CROWN BIOSCIENCE

Mouse I/O RNA-Seq Panel Analysis of Syngeneic Study Samples

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STUDY REPORT

V1.0

1. Summary

In this study, Crown Bioscience's Mouse I/O RNA-Seq Panel assay was performed for 36 samples. Clear clustering on expression signatures was observed by therapeutic conditions. Two comparisons (Hepa16TumorG1 Vs. Hepa16TumorG2 and MC38TumorG1 Vs. MC38TumorG2) were performed to identify differentially expressed genes. Relative cell component fractions were estimated using murine immune reference cell signatures.

2. Introduction

Cancer is a heterogeneous disease, which is highly related to the immune system. With the advance of immunotherapies, it is important to fully understand interactions between cancer and immunity, to correctly predict which patients will respond to specific immuno-oncology (I/O) agents. This also needs to be implemented across the range of murine models used as preclinical I/O study systems. NGS enables in-depth interrogation of the tumor-immune interactions at the gene expression and pathway levels, providing an efficient and robust method to systematically profile and characterize preclinical murine models.

3. Results

3.1. Targeted RNA Sequencing QC

Total RNAs for 36 samples were collected and a cDNA library was constructed using primers for 1080 target genes. The sequencing quality is summarized in Table 1. All 36 samples have passed quality control.

Table 1. Targeted RNA Sequencing Quality Control

Sample ID	Nucleic Acid	Unit	A260 (Abs)	A280 (Abs)	Raw bases (Mb)	Clean bases (Mb)	QCrate (%)	QC
Hepa16-Blood-G1-5795	319.3	ng/µl	7.982	3.846	13543.74	10257.72	75.74	PASS
Hepa16-Blood-G1-5796	511.1	ng/µl	12.779	6.102	10621.55	8104.24	76.3	PASS
Hepa16-Blood-G1-5798	410.7	ng/µl	10.268	4.948	12591.36	9941.3	78.95	PASS
Hepa16-Blood-G2-5794	1670.4	ng/µl	41.76	19.984	13248.39	9663.48	72.94	PASS
Hepa16-Blood-G2-5797	3110.4	ng/µl	77.761	37.343	12228.39	9288.55	75.96	PASS
Hepa16-Blood-G2-5800	1194.6	ng/µl	29.864	14.406	12382.53	9221.57	74.47	PASS
Hepa16-Spleen-G1-5795	1967.5	ng/µl	49.187	23.48	13796.11	8685.93	62.96	PASS
Hepa16-Spleen-G1-5796	2729.7	ng/µl	68.242	32.726	14221.91	10601.94	74.55	PASS
Hepa16-Spleen-G1-5798	1307.9	ng/µl	32.697	15.549	14579.72	10660.6	73.12	PASS
Hepa16-Spleen-G2-5794	2626.9	ng/µl	65.673	31.465	15641.62	12791.58	81.78	PASS
Hepa16-Spleen-G2-5797	2714.2	ng/µl	67.856	32.578	10843.43	8647.1	79.75	PASS
Hepa16-Spleen-G2-5800	3736.2	ng/µl	93.406	44.822	12920.08	10187.99	78.85	PASS
Hepa16-Tumor-G1-5795	724.6	ng/µl	18.115	8.6	13693.68	11154.5	81.46	PASS
Hepa16-Tumor-G1-5796	1662.3	ng/µl	41.558	19.836	8377.23	6063.41	72.38	PASS
Hepa16-Tumor-G1-5798	1762.1	ng/µl	44.054	21.209	12551.04	10167.55	81.01	PASS
Hepa16-Tumor-G2-5794	2415.4	ng/µl	60.384	29.19	9837.02	7959.72	80.92	PASS
Hepa16-Tumor-G2-5797	829.5	ng/µl	20.738	9.916	12409.67	10002.37	80.6	PASS
Hepa16-Tumor-G2-5800	2718.4	ng/µl	67.96	32.727	11259.64	8964.67	79.62	PASS
MC38-Blood-G1-5805	440.6	ng/µl	11.015	5.278	12560.15	9880.27	78.66	PASS
MC38-Blood-G1-5807	2805.5	ng/µl	70.137	33.505	10380.65	8283.74	79.8	PASS
MC38-Blood-G1-5810	202.8	ng/µl	5.071	2.489	13559.34	10482.26	77.31	PASS

Table 1. Targeted RNA Sequencing Quality Control (Continuation)

Sample ID	Nucleic Acid	Unit	A260 (Abs)	A280 (Abs)	Raw bases (Mb)	Clean bases (Mb)	QCrate (%)	QC
MC38-Blood-G2-5806	300.1	ng/µl	7.501	3.626	13614.43	10816.04	79.45	PASS
MC38-Blood-G2-5809	637.8	ng/µl	15.945	7.624	10893.95	8353.78	76.68	PASS
MC38-Blood-G2-5813	274.8	ng/µl	6.87	3.326	11133.4	8729.76	78.41	PASS
MC38-Spleen-G1-5805	1135.6	ng/µl	28.39	13.69	13006.25	10265.57	78.93	PASS
MC38-Spleen-G1-5807	3976.7	ng/µl	99.418	47.632	12563.41	10047.69	79.98	PASS
MC38-Spleen-G1-5810	2568.3	ng/µl	64.206	30.747	14819.61	12018.79	81.1	PASS
MC38-Spleen-G2-5806	1438.5	ng/µl	35.963	17.053	10886.33	8496.87	78.05	PASS
MC38-Spleen-G2-5809	2009.8	ng/µl	50.244	24.03	14482.78	11440.21	78.99	PASS
MC38-Spleen-G2-5813	3117.4	ng/µl	77.935	37.355	8910.56	7199.24	80.79	PASS
MC38-Tumor-G1-5805	1001.9	ng/µl	25.047	11.864	12730.88	10161.85	79.82	PASS
MC38-Tumor-G1-5807	1956.9	ng/µl	48.922	23.454	12031.01	9977.53	82.93	PASS
MC38-Tumor-G1-5810	2599.2	ng/µl	64.981	31.243	13474.47	10863.83	80.63	PASS
MC38-Tumor-G2-5806	4248.5	ng/µl	106.214	51.092	10200.63	8381.28	82.16	PASS
MC38-Tumor-G2-5809	3390.9	ng/µl	84.773	40.622	9820.85	8067.15	82.14	PASS
MC38-Tumor-G2-5813	2135.9	ng/µl	53.397	25.565	12116.25	10112.07	83.46	PASS

3.2. Gene Expression Data Overview

3.2.1. Data normalization

The raw gene expression data were normalized by transcripts per megabase (TPM), and the data distribution of 36 samples is shown in a boxplot (Figure 1) and a density plot (Figure 2).

3.2.2. Cluster analysis

To investigate similarity and dissimilarity at the mRNA expression level for all involved genes, we performed principal component analysis (PCA) on normalized gene expression data (Figure 3) and cluster analysis on all gene signatures (Figure 4). In general, samples within the same experimental groups were clustered together.

3.3. Differentiation Expression Analysis

3.2.1. Differential expressed (DE) genes in comparisons of interest

Differential expression of single genes was assessed by comparing samples from 12 groups of interest by the limma package1. Two comparisons (Hepa16TumorG1 Vs. Hepa16TumorG2 and MC38TumorG1 Vs. MC38TumorG2) were made. For each comparison, we identified differentially expressed (DE) genes (one-way ANOVA, $|FC| > 2$, Benjamini-Hochberg adjusted p value <0.01. FC: fold change) (Figure 5, Supp. Table 1).

3.2.2. Functional analysis on DE genes

Based on these DE genes, we identified significantly enriched Gene Ontology terms (p-value <0.05) (Supp. Table 2), i.e. the most important biological processes that changed in two groups.

4. Figures

Figure 1: Boxplots for all samples. Data were normalized by TPM.

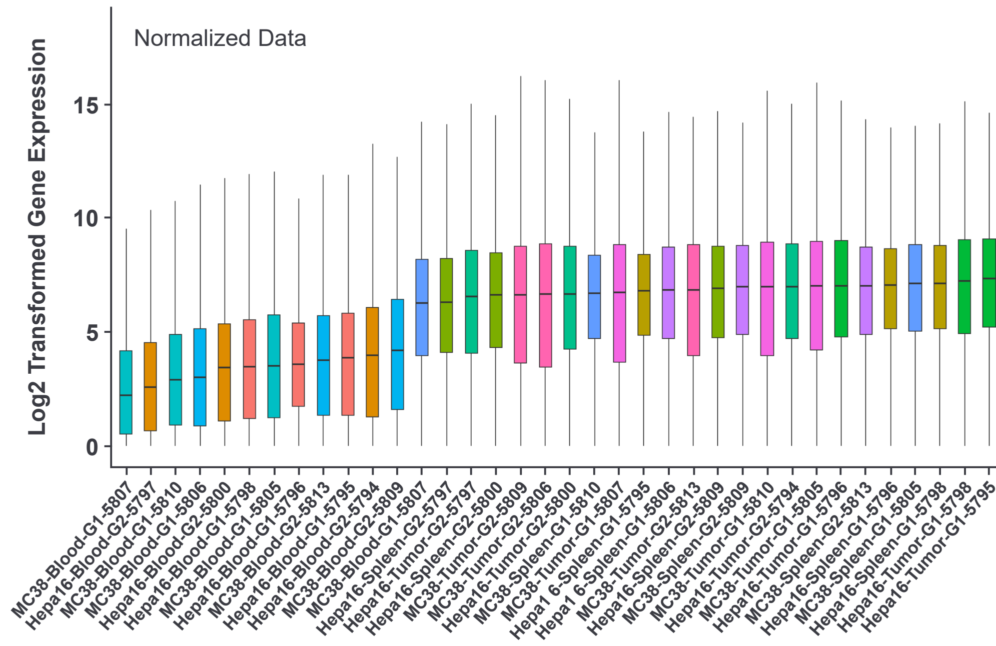


Figure 2: Density plot for each individual sample reveals gene expression data distribution. The vertical dashed line represents the median expression value of all samples.

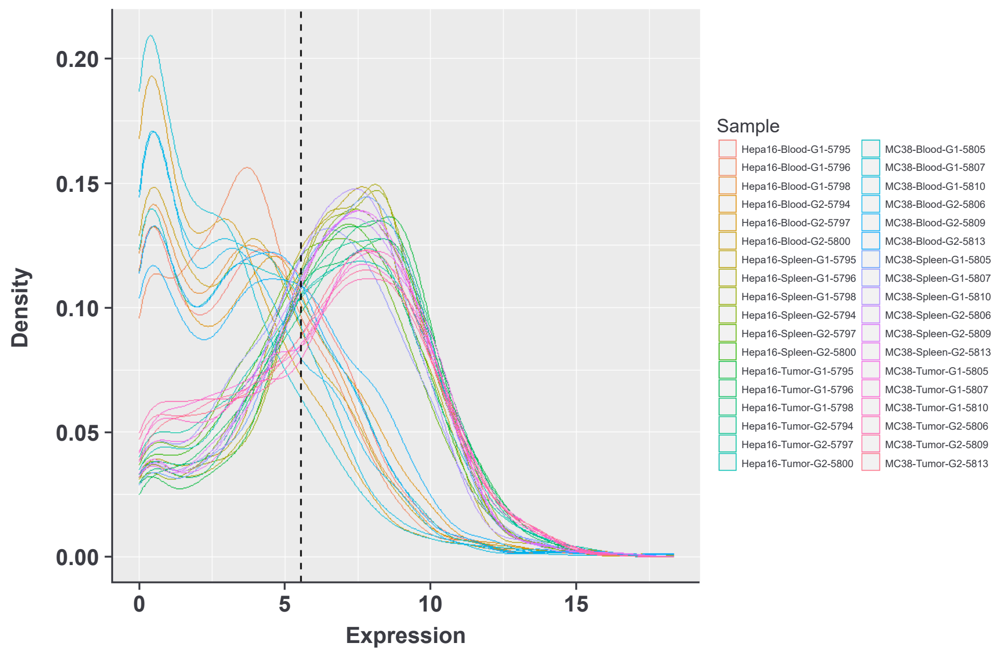
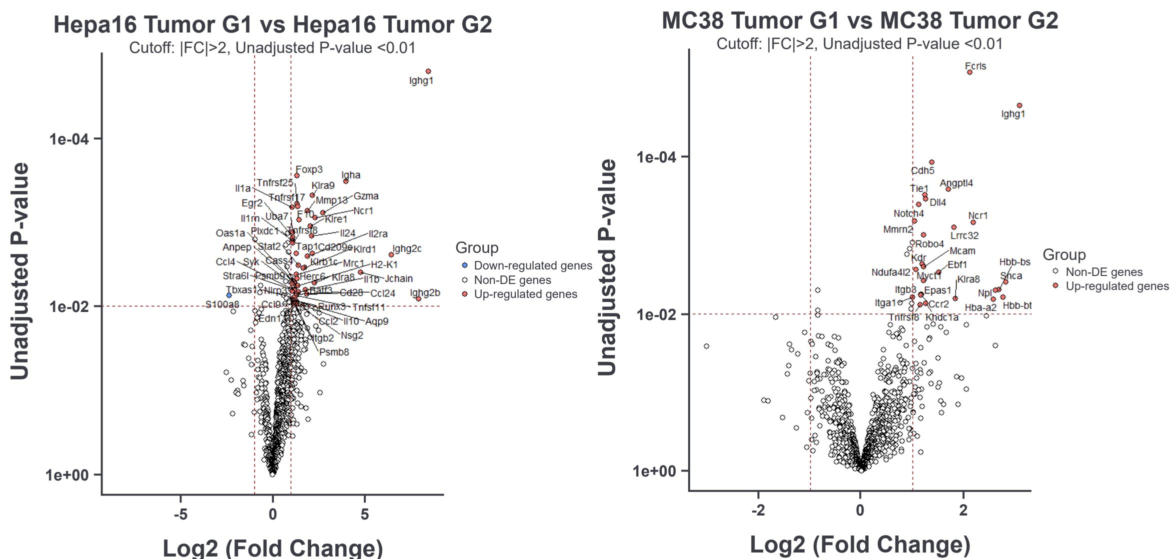


Figure 3: Principle component analysis on all genes.

Figure 4: Heatmap and hierarchical clustering on 99 I/O panel annotation and functional signatures (rows) on 36 samples (columns). Expression levels (low-to-high) are shown in blue-white-red scale.

Figure 5: Volcano plots for differentially expressed genes in two comparisons. All genes with log2- transformed fold change (x axis) and unadjusted p value (y axis) are shown in each volcano plot. Up- and down-regulated genes are highlighted in red and blue, respectively.



5. Methods

5.1. Normalization

Raw mRNA read counts are normalized by TPM. The TPM-normalized data is log(2)- transformed.

5.2. Differential Expression Analysis

5.2.1. Grouping Variable

Differential expression is fit on a per gene or per signature basis using a linear model for analyses without a blocking factor. The statistical model uses the expression value or signature score as the dependent variable and fits a grouping variable as a fixed effect to test for differences in the levels of that grouping variable.

Expression (gene or signature) = μ + Group + ε
P values are adjusted within each analysis, gene, or signature, and on the grouping variable level difference t-test using the Benjamini and Yekutieli False Discovery Rate (FDR) adjustment to account for correlations amongst the tests. All models are fit using the limma package1 in R.

5. References

[1] Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Research, 43(7), e47. https://bioconductor.org/packages/release/bioc/html/limma.html

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