi per flow cell run.
- c. ChIPseq: Generally 15-20mi reads are sufficient if ChIP enrichment is good. For Pol-II and histone mark ChIP-seq 30-40mi SE run is recommended. The coverage also depends on the PCR amplification bias introduced during library preparation for ChIP-seq, therefore PCR steps need to be optimized if required.
- d. Exome analysis: Cost depends on how much coverage you need per exome. Calculate how many samples you can pool based on coverage and total output of flow cell. For exome analysis one needs to run 150×2 high output sequence run.
- e. 16S: large numbers of sample can be sequenced for 16S sequencing (upto 384) therefore either we need to pool Shotgun sequencing samples along with 16S or one has to wait till we get enough samples to start sequence run.
- f. Small RNA: Several samples can be pooled for small RNA sequencing as well if library prepared specifically for small RNA using small RNA enrichment methods.
- g. Check the NGS cost listed on ILS website and calculate your sequencing run cost before giving the work order for NGS run. If required discuss it in detail with the sequencing core facility.

Illumina NextSeq500 sequencing (Cost per fow cell)

High output kits (Regular)	Internal	External (Private)	External (Academic)
75 bp sequencing kit (1x75 bp or 2x37 bp) run (~350-400million reads SE or ~700-800 PE)	\$1,700	\$2,500	\$1,900
150 bp sequencing kit (1x150 bp or 2x75 bp) run (~350-400million reads SE or ~700-800 PE)	\$3,200	\$4,900	\$3,400
300 bp sequencing kit (2x150 bp) run (~350-400million reads SE or ~700-800 PE)	\$5,000	\$5,700	\$5,200
Smaller output kits (only in case of specific requirements):			
150 bp Mid output kit, (~130-150 million SE or 260-300PE)	\$1,000	\$1,500	\$1,200
300 bp Mid output kit, (~130-150 million SE or 260-300PE)	\$1,800	\$2,300	\$2,000

Library preparation cost (recommended to be prepared by the lab itself):

NEB ultra RNA library prep kit with mRNA isolation module (48Rx): Rs 1,80,000/- = 3750/sample + 3000 for USM = 6750/- per sample

TruSeq stranded mRNA seq kit (48Rx): \$2500 = ~3850/- + 3000 for USM = 6850/- per sample

NEB ChIP-seq kit master mix set (12Rx): Rs. 21375/- = 2000/- + 2000 for USM = 4000 /- sample

TruSeq ChIP-seq kit (48Rx): \$3050 = ~4450/- + 2000 for USM = 6000/- per sample

I have put the cost of sequencing in \$ because we purchase in USD and it can be variable depending on the conversion rate.

USM: User supplied material like Ampure beads, plasticware etc