Supplementary Figures and Tables

Systematic comparison and assessment of RNA-seq procedures for gene expression quantitative analysis

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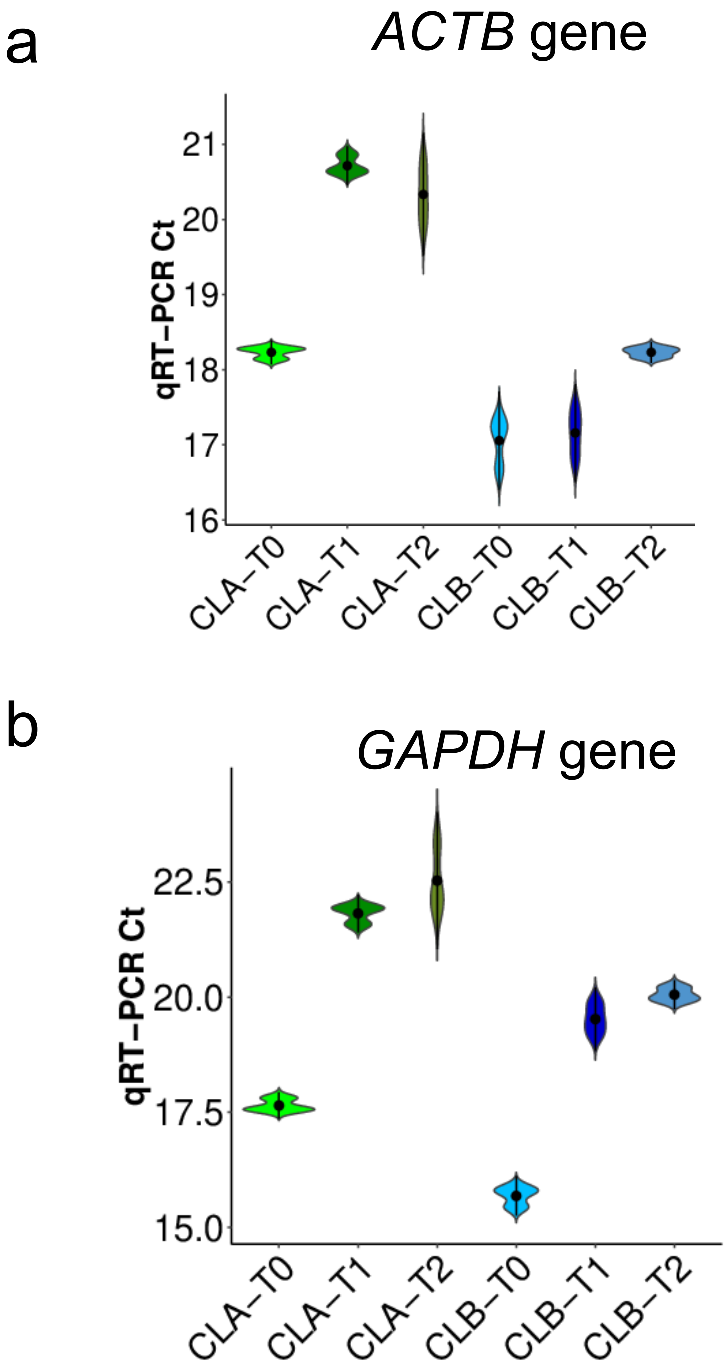
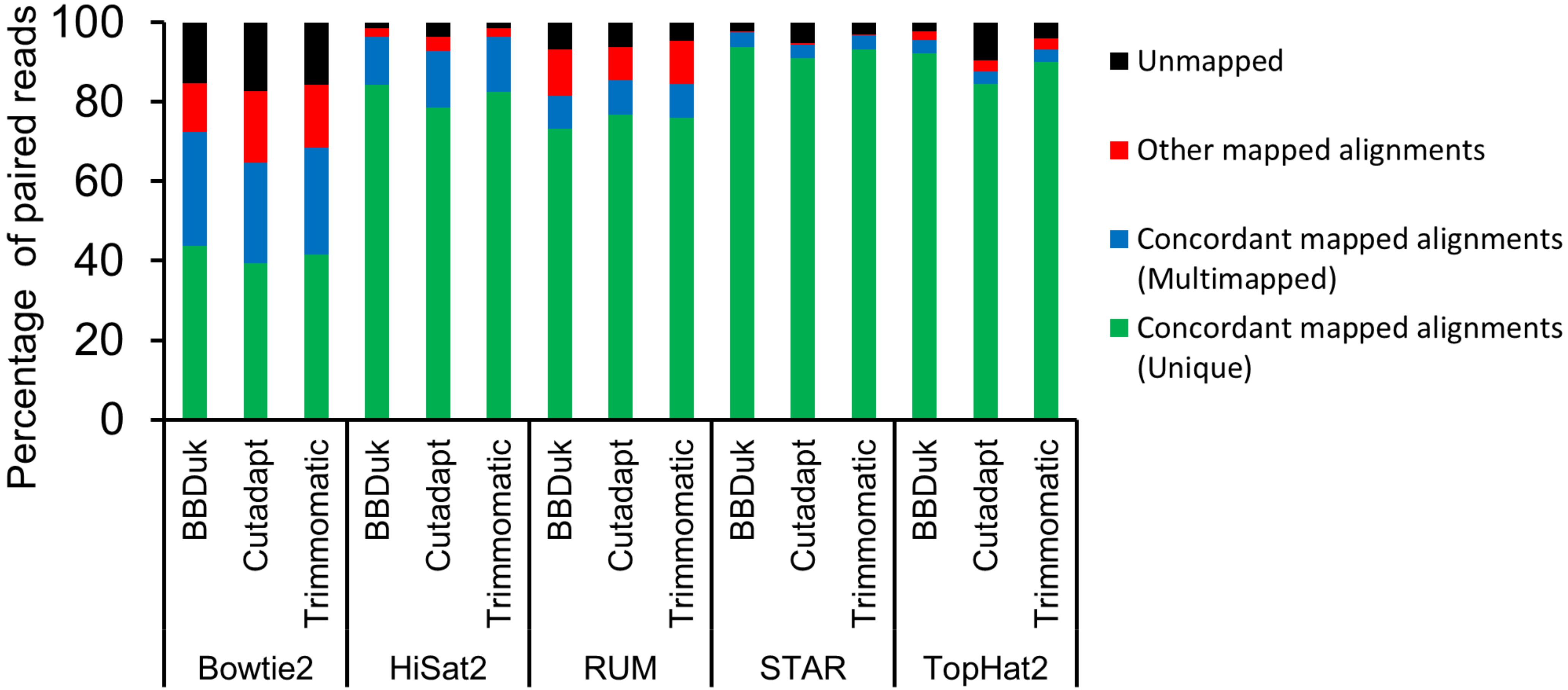
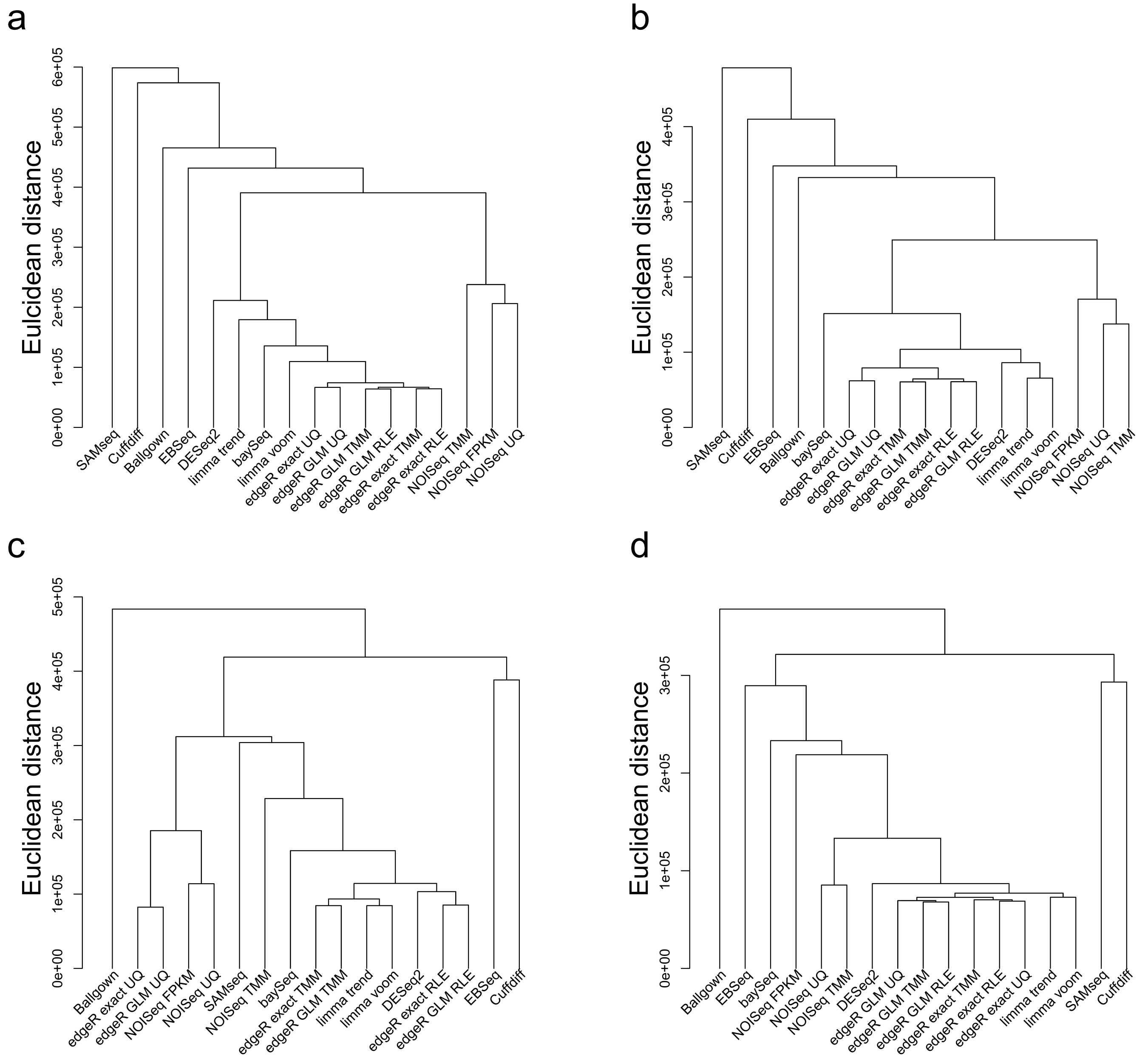
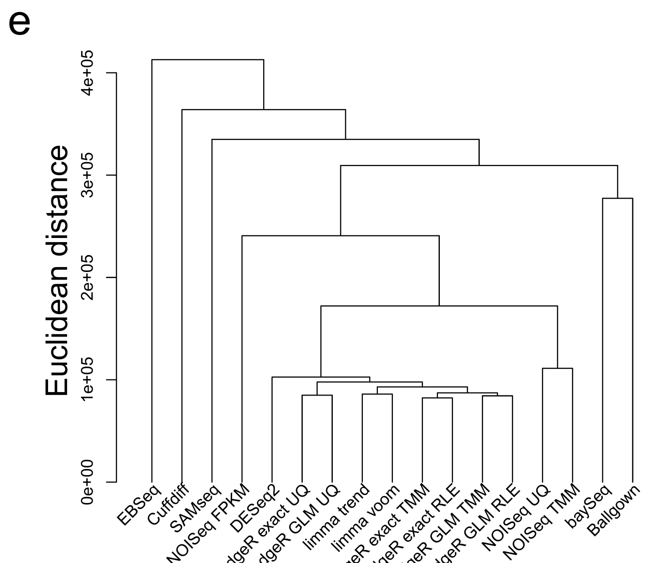


Supplementary Figure S1. qRT-PCR Ct values of the *GAPDH* and *ACTB* genes across all assayed conditions. Violin plots of (**a**) *ACTB* and (**b**) *GAPDH* genes using the Ct values from the qRT-PCR assay considering the KMS12-BM (CLA) and JJN-3 (CLB) cell lines, treated with Amiloride (T1) or TG003 (T2) and controls (T0).

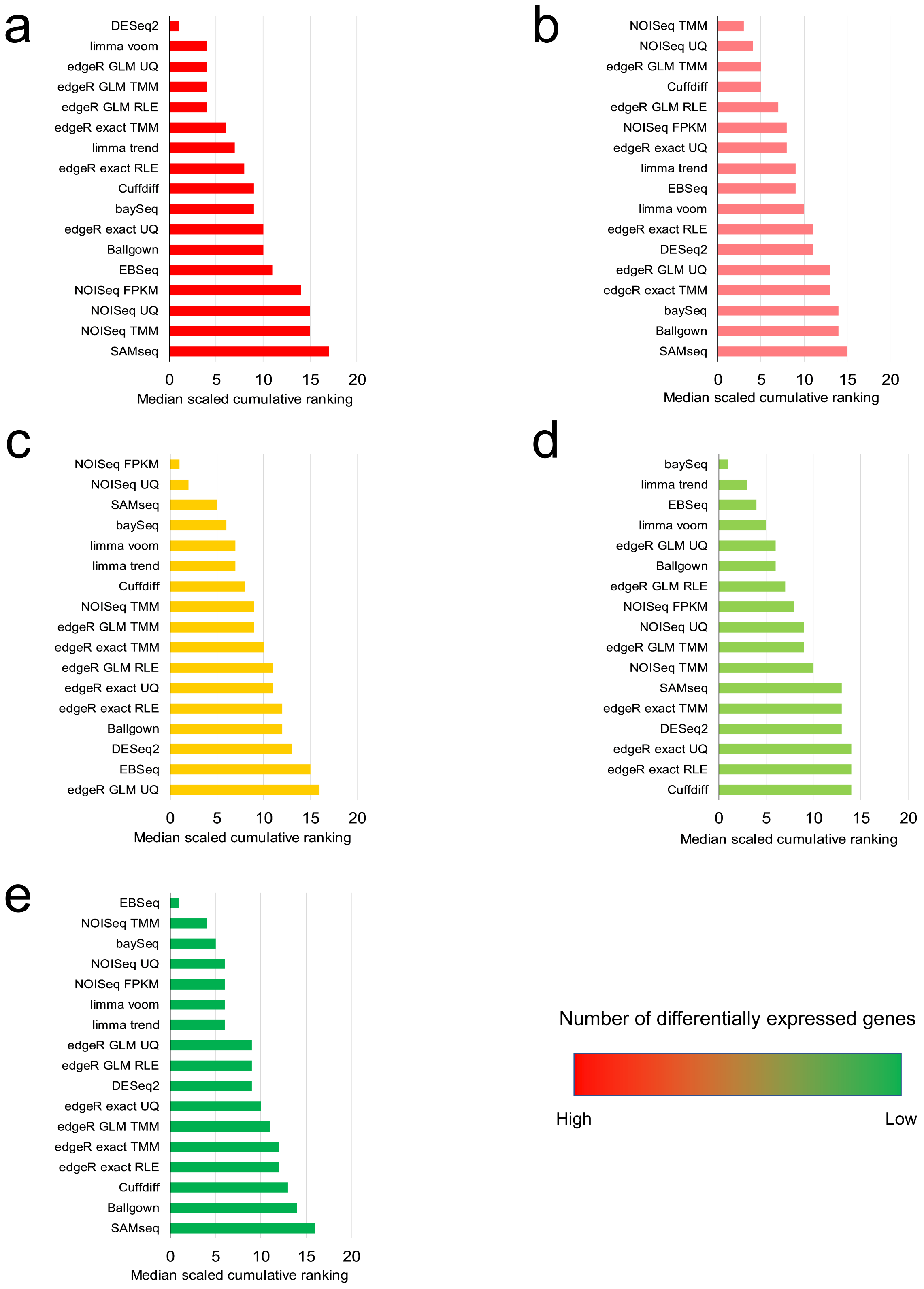


Supplementary Figure S2. Mapping summary of the alignment algorithms. The output of the five alignment methods was analysed considering the previous trimming algorithms (*BBDuk*, *Cutadapt* or *Trimmomatic*). The number of alignments are shown as percentages and are classified into four categories according the alignment type: unmapped alignments, concordant multimapped alignments, concordant uniquely mapped alignments and other alignments.

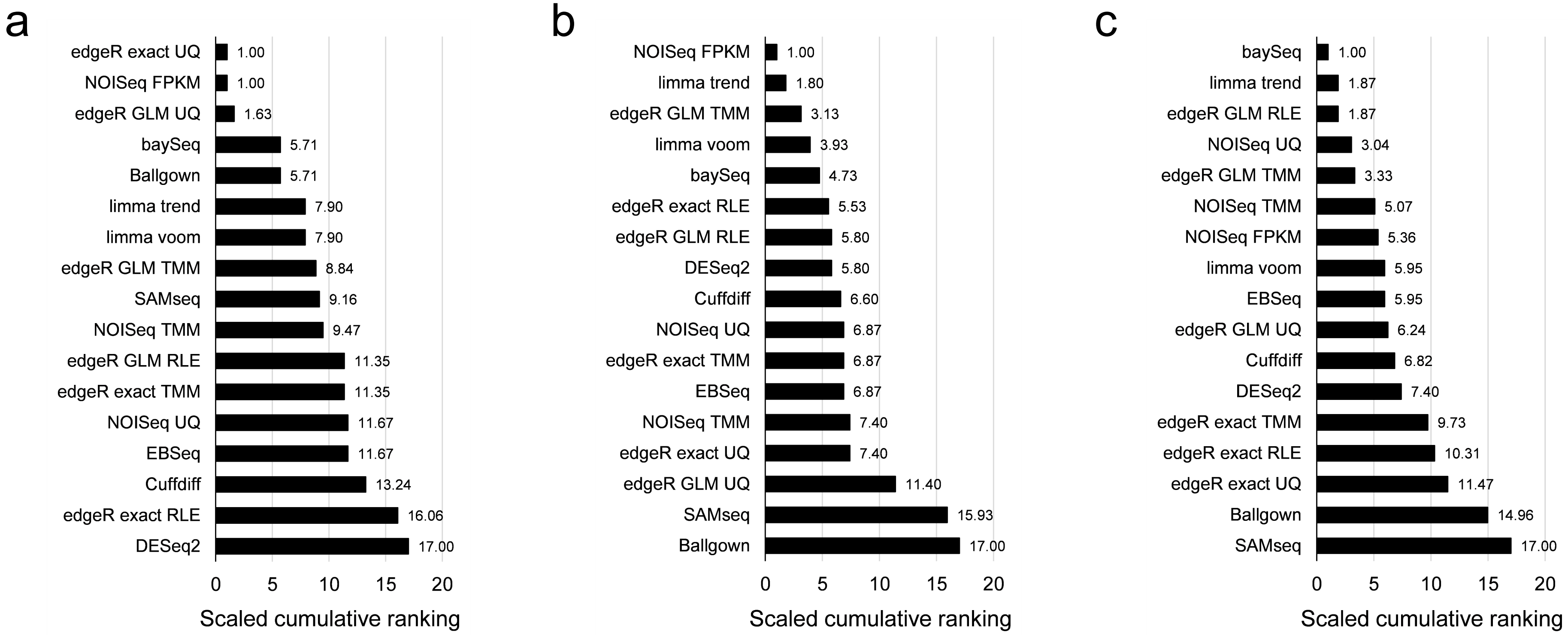




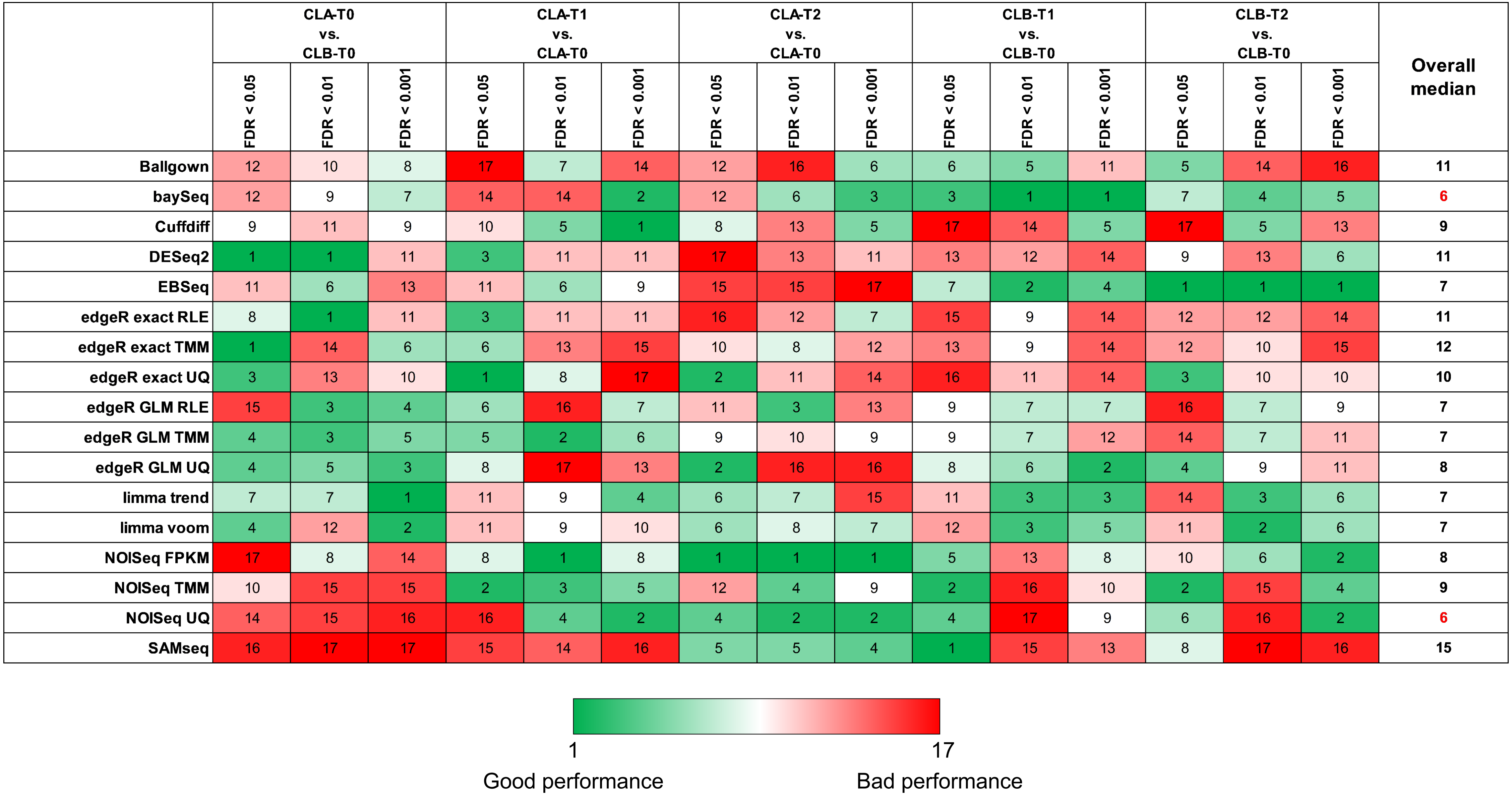
Supplementary Figure S3. Similarity among the 17 RNA-seq differential gene expression detection methods. Each graph represents the Euclidean distance among the 17 methods using dendrograms. (**a**) CLA-T0 vs. CLB-T0, (**b**) CLA-T1 vs. CLA-T0, (**c**) CLA-T2 vs. CLA-T0, (**e**) CLB-T1 vs. CLB-T0 and (**e**) CLB-T2 vs. CLB-T0. CLA = cell line A (KMS12MB); CLB = cell line B (JJN-3); T0 = DMSO; T1 = Amiloride; T2 = TG003



Supplementary Figure S4. Performance of the 17 differential expression methods based on the five contrasts proposed in this work. These five contrasts are defined according to the number of changes in gene expression between the contrasted conditions.(**a**) CLA-T0 vs. CLB-T0, (**b**) CLA-T1 vs. CLA-T0, (**c**) CLA-T2 vs. CLA-T0, (**d**) CLB-T1 vs. CLB-T0 and (**e**) CLB-T2 vs. CLB-T0, where CLA is the KMS12-BM cell line, CLB is the JJN-3 cell line, T0 is DMSO treatment, T1 is Amiloride treatment and T2 is TG003 treatment.

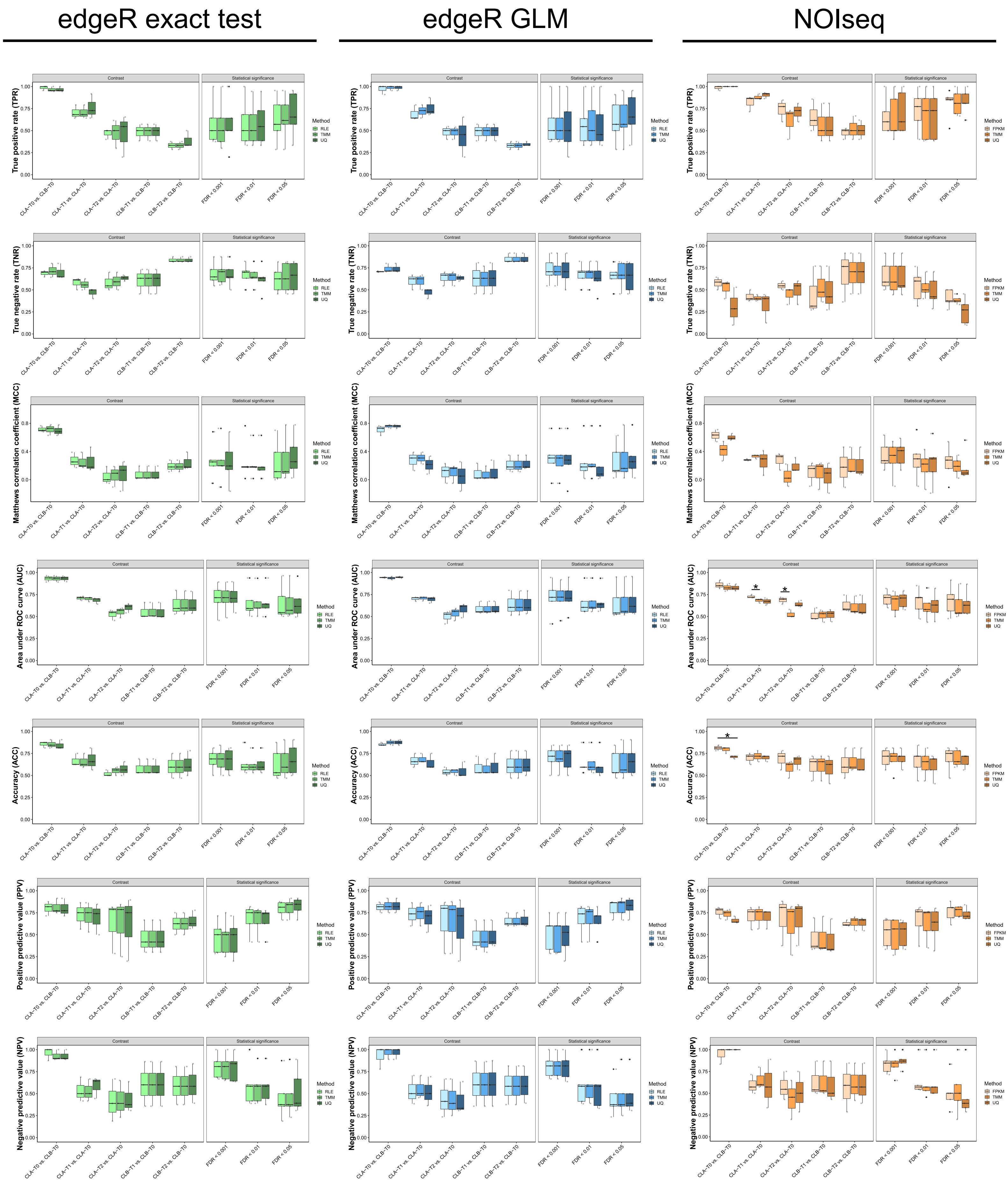


Supplementary Figure S5. Performance of the 17 differential expression methods based on the three FDR cutoffs proposed in this work. (**a**) FDR < 0.05, (**b)** FDR < 0.01 and (**c**) FDR < 0.001.



	CLA-T0 vs. CLB-T0			CLA-T1 vs. CLA-T0			CLA-T2 vs. CLA-T0			CLB-T1 vs. CLB-T0			CLB-T2 vs. CLB-T0			Overall median
	FDR < 0.05	FDR < 0.01	FDR < 0.001	FDR < 0.05	FDR < 0.01	FDR < 0.001	FDR < 0.05	FDR < 0.01	FDR < 0.001	FDR < 0.05	FDR < 0.01	FDR < 0.001	FDR < 0.05	FDR < 0.01	FDR < 0.001	
Ballgown	12	10	8	17	7	14	12	16	6	6	5	11	5	14	16	**11**
baySeq	12	9	7	14	14	2	12	6	3	3	1	1	7	4	5	**6**
Cuffdiff	9	11	9	10	5	1	8	13	5	17	14	5	17	5	13	**9**
DESeq2	1	1	11	3	11	11	17	13	11	13	12	14	9	13	6	**11**
EBSeq	11	6	13	11	6	9	15	15	17	7	2	4	1	1	1	**7**
edgeR exact RLE	8	1	11	3	11	11	16	12	7	15	9	14	12	12	14	**11**
edgeR exact TMM	1	14	6	6	13	15	10	8	12	13	9	14	12	10	15	**12**
edgeR exact UQ	3	13	10	1	8	17	2	11	14	16	11	14	3	10	10	**10**
edgeR GLM RLE	15	3	4	6	16	7	11	3	13	9	7	7	16	7	9	**7**
edgeR GLM TMM	4	3	5	5	2	6	9	10	9	9	7	12	14	7	11	**7**
edgeR GLM UQ	4	5	3	8	17	13	2	16	16	8	6	2	4	9	11	**8**
limma trend	7	7	1	11	9	4	6	7	15	11	3	3	14	3	6	**7**
limma voom	4	12	2	11	9	10	6	8	7	12	3	5	11	2	6	**7**
NOISeq FPKM	17	8	14	8	1	8	1	1	1	5	13	8	10	6	2	**8**
NOISeq TMM	10	15	15	2	3	5	12	4	9	2	16	10	2	15	4	**9**
NOISeq UQ	14	15	16	16	4	2	4	2	2	4	17	9	6	16	2	**6**
SAMseq	16	17	17	15	14	16	5	5	4	1	15	13	8	17	16	**15**

Supplementary Figure S6. Overall performance of the 17 differential expression methods based on the combination of the five contrasts and the three FDR cutoffs proposed in this work. CLA is the KMS12-BM cell line, CLB is the JJN-3 cell line, T0 is DMSO treatment, T1 is Amiloride treatment and T2 is TG003 treatment.



Supplementary Figure S7: Influence of normalization on differentially gene expression detection. Boxplot graphs were performed for the differential expression methods that admitted multiple normalization approaches (edgeR exact test, edgeR GLM and NOIseq) and represent the values of 7 diagnostic test parameters: true positive rate (TPR), true negative rate (TNR), Matthews correlation coefficient (MCC), accuracy (ACC), area under the ROC curve (AUC), positive predictive value (PPV) and negative predictive value. These parameters were measured at three FDR cut-off levels and five experimental contrasts, which are the result of the combination of two cell lines (CLA and CLB) with two treatment options (T1 and T2) and DMSO (T0).The asterisks indicate the significance of the post-hoc Dunn's test: * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. Comparisons without asterisk are statistically insignificant ($p > 0.05$).

Supplementary Table S1. Initial number of reads for each of the analysed samples.

Sample	Input paired reads	Reads Q > 20		
		R1	**R2**	**Mean R1 R2**
CLA-T0-S1	50,946,237	50,042,477	49,158,906	49,600,691.5
CLA-T0-S2	62,571,514	60,745,925	59,593,922	60,169,923.5
CLA-T0-S3	77,906,369	75,745,249	74,632,834	75,189,041.5
CLB-T0-S1	46,810,430	46,097,180	45,536,869	45,817,024.5
CLB-T0-S2	60,376,359	58,310,725	57,124,153	57,717,439
CLB-T0-S3	40,840,185	39,720,525	39,264,085	39,492,305
CLA-T1-S1	60,733,681	58,485,539	56,777,315	57,631,427
CLA-T1-S2	66,024,375	64,206,003	63,222,987	63,714,495
CLA-T1-S3	45,598,113	45,138,641	44,429,554	44,784,097.5
CLB-T1-S1	37,568,155	36,970,927	36,711,895	36,841,411
CLB-T1-S2	58,656,767	57,180,047	56,287,686	56,733,866.5
CLB-T1-S3	64,447,257	62,685,537	62,013,794	62,349,665.5
CLA-T2-S1	48,670,462	46,934,263	45,967,599	46,450,931
CLA-T2-S2	40,156,676	39,052,670	38,459,170	38,755,920
CLA-T2-S3	49,142,091	48,615,422	47,902,731	48,259,076.5
CLB-T2-S1	36,240,231	35,637,569	34,837,372	35,237,470.5
CLB-T2-S2	59,666,947	57,558,513	56,118,366	56,838,439.5
CLB-T2-S3	41,733,924	40,510,376	39,886,662	40,198,519

Supplementary Table S2. List of the 107 genes selected for the pipeline precision analysis and the 32 genes selected (highlighted genes) for the pipeline accuracy analysis.

Selected for qRT-PCR?	Ensembl ID	HGNC symbol	Chromosome	Gene type	Median MAD ranking
Selected	ENSG00000100138	NHP2L1	22	protein coding	7
Selected	ENSG00000105193	RPS16	19	protein coding	7
Selected	ENSG00000068697	LAPTM4A	2	protein coding	8
Selected	ENSG00000127884	ECHS1	10	protein coding	10
Selected	ENSG00000101361	NOP56	20	protein coding	14
Selected	ENSG00000165502	RPL36AL	14	protein coding	14.5
Selected	ENSG00000154723	ATP5J	21	protein coding	16
Selected	ENSG00000143158	MPC2	1	protein coding	16
Selected	ENSG00000138279	ANXA7	10	protein coding	17
Selected	ENSG00000107223	EDF1	9	protein coding	17
NO	ENSG00000163131	CTSS	1	protein coding	17.5
NO	ENSG00000156467	UQCRB	8	protein coding	18.5
NO	ENSG00000130255	RPL36	19	protein coding	19
NO	ENSG00000178913	TAF7	5	protein coding	20
NO	ENSG00000198961	PJA2	5	protein coding	20.5
NO	ENSG00000092820	EZR	6	protein coding	21
NO	ENSG00000114391	RPL24	3	protein coding	21
Selected	ENSG00000142541	RPL13A	19	protein coding	22
NO	ENSG00000164587	RPS14	5	protein coding	22
NO	ENSG00000156482	RPL30	8	protein coding	23
NO	ENSG00000151366	NDUFC2	11	protein coding	24
NO	ENSG00000164919	COX6C	8	protein coding	24.5
NO	ENSG00000172115	CYCS	7	protein coding	25.5
NO	ENSG00000100348	TXN2	22	protein coding	26
Selected	ENSG00000147677	EIF3H	8	protein coding	28
NO	ENSG00000183978	COA3	17	protein coding	29
NO	ENSG00000125743	SNRPD2	19	protein coding	29
NO	ENSG00000265681	RPL17	18	protein coding	30
NO	ENSG00000169976	SF3B5	6	protein coding	30
NO	ENSG00000198242	RPL23A	17	protein coding	31
NO	ENSG00000178952	TUFM	16	protein coding	33
NO	ENSG00000122566	HNRNPA2B1	7	protein coding	34
NO	ENSG00000108107	RPL28	19	protein coding	34
Selected	ENSG00000166710	B2M	15	protein coding	35
NO	ENSG00000170144	HNRNPA3	2	protein coding	35
NO	ENSG00000196776	CD47	3	protein coding	36
NO	ENSG00000099795	NDUFB7	19	protein coding	37
NO	ENSG00000177700	POLR2L	11	protein coding	37
NO	ENSG00000117614	SYF2	1	protein coding	38
NO	ENSG00000115053	NCL	2	protein coding	39
Selected	ENSG00000161016	RPL8	8	protein coding	39
NO	ENSG00000165119	HNRNPK	9	protein coding	40
NO	ENSG00000244038	DDOST	1	protein coding	42
NO	ENSG00000145592	RPL37	5	protein coding	42
NO	ENSG00000147604	RPL7	8	protein coding	43
NO	ENSG00000105583	WDR83OS	19	protein coding	43
NO	ENSG00000164032	H2AFZ	4	protein coding	44
NO	ENSG00000088832	FKBP1A	20	protein coding	45
NO	ENSG00000070756	PABPC1	8	protein coding	45
NO	ENSG00000115128	SF3B14	2	protein coding	46.5
Selected	ENSG00000131495	NDUFA2	5	protein coding	48
NO	ENSG00000096384	HSP90AB1	6	protein coding	50

NO	ENSG00000169714	CNBP	3	protein coding	51
NO	ENSG00000114850	SSR3	3	protein coding	54
NO	ENSG00000118418	HMGN3	6	protein coding	55
NO	ENSG00000153187	HNRNPU	1	protein coding	56
NO	ENSG00000143162	CREG1	1	protein coding	57
NO	ENSG00000144713	RPL32	3	protein coding	57
NO	ENSG00000125835	SNRPB	20	protein coding	57
NO	ENSG00000143106	PSMA5	1	protein coding	59
Selected	ENSG00000142937	RPS8	1	protein coding	59
NO	ENSG00000135486	HNRNPA1	12	protein coding	61
NO	ENSG00000132646	PCNA	20	protein coding	61
NO	ENSG00000174444	RPL4	15	protein coding	64
NO	ENSG00000154518	ATP5G3	2	protein coding	66
NO	ENSG00000181163	NPM1	5	protein coding	66
NO	ENSG00000188612	SUMO2	17	protein coding	66
Selected	ENSG00000166337	TAF10	11	protein coding	66
NO	ENSG00000170027	YWHAG	7	protein coding	67.5
NO	ENSG00000143612	C1orf43	1	protein coding	68
NO	ENSG00000239264	TXNDC5	6	protein coding	68
NO	ENSG00000164096	C4orf3	4	protein coding	72
NO	ENSG00000152795	HNRNPDL	4	protein coding	74
NO	ENSG00000134825	TMEM258	11	protein coding	74
NO	ENSG00000115484	CCT4	2	protein coding	75
NO	ENSG00000142864	SERBP1	1	protein coding	76
Selected	ENSG00000107581	EIF3A	10	protein coding	78
NO	ENSG00000116251	RPL22	1	protein coding	78
NO	ENSG00000103363	TCEB2	16	protein coding	78
NO	ENSG00000134884	ARGLU1	13	protein coding	79
NO	ENSG00000185043	CIB1	15	protein coding	81
NO	ENSG00000166441	RPL27A	11	protein coding	81
NO	ENSG00000069275	NUCKS1	1	protein coding	82
NO	ENSG00000170348	TMED10	14	protein coding	82
NO	ENSG00000108518	PFN1	17	protein coding	83
NO	ENSG00000111897	SERINC1	6	protein coding	83
Selected	ENSG00000008018	PSMB1	6	protein coding	86
NO	ENSG00000196262	PPIA	7	protein coding	87
NO	ENSG00000137154	RPS6	9	protein coding	88
NO	ENSG00000111639	MRPL51	12	protein coding	89
NO	ENSG00000173113	TRMT112	11	protein coding	89
NO	ENSG00000152518	ZFP36L2	2	protein coding	89
NO	ENSG00000106153	CHCHD2	7	protein coding	93
NO	ENSG00000124767	GLO1	6	protein coding	93
Selected	ENSG00000178741	COX5A	15	protein coding	94
NO	ENSG00000133639	BTG1	12	protein coding	95.5
NO	ENSG00000166562	SEC11C	18	protein coding	96
Selected	ENSG00000044574	HSPA5	9	protein coding	97
Selected	ENSG00000115758	ODC1	2	protein coding	98
Selected	ENSG00000182117	NOP10	15	protein coding	100
Selected	ENSG00000119421	NDUFA8	9	protein coding	101
Selected	ENSG00000168264	IRF2BP2	1	protein coding	102
Selected	ENSG00000163956	LRPAP1	4	protein coding	102
Selected	ENSG00000065978	YBX1	1	protein coding	104
Selected	ENSG00000105968	H2AFV	7	protein coding	105
Selected	ENSG00000105887	MTPN	7	protein coding	106
Selected	ENSG00000175130	MARCKSL1	1	protein coding	107
Selected	**ENSG0000007562**	**ACTB**	**7**	**Housekeeping gene**	

Selected	**ENSG00000111640**	**GAPDH**	**12**	**Housekeeping gene**	

*Supplementary Table S2 legend:

Genes selected by the median absolute deviation (MAD) among all control samples:	**Low MAD**
	High MAD
Randomly selected genes	
Selected Housekeeping genes	

Suplementary Table S3. Oligonucleotide probes used for the TaqMan qRT-PCR assay.

Ensembl ID	HGNC ID	Chromosome	TaqMan Gene Expression ID assay	Source	Identifier
ENSG00000008018	PSMB1	6	Hs00427357_m1	ThermoFisher	Cat#4448892
ENSG00000044574	HSPA5	9	Hs00607129_gH	ThermoFisher	Cat#4448892
ENSG00000065978	YBX1	1	Hs00358903_g1	ThermoFisher	Cat#4448892
ENSG00000068697	LAPTM4A	2	Hs01092025_m1	ThermoFisher	Cat#4448892
ENSG0000007562	ACTB	7	Hs01060665_g1	ThermoFisher	Cat#4448892
ENSG00000100138	NHP2L1 (SNU13)	22	Hs03025442_s1	ThermoFisher	Cat#4448892
ENSG00000101361	NOP56	20	Hs00197340_m1	ThermoFisher	Cat#4448892
ENSG00000105193	RPS16	19	Hs01598518_gH	ThermoFisher	Cat#4448892
ENSG00000105887	MTPN	7	Hs00377581_m1	ThermoFisher	Cat#4448892
ENSG00000105968	H2AFV	7	Hs00606542_mH	ThermoFisher	Cat#4448892
ENSG00000107223	EDF1	9	Hs00610152_m1	ThermoFisher	Cat#4448892
ENSG00000107581	EIF3A	10	Hs01025769_m1	ThermoFisher	Cat#4448892
ENSG00000111640	GAPDH	12	Hs02786624_g1	ThermoFisher	Cat#4453320
ENSG00000115758	ODC1	2	Hs00159739_m1	ThermoFisher	Cat#4453320
ENSG00000119421	NDUFA8	9	Hs00204417_m1	ThermoFisher	Cat#4448892
ENSG00000127884	ECHS1	10	Hs00187943_m1	ThermoFisher	Cat#4448892
ENSG00000131495	NDUFA2	5	Hs04187282_g1	ThermoFisher	Cat#4448892
ENSG00000138279	ANXA7	10	Hs00559410_m1	ThermoFisher	Cat#4448892
ENSG00000142541	RPL13A	19	Hs04194366_g1	ThermoFisher	Cat#4448892
ENSG00000142937	RPS8	1	Hs01374307_g1	ThermoFisher	Cat#4448892
ENSG00000143158	MPC2	1	Hs00967250_m1	ThermoFisher	Cat#4448892
ENSG00000147677	EIF3H	8	Hs00186779_m1	ThermoFisher	Cat#4448892
ENSG00000154723	ATP5J	21	Hs01081389_g1	ThermoFisher	Cat#4448892
ENSG00000161016	RPL8	8	Hs00361285_g1	ThermoFisher	Cat#4453320
ENSG00000163956	LRPAP1	4	Hs00936301_m1	ThermoFisher	Cat#4448892
ENSG00000165502	RPL36AL	14	Hs00733231_m1	ThermoFisher	Cat#4448892
ENSG00000166337	TAF10	11	Hs00359540_g1	ThermoFisher	Cat#4448892
ENSG00000166710	B2M	15	Hs00187842_m1	ThermoFisher	Cat#4453320
ENSG00000168264	IRF2BP2	1	Hs02930738_m1	ThermoFisher	Cat#4448892
ENSG00000175130	MARCKSL1	1	Hs00702769_s1	ThermoFisher	Cat#4448892
ENSG00000178741	COX5A	15	Hs00362067_m1	ThermoFisher	Cat#4448892
ENSG00000182117	NOP10	15	Hs00430282_m1	ThermoFisher	Cat#4448892

Supplementary Table S4. Precision analysis results

Median precision (cell lines A and B)						
RANK position	**Median RANK**	**MAD**	**Trimming method**	**Alignment method**	**Counting method**	**Normalization method**
1	48.5	0.5	Cutadapt	Salmon-QMB-AUT		TPM
2	49	0	Cutadapt	Salmon-FMD-AUT		TPM
3	49	1	Cutadapt	Salmon-FMD-ISR		TPM
4	50	1	Cutadapt	Salmon-QMB-ISR		TPM
5	50.5	0.5	Trimmomatic	Salmon-QMB-AUT		TPM
6	51.5	1.5	Cutadapt	Bowtie2	RSEM	TPM
7	51.5	4.5	BBDuk	Salmon-FMD-ISR		TPM
8	53.5	1.5	Trimmomatic	Salmon-QMB-ISR		TPM
9	53.5	4.5	Trimmomatic	Salmon-FMD-AUT		TPM
10	53.5	7.5	BBDuk	Salmon-FMD-AUT		TPM
11	54	3	Cutadapt	TopHat2	HTSeq INTER	TMM
12	54	4	BBDuk	Sailfish-ISR		TPM
13	54	6	Trimmomatic	STAR	RSEM	TPM
14	54.5	2.5	BBDuk	Salmon-QMB-AUT		TPM
15	55	3	Trimmomatic	Salmon-FMD-ISR		TPM
16	55	4	Trimmomatic	Bowtie2	RSEM	TPM
17	55	5	BBDuk	Salmon-QMB-ISR		TPM
18	56.5	2.5	Cutadapt	Sailfish-ISR		TPM
19	56.5	3.5	Cutadapt	Kallisto		TPM
20	56.5	4.5	BBDuk	Bowtie2	RSEM	TPM
21	57	5	Cutadapt	STAR	RSEM	TPM
22	58	10	BBDuk	Kallisto		TPM
23	58.5	7.5	BBDuk	TopHat2	HTSeq INTER	RLE
24	60.5	5.5	BBDuk	TopHat2	HTSeq UNION	RLE
25	61	1	BBDuk	STAR	Stringtie	FPKM
26	61	4	BBDuk	STAR	RSEM	TPM
27	61.5	0.5	Trimmomatic	STAR	Stringtie	FPKM
28	61.5	2.5	Trimmomatic	TopHat2	Cufflinks	FPKM
29	61.5	8.5	Trimmomatic	TopHat2	HTSeq INTER	RLE
30	62	2	Cutadapt	HiSat2	HTSeq UNION	TMM
31	62	4	Cutadapt	STAR	Stringtie	FPKM
32	62	4	Cutadapt	TopHat2	HTSeq UNION	RLE
33	62.5	7.5	Trimmomatic	TopHat2	HTSeq UNION	RLE
34	62.5	7.5	Trimmomatic	Kallisto		TPM
35	62.5	9.5	BBDuk	TopHat2	HTSeq INTER	TMM
36	62.5	12.5	Cutadapt	TopHat2	HTSeq UNION	TMM
37	63	0	Cutadapt	TopHat2	Cufflinks	FPKM
38	63	3	Cutadapt	HiSat2	HTSeq UNION	RLE
39	63	6	BBDuk	HiSat2	HTSeq INTER	TMM
40	63	8	Cutadapt	HiSat2	HTSeq INTER	RLE

41	63	11	Trimmomatic	HiSat2	HTSeq INTER	RLE
42	63.5	4.5	Trimmomatic	STAR	HTSeq INTER	TMM
43	63.5	5.5	Trimmomatic	RUM	HTSeq UNION	RLE
44	63.5	10.5	BBDuk	HiSat2	HTSeq UNION	TMM
45	63.5	12.5	BBDuk	TopHat2	HTSeq UNION	TMM
46	64	1	Trimmomatic	Bowtie2	RSEM	FPKM
47	64	2	BBDuk	STAR	Stringtie	TPM
48	64	3	BBDuk	STAR	Cufflinks	FPKM
49	64	9	Cutadapt	TopHat2	HTSeq INTER	RLE
50	64	10	BBDuk	HiSat2	HTSeq INTER	RLE
51	64.5	5.5	Trimmomatic	HiSat2	HTSeq INTER	TMM
52	64.5	7.5	BBDuk	STAR	HTSeq INTER	RLE
53	65	3	Cutadapt	Bowtie2	RSEM	FPKM
54	65	5	BBDuk	Bowtie2	RSEM	FPKM
55	65	6	BBDuk	HiSat2	HTSeq UNION	RLE
56	65	7	Trimmomatic	HiSat2	HTSeq UNION	RLE
57	65	11	Trimmomatic	Sailfish-ISR		TPM
58	65.5	0.5	Trimmomatic	STAR	RSEM	FPKM
59	65.5	1.5	BBDuk	HiSat2	Cufflinks	FPKM
60	65.5	6.5	Trimmomatic	HiSat2	HTSeq UNION	TMM
61	65.5	9.5	Trimmomatic	TopHat2	HTSeq INTER	TMM
62	66	1	Cutadapt	STAR	RSEM	FPKM
63	66	9	Trimmomatic	RUM	HTSeq INTER	RLE
64	66.5	2.5	BBDuk	TopHat2	Cufflinks	FPKM
65	67.5	0.5	BBDuk	STAR	RSEM	FPKM
66	67.5	4.5	Cutadapt	STAR	Stringtie	TPM
67	67.5	5.5	Cutadapt	STAR	HTSeq UNION	TMM
68	67.5	12.5	Trimmomatic	STAR	Stringtie	TPM
69	68	3	Trimmomatic	STAR	Cufflinks	FPKM
70	68	3	BBDuk	STAR	HTSeq UNION	RLE
71	68	7	BBDuk	STAR	HTSeq UNION	TMM
72	68.5	7.5	Trimmomatic	RUM	HTSeq INTER	TMM
73	68.5	8.5	Trimmomatic	RUM	HTSeq UNION	TMM
74	69	8	Trimmomatic	STAR	HTSeq UNION	TMM
75	69	9	Trimmomatic	TopHat2	HTSeq UNION	TMM
76	70	1	Trimmomatic	HiSat2	Cufflinks	FPKM
77	70	10	BBDuk	RUM	HTSeq UNION	TMM
78	70.5	9.5	Cutadapt	STAR	Cufflinks	FPKM
79	71	6	Trimmomatic	STAR	HTSeq INTER	RLE
80	71	8	BBDuk	STAR	HTSeq INTER	TMM
81	72	13	Cutadapt	RUM	Cufflinks	FPKM
82	72.5	9.5	Trimmomatic	RUM	Cufflinks	FPKM
83	73	5	BBDuk	RUM	HTSeq INTER	TMM
84	73.5	20.5	Cutadapt	STAR	HTSeq INTER	TMM
85	74	4	Cutadapt	HiSat2	Cufflinks	FPKM

86	74	11	Trimmomatic	STAR	HTSeq UNION	RLE
87	75	2	Cutadapt	HiSat2	HTSeq INTER	TMM
88	75.5	14.5	BBDuk	TopHat2	Stringtie	TPM
89	76	0	BBDuk	TopHat2	Stringtie	FPKM
90	76	1	Cutadapt	TopHat2	Stringtie	FPKM
91	76.5	6.5	Cutadapt	TopHat2	Stringtie	TPM
92	78.5	2.5	Trimmomatic	TopHat2	Stringtie	FPKM
93	78.5	8.5	Trimmomatic	TopHat2	Stringtie	TPM
94	80	2	BBDuk	RUM	HTSeq UNION	RLE
95	80.5	19.5	BBDuk	RUM	Cufflinks	FPKM
96	82	0	BBDuk	HiSat2	Stringtie	FPKM
97	82	0	BBDuk	RUM	HTSeq INTER	RLE
98	82.5	9.5	Cutadapt	STAR	HTSeq INTER	RLE
99	84.5	1.5	BBDuk	RUM	Stringtie	FPKM
100	84.5	3.5	BBDuk	HiSat2	Stringtie	TPM
101	84.5	9.5	Cutadapt	RUM	Stringtie	TPM
102	85	6	Cutadapt	RUM	HTSeq INTER	TMM
103	85	11	Cutadapt	STAR	HTSeq UNION	RLE
104	85.5	1.5	BBDuk	RUM	Stringtie	TPM
105	86	6	Cutadapt	RUM	HTSeq UNION	TMM
106	86.5	2.5	Trimmomatic	RUM	Stringtie	FPKM
107	87	4	Cutadapt	HiSat2	Stringtie	FPKM
108	87.5	2.5	Cutadapt	RUM	HTSeq INTER	RLE
109	87.5	4.5	Cutadapt	RUM	HTSeq UNION	RLE
110	87.5	4.5	Cutadapt	RUM	Stringtie	FPKM
111	88	5	Trimmomatic	HiSat2	Stringtie	FPKM
112	88.5	3.5	Cutadapt	HiSat2	Stringtie	TPM
113	90	4	Trimmomatic	HiSat2	Stringtie	TPM
114	90	4	Trimmomatic	RUM	Stringtie	TPM
115	102.5	8.5	Cutadapt	STAR	eXpress	TPM
116	103.5	6.5	Cutadapt	STAR	eXpress	FPKM
117	109	2	BBDuk	STAR	eXpress	FPKM
118	109.5	0.5	Trimmomatic	STAR	eXpress	TPM
119	112.5	2.5	Trimmomatic	STAR	eXpress	FPKM
120	113	3	BBDuk	STAR	eXpress	TPM
121	113.5	9.5	Cutadapt	Bowtie2	eXpress	FPKM
122	114	6	Cutadapt	Bowtie2	eXpress	TPM
123	118.5	3.5	BBDuk	Bowtie2	eXpress	TPM
124	118.5	4.5	BBDuk	Bowtie2	eXpress	FPKM
125	119	4	Trimmomatic	Bowtie2	eXpress	TPM
126	120.5	0.5	Trimmomatic	Bowtie2	eXpress	FPKM
127	139.5	11.5	BBDuk	RUM	Stringtie	Coverage
128	140	6	BBDuk	STAR	eXpress	Eff_Counts
129	140	10	Trimmomatic	HiSat2	HTSeq INTER	Raw
130	141	3	BBDuk	HiSat2	Stringtie	Coverage

131	141	11	Trimmomatic	HiSat2	HTSeq UNION	Raw
132	141	14	Trimmomatic	RUM	Stringtie	Coverage
133	141.5	2.5	BBDuk	TopHat2	Stringtie	Coverage
134	141.5	5.5	BBDuk	STAR	eXpress	Est_Count
135	142	10	BBDuk	Bowtie2	eXpress	Eff_Counts
136	143	6	BBDuk	HiSat2	HTSeq INTER	Raw
137	143	10	BBDuk	Salmon-FMD-ISR		NumReads
138	143	11	BBDuk	Salmon-FMD-AUT		NumReads
139	143.5	0.5	BBDuk	STAR	Stringtie	Coverage
140	143.5	7.5	BBDuk	HiSat2	HTSeq UNION	Raw
141	144.5	3.5	BBDuk	Bowtie2	eXpress	Est_Count
142	145	3	BBDuk	Kallisto		Est-Counts
143	145.5	18.5	Trimmomatic	Sailfish-ISR		NumReads
144	146	4	BBDuk	STAR	HTSeq INTER	Raw
145	146	5	BBDuk	Salmon-QMB-AUT		NumReads
146	146	9	Trimmomatic	STAR	eXpress	Eff_Counts
147	146	13	Trimmomatic	HiSat2	Stringtie	Coverage
148	146.5	3.5	BBDuk	Sailfish-ISR		NumReads
149	146.5	4.5	BBDuk	TopHat2	HTSeq INTER	Raw
150	147	4	BBDuk	Salmon-QMB-ISR		NumReads
151	147	7	BBDuk	TopHat2	HTSeq UNION	Raw
152	147.5	5.5	BBDuk	STAR	HTSeq UNION	Raw
153	147.5	13.5	Trimmomatic	RUM	HTSeq INTER	Raw
154	148.5	16.5	Trimmomatic	TopHat2	Stringtie	Coverage
155	149	6	Trimmomatic	STAR	eXpress	Est_Count
156	149	13	Trimmomatic	RUM	HTSeq UNION	Raw
157	151	9	Trimmomatic	Salmon-FMD-ISR		NumReads
158	151.5	8.5	Trimmomatic	Salmon-FMD-AUT		NumReads
159	151.5	8.5	BBDuk	RUM	HTSeq INTER	Raw
160	154	11	Trimmomatic	Kallisto		Est-Counts
161	154.5	7.5	BBDuk	RUM	HTSeq UNION	Raw
162	154.5	9.5	Trimmomatic	Salmon-QMB-ISR		NumReads
163	155	11	Trimmomatic	TopHat2	HTSeq INTER	Raw
164	155.5	12.5	Trimmomatic	STAR	HTSeq INTER	Raw
165	156	13	Trimmomatic	STAR	Stringtie	Coverage
166	156.5	2.5	Cutadapt	STAR	eXpress	Est_Count
167	157	9	Trimmomatic	TopHat2	HTSeq UNION	Raw
168	157	12	Trimmomatic	STAR	HTSeq UNION	Raw
169	157.5	0.5	Trimmomatic	Bowtie2	eXpress	Eff_Counts
170	159.5	0.5	Cutadapt	Kallisto		Est-Counts
171	160	8	Cutadapt	Salmon-FMD-AUT		NumReads
172	160.5	7.5	Cutadapt	Salmon-FMD-ISR		NumReads
173	162	3	Cutadapt	Sailfish-ISR		NumReads
174	162.5	4.5	Cutadapt	Salmon-QMB-AUT		NumReads

175	162.5	4.5	Cutadapt	Salmon-QMB-ISR		NumReads
176	162.5	6.5	Cutadapt	HiSat2	HTSeq INTER	Raw
177	163	7	Cutadapt	HiSat2	HTSeq UNION	Raw
178	163.5	5.5	Trimmomatic	Bowtie2	eXpress	Est_Count
179	164	7	Cutadapt	STAR	eXpress	Eff_Counts
180	164.5	0.5	Cutadapt	Bowtie2	eXpress	Est_Count
181	165	5	Cutadapt	RUM	Stringtie	Coverage
182	167.5	0.5	Cutadapt	HiSat2	Stringtie	Coverage
183	167.5	2.5	Cutadapt	TopHat2	Stringtie	Coverage
184	169.5	0.5	Cutadapt	RUM	HTSeq INTER	Raw
185	169.5	1.5	Cutadapt	STAR	Stringtie	Coverage
186	169.5	3.5	Cutadapt	Bowtie2	eXpress	Eff_Counts
187	170.5	4.5	Cutadapt	TopHat2	HTSeq INTER	Raw
188	171	1	Cutadapt	RUM	HTSeq UNION	Raw
189	172.5	0.5	Cutadapt	STAR	HTSeq INTER	Raw
190	173	1	Cutadapt	STAR	HTSeq UNION	Raw
191	173.5	4.5	Cutadapt	TopHat2	HTSeq UNION	Raw
192	177	12	Trimmomatic	Salmon-QMB-AUT		NumReads

Supplementary Table S5. Accuracy analysis results.

Median accuracy (cell lines A and B)

RANK position	Median RANK	MAD	Trimming method	Alignment method	Counting method	Normalization method
1	27.5	18.5	Trimmomatic	Sailfish-ISR		NumReads
2	56	18.5	Trimmomatic	RUM	HTSeq UNION	TMM
3	56.5	30	BBDuk	RUM	HTSeq UNION	TMM
4	56.75	25.75	Cutadapt	RUM	HTSeq INTER	TMM
5	57.75	20.75	Trimmomatic	RUM	HTSeq INTER	TMM
6	58.25	24.25	Cutadapt	RUM	HTSeq UNION	TMM
7	62	32	BBDuk	RUM	HTSeq INTER	TMM
8	62	37.5	BBDuk	STAR	HTSeq UNION	TMM
9	64.75	8.25	Trimmomatic	STAR	HTSeq UNION	TMM
10	66.75	14.75	Trimmomatic	TopHat2	HTSeq UNION	TMM
11	67.75	22.75	Cutadapt	TopHat2	HTSeq UNION	TMM
12	68	23.5	BBDuk	TopHat2	HTSeq UNION	TMM
13	69.25	27.25	BBDuk	HiSat2	HTSeq UNION	TMM
14	69.5	20.5	Trimmomatic	HiSat2	HTSeq UNION	TMM
15	70.5	20.5	BBDuk	TopHat2	HTSeq INTER	TMM
16	71	12	Trimmomatic	STAR	HTSeq INTER	TMM
17	72.25	39.25	BBDuk	STAR	HTSeq INTER	TMM
18	72.5	24.5	Cutadapt	HiSat2	HTSeq INTER	TMM
19	73	19.5	Trimmomatic	TopHat2	HTSeq INTER	TMM
20	73.75	41.25	Trimmomatic	RUM	HTSeq UNION	RLE
21	76.25	26.75	Trimmomatic	HiSat2	HTSeq INTER	TMM
22	76.5	28	Cutadapt	STAR	HTSeq UNION	RLE
23	77.25	27.25	Cutadapt	STAR	HTSeq INTER	RLE
24	79	51	BBDuk	RUM	HTSeq UNION	RLE
25	80.25	51.75	BBDuk	RUM	HTSeq INTER	RLE
26	80.75	39.25	Trimmomatic	RUM	HTSeq INTER	RLE
27	81.75	36.75	Trimmomatic	RUM	Stringtie	Coverage
28	82.5	15.5	BBDuk	STAR	Stringtie	TPM
29	82.5	36	Cutadapt	RUM	Cufflinks	FPKM
30	82.75	13.25	Cutadapt	STAR	Cufflinks	FPKM
31	83.25	20.75	Cutadapt	STAR	HTSeq UNION	TMM
32	85.75	27.25	Trimmomatic	Bowtie2	eXpress	Eff_Counts
33	86	38.5	Trimmomatic	HiSat2	HTSeq INTER	Raw
34	86	43.5	Trimmomatic	RUM	HTSeq INTER	Raw
35	86.25	1.75	Cutadapt	STAR	HTSeq INTER	TMM
36	86.75	38.25	Trimmomatic	HiSat2	HTSeq UNION	Raw
37	87.25	44.75	Trimmomatic	RUM	HTSeq UNION	Raw
38	87.5	26.5	BBDuk	HiSat2	HTSeq INTER	TMM
39	88	41	Cutadapt	RUM	HTSeq INTER	RLE
40	88.5	32	BBDuk	RUM	Stringtie	Coverage

41	88.5	41.5	Trimmomatic	TopHat2	Stringtie	Coverage
42	88.75	19.25	Cutadapt	HiSat2	Cufflinks	FPKM
43	88.75	44.25	Cutadapt	RUM	HTSeq UNION	RLE
44	89.5	16	Trimmomatic	STAR	Cufflinks	FPKM
45	89.5	46.5	BBDuk	RUM	HTSeq INTER	Raw
46	89.75	40.75	Trimmomatic	HiSat2	Stringtie	Coverage
47	90	65	BBDuk	RUM	Cufflinks	FPKM
48	90.25	35.75	Trimmomatic	STAR	Stringtie	FPKM
49	90.25	61.25	BBDuk	STAR	HTSeq UNION	RLE
50	91	20	Trimmomatic	STAR	Stringtie	TPM
51	91.75	46.25	BBDuk	RUM	HTSeq UNION	Raw
52	92	19.5	Trimmomatic	HiSat2	Cufflinks	FPKM
53	92	42	BBDuk	STAR	Stringtie	FPKM
54	92	47.5	Trimmomatic	RUM	Cufflinks	FPKM
55	92.25	5.25	Trimmomatic	STAR	eXpress	FPKM
56	92.5	39.5	Trimmomatic	Kallisto		Est-Counts
57	93	30.5	Cutadapt	HiSat2	HTSeq UNION	TMM
58	93.5	44	Trimmomatic	Salmon-FMD-ISR		NumReads
59	93.75	24.25	Trimmomatic	STAR	HTSeq INTER	RLE
60	94	25	BBDuk	Bowtie2	eXpress	Eff_Counts
61	94.25	25.25	Trimmomatic	STAR	HTSeq UNION	RLE
62	94.25	43.75	Trimmomatic	Salmon-FMD-AUT		NumReads
63	94.25	47.25	Trimmomatic	Salmon-QMB-ISR		NumReads
64	94.25	49.75	BBDuk	HiSat2	HTSeq UNION	RLE
65	94.5	32	Trimmomatic	STAR	eXpress	Eff_Counts
66	94.75	28.25	Trimmomatic	Bowtie2	eXpress	Est_Count
67	94.75	38.75	Cutadapt	STAR	Stringtie	FPKM
68	94.75	41.25	Trimmomatic	STAR	Stringtie	Coverage
69	95	45	Trimmomatic	STAR	HTSeq INTER	Raw
70	95	46	Trimmomatic	TopHat2	HTSeq UNION	Raw
71	95	46.5	Trimmomatic	STAR	HTSeq UNION	Raw
72	95	61	BBDuk	STAR	HTSeq INTER	RLE
73	95.25	4.25	Cutadapt	HiSat2	Stringtie	TPM
74	95.5	33	Trimmomatic	STAR	eXpress	Est_Count
75	95.75	25.25	Cutadapt	STAR	Stringtie	TPM
76	96	45.5	Trimmomatic	TopHat2	HTSeq INTER	Raw
77	96.5	5.5	Cutadapt	RUM	Stringtie	TPM
78	97	21.5	BBDuk	Bowtie2	RSEM	FPKM
79	97.25	36.75	Cutadapt	HiSat2	HTSeq INTER	RLE
80	97.5	0	Trimmomatic	Bowtie2	eXpress	FPKM
81	97.5	52	BBDuk	HiSat2	HTSeq INTER	RLE
82	98	25	BBDuk	Bowtie2	eXpress	Est_Count
83	98.25	27.75	Cutadapt	STAR	RSEM	FPKM
84	98.75	28.75	BBDuk	HiSat2	Cufflinks	FPKM
85	99.25	20.75	Trimmomatic	HiSat2	Stringtie	FPKM

86	99.25	32.25	Trimmomatic	STAR	RSEM	FPKM
87	99.75	22.75	Cutadapt	Bowtie2	RSEM	FPKM
88	99.75	38.25	Cutadapt	HiSat2	HTSeq UNION	RLE
89	100	29.5	Trimmomatic	TopHat2	Cufflinks	FPKM
90	100.25	13.75	BBDuk	RUM	Stringtie	TPM
91	100.25	24.75	Trimmomatic	Bowtie2	RSEM	FPKM
92	101.25	24.75	BBDuk	STAR	Cufflinks	FPKM
93	101.25	31.25	BBDuk	TopHat2	Cufflinks	FPKM
94	101.5	26	BBDuk	STAR	RSEM	FPKM
95	101.5	46	BBDuk	TopHat2	HTSeq INTER	RLE
96	101.75	38.75	Cutadapt	RUM	Stringtie	FPKM
97	102	31	Cutadapt	TopHat2	Cufflinks	FPKM
98	102.5	37.5	BBDuk	TopHat2	Stringtie	Coverage
99	102.5	41	Trimmomatic	HiSat2	HTSeq UNION	RLE
100	102.75	4.25	BBDuk	HiSat2	Stringtie	TPM
101	102.75	10.75	Trimmomatic	HiSat2	Stringtie	TPM
102	102.75	35.25	BBDuk	RUM	Stringtie	FPKM
103	102.75	44.75	Trimmomatic	TopHat2	HTSeq INTER	RLE
104	102.75	49.25	Cutadapt	TopHat2	HTSeq INTER	RLE
105	103	24	Cutadapt	HiSat2	Stringtie	FPKM
106	103.25	0.75	Trimmomatic	Bowtie2	RSEM	TPM
107	103.25	2.75	Trimmomatic	TopHat2	Stringtie	TPM
108	103.25	36.25	BBDuk	HiSat2	Stringtie	Coverage
109	103.75	3.25	BBDuk	STAR	eXpress	FPKM
110	104	30	Trimmomatic	TopHat2	Stringtie	FPKM
111	104.25	28.75	BBDuk	HiSat2	Stringtie	FPKM
112	104.25	43.75	Trimmomatic	HiSat2	HTSeq INTER	RLE
113	104.5	1.5	Cutadapt	STAR	eXpress	FPKM
114	104.5	4	Cutadapt	Bowtie2	RSEM	TPM
115	104.5	8.5	Trimmomatic	RUM	Stringtie	TPM
116	105.25	6.75	BBDuk	Salmon-QMB-AUT		TPM
117	105.25	33.75	BBDuk	STAR	Stringtie	Coverage
118	105.25	44.75	BBDuk	HiSat2	HTSeq INTER	Raw
119	105.25	56.75	Cutadapt	RUM	Stringtie	Coverage
120	105.5	15.5	BBDuk	TopHat2	Stringtie	TPM
121	106	7	BBDuk	Salmon-QMB-ISR		TPM
122	106	10.5	BBDuk	Sailfish-ISR		TPM
123	106	13	Cutadapt	STAR	RSEM	TPM
124	106	47	BBDuk	TopHat2	HTSeq UNION	RLE
125	106	51	Cutadapt	TopHat2	HTSeq UNION	RLE
126	106.25	3.75	Trimmomatic	STAR	eXpress	TPM
127	106.25	16.25	BBDuk	Bowtie2	eXpress	FPKM
128	106.25	43.25	BBDuk	STAR	HTSeq INTER	Raw
129	106.5	61.5	Cutadapt	HiSat2	HTSeq INTER	Raw
130	106.75	44.25	Trimmomatic	TopHat2	HTSeq UNION	RLE

131	106.75	61.25	Cutadapt	HiSat2	HTSeq UNION	Raw
132	107	0	Cutadapt	TopHat2	Stringtie	TPM
133	107.75	21.75	Cutadapt	TopHat2	HTSeq INTER	TMM
134	107.75	27.75	BBDuk	STAR	eXpress	Eff_Counts
135	107.75	42.75	BBDuk	STAR	HTSeq UNION	Raw
136	108.25	31.25	Trimmomatic	RUM	Stringtie	FPKM
137	108.25	43.25	BBDuk	HiSat2	HTSeq UNION	Raw
138	108.5	6	BBDuk	Bowtie2	RSEM	TPM
139	108.5	11	Trimmomatic	Salmon-FMD-ISR		TPM
140	108.75	63.25	Cutadapt	HiSat2	Stringtie	Coverage
141	109	6.5	BBDuk	Salmon-FMD-AUT		TPM
142	109	7.5	Cutadapt	STAR	eXpress	TPM
143	109.25	31.75	Cutadapt	TopHat2	Stringtie	FPKM
144	109.25	61.75	Cutadapt	RUM	HTSeq UNION	Raw
145	109.5	7	BBDuk	Salmon-FMD-ISR		TPM
146	109.75	21.75	BBDuk	TopHat2	Stringtie	FPKM
147	110	11.5	Trimmomatic	Salmon-QMB-AUT		TPM
148	110	47.5	BBDuk	TopHat2	HTSeq INTER	Raw
149	110	49.5	BBDuk	Salmon-QMB-ISR		NumReads
150	110.25	49.25	BBDuk	Sailfish-ISR		NumReads
151	110.5	6.5	Trimmomatic	Bowtie2	eXpress	TPM
152	110.5	46.5	BBDuk	TopHat2	HTSeq UNION	Raw
153	110.5	48.5	BBDuk	Salmon-QMB-AUT		NumReads
154	110.5	52.5	Cutadapt	STAR	eXpress	Est_Count
155	110.75	19.25	BBDuk	STAR	RSEM	TPM
156	110.75	59.25	Cutadapt	RUM	HTSeq INTER	Raw
157	111	7.5	BBDuk	STAR	eXpress	TPM
158	111	10.5	Trimmomatic	Salmon-FMD-AUT		TPM
159	111.5	9	Trimmomatic	Salmon-QMB-ISR		TPM
160	112.25	54.25	Cutadapt	Bowtie2	eXpress	Est_Count
161	112.5	6	BBDuk	Bowtie2	eXpress	TPM
162	112.75	45.25	BBDuk	Kallisto		Est-Counts
163	113	14	Cutadapt	Salmon-QMB-ISR		TPM
164	113.5	57.5	Cutadapt	TopHat2	Stringtie	Coverage
165	113.5	63.5	Cutadapt	STAR	HTSeq UNION	Raw
166	113.75	61.75	Cutadapt	STAR	Stringtie	Coverage
167	114	25.5	BBDuk	STAR	eXpress	Est_Count
168	114	52.5	Cutadapt	STAR	eXpress	Eff_Counts
169	114.25	42.25	BBDuk	Salmon-FMD-ISR		NumReads
170	114.25	62.75	Cutadapt	STAR	HTSeq INTER	Raw
171	114.5	42.5	BBDuk	Salmon-FMD-AUT		NumReads
172	114.5	67	Cutadapt	Salmon-QMB-ISR		NumReads
173	114.75	10.75	Trimmomatic	STAR	RSEM	TPM
174	114.75	55.25	Cutadapt	Bowtie2	eXpress	Eff_Counts

175	115.25	14.25	Cutadapt	Salmon-QMB-AUT		TPM
176	115.25	66.25	Cutadapt	Salmon-QMB-AUT		NumReads
177	115.5	61	Cutadapt	TopHat2	HTSeq INTER	Raw
178	115.5	61	Cutadapt	TopHat2	HTSeq UNION	Raw
179	115.5	66	Cutadapt	Sailfish-ISR		NumReads
180	116.75	16.25	Cutadapt	Sailfish-ISR		TPM
181	117	9.5	Cutadapt	Salmon-FMD-ISR		TPM
182	117.5	16	Cutadapt	Bowtie2	eXpress	FPKM
183	117.75	63.25	Cutadapt	Salmon-FMD-ISR		NumReads
184	118	11	Cutadapt	Salmon-FMD-AUT		TPM
185	118.5	62.5	Cutadapt	Salmon-FMD-AUT		NumReads
186	118.75	59.75	Cutadapt	Kallisto		Est-Counts
187	119.25	30.25	BBDuk	Kallisto		TPM
188	119.75	72.25	Trimmomatic	Salmon-QMB-AUT		NumReads
189	120.25	25.25	Trimmomatic	Kallisto		TPM
190	125	25.5	Cutadapt	Kallisto		TPM
191	129.5	11	Trimmomatic	Sailfish-ISR		TPM
192	133.25	21.75	Cutadapt	Bowtie2	eXpress	TPM

Supplementary Table S6. Overall ranking of the 192 pipelines based on the sum of the precision and accuracy in the two cell lines.

Overall RANK (precision and accuracy, cell lines A and B)

RANK position	Sum RANK	MAD	Trimming method	Alignment method	Counting method	Normalization method
1	249	8.5	Trimmomatic	RUM	HTSeq UNION	TMM
2	252.5	8.75	Trimmomatic	RUM	HTSeq INTER	TMM
3	253	13.25	BBDuk	RUM	HTSeq UNION	TMM
4	260	19.25	BBDuk	STAR	HTSeq UNION	TMM
5	260.5	15	Cutadapt	TopHat2	HTSeq UNION	TMM
6	263	15.75	BBDuk	TopHat2	HTSeq UNION	TMM
7	265.5	16	BBDuk	HiSat2	HTSeq UNION	TMM
8	266	11	BBDuk	TopHat2	HTSeq INTER	TMM
9	267.5	8	Trimmomatic	STAR	HTSeq UNION	TMM
10	269	4.5	Trimmomatic	STAR	HTSeq INTER	TMM
11	270	11.5	Trimmomatic	HiSat2	HTSeq UNION	TMM
12	270	13	BBDuk	RUM	HTSeq INTER	TMM
13	271.5	10.75	Trimmomatic	TopHat2	HTSeq UNION	TMM
14	274.5	18.25	Trimmomatic	RUM	HTSeq UNION	RLE
15	277	10.75	Trimmomatic	TopHat2	HTSeq INTER	TMM
16	281.5	10.25	Trimmomatic	HiSat2	HTSeq INTER	TMM
17	283.5	6	Cutadapt	RUM	HTSeq INTER	TMM
18	286.5	23	BBDuk	STAR	HTSeq INTER	TMM
19	288.5	6	Cutadapt	RUM	HTSeq UNION	TMM
20	293	2.5	BBDuk	STAR	Stringtie	TPM
21	293.5	16.75	Trimmomatic	RUM	HTSeq INTER	RLE
22	295	12	Cutadapt	HiSat2	HTSeq INTER	TMM
23	301	6	BBDuk	HiSat2	HTSeq INTER	TMM
24	301.5	5.5	Cutadapt	STAR	HTSeq UNION	TMM
25	303.5	3.75	Trimmomatic	STAR	Stringtie	FPKM
26	306	6	BBDuk	STAR	Stringtie	FPKM
27	306.5	9.5	Cutadapt	STAR	Cufflinks	FPKM
28	309	19.25	Cutadapt	RUM	Cufflinks	FPKM
29	310	2	Cutadapt	HiSat2	HTSeq UNION	TMM
30	312	25.25	Cutadapt	Bowtie2	RSEM	TPM
31	313.5	5	Cutadapt	STAR	Stringtie	FPKM
32	315	4.25	Trimmomatic	STAR	Cufflinks	FPKM
33	316.5	21	BBDuk	STAR	HTSeq UNION	RLE
34	316.5	22.5	Trimmomatic	Bowtie2	RSEM	TPM
35	317	12.5	Trimmomatic	STAR	Stringtie	TPM
36	318	26	BBDuk	RUM	HTSeq UNION	RLE
37	318.5	13.25	BBDuk	HiSat2	HTSeq UNION	RLE
38	319	19	BBDuk	STAR	HTSeq INTER	RLE
39	319.5	4.75	Cutadapt	STAR	HTSeq INTER	TMM

40	319.5	15.75	Cutadapt	STAR	HTSeq INTER	RLE
41	319.5	23.25	BBDuk	Salmon-QMB-AUT		TPM
42	320	7.5	BBDuk	TopHat2	HTSeq INTER	RLE
43	320	22.75	BBDuk	Sailfish-ISR		TPM
44	320.5	8	Cutadapt	HiSat2	HTSeq INTER	RLE
45	321	24.25	Trimmomatic	Salmon-QMB-AUT		TPM
46	322	24.5	BBDuk	Salmon-QMB-ISR		TPM
47	322	27.75	BBDuk	Salmon-FMD-ISR		TPM
48	323	5.75	Trimmomatic	TopHat2	Cufflinks	FPKM
49	323	14.25	BBDuk	HiSat2	HTSeq INTER	RLE
50	323	15.25	Cutadapt	STAR	HTSeq UNION	RLE
51	323.5	17.5	Cutadapt	TopHat2	HTSeq INTER	TMM
52	324	1.75	Trimmomatic	HiSat2	Cufflinks	FPKM
53	324	7.75	BBDuk	Bowtie2	RSEM	FPKM
54	324.5	25	BBDuk	RUM	HTSeq INTER	RLE
55	325	27.25	BBDuk	Salmon-FMD-AUT		TPM
56	325.5	3	Cutadapt	HiSat2	HTSeq UNION	RLE
57	325.5	4.25	Cutadapt	HiSat2	Cufflinks	FPKM
58	326	20.5	Cutadapt	STAR	RSEM	TPM
59	326	25	Cutadapt	Salmon-QMB-ISR		TPM
60	326.5	4.5	Cutadapt	STAR	Stringtie	TPM
61	327	22.75	Trimmomatic	Salmon-FMD-ISR		TPM
62	327.5	26.5	Cutadapt	Salmon-QMB-AUT		TPM
63	328.5	2.75	Cutadapt	STAR	RSEM	FPKM
64	328.5	3	BBDuk	HiSat2	Cufflinks	FPKM
65	328.5	6.25	Trimmomatic	Bowtie2	RSEM	FPKM
66	328.5	8.5	Trimmomatic	TopHat2	HTSeq INTER	RLE
67	329	18.75	Trimmomatic	RUM	Cufflinks	FPKM
68	329	25.75	Trimmomatic	Salmon-FMD-AUT		TPM
69	329.5	1	Trimmomatic	STAR	RSEM	FPKM
70	329.5	6	Trimmomatic	STAR	HTSeq INTER	RLE
71	329.5	7.5	Cutadapt	Bowtie2	RSEM	FPKM
72	330	4	Cutadapt	TopHat2	Cufflinks	FPKM
73	330	25.25	Trimmomatic	Salmon-QMB-ISR		TPM
74	330	25.25	BBDuk	Bowtie2	RSEM	TPM
75	330.5	7.75	BBDuk	STAR	Cufflinks	FPKM
76	332	29.75	Cutadapt	Salmon-FMD-ISR		TPM
77	333	5.5	BBDuk	TopHat2	HTSeq UNION	RLE
78	333.5	9.75	Cutadapt	TopHat2	HTSeq INTER	RLE
79	334	29	Cutadapt	Salmon-FMD-AUT		TPM
80	334.5	11	Trimmomatic	HiSat2	HTSeq INTER	RLE
81	335	7	Trimmomatic	HiSat2	HTSeq UNION	RLE
82	335.5	3	BBDuk	TopHat2	Cufflinks	FPKM
83	336	5.5	Cutadapt	TopHat2	HTSeq UNION	RLE
84	336.5	11	Trimmomatic	STAR	HTSeq UNION	RLE

85	337.5	28	Trimmomatic	STAR	RSEM	TPM
86	338	4.25	BBDuk	STAR	RSEM	FPKM
87	338.5	7.5	Trimmomatic	TopHat2	HTSeq UNION	RLE
88	341	37.5	BBDuk	RUM	Cufflinks	FPKM
89	343.5	17.25	BBDuk	STAR	RSEM	TPM
90	346	59	Trimmomatic	Sailfish-ISR		NumReads
91	346.5	23.25	Cutadapt	Sailfish-ISR		TPM
92	351	21.5	Cutadapt	RUM	HTSeq INTER	RLE
93	352.5	23.75	Cutadapt	RUM	HTSeq UNION	RLE
94	354.5	20.5	BBDuk	Kallisto		TPM
95	362	5.5	Cutadapt	RUM	Stringtie	TPM
96	362	14.5	BBDuk	TopHat2	Stringtie	TPM
97	363	23.25	Cutadapt	Kallisto		TPM
98	363.5	9.5	Trimmomatic	TopHat2	Stringtie	TPM
99	365	3.5	Trimmomatic	TopHat2	Stringtie	FPKM
100	365.5	20	Trimmomatic	Kallisto		TPM
101	367	12	Cutadapt	TopHat2	Stringtie	TPM
102	367.5	3.5	Cutadapt	HiSat2	Stringtie	TPM
103	370.5	1.25	Cutadapt	TopHat2	Stringtie	FPKM
104	371.5	1.5	BBDuk	RUM	Stringtie	TPM
105	371.5	6	BBDuk	TopHat2	Stringtie	FPKM
106	372.5	3.25	BBDuk	HiSat2	Stringtie	FPKM
107	374.5	7.25	Trimmomatic	HiSat2	Stringtie	FPKM
108	374.5	8.75	BBDuk	HiSat2	Stringtie	TPM
109	374.5	9.25	BBDuk	RUM	Stringtie	FPKM
110	378.5	14.5	Cutadapt	RUM	Stringtie	FPKM
111	380	6	Cutadapt	HiSat2	Stringtie	FPKM
112	385.5	4	Trimmomatic	HiSat2	Stringtie	TPM
113	389	5	Trimmomatic	RUM	Stringtie	TPM
114	389	32.25	Trimmomatic	Sailfish-ISR		TPM
115	389.5	6	Trimmomatic	RUM	Stringtie	FPKM
116	409.5	8.75	Trimmomatic	STAR	eXpress	FPKM
117	416	3.5	Cutadapt	STAR	eXpress	FPKM
118	423	7.5	Cutadapt	STAR	eXpress	TPM
119	425.5	2	BBDuk	STAR	eXpress	FPKM
120	431.5	0.5	Trimmomatic	STAR	eXpress	TPM
121	436	11.25	Trimmomatic	Bowtie2	eXpress	FPKM
122	445.5	18.25	Trimmomatic	RUM	Stringtie	Coverage
123	448	4.25	BBDuk	STAR	eXpress	TPM
124	449.5	4.5	BBDuk	Bowtie2	eXpress	FPKM
125	452	12.75	Trimmomatic	HiSat2	HTSeq INTER	Raw
126	455.5	13.5	Trimmomatic	HiSat2	HTSeq UNION	Raw
127	456	15.25	BBDuk	RUM	Stringtie	Coverage
128	459	4	Trimmomatic	Bowtie2	eXpress	TPM
129	462	3.5	BBDuk	Bowtie2	eXpress	TPM

130	462	10.75	Cutadapt	Bowtie2	eXpress	FPKM
131	467	15.75	Trimmomatic	RUM	HTSeq INTER	Raw
132	471.5	14.25	Trimmomatic	HiSat2	Stringtie	Coverage
133	472	16.5	BBDuk	Bowtie2	eXpress	Eff_Counts
134	472.5	15	Trimmomatic	RUM	HTSeq UNION	Raw
135	474	17.5	Trimmomatic	TopHat2	Stringtie	Coverage
136	481	14.25	Trimmomatic	STAR	eXpress	Eff_Counts
137	482	12	BBDuk	RUM	HTSeq INTER	Raw
138	485	12.5	BBDuk	Bowtie2	eXpress	Est_Count
139	486.5	22.5	Trimmomatic	Bowtie2	eXpress	Eff_Counts
140	488	2.5	BBDuk	TopHat2	Stringtie	Coverage
141	488.5	3	BBDuk	HiSat2	Stringtie	Coverage
142	489	11.25	Trimmomatic	Salmon-FMD-ISR		NumReads
143	489	13.25	Trimmomatic	STAR	eXpress	Est_Count
144	491.5	11	Trimmomatic	Salmon-FMD-AUT		NumReads
145	492.5	12	BBDuk	RUM	HTSeq UNION	Raw
146	493	16.5	Trimmomatic	Kallisto		Est-Counts
147	494.5	6	Cutadapt	Bowtie2	eXpress	TPM
148	495.5	6	BBDuk	STAR	eXpress	Eff_Counts
149	496.5	6.5	BBDuk	HiSat2	HTSeq INTER	Raw
150	497.5	2.5	BBDuk	STAR	Stringtie	Coverage
151	497.5	11.25	Trimmomatic	Salmon-QMB-ISR		NumReads
152	501	14	Trimmomatic	STAR	HTSeq INTER	Raw
153	501.5	16.5	Trimmomatic	STAR	Stringtie	Coverage
154	502	12.25	Trimmomatic	TopHat2	HTSeq INTER	Raw
155	503.5	7.75	BBDuk	HiSat2	HTSeq UNION	Raw
156	504	12.5	Trimmomatic	TopHat2	HTSeq UNION	Raw
157	504	13.75	Trimmomatic	STAR	HTSeq UNION	Raw
158	504.5	4	BBDuk	STAR	HTSeq INTER	Raw
159	510.5	5.5	BBDuk	STAR	HTSeq UNION	Raw
160	511	5.5	BBDuk	STAR	eXpress	Est_Count
161	513	7.75	BBDuk	TopHat2	HTSeq INTER	Raw
162	513	9	BBDuk	Salmon-QMB-AUT		NumReads
163	513.5	8.25	BBDuk	Sailfish-ISR		NumReads
164	514	8.25	BBDuk	Salmon-QMB-ISR		NumReads
165	514.5	11.75	BBDuk	Salmon-FMD-ISR		NumReads
166	515	8.5	BBDuk	TopHat2	HTSeq UNION	Raw
167	515	12.5	BBDuk	Salmon-FMD-AUT		NumReads
168	515.5	8	BBDuk	Kallisto		Est-Counts
169	516.5	23	Trimmomatic	Bowtie2	eXpress	Est_Count
170	534	4.5	Cutadapt	STAR	eXpress	Est_Count
171	538	6.5	Cutadapt	HiSat2	HTSeq INTER	Raw
172	539.5	7	Cutadapt	HiSat2	HTSeq UNION	Raw
173	540.5	5	Cutadapt	RUM	Stringtie	Coverage
174	552.5	2.5	Cutadapt	HiSat2	Stringtie	Coverage

175	553.5	1.25	Cutadapt	Bowtie2	eXpress	Est_Count
176	554	11.75	Cutadapt	Salmon-QMB-ISR		NumReads
177	555	11.25	Cutadapt	Sailfish-ISR		NumReads
178	555.5	11.75	Cutadapt	Salmon-QMB-AUT		NumReads
179	556	7	Cutadapt	STAR	eXpress	Eff_Counts
180	556.5	9.75	Cutadapt	Kallisto		Est-Counts
181	556.5	14	Cutadapt	Salmon-FMD-ISR		NumReads
182	557	14.5	Cutadapt	Salmon-FMD-AUT		NumReads
183	560.5	0.5	Cutadapt	RUM	HTSeq INTER	Raw
184	560.5	1	Cutadapt	RUM	HTSeq UNION	Raw
185	562	3	Cutadapt	TopHat2	Stringtie	Coverage
186	566.5	3.75	Cutadapt	STAR	Stringtie	Coverage
187	568.5	3.5	Cutadapt	Bowtie2	eXpress	Eff_Counts
188	572	5.25	Cutadapt	TopHat2	HTSeq INTER	Raw
189	573	2.5	Cutadapt	STAR	HTSeq UNION	Raw
190	573.5	2.5	Cutadapt	STAR	HTSeq INTER	Raw
191	578	4.5	Cutadapt	TopHat2	HTSeq UNION	Raw
192	593.5	13.5	Trimmomatic	Salmon-QMB-AUT		NumReads

Supplementary Table S7. Results of the trimming algorithms. Percentage of surviving reads and alignment rate for each of the analysed samples.

Sample*	**Percentage of surviving reads**			**Alignment rate (%)****		
	Trimmomatic	**Cutadapt**	**BBDuk**	**Trimmomatic**	**Cutadapt**	**BBDuk**
CLA-T0-S1	83.5	95.9	92.7	94,9 ± 0,4	90,5 ± 3,1	97,0 ± 1,2
CLA-T0-S 2	84.4	95.0	88.8	96,9 ± 0,8	95,0 ± 1,9	98,0 ± 0,7
CLA-T0-S3	84.2	95.4	89.6	96,8 ± 1,6	94,0 ± 2,1	97,9 ± 1,1
CLB-T0-S1	88.9	96.8	93.9	96,1 ± 0,5	94,0 ± 2,4	97,6 ± 0,8
CLB-T0-S2	79.4	94.5	85.6	95,8 ± 1,2	93,8 ± 2,5	97,3 ± 1,4
CLB-T0-S3	83.2	95.6	89.9	96,0 ± 1,1	93,1 ± 2,6	97,8 ± 0,8
Median CLA	**84.2**	**95.4**	**89.6**	**96,1 ± 1,4**	**93,1 ± 2,6**	**97,4 ± 1,3**
Median CLB	**83.2**	**95.6**	**89.9**	**96,0 ± 1,0**	**93,8 ± 2,5**	**97,6 ± 1,0**
Overall median	**83.8**	**95.5**	**89.8**	**96,1 ± 1,2**	**93,4 ± 2,5**	**97,5 ± 1,2**

*CLA = Cell line A (KMS12-BM); CLB = Cell line B (JJN-3); T0 = DMSO

** Values are represented as median ± median absolute deviation (MAD)

Suppplementary Table S8. Kruskal-Wallis and Dunn’s test results for raw gene expression quantification based on the trimming algorithms.

Contrast	Chi squared statistic	Degrees of freedom	*p*-value	Pairwise contrast	Dunn's pairwise z test statistic	Dunn's test FDR
Kruskal-Wallis	3.07E+00	2	2.20E-01	Trimmomatic vs. Cutadapt	1.59E+00	1.69E-01
				Trimmomatic vs. BBDuk	1.50E-01	4.40E-01
				Cutadapt vs. BBDuk	-1.44E+00	1.13E-01

Suppplementary Table S9. Kruskal-Wallis and Dunn’s test results for raw gene expression quantification based on the alignment algorithms.

Contrast	**Chi squared statistic**	**Degrees of freedom**	***p*-value**	**Pairwise contrast**	**Dunn's pairwise z test statistic**	**Dunn's test FDR**
Kruskal-Wallis	3.85E+00	4	4.30E-01	Bowtie2 vs. HiSat2	1.63E+00	2.60E-01
				Bowtie2 vs. RUM	1.87E+00	3.10E-01
				HiSat2 vs. RUM	2.77E-01	4.89E-01
				Bowtie2 vs. STAR	1.52E+00	2.14E-01
				HiSat2 vs. STAR	-2.76E-01	4.35E-01
				RUM vs. STAR	-5.83E-01	4.66E-01
				Bowtie2 vs. Tophat2	1.31E+00	2.38E-01
				HiSat2 vs. Tophat2	-3.66E-01	5.10E-01
				RUM vs. Tophat2	-6.43E-01	5.20E-01
				STAR vs. Tophat2	-1.30E-01	4.48E-01

Suppplementary Table S10. Kruskal-Wallis and Dunn’s test results for raw gene expression quantification based on the counting method.

Contrast	Chi squared statistic	Degrees of freedom	*p*-value*	Pairwise contrast	Dunn's pairwise z test statistic	Dunn's test FDR*
Kruskal-Wallis	3.38E+01	5	< 0.01	Cufflinks vs. eXpress	-4.22E+00	6.15E-05
				Cufflinks vs. HTSeq INTER	-1.08E+00	2.11E-01
				eXpress vs. HTSeq INTER	4.30E+00	6.47E-05
				Cufflinks vs. HTSeq UNION	-1.02E+00	2.09E-01
				eXpress vs. HTSeq UNION	4.36E+00	9.56E-05
				HTSeq INTER vs. HTSeq UNION	7.43E-02	4.70E-01
				Cufflinks vs. RSEM	-2.91E-01	4.13E-01
				eXpress vs. RSEM	3.88E+00	1.94E-04
				HTSeq INTER vs. RSEM	7.19E-01	2.95E-01
				HTSeq UNION vs. RSEM	6.67E-01	2.91E-01
				Cufflinks vs. Stringtie	-2.69E+00	1.08E-02
				eXpress vs. Stringtie	2.26E+00	1.99E-02
				HTSeq INTER vs. Stringtie	-2.28E+00	2.13E-02
				HTSeq UNION vs. Stringtie	-2.35E+00	2.33E-02
				RSEM vs. Stringtie	-2.33E+00	2.12E-02

* Statistically significant comparisons (< 0.05) are shown in green

Suppplementary Table S11. Kruskal-Wallis and Dunn’s test results for raw gene expression quantification based on the normalization approaches.

Contrast	Chi squared statistic	Degrees of freedom	*p*-value*	Pairwise contrast	Dunn's pairwise z test statistic	Dunn's test FDR*
Kruskal-Wallis	1.51E+02	8	< 0.01	Coverage vs. Eff_Counts	-7.65E-02	4.97E-01
				Coverage vs. Est_Count	-3.86E-01	4.50E-01
				Eff_Counts vs. Est_Count	-2.50E-01	4.81E-01
				Coverage vs. FPKM	3.87E+00	1.09E-04
				Eff_Counts vs. FPKM	3.01E+00	2.23E-03
				Est_Count vs. FPKM	3.92E+00	1.01E-04
				Coverage vs. NumReads	-4.94E-01	4.30E-01
				Eff_Counts vs. NumReads	-3.17E-01	4.66E-01
				Est_Count vs. NumReads	-4.98E-02	4.80E-01
				FPKM vs. NumReads	-4.82E+00	2.36E-06
				Coverage vs. Raw	-4.18E-01	4.51E-01
				Eff_Counts vs. Raw	-2.40E-01	4.71E-01
				Est_Count vs. Raw	5.76E-02	4.91E-01
				FPKM vs. Raw	-5.45E+00	9.82E-08
				NumReads vs. Raw	1.32E-01	4.88E-01
				Coverage vs. RLE	4.74E+00	3.27E-06
				Eff_Counts vs. RLE	3.75E+00	1.58E-04
				Est_Count vs. RLE	4.72E+00	3.28E-06
				FPKM vs. RLE	1.46E+00	1.04E-01
				NumReads vs. RLE	5.67E+00	4.33E-08
				Raw vs. RLE	6.31E+00	9.93E-10
				Coverage vs. TMM	6.94E+00	2.36E-11
				Eff_Counts vs. TMM	5.46E+00	1.08E-07
				Est_Count vs. TMM	6.71E+00	8.62E-11
				FPKM vs. TMM	4.42E+00	1.21E-05
				NumReads vs. TMM	8.03E+00	8.43E-15
				Raw vs. TMM	9.01E+00	3.69E-18
				RLE vs. TMM	2.70E+00	5.45E-03
				Coverage vs. TPM	3.84E+00	1.14E-04
				Eff_Counts vs. TPM	2.97E+00	2.43E-03
				Est_Count vs. TPM	3.89E+00	1.06E-04
				FPKM vs. TPM	-1.37E-01	5.01E-01
				NumReads vs. TPM	4.82E+00	2.59E-06
				Raw vs. TPM	5.50E+00	1.00E-07
				RLE vs. TPM	-1.63E+00	7.81E-02
				TMM vs. TPM	-4.67E+00	3.88E-06

* Statistically significant comparisons (p-value or FDR < 0.05) are shown in green

Suppplementary Table S12. Kruskal-Wallis and Dunn’s test results for raw gene expression quantification based on the pseudoalignment algorithms.

Contrast	**Chi squared statistic**	**Degrees of freedom**	***p*-value**	**Pairwise contrast**	**Dunn's pairwise z test statistic**	**Dunn's test FDR**
Kruskal-Wallis	1.08E+00	2	5.80E-01	Kallisto *vs.* Sailfish	8.08E-01	3.14E-01
				Kallisto *vs.* Salmon	1.02E+00	4.63E-01
				Sailfish *vs.* Salmon	-4.33E-03	4.98E-01

Suppplementary Table S13. Kruskal-Wallis and Dunn’s results for the pipelines based on pseudoaligner and classic aligner data.

Contrast	Chi squared statistic	Degrees of freedom	*p*-value	Pairwise contrast	Dunn's pairwise z test statistic	Dunn's test FDR
Kruskal-Wallis	9.51E+00	7	2.20E-01	Bowtie2 vs. HiSat2	1.58E+00	2.00E-01
				Bowtie2 vs. Kallisto	-6.64E-01	3.94E-01
				HiSat2 vs. Kallisto	-1.75E+00	2.79E-01
				Bowtie2 vs. RUM	1.76E+00	3.63E-01
				HiSat2 vs. RUM	2.14E-01	4.65E-01
				Kallisto vs. RUM	1.88E+00	4.25E-01
				Bowtie2 vs. Sailfish	2.65E-02	5.08E-01
				HiSat2 vs. Sailfish	-1.02E+00	3.06E-01
				Kallisto vs. Sailfish	5.64E-01	4.01E-01
				RUM vs. Sailfish	-1.15E+00	2.70E-01
				Bowtie2 vs. Salmon	2.93E-02	5.26E-01
				HiSat2 vs. Salmon	-1.68E+00	2.58E-01
				Kallisto vs. Salmon	7.06E-01	3.96E-01
				RUM vs. Salmon	-1.89E+00	8.30E-01
				Sailfish vs. Salmon	-7.39E-03	4.97E-01
				Bowtie2 vs. STAR	1.50E+00	2.09E-01
				HiSat2 vs. STAR	-2.44E-01	4.71E-01
				Kallisto vs. STAR	1.68E+00	2.18E-01
				RUM vs. STAR	-4.81E-01	4.20E-01
				Sailfish vs. STAR	9.26E-01	3.31E-01
				Salmon vs. STAR	1.62E+00	2.11E-01
				Bowtie2 vs. Tophat2	1.19E+00	2.71E-01
				HiSat2 vs. Tophat2	-4.44E-01	4.18E-01
				Kallisto vs. Tophat2	1.50E+00	1.89E-01
				RUM vs. Tophat2	-6.57E-01	3.76E-01
				Sailfish vs. Tophat2	7.68E-01	3.87E-01
				Salmon vs. Tophat2	1.27E+00	2.61E-01
				STAR vs. Tophat2	-2.48E-01	4.89E-01