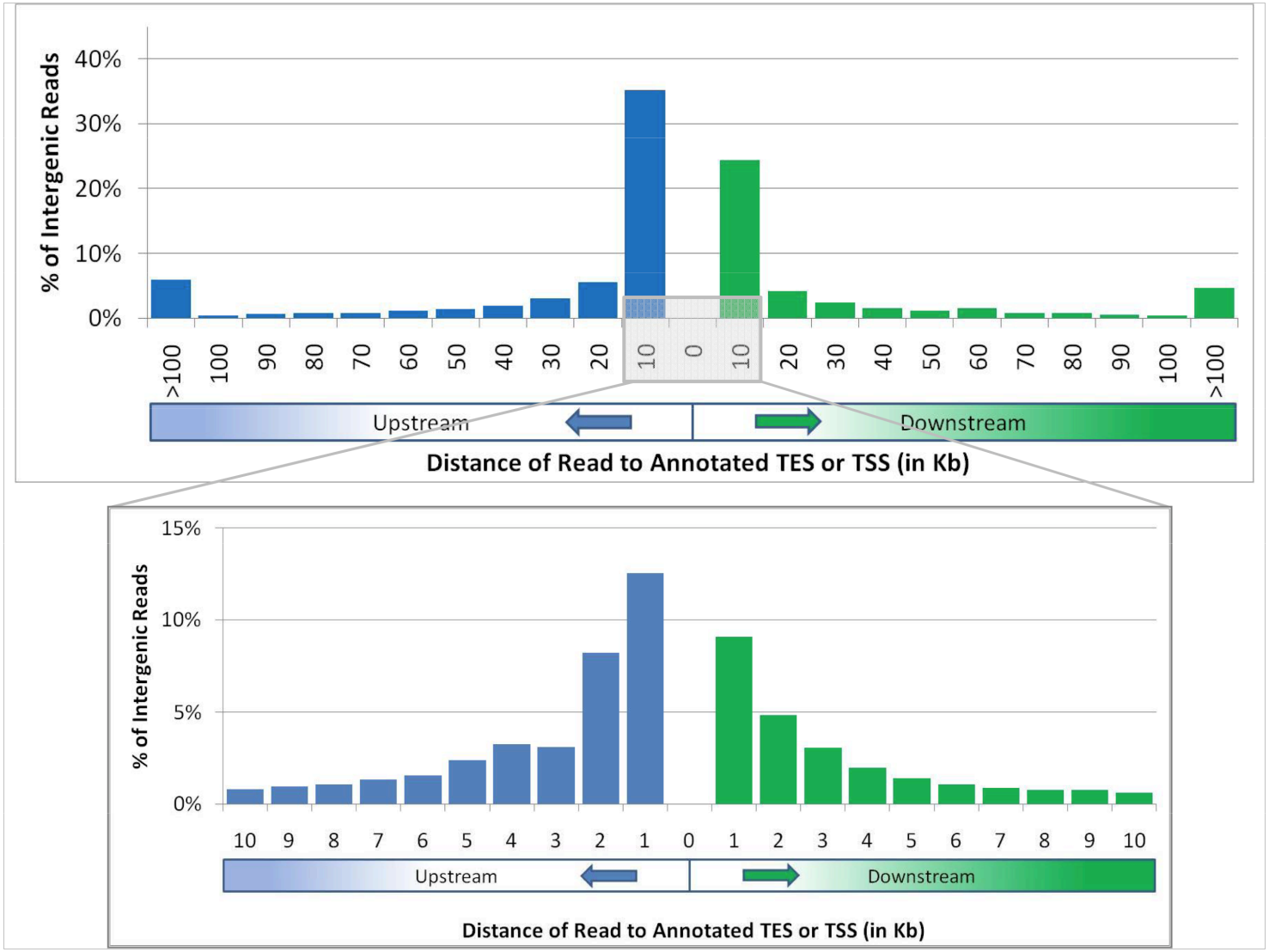
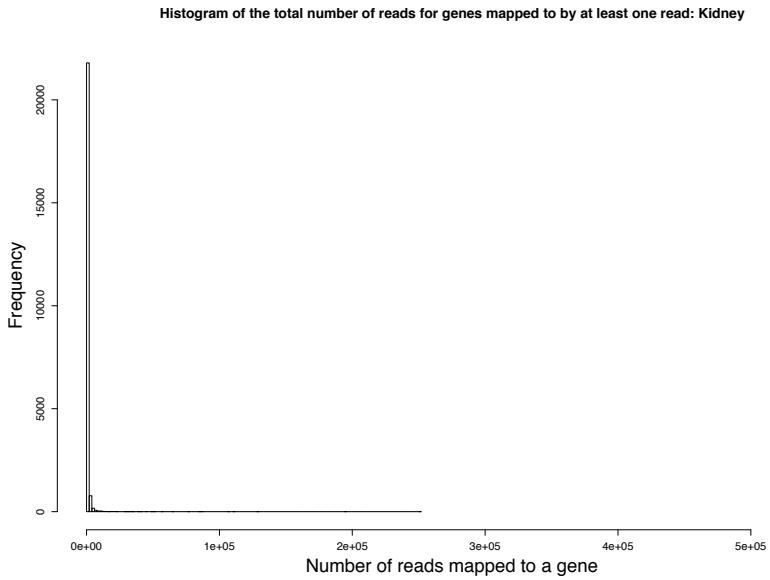
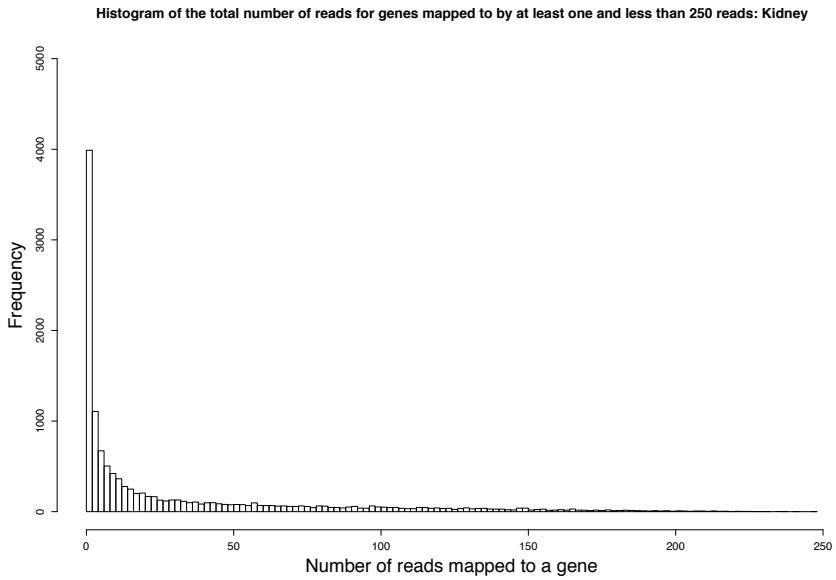
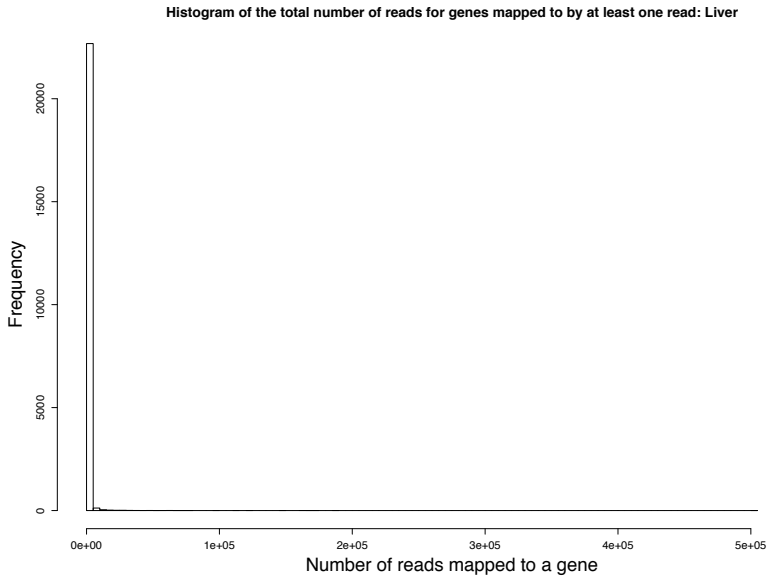
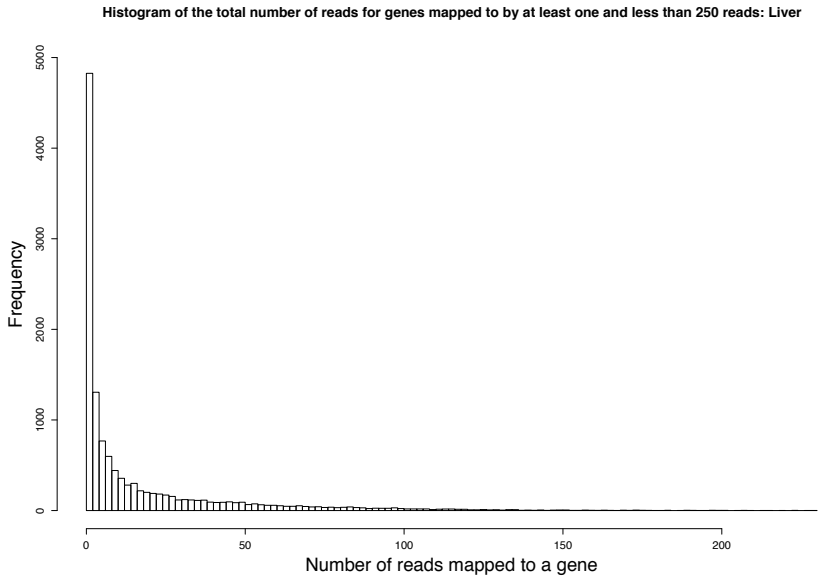


Supplementary Figure 1: Locations of intergenic reads relative to known genes. The histogram shows the location of reads mapped outside genic regions (as annotated by Ensembl v.48) relative to the nearest transcription start site (TSS) or transcription end site (TES) of a gene. In total, 10.6% of such reads are mapped over 100 kb away from either a TSS or a TES, suggesting the existence of novel transcribed regions.

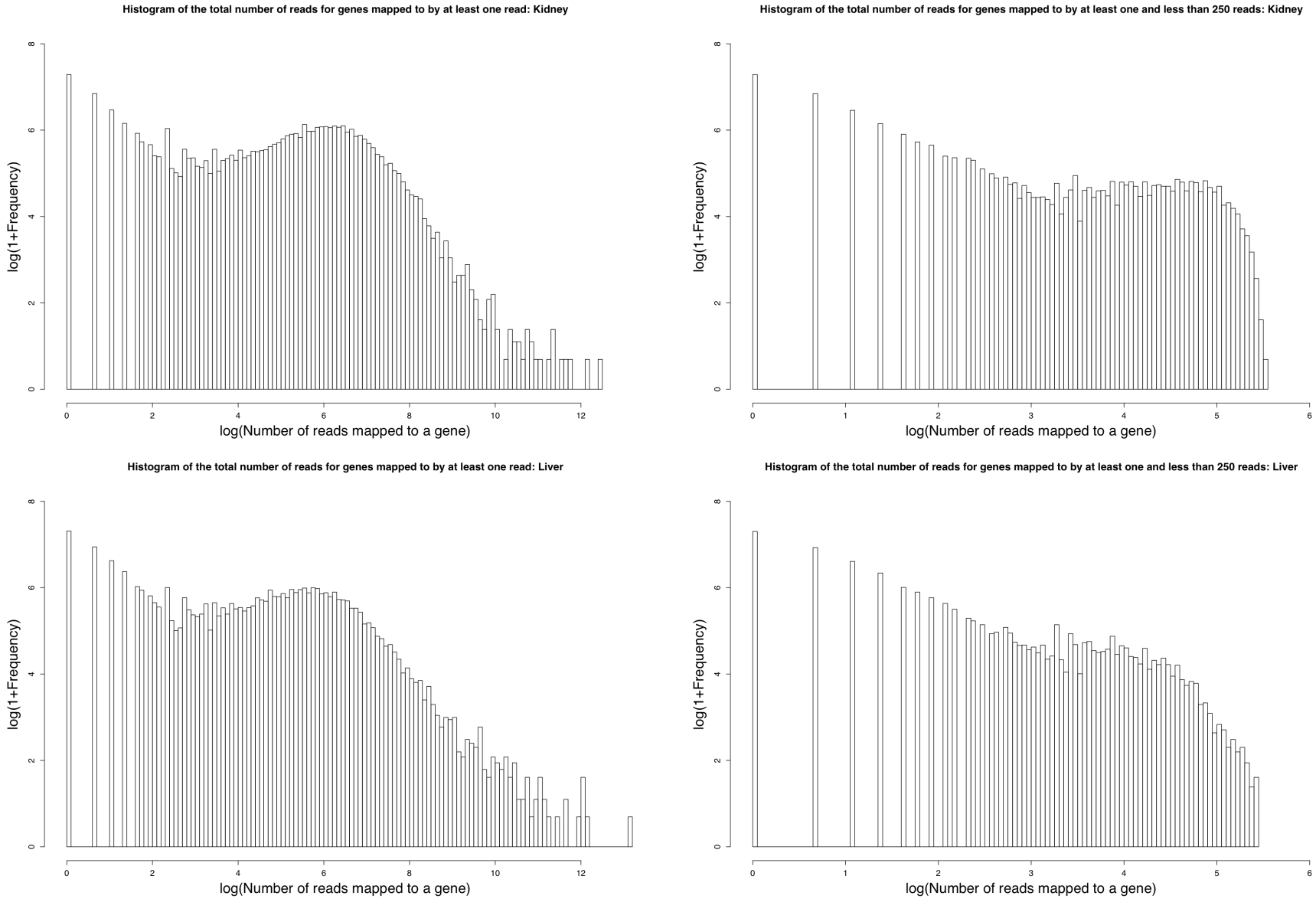




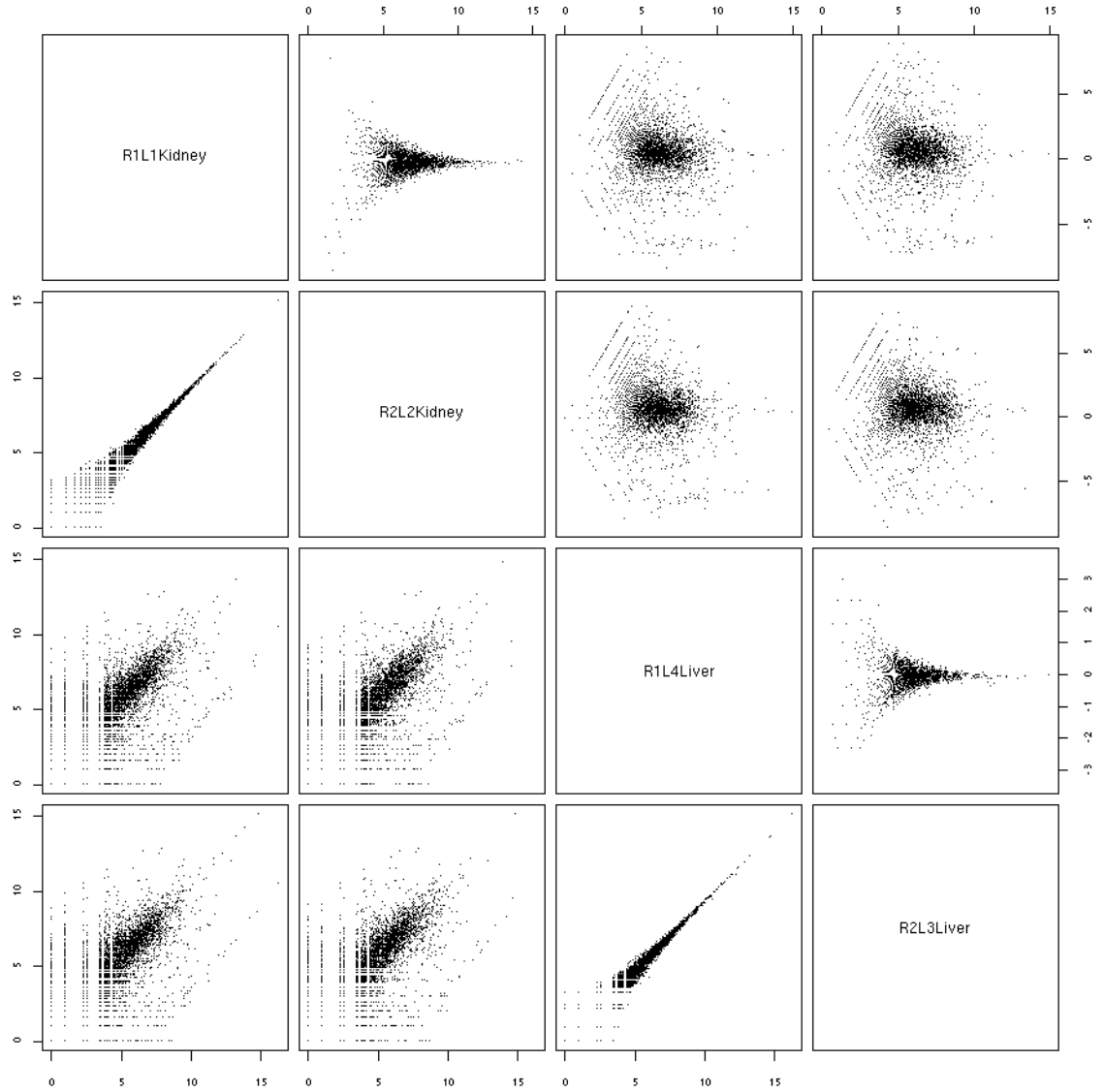




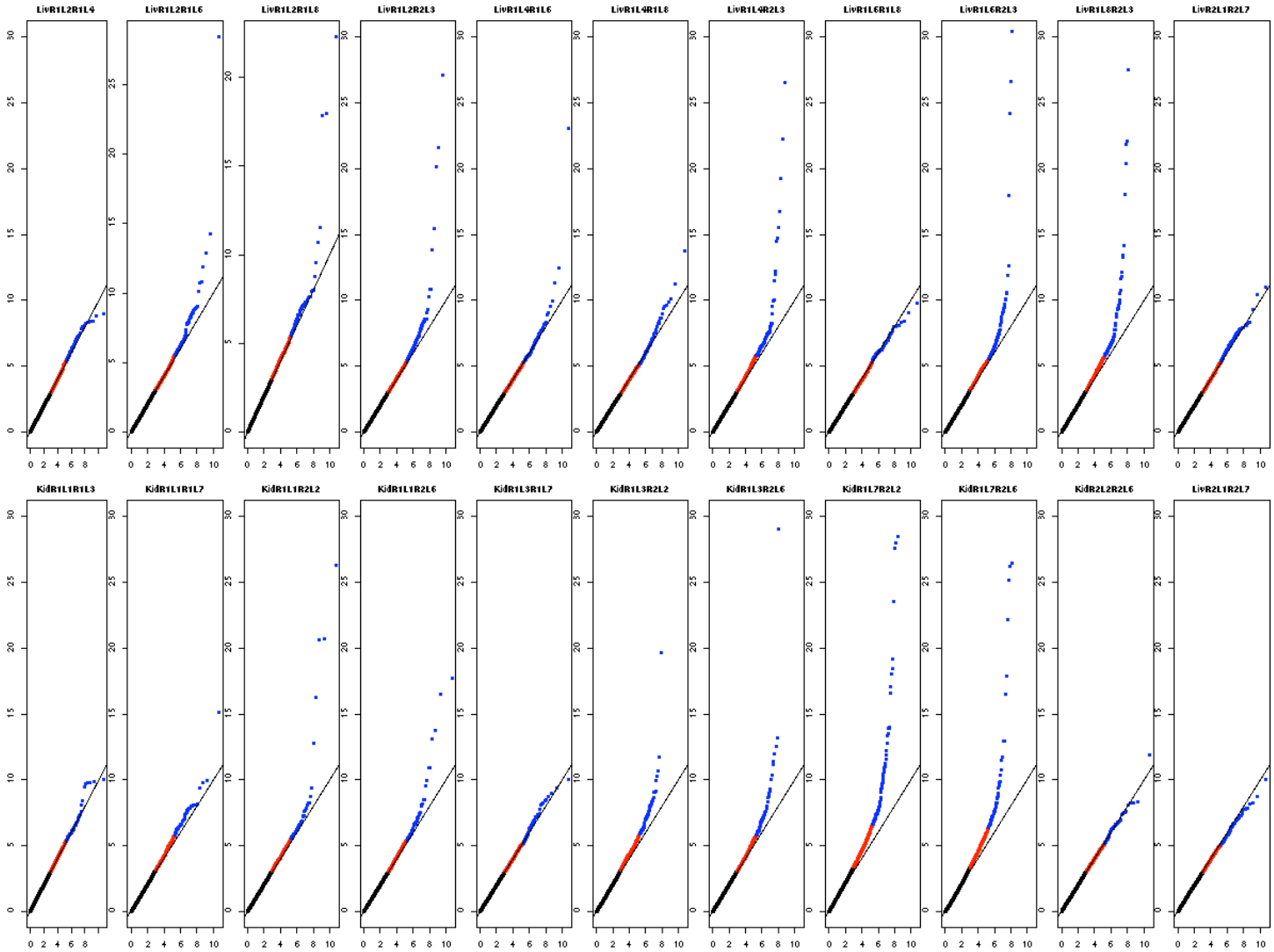
Supplementary Figure 2a: Histograms of the distribution of reads across genes mapped to by at least one read across all 14 lanes of data. In the top row, a histogram of the (total) number of reads mapped to each of these genes for the kidney sample is plotted on the left, and on the right the same plot is shown for all genes that are mapped to by at most 250 reads across all 14 lanes. In the second row, similar plots are shown for the liver sample.



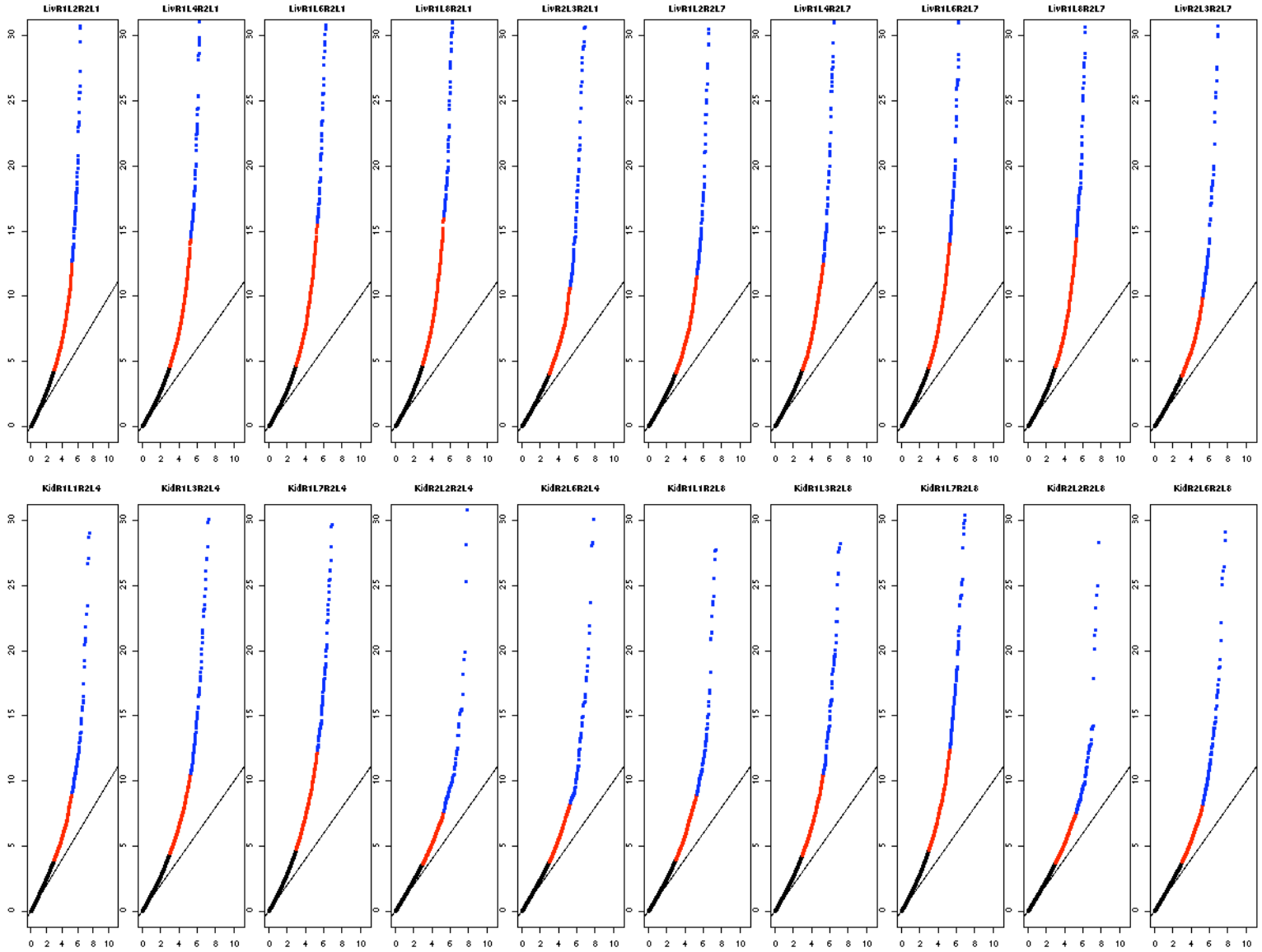
Supplementary Figure 2b: Histograms of the distribution of reads across genes mapped to by at least one read across all 14 lanes of data on the log scale. In the top row, a histogram of the (total) number of reads mapped to each of these genes for the kidney sample is plotted on the left, and on the right the same plot is shown for all genes that are mapped to by at most 250 reads across all 14 lanes. In the second row, similar plots are shown for the liver sample.



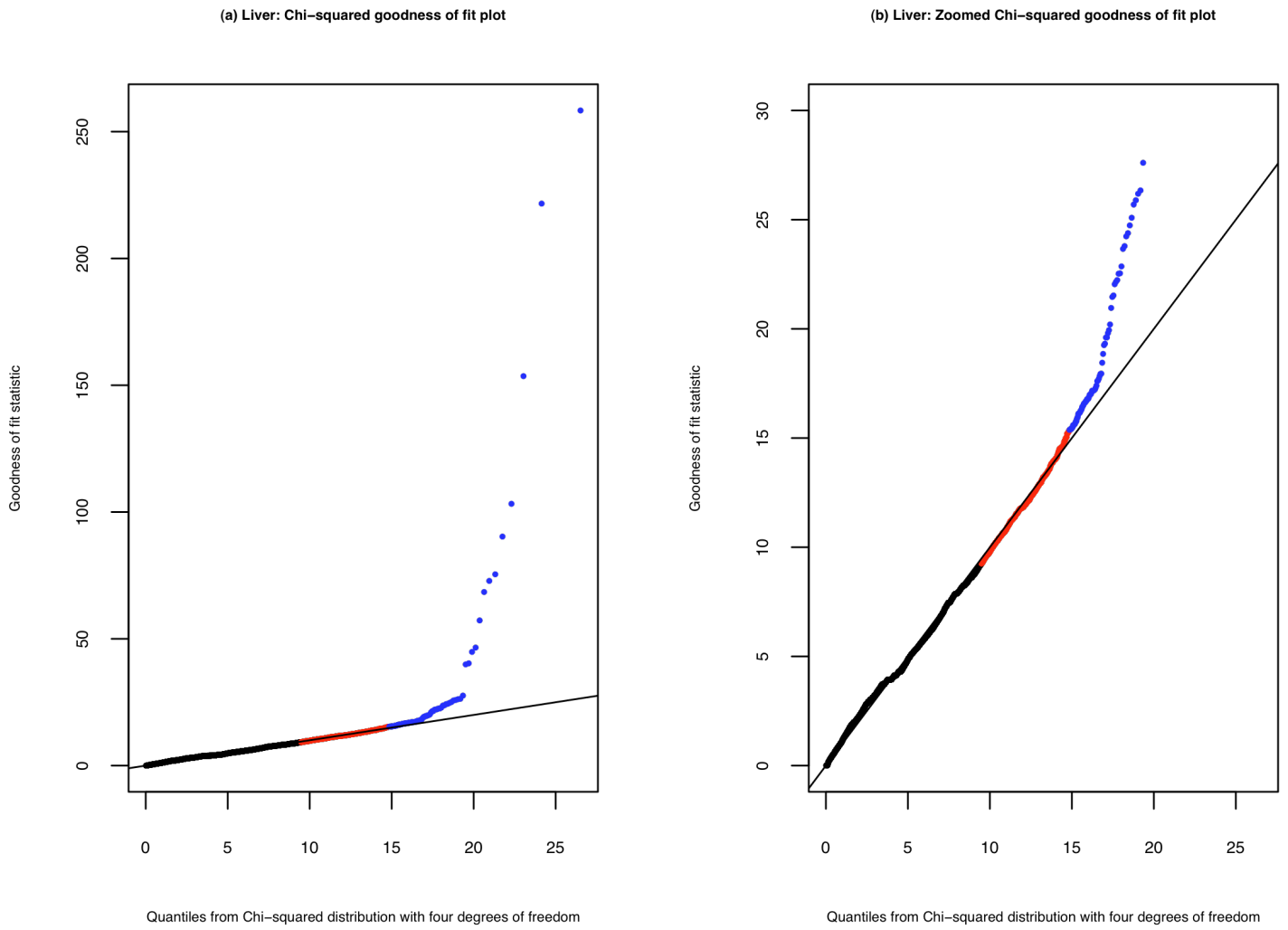
Supplementary Figure 3: Scatterplots and MA plots comparing the number of counts in different lanes. In the first row, the ($\log_2$) counts observed for each gene in Run 1, Lane 1 are plotted on the y-axis, and in the first column, the ($\log_2$) counts for each gene in Run 1, Lane 1 are plotted on the x-axis. Counts for the other samples are plotted similarly and the sample and run/lane that the sample was sequenced in are indicated on the diagonal. In the lower diagonal, scatterplots of the counts associated with each gene are plotted and, in the upper diagonal, MA plots are generated. When the same sample is sequenced in different lanes, a very high correlation can be observed in both the scatter- and MA-plots. To ease interpretation, only genes that were mapped to by at least one read are included.



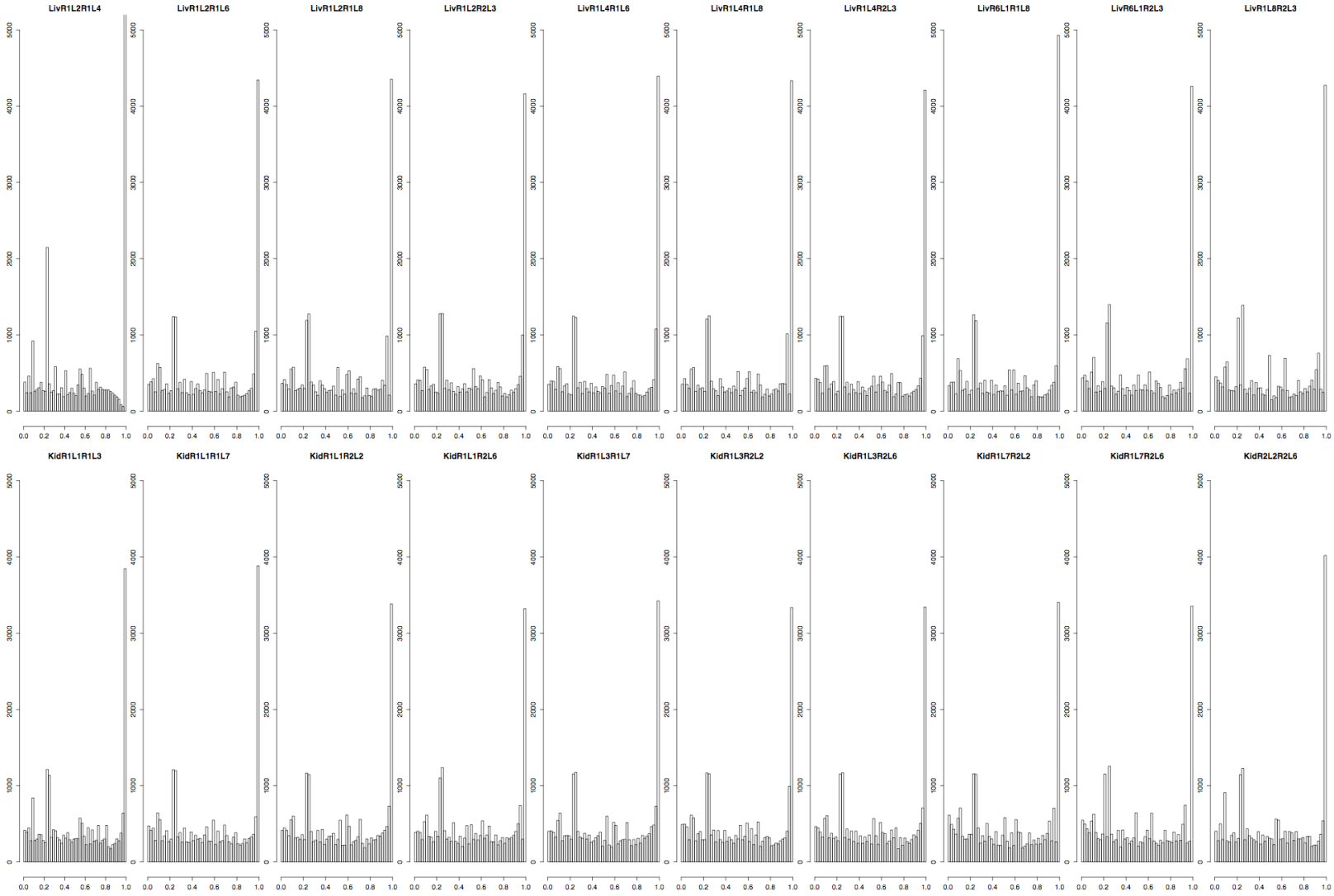
Supplementary Figure 4: *qq*-plots comparing the hypergeometric *p* values for all 22 two-way (lane-vs-lane) comparisons between samples sequenced at the same concentration. In each panel, on the *x* axis the negative log quantiles from a Uniform[0,1) distribution are plotted and on the *y* axis the negative log p values obtained in each pairwise comparison are shown. Points in red are above the 95th percentile and points in blue are above the 99.5th percentile. If the plotted values deviate from the line $y=x$ (plotted in black), there is evidence of a lane effect. The first row gives the 11 combinations for the liver (the first ten columns show the pairwise comparisons for samples sequenced at a concentration of 3 pM, the last column shows the comparison for samples sequenced at 1.5 pM). The second row gives the 11 combinations for the kidney (again, the first 10 columns show the comparisons for samples sequenced at a concentration of 3 pM and the final column shows the single comparison that is possible for samples sequenced at a concentration of 1.5 pM). The title of each histogram indicates the samples that are being compared.



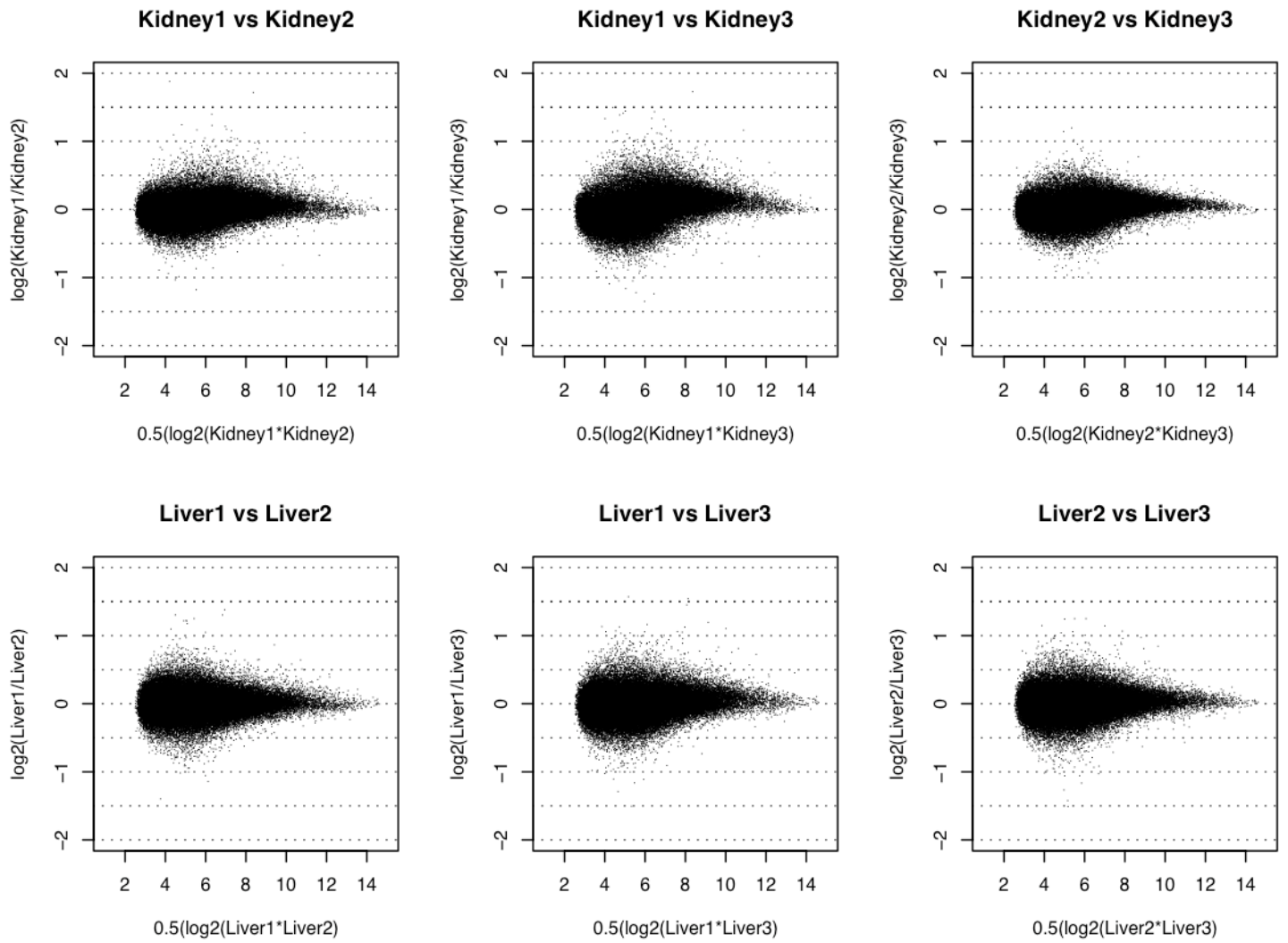
Supplementary Figure 5: *qq*-plots comparing the hypergeometric *p* values for all 20 two-way (lane-vs-lane) comparisons between samples sequenced at different concentrations (i.e., the same sample was sequenced at a concentration of 3 pM in one lane and a concentration of 1.5 pM in another lane). In each panel, on the *x* axis the negative log quantiles from a Uniform[0,1) distribution are plotted and on the *y* axis the negative log p values obtained in each pairwise comparison are shown. Points in red are above the 95th percentile and points in blue are above the 99.5th percentile. If the plotted values deviate from the line $y=x$ (plotted in black), there is evidence of a lane effect. The first row gives the ten combinations for the liver sample and the second row gives the ten combinations for the kidney samples. The title of each histogram indicates the samples that are being compared.



Supplementary Figure 6: Panels (a) and (b) show the *qq*-plot generated (on two different scales) when the goodness-of-fit statistic is used to assess the fit of the Poisson model to the liver data sequenced at a concentration of 3 pM. The goodness-of-fit statistics are plotted on the *y* axis and the quantiles from a chi-squared distribution with 4 degrees of freedom are shown on the *x* axis. Points in red are those above the 95th percentile and those in blue are above the 99.5th percentile. If the plotted values deviate from the line $y=x$ (plotted in black), there is evidence that the Poisson model does not fit the data well. Only a very small number of genes (especially those above the 99.5th percentile) deviate from this line, indicating the Poisson model is appropriate for the overwhelming majority of the data



Supplementary Figure 7: *p* values for all 20 two-way (lane-vs-lane) comparisons to find differentially expressed genes between lanes used to sequence the same sample. The *p* values were determined using the Poisson likelihood ratio statistic. The title of each histogram indicates the samples that are being compared. It is worth noting that a number of spikes appear in the histogram that, at first glance, would indicate a deviation from Uniformity. However, these are due to discretisation of the *p* values and, more importantly, an excess of small *p* values is not observed - this would be worrying since it would indicate that genes were being (incorrectly) called as differentially expressed when two lanes used to sequence the same sample were compared.



Supplementary Figure 8: MA plots for assessing the performance of the Affymetrix data. MA plots for each of the within tissue array-array comparisons are plotted. Data from the arrays used to measure the expression of the kidney sample are plotted on the top and data from the arrays used to measure the liver sample's expression are plotted on the bottom. For each plot, the $\log_2$ ratio for each probe set and the average $\log_2$ sum are plotted on the *y* and *x* axes respectively (the intensity value for each probe set was obtained by applying the RMA algorithm). The plots suggest there are no significant data quality issues in the array data.

				Run 1			
	L1 - kidney	L2 - liver	L3 - kidney	L4 - liver	L6 - liver	L7 - kidney	L8 - liver
Concentration (pM)	3	3	3	3	3	3	3
Number of reads	13017169	14003322	13401343	14230879	13525355	12848201	13096715
Number of mapped reads	5025044	5142214	5199295	5167290	4997324	4901266	4822319
Mapped to chrs 1-24	3261380	3460175	3369521	3480325	3363455	3179248	3249417
Mapped within Ensembl Genes	2706150	2847704	2792026	2861877	2761468	2630987	2668148
Mapped within Ensembl Exons	1926217	1815816	1981182	1821860	1752042	1861126	1692041

				Run 2			
	L1 - liver	L2 - kidney	L3 - liver	L4 - kidney	L6 - kidney	L7 - liver	L8 - kidney
Concentration (pM)	1.5	3	3	1.5	3	1.5	1.5
Number of reads	9096595	13687929	14761931	8843158	13449864	9341101	8449276
Number of mapped reads	4138533	5293547	5320141	4394988	5422895	4437111	4266893
Mapped to chrs 1-24	2794909	3456114	3591760	2885222	3533100	2989819	2799046
Mapped within Ensembl Genes	2328896	2875214	2959436	2416834	2938079	2488832	2345160
Mapped within Ensembl Exons	1532142	2055876	1896001	1751854	2096458	1634684	1701056

Supplementary Table 1: Summary of the number of reads mapped to each lane, the number of reads mapped to the genome, the number of reads mapped to either autosomal or sex chromosomes, the number mapped within Ensembl genes, and the number mapped to exons in the Ensembl database (version 48).

Tissue	# mapped to exons within a gene	# mapped to adjacent exons	# mapped to non-adjacent exons	# conventional splicing events with > 1X coverage	# exon-skipping events with > 1X coverage
Kidney	239,699	237,071	2,628	35,325	1,364
Liver	167,317	165,683	1,634	16,969	634

Supplementary Table 4: For each tissue, we show the number of non-aligned sequence reads (NoHits) that align to the 3' end of one exon and the 5' end of another exons from the Exon-Edge Database (EEDB).

Liver

	No genes	Percentage
Five Lanes	20080	100
Four Lanes	19695	97.9
Three Lanes	19170	95.5
Two Lanes	18390	91.6
One Lane	16973	84.5

Kidney

	No genes	Percentage
Five Lanes	20921	100
Four Lanes	20552	98.2
Three Lanes	20064	96.0
Two Lanes	19355	92.5
One Lane	18080	86.4

Supplementary Table 5: The number of genes mapped to by at least one read as a function of the number of lanes analyzed. For the liver sample, to determine the number of genes mapped to by at least one read when four, three, two or one lane was used, we took the average number of genes mapped to by all permutations of four lanes chosen from five, three lanes chosen from five, two lanes chosen from five and one lane chosen from five respectively. The kidney sample was analyzed similarly.