

Data Summary

- RNA from **22** melanoma cell-lines
(A04,C008,C021,C022,C025,C037,C052,C067,C077,
,C084,C086,C089,C092,CHL-
1,COLO792,D10,D22,D24,D35,D38,M002,MEWO)
- 3 biological replicates each
- Samples were spiked in using the ERCCv92 Mix 1 to get the dynamic range of detection.
- Stranded-RNAseq libraries were prepared
- 3 multiplexed libraries were prepared (each one containing a biological replicate of all of the lines)
- 66 RNA-seq datasets in total

Initial sequencing QC

- PCR duplicate counts based on bwa-aln alignments revealed a **PCR amplification artifact** for all the biological replicates from the cell-line **mC022. (duplicate rate ranging from 49-94%).**
- After speaking with Marco about this since that the initial RNA used was very degraded and Sequencing facility performed additional PCR cycles on this samples. Confirming the origin of the duplicates.

RNA-seq analysis Pipeline

Splice Aware Mapping

- Tophat2 against (GRCh37d5 +ERCCv92) and (ENSEMBL v75 annotation)

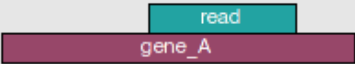
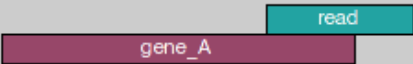


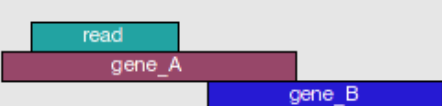
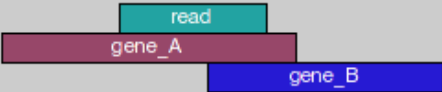
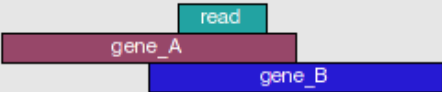
Fragment count

- htseq-count (MQ>10 ENSEMBL v75 genes)intersection non-empty

Quality Assessment

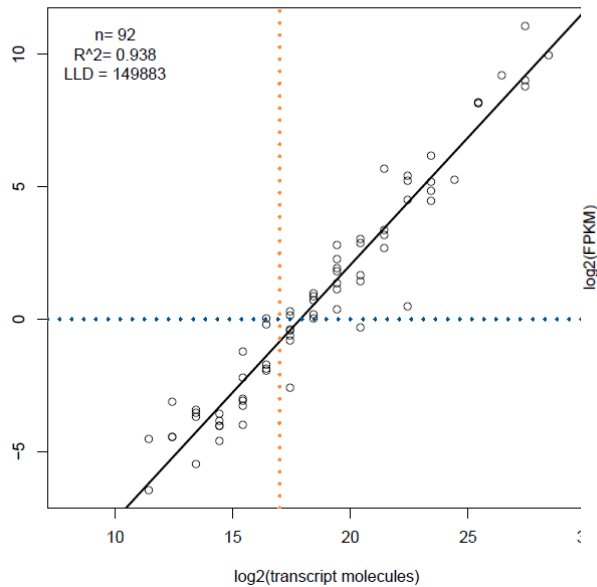
- Hierarchical clustering of the samples based on the $\log_2(\text{Fragments Per Kilobase per Million reads mapped FPKM})$

HTSeq

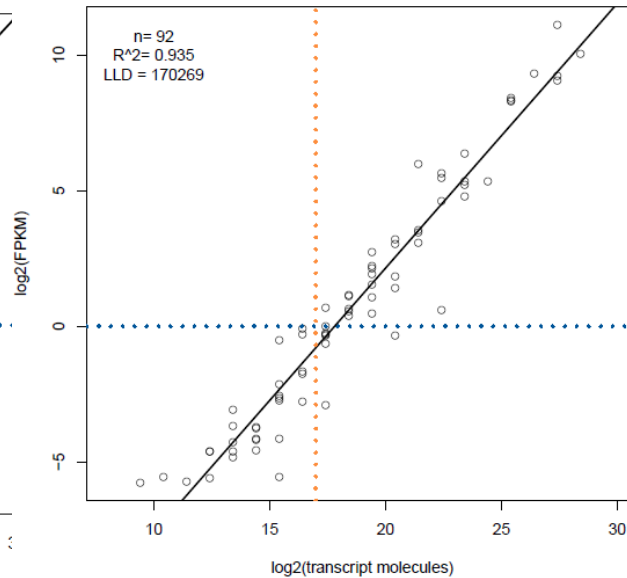
	union	intersection_strict	intersection_nonempty
	gene_A	gene_A	gene_A
	gene_A	no_feature	gene_A
	gene_A	no_feature	gene_A
	gene_A	gene_A	gene_A
	gene_A	gene_A	gene_A
	ambiguous	gene_A	gene_A
	ambiguous	ambiguous	ambiguous

Representative spike-in plots

Sample mA04_A ERCC Mix 1Dose Response



Sample mC008_C ERCC Mix 1Dose Response



Sample mC022_B ERCC Mix 1Dose Response

