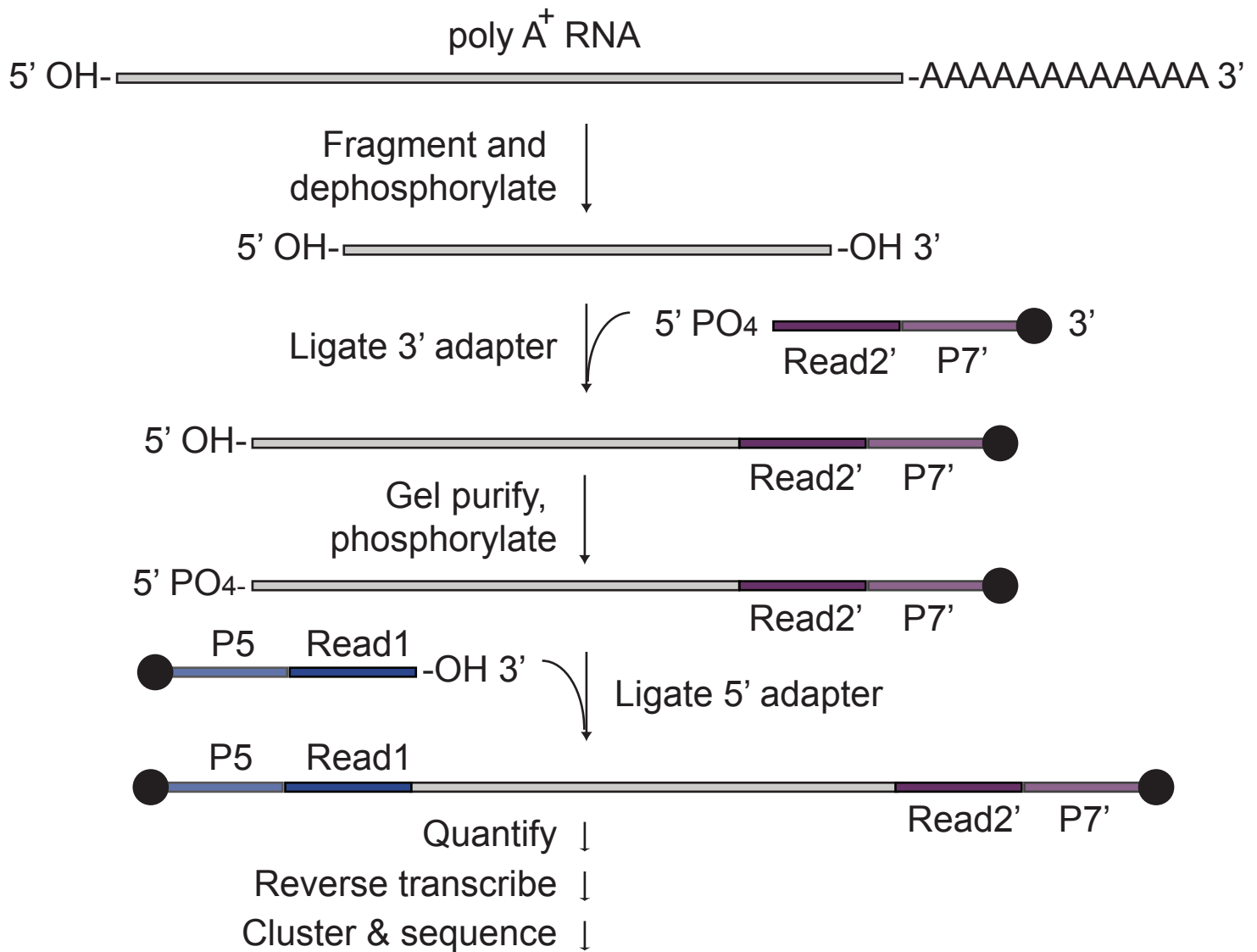


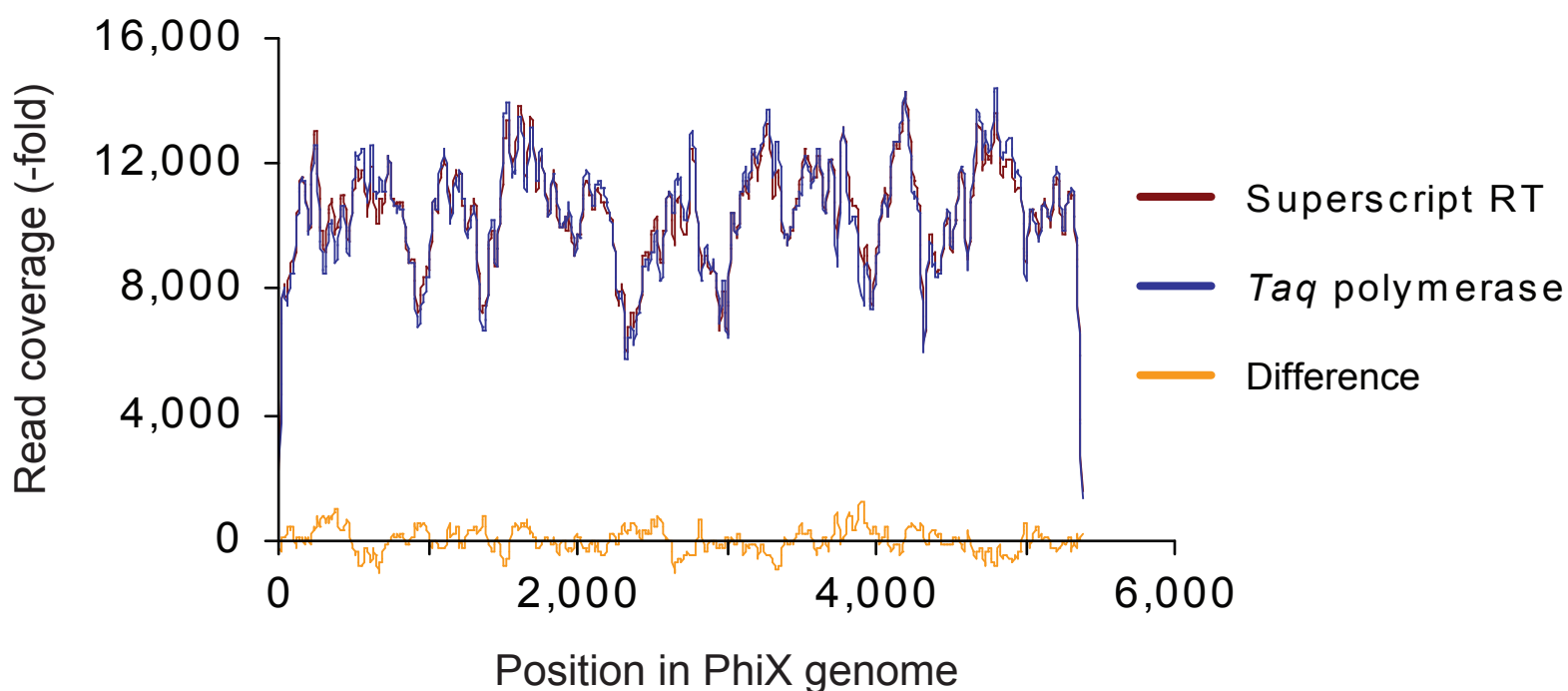
Supplementary Figure 1



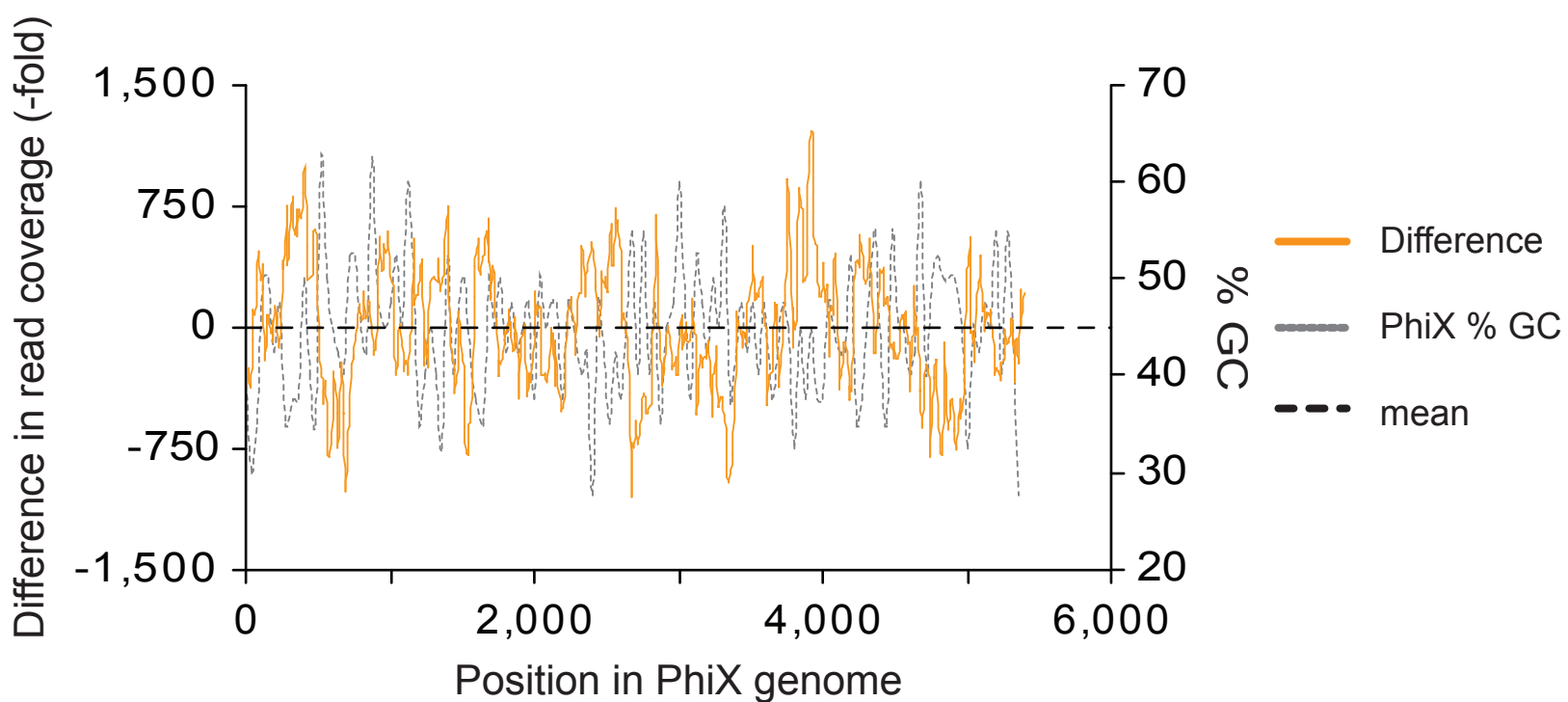
Supplementary Figure 1. Library preparation for Flowcell RT libraries. P5 and P7 are the oligonucleotide sequences present on the Illumina flowcell surface, and P7' is the reverse complementary sequence of P7. Read 1 corresponds to the nucleotide sequence of the Illumina read 1 sequencing primer, and Read 2' corresponds to the reverse complementary nucleotide sequence of the Illumina read 2 sequencing primer. Adapters possess blocking groups, indicated by a black circle, to prevent side reactions: the 3' adapter (red) has a 3' dideoxy cytosine, and the 5' adapter (blue) has an amino C6 modification at its 5' terminus.

Supplementary Figure 2

a)

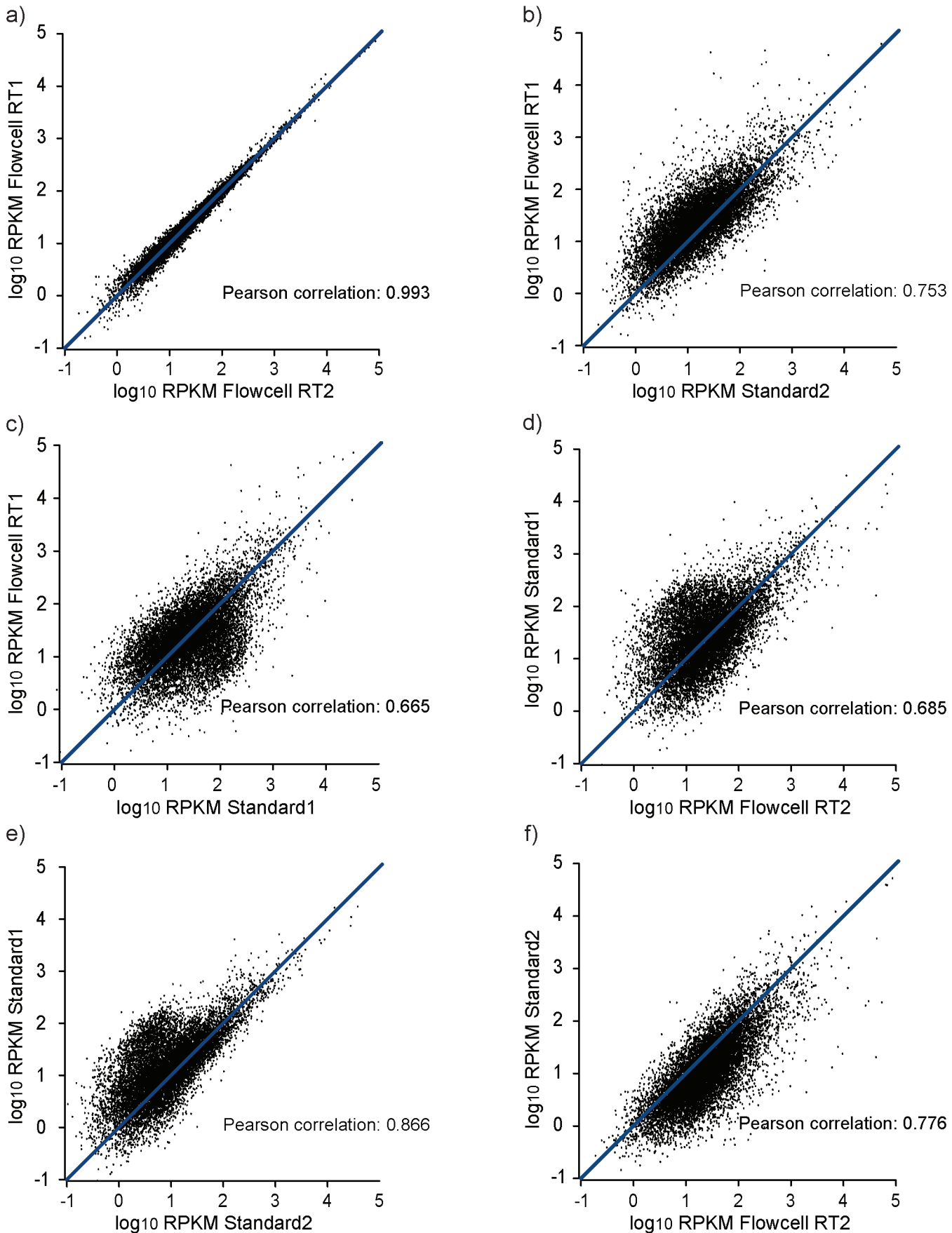


b)



Supplementary Figure 2. Cluster seeding with reverse transcriptase. a) Normalized coverage plot of PhiX genome with 1st strand extension performed by Superscript Reverse Transcriptase (RT, red), Illumina-supplied Taq DNA polymerase (blue), and the difference between the two (light orange). The same aliquot of PhiX was used in each case, at the same loading concentration. Coverage is plotted in 10-base bins, using the first 37 bases of reads. b) An expanded version of the 'difference trace' is shown with % GC for the PhiX genome in 40-base bins superimposed. Spearman correlations were calculated for various bin sizes, with a 10-base shift between bins.

Supplementary Figure 3

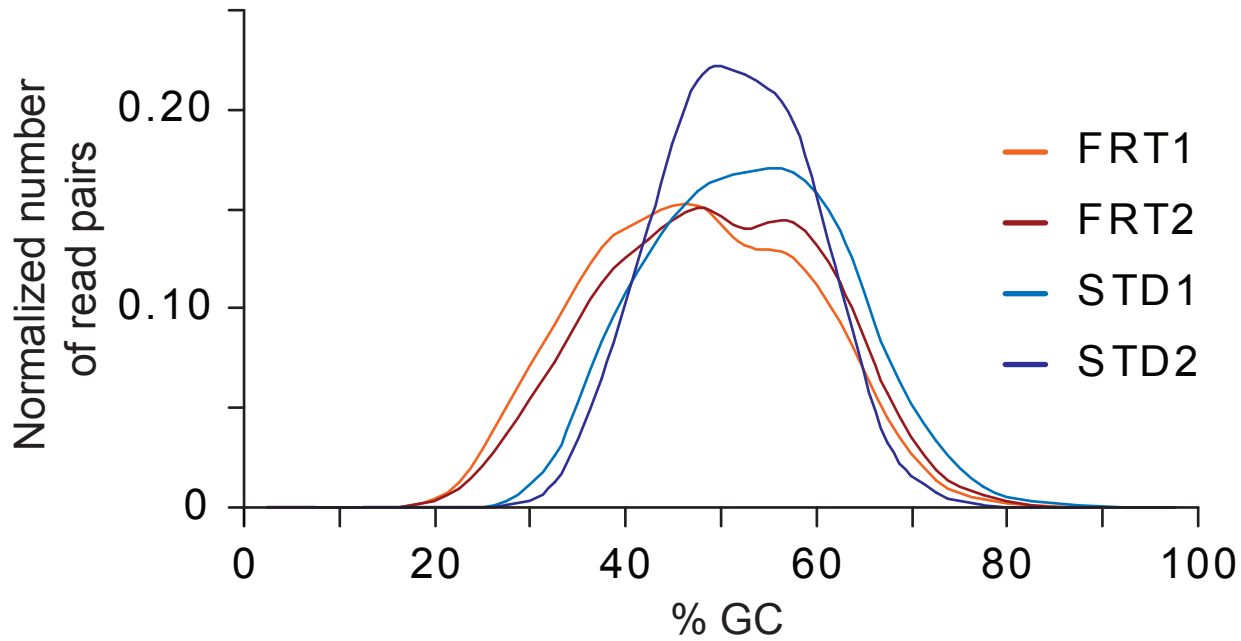


Supplementary Figure 3. Correlation plots for FRT-seq and standard RNA-seq libraries.

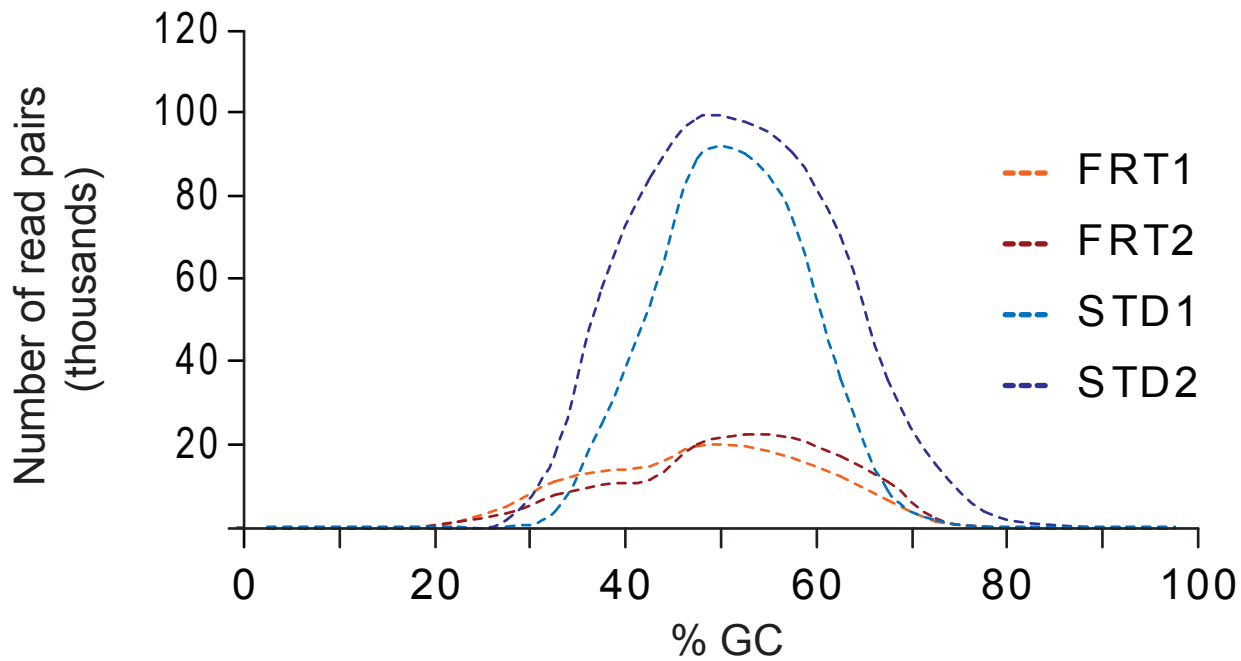
To illustrate library reproducibility, we plotted sequence data obtained from all combinations of libraries. a) FRT1 against FRT2, b) FRT1 against STD2, c) FRT1 against STD1, d) STD1 against FRT2, e) STD1 against STD2, f) STD2 against FRT2. The blue line indicates perfect correlation. RPKM = reads per kilobase of sequence per million reads.

Supplementary Figure 4

a)

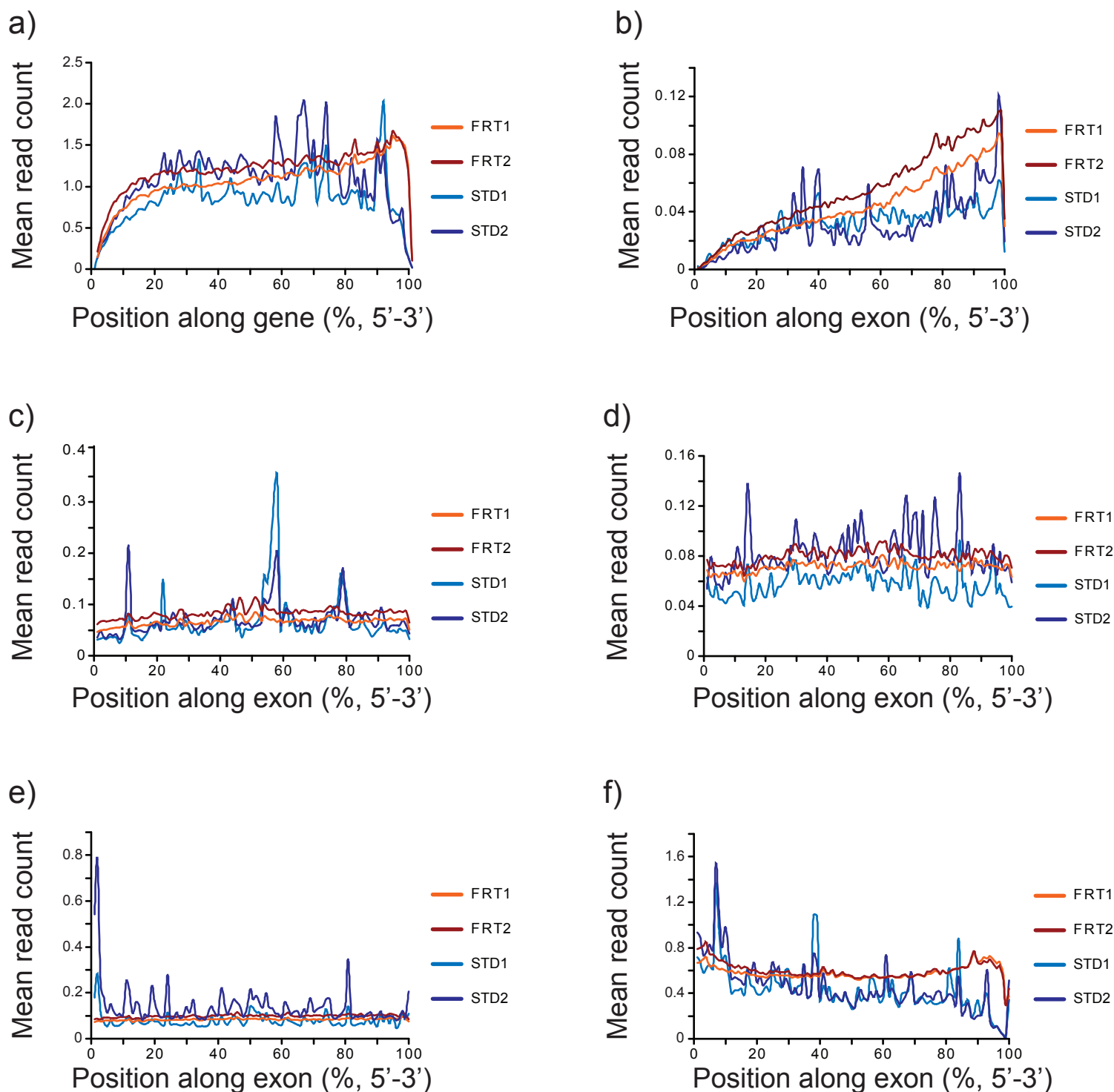


b)



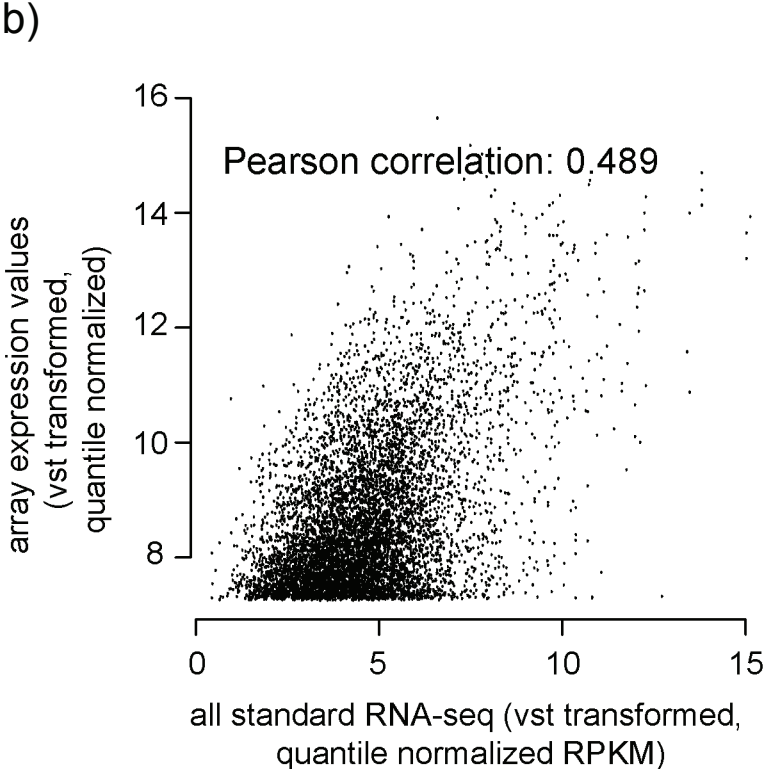
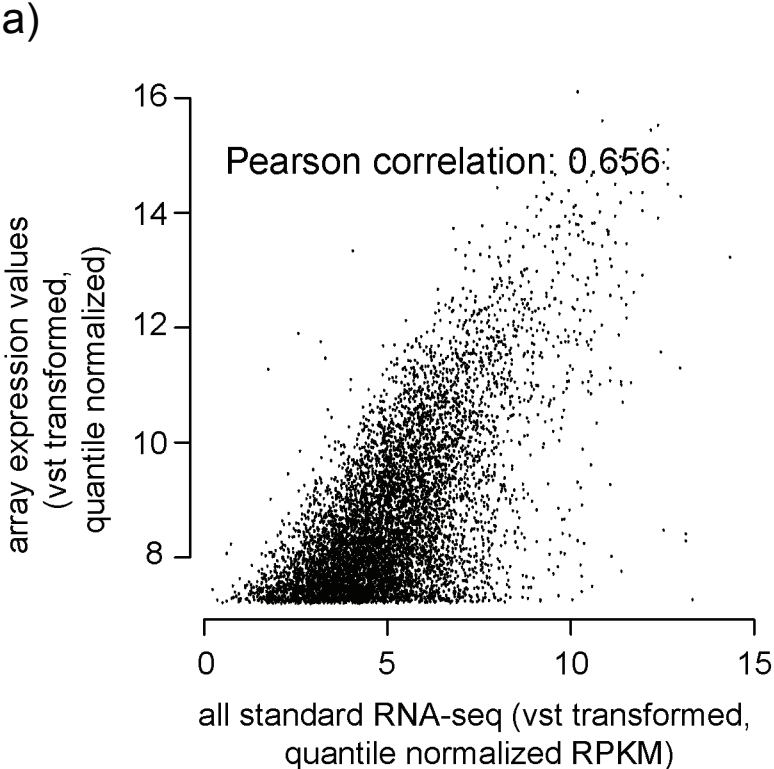
Supplementary Figure 4. Effect of template GC on read depth. Mapped sequences were divided into bins based upon the % GC of the entire fragment. This was plotted against **a)** normalized read count for all reads and **b)** read count for duplicate reads.

Supplementary Figure 5



Supplementary Figure 5. Distribution of reads plotted across transcripts. Mean values of read count for all FRT lanes and all standard RNA-seq libraries in both orientations (Y-axis) are plotted against the position of mapped reads along the entire length of the exon / gene to which they map, expressed as a percentile. **a)** for entire genes. Panes **b)** - **f)** show data by exon for genes with 5 exons or more. Pane **b)** shows the first (5') exon, **c)** shows the second exon, **d)** shows the third exon, **e)** shows the penultimate exon and **f)** shows the 3' exon.

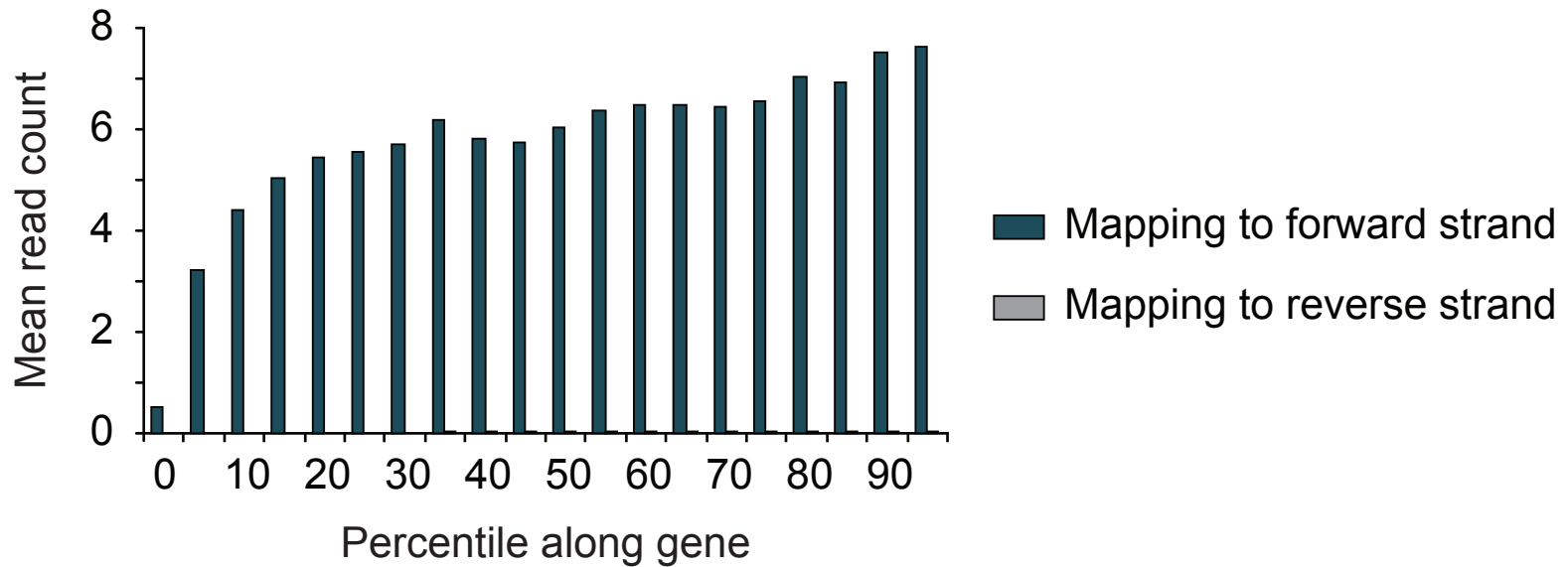
Supplementary Figure 6



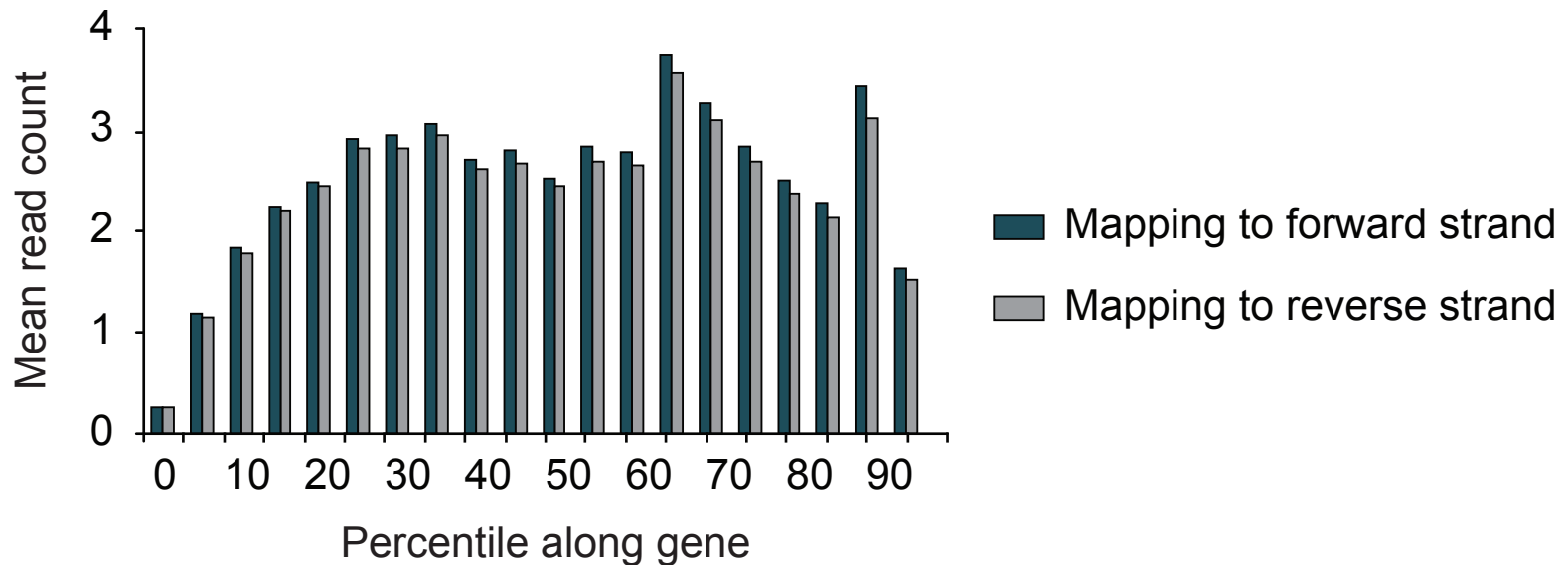
Supplementary Figure 6. Microarray correlations for FRT-seq and Standard libraries. To assess the quantitative nature of FRT-seq, and standard RNA-seq libraries, we plotted transcript counts derived from microarray data against a) both FRT-seq libraries combined, and b) both standard RNA-seq libraries combined.

Supplementary Figure 7

a)



b)



Supplementary Figure 7. Distribution of reads plotted across transcripts for forward and reverse strands. Mean values of read count in forward and reverse orientations for all FRT lanes and all standard RNA-seq libraries (Y-axis) are plotted against the position of mapped reads along the entire length of the gene to which they map, expressed as a percentile. **a)** mean values for both flowcell RT libraries and **b)** mean values for both standard RNA-seq libraries.

Supplementary Figure 8

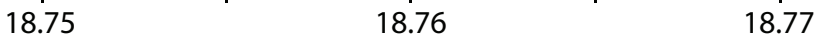
Mapping on forward strand,
quality ≥ 30



Ensembl(+)



Coordinates (Mb)



Mapping on reverse strand,
quality ≥ 30



Ensembl(-)



Supplementary Figure 8. Strand specificity of Flowcell RT using zebrafish ovary poly A+ RNA. Sequences generated by Flowcell RT were mapped against the zebrafish genome version Zv8, and .wig files were generated. These are shown displayed in IGB, though the colours were modified for clarity (dark red). Unlike the commercial human placental poly A+ RNA sample used for other experiments, very few sequences mapped to intronic regions.

Supplementary Table 1. Oligonucleotide sequences.

RNA bases are shown in blue, DNA bases in black.

RNA_P7pr_v7.2 pAGAUCCGAAGAGCGGUUCAG CAGGAATGCCGAGACCGATCTCGTATGCCGTCTTCTGCTTGddC 3'

RNA_P5_v7.1 [AmC6] AATGATACGGCGACCACCGAGATCTACACTCTTCCCT ACACGACGCUCUCCGAUCU 3'

Supplementary Table 2. Spearman correlations between %GC and read coverage

For reverse transcriptase, *Taq* polymerase and for the difference between the two sets of values (RT-Taq). % GC was calculated for padded 10bp sliding windows. Padding is added to both sides of the 10 bp window. For example, Pad = 45 corresponds to a fragment size of 100 bp. Correlations were calculated for the entire PhiX genome, for both reverse transcriptase, *Taq* polymerase, and the difference in read count for the two enzymes. The genome was then divided at either side of the mean % GC (44.7 %) into low and high % GC and the correlations were recalculated.

	Entire PhiX genome			Low GC (<44.7%)				High GC (>44.7%)			
Pad	Reverse transcriptase	Taq polymerase	RT - Taq	Reverse transcriptase	Taq polymerase	RT - Taq	% of sequences in bin	Reverse transcriptase	Taq polymerase	RT - Taq	% of sequences in bin
5	0.2357	0.2758	-0.3027	0.4000	0.4252	-0.2584	34.69	0.0199	0.0467	-0.1524	65.31
10	0.2499	0.3069	-0.3811	0.3167	0.3480	-0.2793	44.16	-0.0366	-0.0043	-0.1284	55.84
15	0.2852	0.3447	-0.4122	0.3080	0.3494	-0.2967	37.48	-0.0074	0.0326	-0.1902	62.52
20	0.2926	0.3633	-0.4632	0.2586	0.3102	-0.3086	44.90	0.0319	0.0695	-0.1944	55.10
25	0.3111	0.3814	-0.4778	0.3172	0.3521	-0.2885	39.89	0.1273	0.1554	-0.2427	60.11
30	0.3265	0.3972	-0.4954	0.3033	0.3661	-0.3506	46.38	0.0412	0.0730	-0.1772	53.62
35	0.3315	0.4023	-0.5102	0.2916	0.3167	-0.2037	39.70	0.1029	0.1383	-0.2472	60.30
40	0.3198	0.3943	-0.5263	0.3201	0.3527	-0.2429	46.75	0.0337	0.0632	-0.1767	53.25
45	0.3001	0.3794	-0.5309	0.2897	0.3232	-0.2605	42.86	0.0462	0.0855	-0.2758	57.14
50	0.2975	0.3765	-0.5244	0.3601	0.3982	-0.2844	48.05	0.0289	0.0566	-0.2180	51.95
55	0.2794	0.3607	-0.5173	0.3815	0.4226	-0.2122	44.34	-0.0160	0.0031	-0.1407	55.66
60	0.2742	0.3568	-0.5163	0.3868	0.4430	-0.3418	49.35	-0.0426	-0.0210	-0.1551	50.65
65	0.2640	0.3491	-0.5169	0.4140	0.4627	-0.3208	44.53	-0.0437	-0.0282	-0.1061	55.47
70	0.2725	0.3545	-0.5123	0.3878	0.4371	-0.3323	48.79	-0.0239	-0.0376	-0.0034	51.21
75	0.2642	0.3474	-0.5123	0.4564	0.4990	-0.3222	45.08	0.0035	0.0063	-0.0600	54.92
80	0.2785	0.3630	-0.5217	0.3655	0.4181	-0.3471	43.23	0.0075	0.0103	-0.0650	56.77
85	0.2693	0.3573	-0.5286	0.4071	0.4738	-0.4207	45.83	0.0329	0.0431	-0.0830	54.17
90	0.2883	0.3752	-0.5352	0.4669	0.5347	-0.4669	43.78	0.1516	0.1695	-0.1761	56.22
95	0.2814	0.3717	-0.5421	0.4440	0.5190	-0.4695	46.75	0.1724	0.1798	-0.1312	53.25
100	0.2944	0.3832	-0.5427	0.4393	0.5033	-0.4315	45.64	0.1425	0.1409	-0.1117	54.36

Supplementary Table 3. Pearson correlations between lanes and libraries.

Pearson correlations between transcript counts for individual lanes, within libraries and between libraries, to test for library prep and sequencing reproducibility.

		FRT-seq 1							FRT-seq 2						
library	lane	2664_1	2664_2	2664_3	2664_5	2664_6	2664_7	2664_8	2665_1	2665_2	2665_3	2665_5	2665_6	2665_7	2665_8
FRT-seq 1	2664_1	1.0000	0.9999	0.9997	0.9987	0.9997	0.9997	0.9998	0.9920	0.9927	0.9929	0.9925	0.9923	0.9928	0.9930
	2664_2	0.9999	1.0000	0.9999	0.9985	0.9996	0.9997	0.9997	0.9918	0.9927	0.9928	0.9923	0.9920	0.9925	0.9928
	2664_3	0.9997	0.9999	1.0000	0.9982	0.9997	0.9997	0.9997	0.9921	0.9930	0.9932	0.9927	0.9924	0.9928	0.9931
	2664_5	0.9987	0.9985	0.9982	1.0000	0.9978	0.9976	0.9981	0.9883	0.9892	0.9889	0.9883	0.9880	0.9888	0.9886
	2664_6	0.9997	0.9996	0.9997	0.9978	1.0000	0.9999	0.9999	0.9941	0.9946	0.9949	0.9946	0.9945	0.9949	0.9951
	2664_7	0.9997	0.9997	0.9997	0.9976	0.9999	1.0000	0.9999	0.9938	0.9943	0.9946	0.9944	0.9943	0.9946	0.9949
	2664_8	0.9998	0.9997	0.9997	0.9981	0.9999	0.9999	1.0000	0.9931	0.9935	0.9939	0.9937	0.9936	0.9941	0.9943
	FRT-seq 2	2665_1	0.9920	0.9918	0.9921	0.9883	0.9941	0.9938	0.9931	1.0000	0.9997	0.9998	0.9997	0.9996	0.9993
2665_2		0.9927	0.9927	0.9930	0.9892	0.9946	0.9943	0.9935	0.9997	1.0000	0.9998	0.9995	0.9990	0.9988	0.9984
2665_3		0.9929	0.9928	0.9932	0.9889	0.9949	0.9946	0.9939	0.9998	0.9998	1.0000	0.9998	0.9995	0.9992	0.9989
2665_5		0.9925	0.9923	0.9927	0.9883	0.9946	0.9944	0.9937	0.9997	0.9995	0.9998	1.0000	0.9999	0.9998	0.9996
2665_6		0.9923	0.9920	0.9924	0.9880	0.9945	0.9943	0.9936	0.9996	0.9990	0.9995	0.9999	1.0000	0.9999	0.9997
2665_7		0.9928	0.9925	0.9928	0.9888	0.9949	0.9946	0.9941	0.9993	0.9988	0.9992	0.9998	0.9999	1.0000	0.9998
2665_8		0.9930	0.9928	0.9931	0.9886	0.9951	0.9949	0.9943	0.9989	0.9984	0.9989	0.9996	0.9997	0.9998	1.0000
STD RNA-seq 1		3202_1	0.6636	0.6615	0.6616	0.6683	0.6664	0.6640	0.6643	0.6859	0.6839	0.6833	0.6833	0.6835	0.6846
	3202_2	0.6627	0.6605	0.6607	0.6674	0.6655	0.6631	0.6634	0.6851	0.6830	0.6825	0.6825	0.6828	0.6838	0.6800
	3202_3	0.6620	0.6598	0.6600	0.6667	0.6648	0.6624	0.6627	0.6845	0.6825	0.6819	0.6819	0.6821	0.6832	0.6794
	3202_5	0.6652	0.6631	0.6632	0.6698	0.6681	0.6657	0.6660	0.6878	0.6858	0.6853	0.6852	0.6855	0.6865	0.6829
STD RNA-seq 2	3202_6	0.7518	0.7490	0.7508	0.7578	0.7575	0.7540	0.7539	0.7799	0.7755	0.7755	0.7763	0.7789	0.7790	0.7725
	3202_7	0.7521	0.7492	0.7511	0.7581	0.7578	0.7543	0.7542	0.7803	0.7760	0.7759	0.7767	0.7792	0.7793	0.7728
	3202_8	0.7516	0.7487	0.7506	0.7576	0.7573	0.7538	0.7537	0.7797	0.7753	0.7753	0.7761	0.7786	0.7788	0.7723
All FRT-seq 1		1.0000	0.9998	0.9998	0.9984	0.9998	0.9998	1.0000	0.9926	0.9932	0.9934	0.9932	0.9930	0.9935	0.9937
All FRT-seq 2		0.9928	0.9925	0.9929	0.9887	0.9949	0.9946	0.9940	0.9997	0.9993	0.9997	0.9999	0.9999	0.9999	0.9997
All FRT-seq		0.9979	0.9977	0.9979	0.9949	0.9989	0.9988	0.9986	0.9975	0.9975	0.9979	0.9981	0.9981	0.9984	0.9985
all STD RNA-seq 1		0.6645	0.6624	0.6625	0.6691	0.6674	0.6650	0.6653	0.6870	0.6850	0.6844	0.6844	0.6846	0.6857	0.6820
all STD RNA-seq 2		0.7518	0.7489	0.7508	0.7577	0.7575	0.7540	0.7539	0.7799	0.7755	0.7755	0.7763	0.7788	0.7789	0.7724
all STD RNA-seq		0.7359	0.7333	0.7344	0.7415	0.7404	0.7373	0.7374	0.7622	0.7588	0.7586	0.7590	0.7605	0.7611	0.7557

STD RNA-seq 1				STD RNA-seq 2								
3202_1	3202_2	3202_3	3202_5	3202_6	3202_7	3202_8	FRT-seq 1	FRT-seq 2	all FRT-seq	STD RNA-seq 1	STD RNA-seq 2	all STD RNA-seq
0.6636	0.6627	0.6620	0.6652	0.7518	0.7521	0.7516	1.0000	0.9928	0.9979	0.6645	0.7518	0.7359
0.6615	0.6605	0.6598	0.6631	0.7490	0.7492	0.7487	0.9998	0.9925	0.9977	0.6624	0.7489	0.7333
0.6616	0.6607	0.6600	0.6632	0.7508	0.7511	0.7506	0.9998	0.9929	0.9979	0.6625	0.7508	0.7344
0.6683	0.6674	0.6667	0.6698	0.7578	0.7581	0.7576	0.9984	0.9887	0.9949	0.6691	0.7577	0.7415
0.6664	0.6655	0.6648	0.6681	0.7575	0.7578	0.7573	0.9998	0.9949	0.9989	0.6674	0.7575	0.7404
0.6640	0.6631	0.6624	0.6657	0.7540	0.7543	0.7538	0.9998	0.9946	0.9988	0.6650	0.7540	0.7373
0.6643	0.6634	0.6627	0.6660	0.7539	0.7542	0.7537	1.0000	0.9940	0.9986	0.6653	0.7539	0.7374
0.6859	0.6851	0.6845	0.6878	0.7799	0.7803	0.7797	0.9926	0.9997	0.9975	0.6870	0.7799	0.7622
0.6839	0.6830	0.6825	0.6858	0.7755	0.7760	0.7753	0.9932	0.9993	0.9975	0.6850	0.7755	0.7588
0.6833	0.6825	0.6819	0.6853	0.7755	0.7759	0.7753	0.9934	0.9997	0.9979	0.6844	0.7755	0.7586
0.6833	0.6825	0.6819	0.6852	0.7763	0.7767	0.7761	0.9932	0.9999	0.9981	0.6844	0.7763	0.7590
0.6835	0.6828	0.6821	0.6855	0.7789	0.7792	0.7786	0.9930	0.9999	0.9981	0.6846	0.7788	0.7605
0.6846	0.6838	0.6832	0.6865	0.7790	0.7793	0.7788	0.9935	0.9999	0.9984	0.6857	0.7789	0.7611
0.6809	0.6800	0.6794	0.6829	0.7725	0.7728	0.7723	0.9937	0.9997	0.9985	0.6820	0.7724	0.7557
1.0000	0.9996	0.9996	0.9995	0.8645	0.8640	0.8648	0.6640	0.6836	0.6742	0.9999	0.8646	0.9604
0.9996	1.0000	0.9996	0.9996	0.8667	0.8662	0.8670	0.6631	0.6828	0.6733	0.9997	0.8668	0.9614
0.9996	0.9996	1.0000	0.9996	0.8667	0.8662	0.8670	0.6624	0.6821	0.6726	0.9997	0.8668	0.9614
0.9995	0.9996	0.9996	1.0000	0.8666	0.8661	0.8669	0.6656	0.6855	0.6760	0.9999	0.8667	0.9614
0.8645	0.8667	0.8667	0.8666	1.0000	0.9999	0.9999	0.7530	0.7764	0.7644	0.8657	1.0000	0.9702
0.8640	0.8662	0.8662	0.8661	0.9999	1.0000	0.9999	0.7532	0.7768	0.7647	0.8652	0.9999	0.9700
0.8648	0.8670	0.8670	0.8669	0.9999	0.9999	1.0000	0.7527	0.7762	0.7641	0.8660	1.0000	0.9704
0.6640	0.6631	0.6624	0.6656	0.7530	0.7532	0.7527	1.0000	0.9934	0.9983	0.6649	0.7529	0.7367
0.6836	0.6828	0.6821	0.6855	0.7764	0.7768	0.7762	0.9934	1.0000	0.9983	0.6847	0.7764	0.7592
0.6742	0.6733	0.6726	0.6760	0.7644	0.7647	0.7641	0.9983	0.9983	1.0000	0.6752	0.7643	0.7479
0.9999	0.9997	0.9997	0.9999	0.8657	0.8652	0.8660	0.6649	0.6847	0.6752	1.0000	0.8658	0.9610
0.8646	0.8668	0.8668	0.8667	1.0000	0.9999	1.0000	0.7529	0.7764	0.7643	0.8658	1.0000	0.9703
0.9604	0.9614	0.9614	0.9614	0.9702	0.9700	0.9704	0.7367	0.7592	0.7479	0.9610	0.9703	1.0000

Supplementary Table 4. Duplicate reads for each library.

Reverse complementary duplicates are chiefly an artefact of the PCR step in a library prep that is not strand-specific. ‘% read pairs with ≥ 1 duplicate’ indicates the absolute percentage of duplicate sequences in the different libraries, whereas ‘% transcriptomic positions with ≥ 1 mapped duplicate’ indicates the % of sites to which duplicate sequences map.

	Lane ID	Total mapped read pairs	Total transcriptomic positions with mapped read pairs	Read pairs with ≥ 1 duplicate	Transcriptomic positions with ≥ 1 mapped duplicate	Read pairs with ≥ 1 reverse complementary duplicate	% read pairs with ≥ 1 duplicate	% transcriptomic positions with ≥ 1 mapped duplicate	% read pairs with ≥ 1 reverse complementary duplicate
FRT-seq library 1	2664_1	1324788	1271481	80333	27026	0	6.06	2.13	0.00
	2664_2	1262486	1212363	76650	26527	0	6.07	2.19	0.00
	2664_3	1263575	1216929	70973	24327	2	5.62	2.00	0.00
	2664_5	1140866	1103475	57195	19804	0	5.01	1.79	0.00
	2664_6	1377672	1320307	88164	30799	4	6.40	2.33	0.00
	2664_7	1539237	1471781	101659	34203	0	6.60	2.32	0.00
	2664_8	1404722	1343867	93517	32662	2	6.66	2.43	0.00
	FRT-seq library 2	2665_1	1398046	1327633	99010	28597	0	7.08	2.15
2665_2		1169470	1115347	76348	22225	0	6.53	1.99	0.00
2665_3		1438074	1365830	101321	29077	0	7.05	2.13	0.00
2665_5		1627890	1540787	121056	33953	0	7.44	2.20	0.00
2665_6		1667040	1577096	125146	35202	7	7.51	2.23	0.00
2665_7		1589797	1505822	117302	33327	2	7.38	2.21	0.00
2665_8		1384241	1314180	98375	28314	0	7.11	2.15	0.00
STD RNA-seq library 1		3202_1	1885143	446241	1766768	327866	572	93.72	73.47
	3202_2	2015908	453679	1900588	338359	602	94.28	74.58	0.03
	3202_3	2014349	452612	1899698	337961	562	94.31	74.67	0.03
	3202_5	1968366	450628	1852300	334562	592	94.10	74.24	0.03
STD RNA-seq library 2	3202_6	1414876	985120	569216	139460	187	40.23	14.16	0.01
	3202_7	1371305	960292	544014	133001	190	39.67	13.85	0.01
	3202_8	1309552	923075	511800	125323	179	39.08	13.58	0.01

Supplementary Table 5. Sequence count in all orientations for sequences obtained from all libraries.

0 = not mapped, dg = other read maps to a different gene. Poly T is the reverse complement of the 3' poly A tail that is present on the 3' end of mature mRNA strands, and would prevent read 2 from mapping. A fragment consisting mainly of poly A would generate poly T in read 2 and some poly A in read 1, so would fail to map. We used only those reads for which a mapping score ≥ 30 was obtained.

+ - Read 1 maps to sense strand and read 2 maps to antisense strand;

- + Read 2 maps to sense strand and read 1 maps to antisense strand;

++ Both reads map to the sense strand;

-- Both reads map to the antisense strand.

A mapping score threshold of 30 was used. For + and - orientations, data was required to be paired, whereas for dg and null read pairs it was not.

FRT-seq

library	Lane	+/+	+/-	-/+	-/-	+/0	-/0	0/+	0/-	+ / poly T	- / poly	poly T / +	poly T / -	+ / dg	- / dg	dg / +	dg / -
FRT-seq 1	2664_1	0	965003	3127	0	68887	818	843	36260	1798	59	6	291	474	4	7	159
FRT-seq 1	2664_2	0	920633	3122	0	75381	761	790	34832	1684	67	4	266	432	4	4	187
FRT-seq 1	2664_3	0	922121	3183	0	87912	870	746	32498	1736	52	7	254	461	1	16	217
FRT-seq 1	2664_5	0	814800	2835	0	61704	683	686	30210	1620	74	2	264	427	2	9	163
FRT-seq 1	2664_6	0	1006475	3324	0	81225	889	851	36916	1823	37	5	276	496	2	7	193
FRT-seq 1	2664_7	0	1120329	3725	0	78684	948	956	41478	1992	52	9	302	578	4	9	218
FRT-seq 1	2664_8	0	1017022	3463	0	75698	807	895	38408	1788	52	3	260	500	1	7	194
FRT-seq 2	2665_1	0	1052227	3427	0	66819	839	808	39279	1360	30	7	211	509	3	8	191
FRT-seq 2	2665_2	0	876144	2887	0	70695	708	650	31523	1126	33	6	202	400	2	12	223
FRT-seq 2	2665_3	0	1081283	3539	0	72861	827	817	37636	1459	39	4	250	528	2	11	240
FRT-seq 2	2665_5	0	1224159	4014	0	76586	965	909	45285	1497	52	6	231	580	2	6	284
FRT-seq 2	2665_6	0	1254076	4039	0	77664	1002	976	45518	1494	48	8	262	620	5	9	248
FRT-seq 2	2665_7	0	1196943	3890	0	74618	919	902	43807	1392	35	6	270	568	4	5	229
FRT-seq 2	2665_8	0	1045435	3296	0	65035	846	801	38259	1237	38	3	219	518	3	11	174
Total		0	1.4E+07	47871	0	1E+06	11882	11630	531909	22006	668	76	3558	7091	39	121	2920
% of total		0.00	89.65	0.30	0.00	6.39	0.07	0.07	3.29	0.14	0.00	0.00	0.02	0.04	0.00	0.00	0.02

STD RNA-seq

library	Lane	+/+	+/-	-/+	-/-	+ / 0	- / 0	0 / +	0 / -	+ / poly T	- / poly	poly T / +	poly T / -	+ / dg	- / dg	dg / +	dg / -
STD RNA-seq 1	3202_8	0	383622	4E+05	0	13802	9076	11883	8526	109	81	53	128	202	66	171	34
STD RNA-seq 1	3202_7	0	414900	4E+05	0	14579	9865	12464	9068	95	57	72	112	207	54	202	37
STD RNA-seq 1	3202_6	0	415561	4E+05	0	14769	9748	12355	9170	101	64	75	106	222	46	192	47
STD RNA-seq 1	3202_5	0	410308	4E+05	0	14500	9546	12042	9071	95	58	60	110	212	61	213	40
STD RNA-seq 2	3202_3	0	550861	5E+05	0	18121	7576	16628	7368	80	53	25	51	88	72	89	54
STD RNA-seq 2	3202_2	0	534373	5E+05	0	17441	7358	16040	7013	68	48	38	46	86	61	77	74
STD RNA-seq 2	3202_1	0	511475	5E+05	0	16533	6986	14996	6827	69	50	32	50	98	62	76	52
Total		0	3221100	3E+06	0	109745	60155	96408	57043	617	411	355	603	1115	422	1020	338
% of total		0.00	48.58	46.47	0.00	1.66	0.91	1.45	0.86	0.01	0.01	0.01	0.01	0.02	0.01	0.02	0.01

Supplementary Table 6. Hits in exons, introns and intergenic regions. Only paired reads were considered, but each read was assigned to the categories separately.

+ - Read 1 maps to sense strand and read 2 maps to antisense strand;

- + Read 2 maps to sense strand and read 1 maps to antisense strand;

Within Exon = read pair maps to transcriptome within 250bp and the read being considered falls entirely within an exon

Within Intron = read pair maps to transcriptome within 250bp and the read being considered falls entirely within an intron

Exon-Exon = read pair maps to transcriptome within 250bp and the read being considered overlaps boundary between exons

Exon-Intron = read pair maps to genome and the read being considered overlaps an internal exon-intron boundary

Exon-Intergenic = read pair maps to genome and the read being considered overlaps first or last exon – intergenic boundary

Promoter = read pair maps to genome 1kb upstream from 1st exon

Intergenic = read pair maps to genome and falls outside of genes

			Within Exon + -	Within Exon - +	Within Intron + -	Within Intron - +	Exon Exon + -	Exon Exon - +	Exon Intron + -	Exon Intron - +	Exon Intergenic + -	Exon Intergenic - +	Promoter + -	Promoter - +	Intergenic
FRT-seq library 1	2664_1	sequence count	1741845	7594	235593	22222	263658	130	0	0	9687	285	7520	5374	274478
		% of total	67.82%	0.30%	9.17%	0.87%	10.27%	0.01%	0.00%	0.00%	0.38%	0.01%	0.29%	0.21%	10.69%
		% by orientation	77.13%	21.33%	10.43%	62.41%	11.68%	0.37%	0.00%	0.00%	0.43%	0.80%	0.33%	15.09%	N/A
		% within location	99.57%	0.43%	91.38%	8.62%	99.95%	0.05%	-	-	97.14%	2.86%	58.32%	41.68%	100%
	2664_2	sequence count	1669821	7497	227442	21696	251878	124	0	0	9172	324	7370	4823	261565
		% of total	67.83%	0.29%	8.86%	0.84%	9.81%	0.00%	0.00%	0.00%	0.36%	0.01%	0.29%	0.19%	10.18%
		% by orientation	77.10%	21.75%	10.50%	62.95%	11.63%	0.36%	0.00%	0.00%	0.42%	0.94%	0.34%	13.99%	N/A
		% within location	99.55%	0.45%	91.29%	8.71%	99.95%	0.05%	-	-	96.59%	3.41%	60.44%	39.56%	100%
	2664_3	sequence count	1685578	7722	229210	21990	253153	109	0	0	9417	306	7132	4414	261085
		% of total	67.96%	0.30%	8.92%	0.86%	9.86%	0.00%	0.00%	0.00%	0.37%	0.01%	0.28%	0.17%	10.17%
		% by orientation	77.16%	22.36%	10.49%	63.66%	11.59%	0.32%	0.00%	0.00%	0.43%	0.89%	0.33%	12.78%	N/A
		% within location	99.54%	0.46%	91.25%	8.75%	99.96%	0.04%	-	-	96.85%	3.15%	61.77%	38.23%	100%
	2664_5	sequence count	1484038	6780	211368	20680	217414	109	0	0	8622	247	5932	3707	239478
		% of total	67.51%	0.26%	8.23%	0.81%	8.47%	0.00%	0.00%	0.00%	0.34%	0.01%	0.23%	0.14%	9.32%
		% by orientation	77.00%	21.51%	10.97%	65.60%	11.28%	0.35%	0.00%	0.00%	0.45%	0.78%	0.31%	11.76%	N/A
		% within location	99.55%	0.45%	91.09%	8.91%	99.95%	0.05%	-	-	97.22%	2.78%	61.54%	38.46%	100%

FRT-seq library 1	2664_6	sequence count	1822759	8043	243879	23474	277061	127	0	0	10016	298	8320	5234	280559
		% of total	68.02%	0.31%	9.50%	0.91%	10.79%	0.00%	0.00%	0.00%	0.39%	0.01%	0.32%	0.20%	10.92%
		% by orientation	77.17%	21.63%	10.32%	63.14%	11.73%	0.34%	0.00%	0.00%	0.42%	0.80%	0.35%	14.08%	N/A
		% within location	99.56%	0.44%	91.22%	8.78%	99.95%	0.05%	-	-	97.11%	2.89%	61.38%	38.62%	100%
	2664_7	sequence count	2018819	9010	270090	25995	306646	141	0	0	11126	339	8861	6074	316668
		% of total	67.89%	0.35%	10.52%	1.01%	11.94%	0.01%	0.00%	0.00%	0.43%	0.01%	0.35%	0.24%	12.33%
		% by orientation	77.19%	21.68%	10.33%	62.55%	11.72%	0.34%	0.00%	0.00%	0.43%	0.82%	0.34%	14.62%	N/A
		% within location	99.56%	0.44%	91.22%	8.78%	99.95%	0.05%	-	-	97.04%	2.96%	59.33%	40.67%	100%
	2664_8	sequence count	1838485	8266	248659	23778	278350	147	0	0	10142	318	7840	5293	290639
% of total		67.79%	0.32%	9.68%	0.93%	10.84%	0.01%	0.00%	0.00%	0.39%	0.01%	0.31%	0.21%	11.32%	
% by orientation		77.13%	21.87%	10.43%	62.90%	11.68%	0.39%	0.00%	0.00%	0.43%	0.84%	0.33%	14.00%	N/A	
% within location		99.55%	0.45%	91.27%	8.73%	99.95%	0.05%	-	-	96.96%	3.04%	59.70%	40.30%	100%	

			Within Exon + -	Within Exon - +	Within Intron + -	Within Intron - +	Exon Exon + -	Exon Exon - +	Exon Intron + -	Exon Intron - +	Exon Intergenic + -	Exon Intergenic - +	Promoter + -	Promoter - +	Intergenic
FRT-seq library 2	2665_1	sequence count	1872024	8145	230363	21418	298644	167	0	0	9335	311	8541	5737	280811
		% of total	68.43%	0.32%	8.97%	0.83%	11.63%	0.01%	0.00%	0.00%	0.36%	0.01%	0.33%	0.22%	10.93%
		% by orientation	77.39%	22.77%	9.52%	59.86%	12.35%	0.47%	0.00%	0.00%	0.39%	0.87%	0.35%	16.03%	N/A
		% within location	99.57%	0.43%	91.49%	8.51%	99.94%	0.06%	-	-	96.78%	3.22%	59.82%	40.18%	100%
	2665_2	sequence count	1575623	6854	200153	18911	249473	106	0	0	7859	259	6789	4539	236432
		% of total	68.30%	0.27%	7.79%	0.74%	9.71%	0.00%	0.00%	0.00%	0.31%	0.01%	0.26%	0.18%	9.21%
		% by orientation	77.24%	22.35%	9.81%	61.66%	12.23%	0.35%	0.00%	0.00%	0.39%	0.84%	0.33%	14.80%	N/A
		% within location	99.57%	0.43%	91.37%	8.63%	99.96%	0.04%	-	-	96.81%	3.19%	59.93%	40.07%	100%
2665_3	sequence count	1926512	8289	240042	22337	308731	167	0	0	9534	336	8615	5658	288761	
	% of total	68.34%	0.32%	9.35%	0.87%	12.02%	0.01%	0.00%	0.00%	0.37%	0.01%	0.34%	0.22%	11.24%	
	% by orientation	77.26%	22.53%	9.63%	60.72%	12.38%	0.45%	0.00%	0.00%	0.38%	0.91%	0.35%	15.38%	N/A	
	% within location	99.57%	0.43%	91.49%	8.51%	99.95%	0.05%	-	-	96.60%	3.40%	60.36%	39.64%	100%	
2665_5	sequence count	2176465	9505	268101	24845	347591	144	0	0	10653	421	9793	6752	325557	
	% of total	68.45%	0.37%	10.44%	0.97%	13.53%	0.01%	0.00%	0.00%	0.41%	0.02%	0.38%	0.26%	12.68%	
	% by orientation	77.38%	22.81%	9.53%	59.63%	12.36%	0.35%	0.00%	0.00%	0.38%	1.01%	0.35%	16.20%	N/A	
	% within location	99.57%	0.43%	91.52%	8.48%	99.96%	0.04%	-	-	96.20%	3.80%	59.19%	40.81%	100%	
2665_6	sequence count	2226092	9659	272290	25206	357368	182	0	0	10875	378	9963	6819	331166	
	% of total	68.50%	0.38%	10.60%	0.98%	13.91%	0.01%	0.00%	0.00%	0.42%	0.01%	0.39%	0.27%	12.89%	
	% by orientation	77.39%	22.86%	9.47%	59.67%	12.42%	0.43%	0.00%	0.00%	0.38%	0.89%	0.35%	16.14%	N/A	
	% within location	99.57%	0.43%	91.53%	8.47%	99.95%	0.05%	-	-	96.64%	3.36%	59.37%	40.63%	100%	
2665_7	sequence count	2128194	9184	260537	24087	339603	182	0	0	10423	346	9314	6527	314858	
	% of total	68.58%	0.36%	10.14%	0.94%	13.22%	0.01%	0.00%	0.00%	0.41%	0.01%	0.36%	0.25%	12.26%	
	% by orientation	77.44%	22.77%	9.48%	59.73%	12.36%	0.45%	0.00%	0.00%	0.38%	0.86%	0.34%	16.19%	N/A	
	% within location	99.57%	0.43%	91.54%	8.46%	99.95%	0.05%	-	-	96.79%	3.21%	58.80%	41.20%	100%	
2665_8	sequence count	1855899	7851	223763	20696	298774	141	0	0	8994	286	8651	5726	271218	
	% of total	68.69%	0.31%	8.71%	0.81%	11.63%	0.01%	0.00%	0.00%	0.35%	0.01%	0.34%	0.22%	10.56%	
	% by orientation	77.46%	22.63%	9.34%	59.64%	12.47%	0.41%	0.00%	0.00%	0.38%	0.82%	0.36%	16.50%	N/A	
	% within location	99.58%	0.42%	91.53%	8.47%	99.95%	0.05%	-	-	96.92%	3.08%	60.17%	39.83%	100%	

			Within Exon + -	Within Exon - +	Within Intron + -	Within Intron - +	Exon Exon + -	Exon Exon - +	Exon Intron + -	Exon Intron - +	Exon Intergenic + -	Exon Intergenic - +	Promoter + -	Promoter - +	Intergenic
STD RNA-seq library 1	3202_1	sequence count	675885	653835	353482	197559	104193	105737	0	0	4133	3726	6701	6577	914850
		% of total	22.33%	25.46%	13.76%	7.69%	4.06%	4.12%	0.00%	0.00%	0.16%	0.15%	0.26%	0.26%	35.62%
		% by orientation	59.06%	67.58%	30.89%	20.42%	9.10%	10.93%	0.00%	0.00%	0.36%	0.39%	0.59%	0.68%	N/A
		% within location	50.83%	49.17%	64.15%	35.85%	49.63%	50.37%	-	-	52.59%	47.41%	50.47%	49.53%	100%
STD RNA-seq library 1	3202_2	sequence count	730224	698710	373789	206692	114470	114714	0	0	4286	3936	7302	6895	977422
		% of total	22.55%	27.20%	14.55%	8.05%	4.46%	4.47%	0.00%	0.00%	0.17%	0.15%	0.28%	0.27%	38.06%
		% by orientation	59.36%	67.77%	30.39%	20.05%	9.31%	11.13%	0.00%	0.00%	0.35%	0.38%	0.59%	0.67%	N/A
		% within location	51.10%	48.90%	64.39%	35.61%	49.95%	50.05%	-	-	52.13%	47.87%	51.43%	48.57%	100%
STD RNA-seq library 1	3202_3	sequence count	732604	700157	372792	205842	114179	114939	0	0	4564	4025	7112	7126	973916
		% of total	22.63%	27.26%	14.51%	8.01%	4.45%	4.48%	0.00%	0.00%	0.18%	0.16%	0.28%	0.28%	37.92%
		% by orientation	59.50%	67.84%	30.28%	19.94%	9.27%	11.14%	0.00%	0.00%	0.37%	0.39%	0.58%	0.69%	N/A
		% within location	51.13%	48.87%	64.43%	35.57%	49.83%	50.17%	-	-	53.14%	46.86%	49.95%	50.05%	100%
STD RNA-seq library 1	3202_5	sequence count	723154	685887	362188	199917	113014	112991	0	0	4250	3819	7061	6986	948939
		% of total	22.83%	26.70%	14.10%	7.78%	4.40%	4.40%	0.00%	0.00%	0.17%	0.15%	0.27%	0.27%	36.95%
		% by orientation	59.78%	67.94%	29.94%	19.80%	9.34%	11.19%	0.00%	0.00%	0.35%	0.38%	0.58%	0.69%	N/A
		% within location	51.32%	48.68%	64.43%	35.57%	50.01%	49.99%	-	-	52.67%	47.33%	50.27%	49.73%	100%
STD RNA-seq library 2	3202_6	sequence count	935413	887241	188999	98435	174707	167323	0	0	2704	2233	3693	3549	348386
		% of total	33.26%	34.54%	7.36%	3.83%	6.80%	6.51%	0.00%	0.00%	0.11%	0.09%	0.14%	0.14%	13.56%
		% by orientation	71.65%	76.57%	14.48%	8.49%	13.38%	14.44%	0.00%	0.00%	0.21%	0.19%	0.28%	0.31%	N/A
STD RNA-seq library 2	3202_7	sequence count	907102	858297	183118	94266	169591	162759	0	0	2672	2183	3442	3484	337614
		% of total	33.29%	33.42%	7.13%	3.67%	6.60%	6.34%	0.00%	0.00%	0.10%	0.08%	0.13%	0.14%	13.14%
		% by orientation	71.66%	76.57%	14.47%	8.41%	13.40%	14.52%	0.00%	0.00%	0.21%	0.19%	0.27%	0.31%	N/A
STD RNA-seq library 2	3202_8	sequence count	866305	817218	174053	90311	161370	154596	0	0	2561	2138	3315	3212	322450
		% of total	33.35%	31.82%	6.78%	3.52%	6.28%	6.02%	0.00%	0.00%	0.10%	0.08%	0.13%	0.13%	12.55%
		% by orientation	71.74%	76.56%	14.41%	8.46%	13.36%	14.48%	0.00%	0.00%	0.21%	0.20%	0.27%	0.30%	N/A
	% within location	51.46%	48.54%	65.84%	34.16%	51.07%	48.93%	-	-	54.50%	45.50%	50.79%	49.21%	100%	