Assessment of transcript reconstruction methods for RNA-seq

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Supplement

Contents

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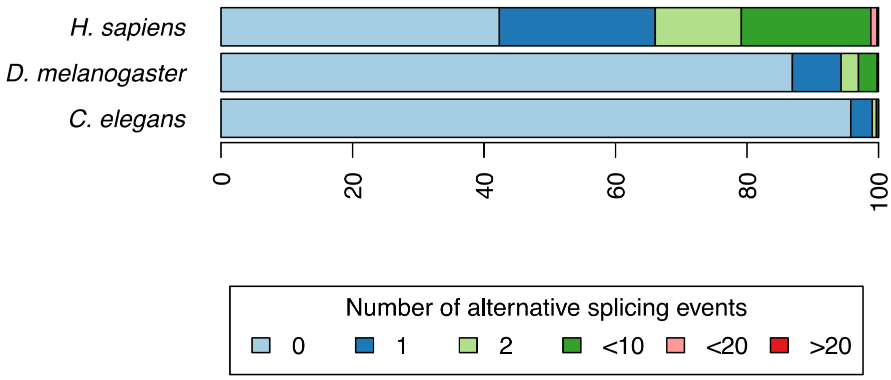
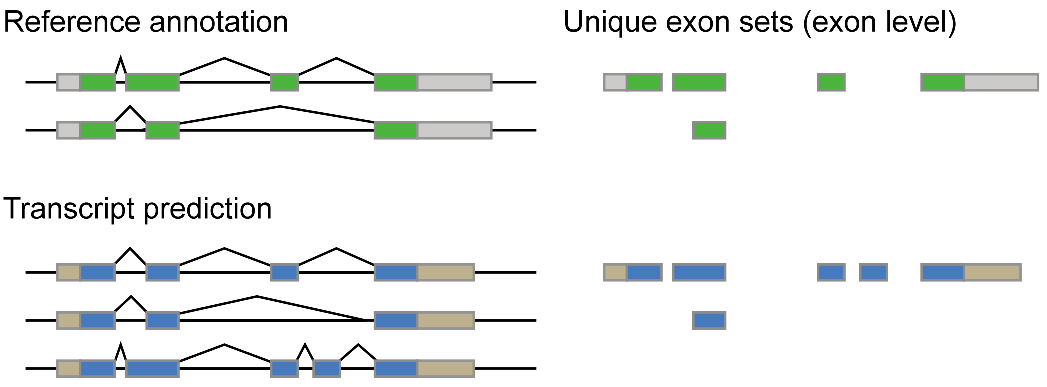
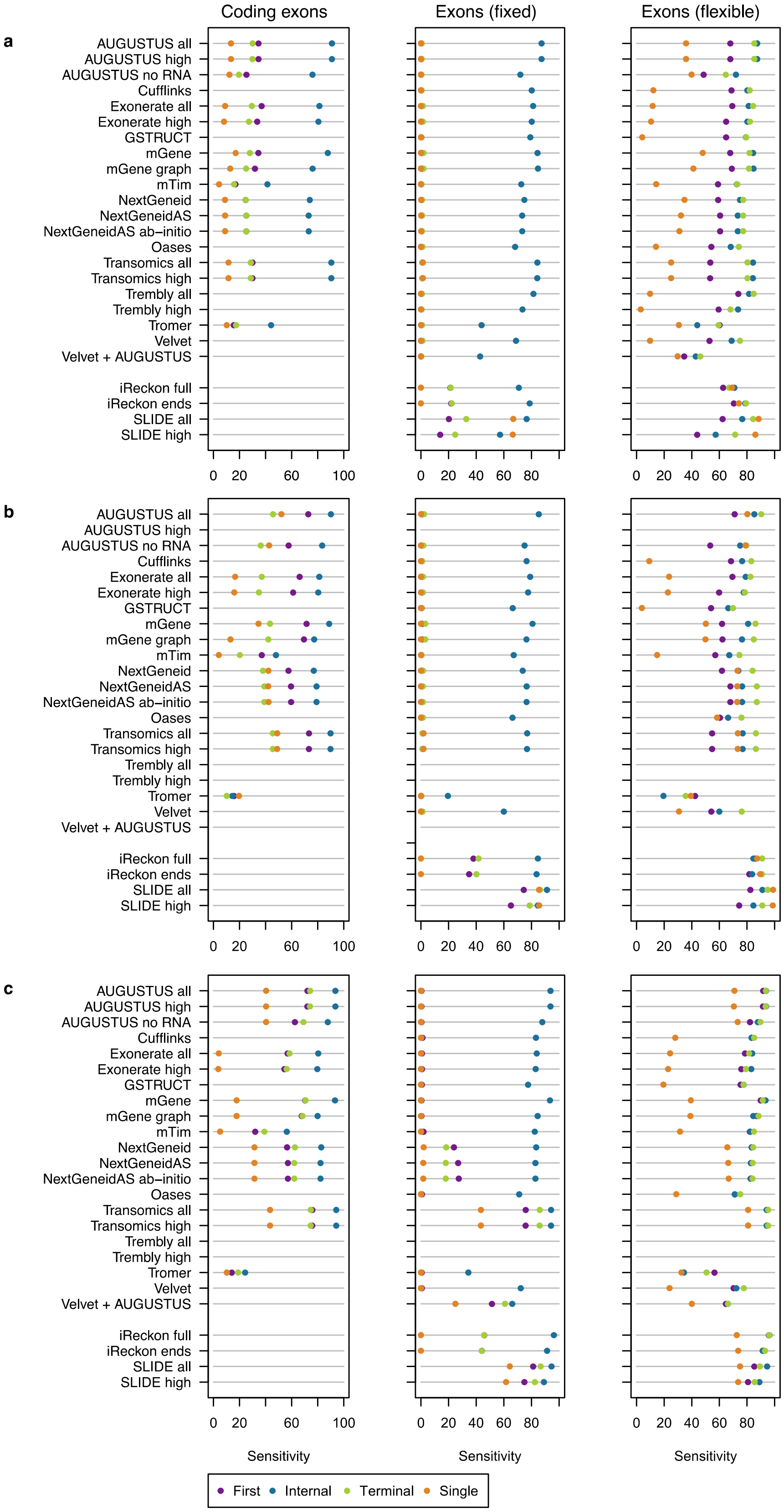


Supplementary Figure 1. Frequency of alternative splicing events. Bars show the percentage of genes with the indicated number of alternative splicing events in the reference annotation. Events were counted by analysis of annotated transcripts to identify skipped exons, retained introns, and alternative donor and acceptor sites.

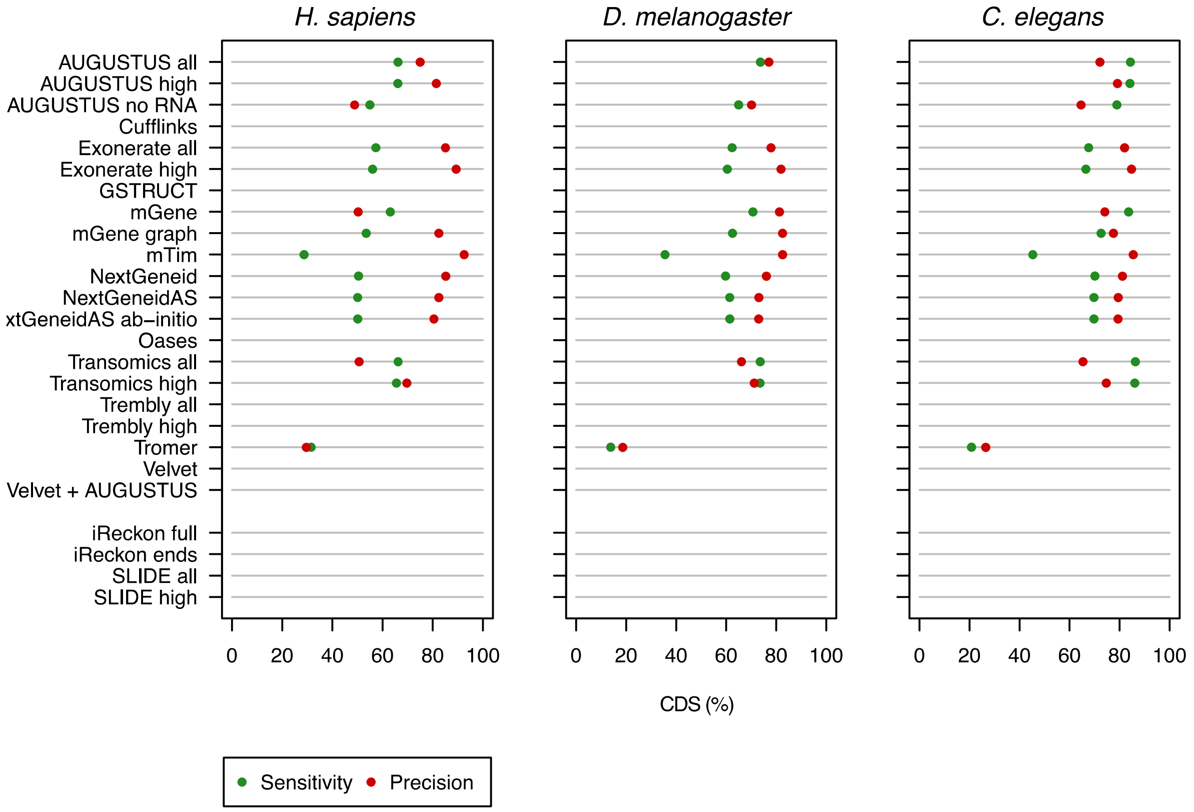


Level	Feature	fixed mode Sensitivity	fixed mode Precision	flexible mode Sensitivity	flexible mode Precision
Exon	CDS	1.00	0.83	NA	NA
	Exon	0.80	0.67	1.00	0.83
Transcript	CDS	0.50	0.33	NA	NA
	Exon	0.00	0.00	0.50	0.33
Gene	CDS	1.00	1.00	NA	NA
	Exon	0.00	0.00	1.00	1.00

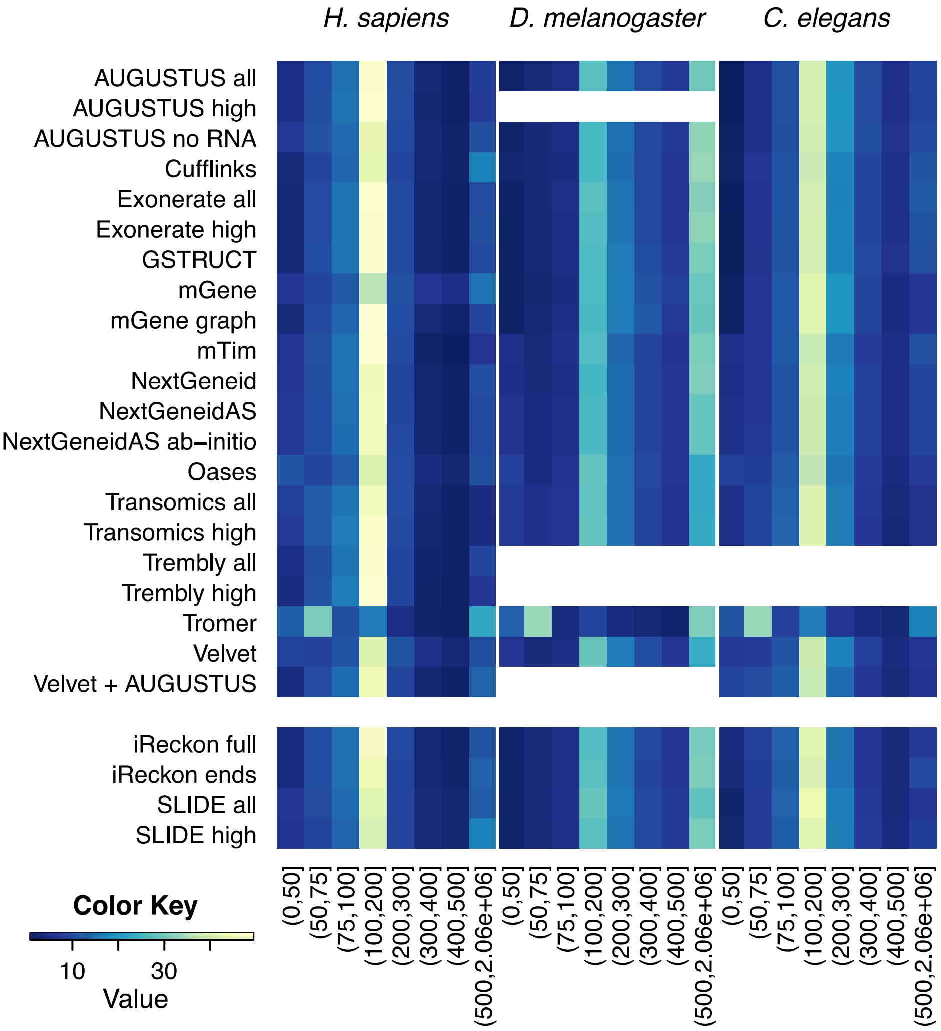
Supplementary Figure 2. Structural validation strategy. Transcript models were validated against annotated isoforms. For exon-level evaluation, transcripts were collapsed into unique exon sets, i.e. exons shared between transcript isoforms are counted once. Sensitivity (a.k.a. recall) was calculated as the proportion of reference features (exons, transcripts, or genes) matched by a reported feature. Precision was calculated as the proportion of reported features matching a reference feature. We primarily used a flexible evaluation strategy where exact agreement between transcript boundaries was not required. For comparison, certain analyses were also carried out using a fixed evaluation mode, where annotated and predicted exons were required to match exactly. See Methods for further details.



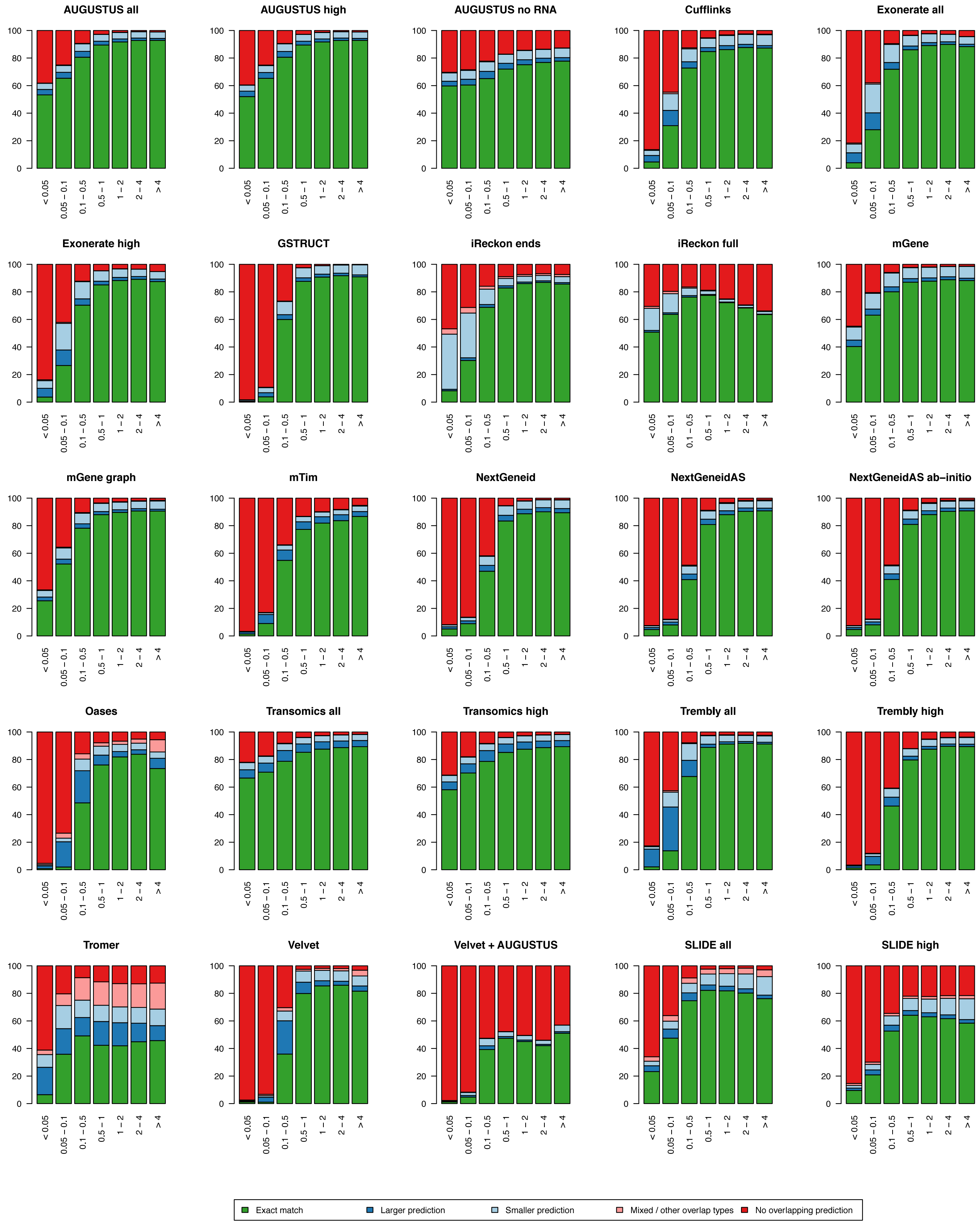
Supplementary Figure 3. Influence of exon rank on detection performance. Results are shown for *D. melanogaster* (a) and *C. elegans* (b). Annotated exons were classified as first, internal, terminal or single (i.e., those comprising an entire transcript) and sensitivity calculated separately for each class. Exon boundaries were required to be predicted exactly as annotated (left, center) or according to relaxed criteria for the external transcript boundaries (right). Programs run with reference annotation are grouped separately (lower tracks). As SLIDE is provided with full gene annotation as a requirement, those protocols do not display a strong preference for internal exons. Several methods were unable to accurately determine the strand orientation for unspliced transcripts, resulting in low sensitivity for constituent exons.



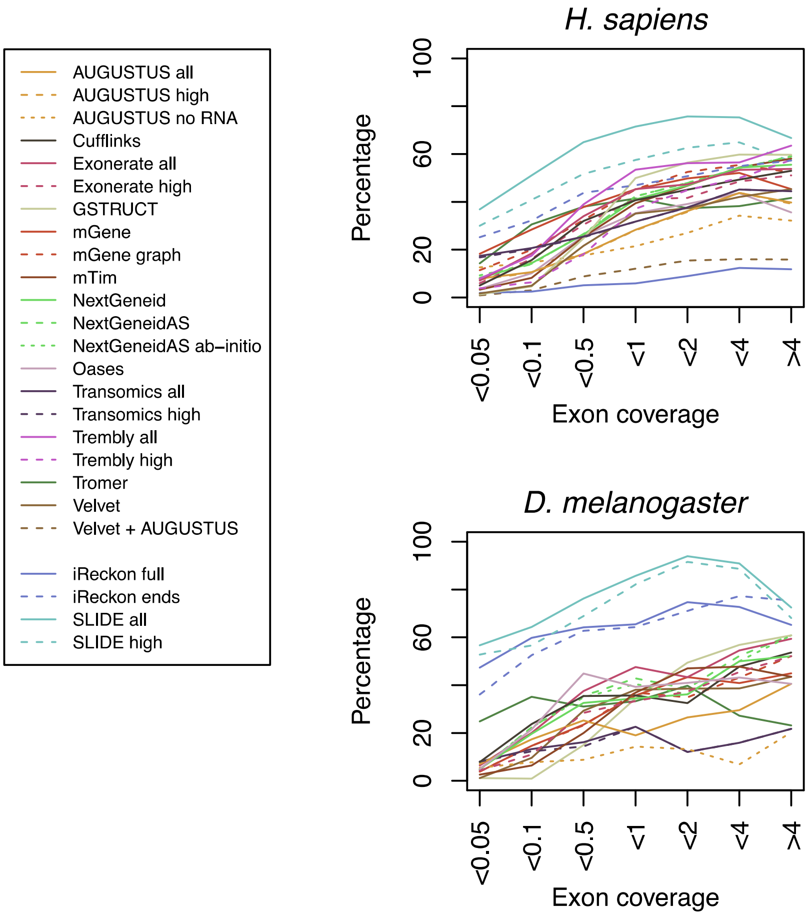
Supplementary Figure 4. Performance at detecting individual coding exons. Points indicate the percentage of reference coding exons with a matching feature in the submitted transcript models (recall, green), and the proportion of reported coding exons that agree with annotation (precision, red).



Supplementary Figure 5. Exon length distributions in transcriptome assembly results. Colors indicate percentage of exons within the indicated length intervals.



Supplementary Figure 6. Internal exon detection rate stratified by read coverage. Bars indicate the percentage of annotated internal exons (of human protein-coding genes) that overlap with reported exons. Reference exons were binned by read coverage (*x* axis) and further classified based on overlap with predicted exons (inset legend). Specifically, the classes represent exons with a perfectly matching prediction (green); exons for which all overlapping predictions span a larger region, including the entire reference exon (dark blue); exons for which all overlapping predictions are contained within the reference exon (light blue); and exons with other or multiple overlap types (pink). Note the decrease in detection performance at high read coverage for Oases, Velvet and SLIDE, as well as the high frequency of imperfect overlaps for Tromer. See also Figure 2b.



Supplementary Figure 7. Influence of sequencing coverage on non-coding exon-level sensitivity. Annotated exons of non-coding transcripts were binned according to RNA-seq read coverage and method sensitivities were calculated for each bin separately.

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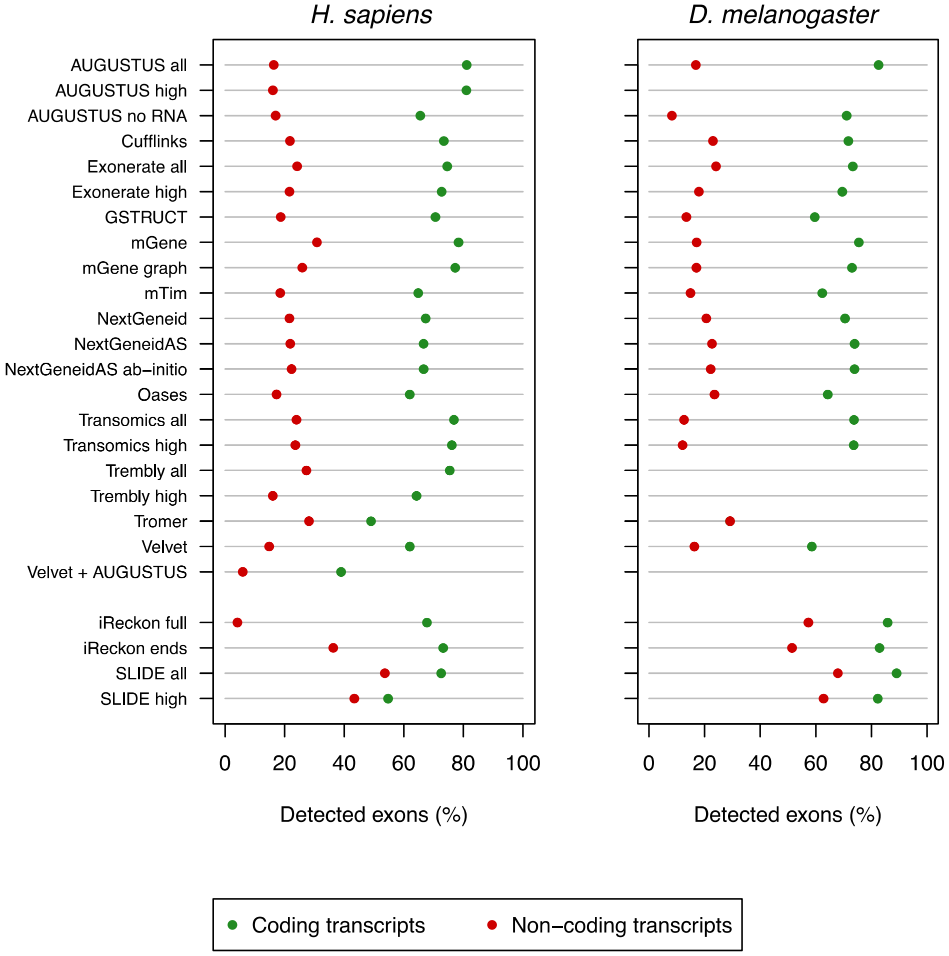
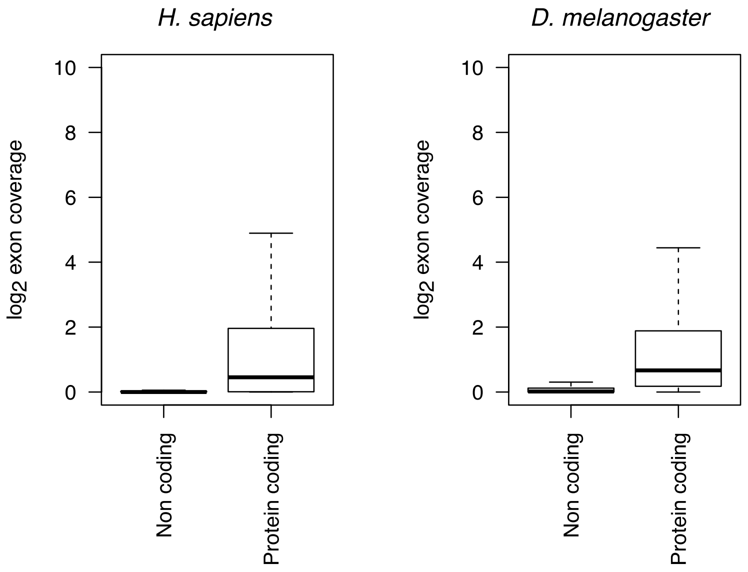


Supplementary Figure 8. Exon detection sensitivity relative to coding potential. Percentage of detected exons belonging to coding (green) and non-coding (red) transcripts in *H. sapiens* and *D. melanogaster*.



Supplementary Figure 9. RNA-seq read coverage for exons of coding and non-coding transcripts.

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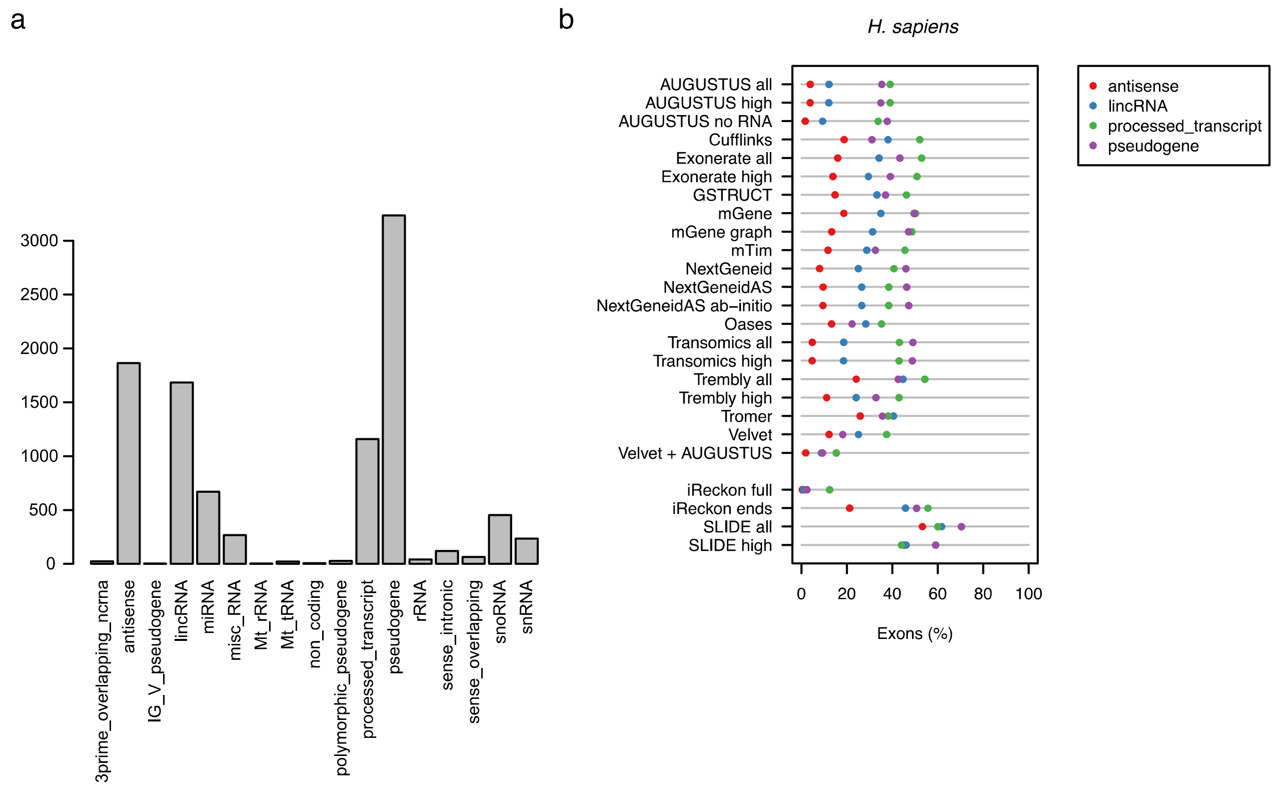
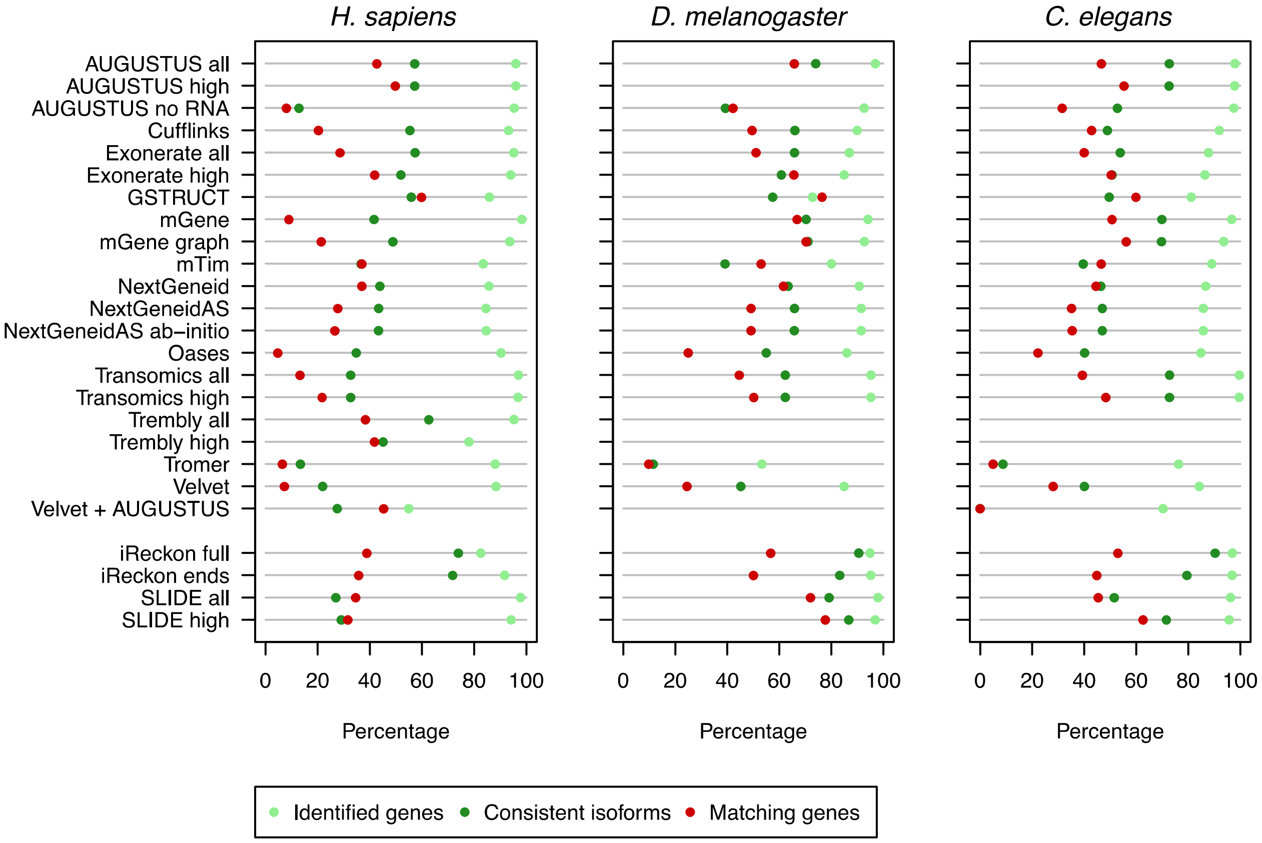
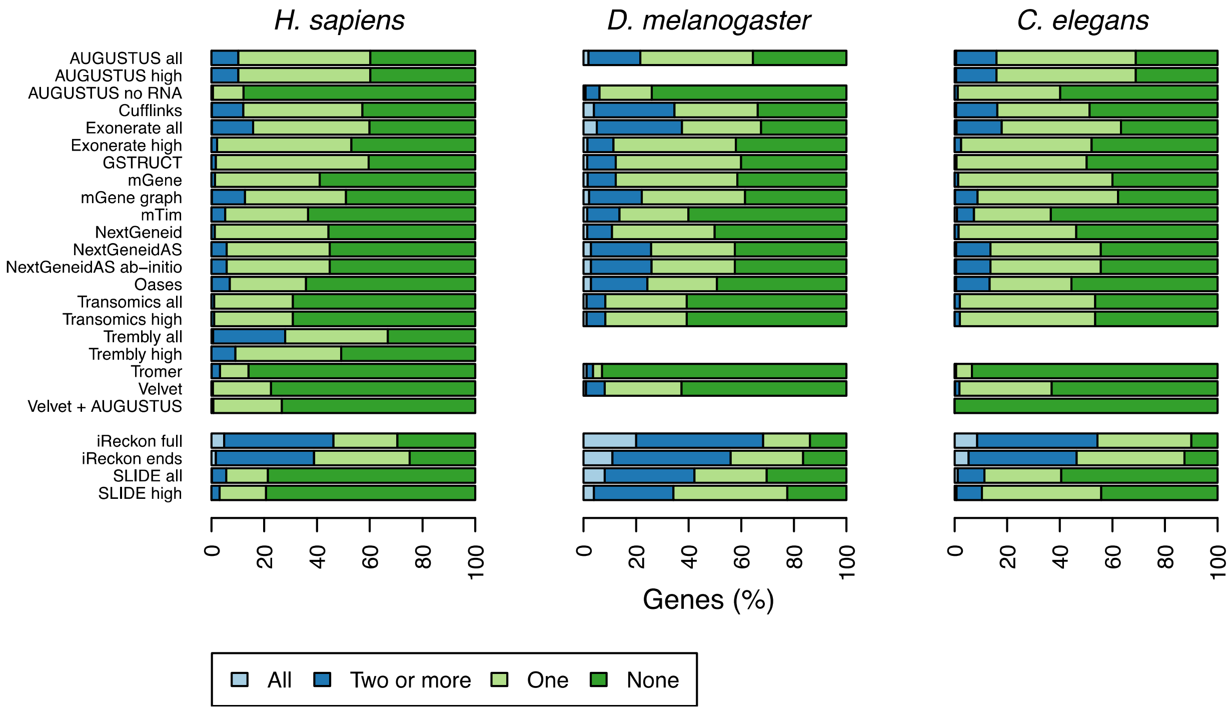


Supplementary Figure 10. Exon detection sensitivity for non-coding genes. (a) Non-coding transcripts expressed in the human RNA-seq data set, annotated by gene biotype. (b) Detected exons from transcripts of non-coding gene biotypes. Small RNAs were excluded, as they are underrepresented in data sets derived from standard mRNA-seq protocols that incorporate poly(A) selection. Alternative library construction protocols are required to specifically interrogate small RNA populations.



Supplementary Figure 11. Gene detection performance. Points indicate the percentage of reference genes with a matching assembled transcript (recall, green) and reported genes with at least one transcript matching the reference (precision, red).



Supplementary Figure 12. Number of isoforms detected per gene. Genes with at least three annotated splice products for which various subsets have been reported.

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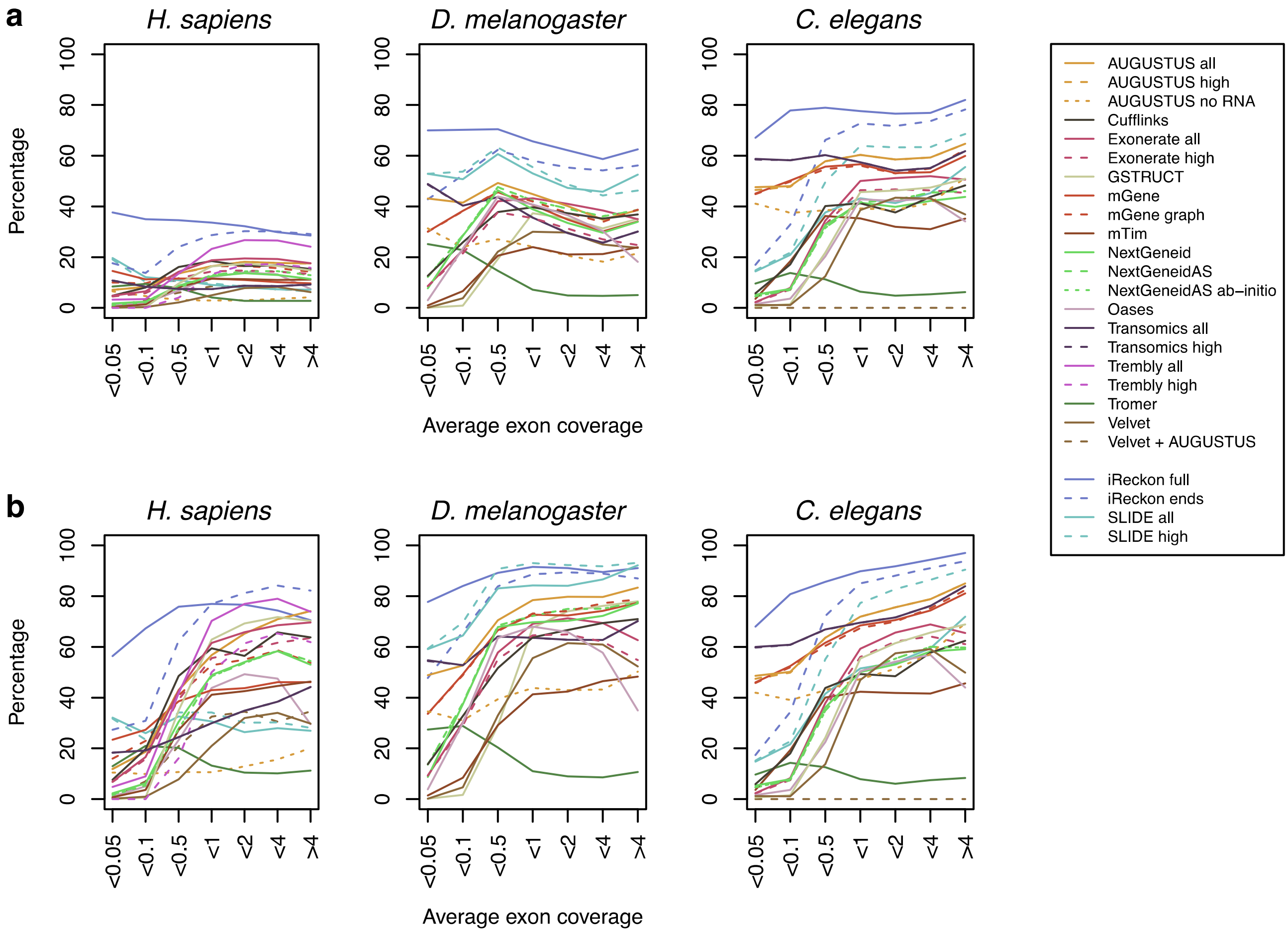
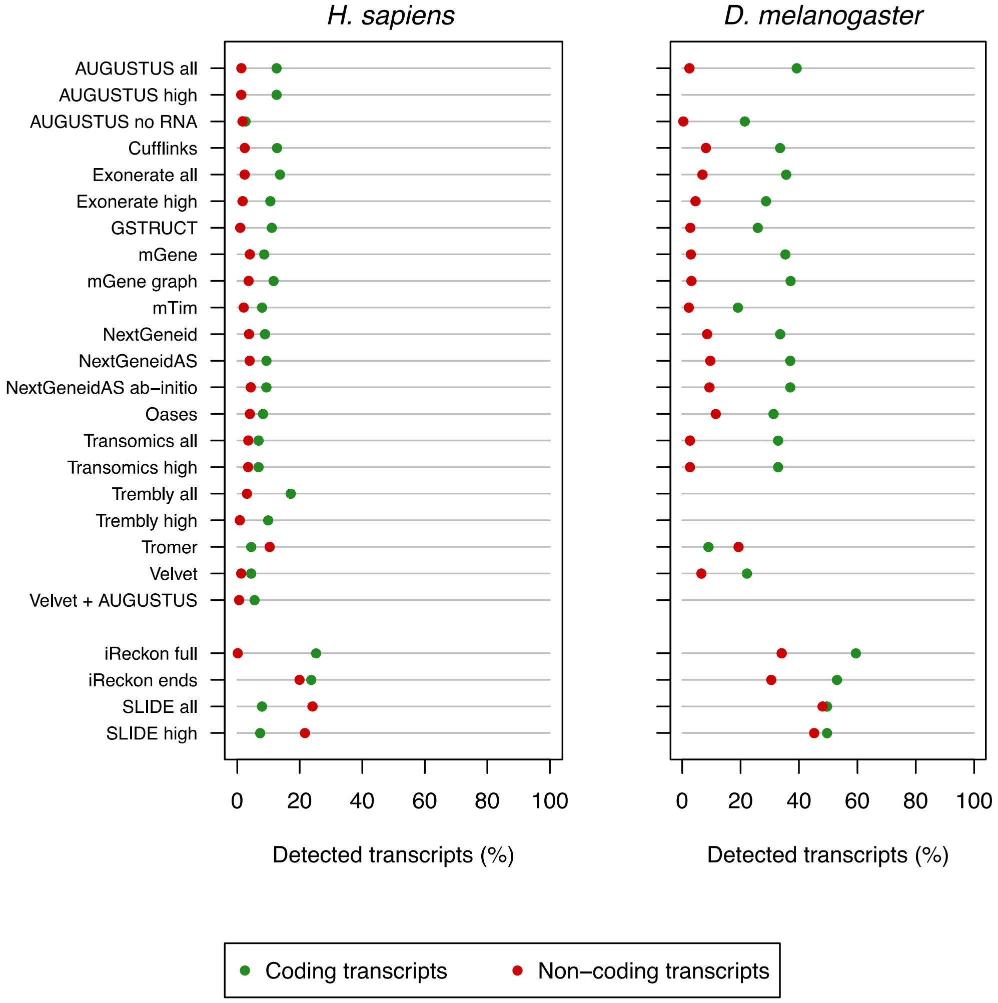
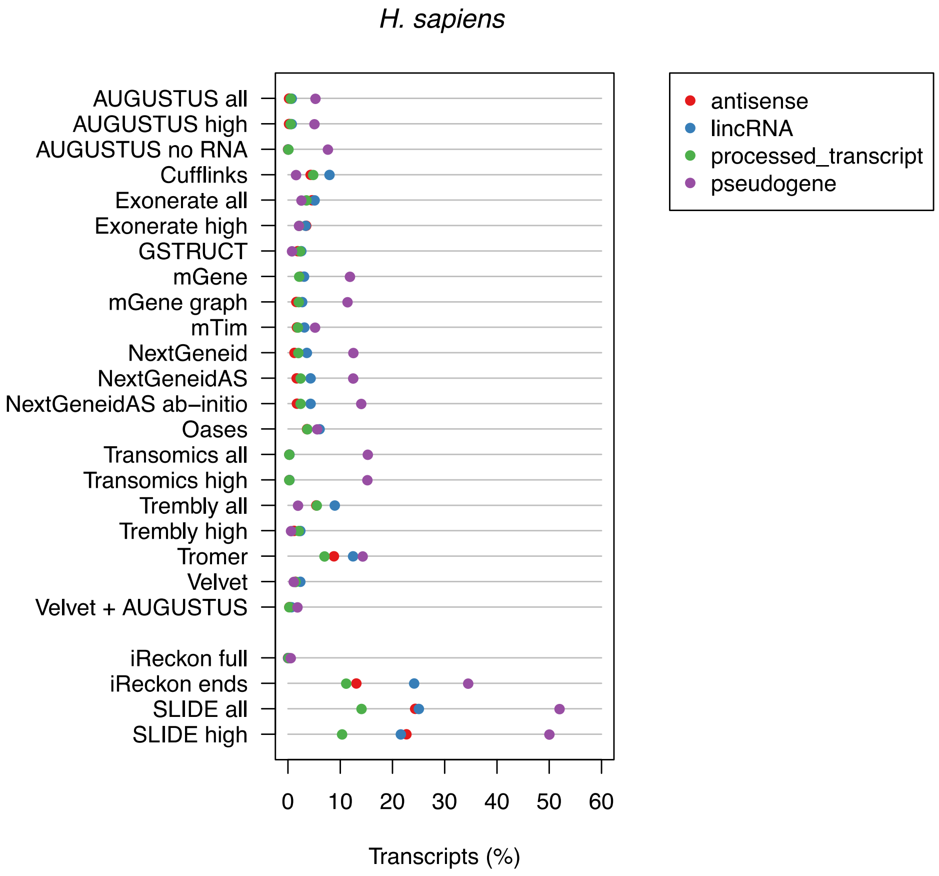


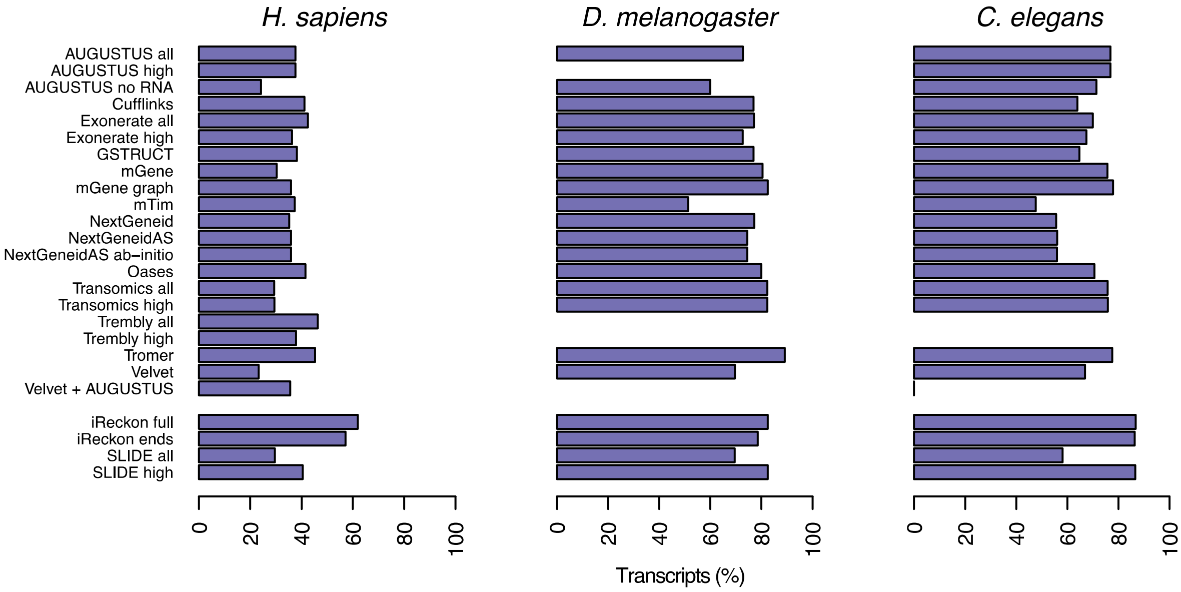
Supplementary Figure 13. Influence of read depth on transcript-level (a) and gene-level (b) sensitivity. Annotated transcripts and genes were binned according to RNA-seq read coverage and method sensitivities were calculated for each bin separately.



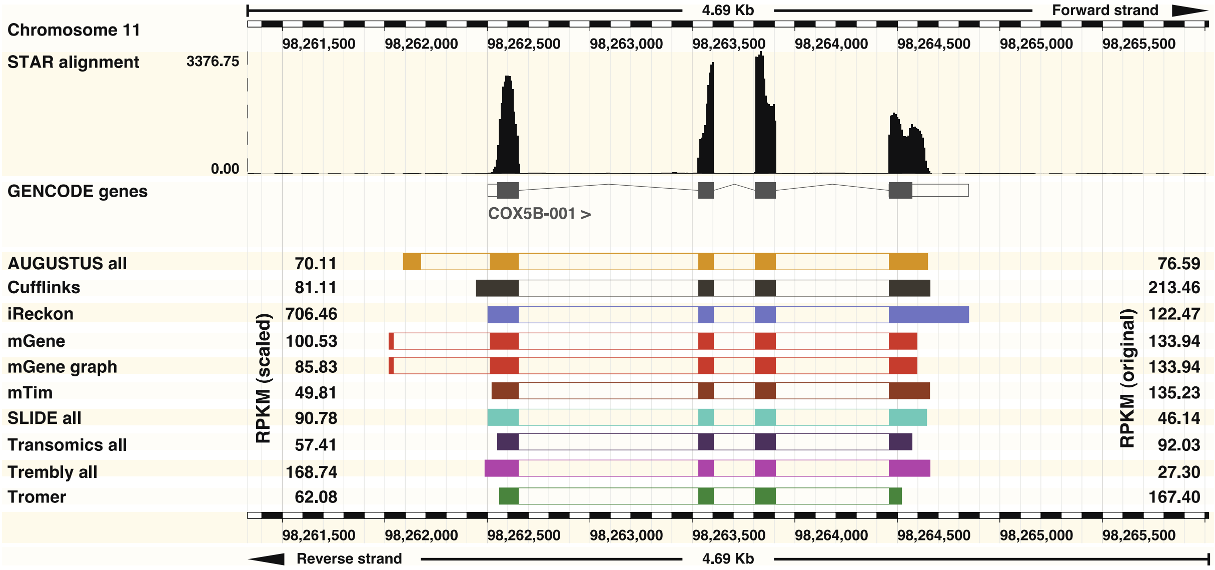
Supplementary Figure 14. Transcript level performance for coding and non-coding transcripts. Percentage of transcripts annotated in *H. sapiens* and *D. melanogaster* matching a reported transcript. Note, SLIDE shows higher sensitivity for non-coding transcripts as these tend to be shorter than protein coding transcripts. For transcripts with a given number of exons SLIDE identifies more protein coding than non-coding transcripts.



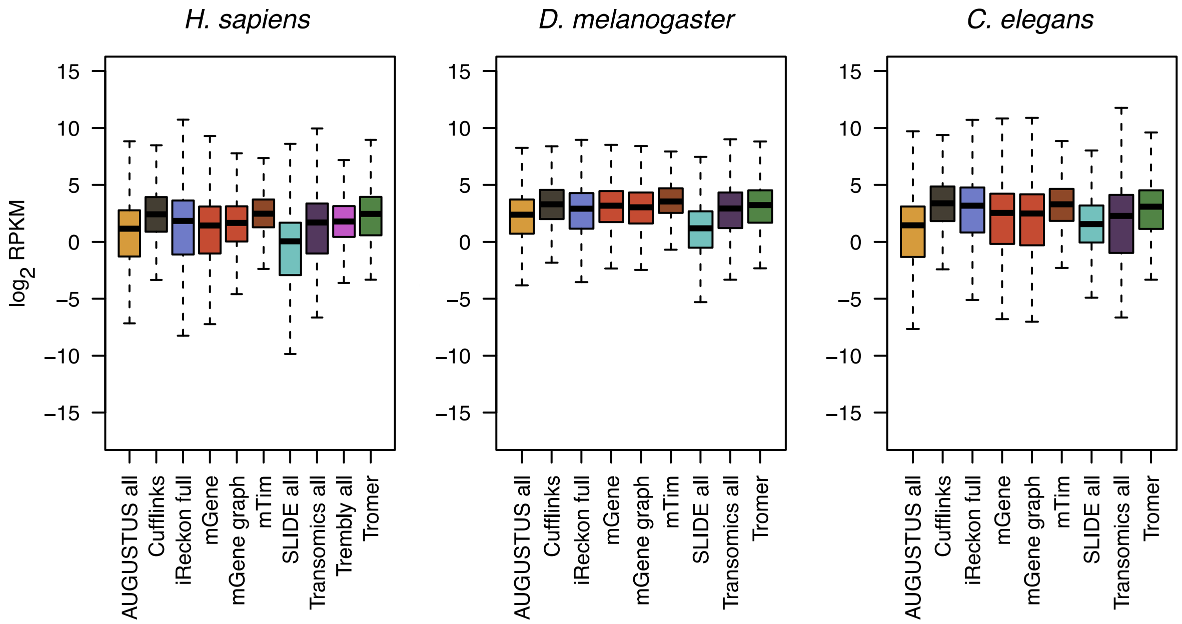
Supplementary Figure 15. Transcript detection sensitivity for non-coding genes. Transcripts identified in the human data set annotated by non-coding gene biotype.



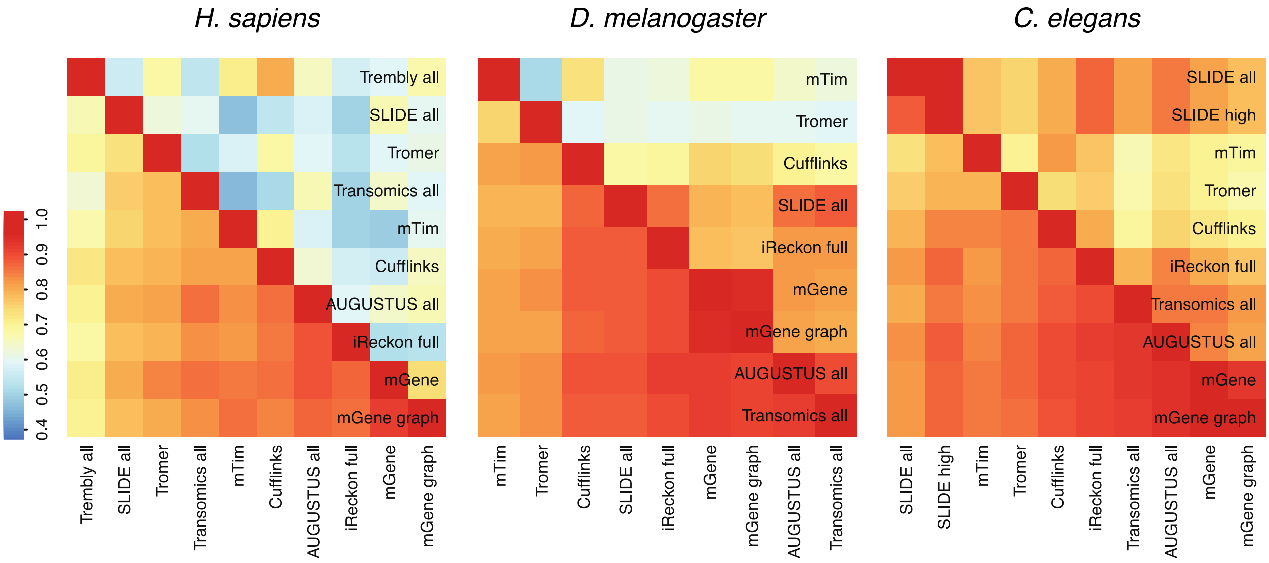
Supplementary Figure 16. Transcript assembly performance. Percentage of transcripts, for which all exons have been identified, that were correctly assembled to a full-length annotated splice variant.



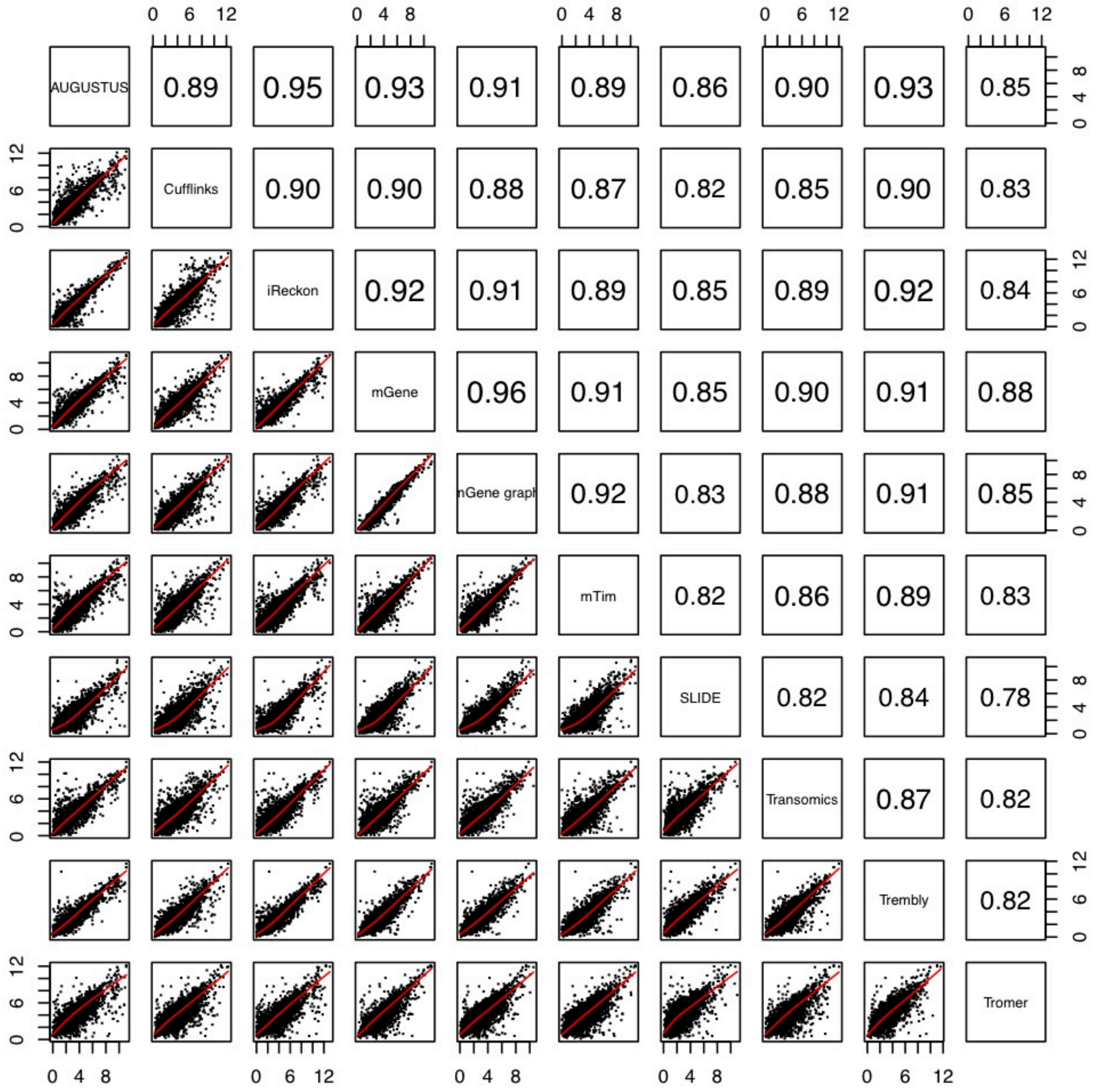
Supplementary Figure 17. Transcript predictions and expression level estimates (in RPKM) at the *COX5B* locus. Upper tracks depict RNA-seq read coverage (from STAR alignments; see Methods) and annotated genes. Exon predictions from the 10 methods that provided RPKM values are illustrated below the annotated gene by colored boxes. Exons reported as part the same transcript isoform are connected. iReckon full does not predict retained introns for this gene. Original and median-scaled RPKMs are presented to the right and left, respectively.



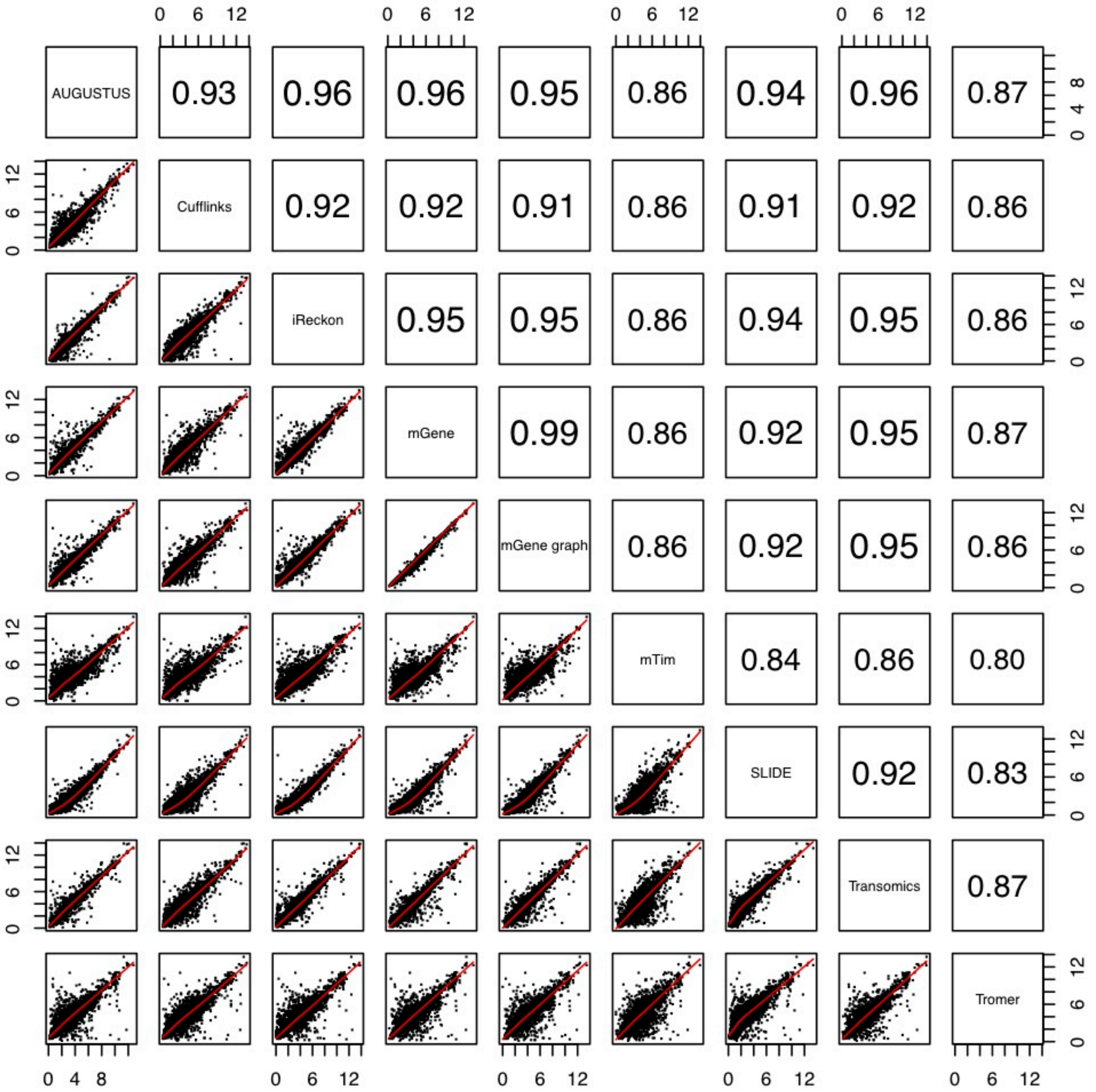
Supplementary Figure 18. Distribution of gene expression values (RPKM) for each method. Results are shown for annotated genes only. Where multiple transcripts were reported for the same gene, the highest RPKM value was used, corresponding to the predominant transcript identified by each method.



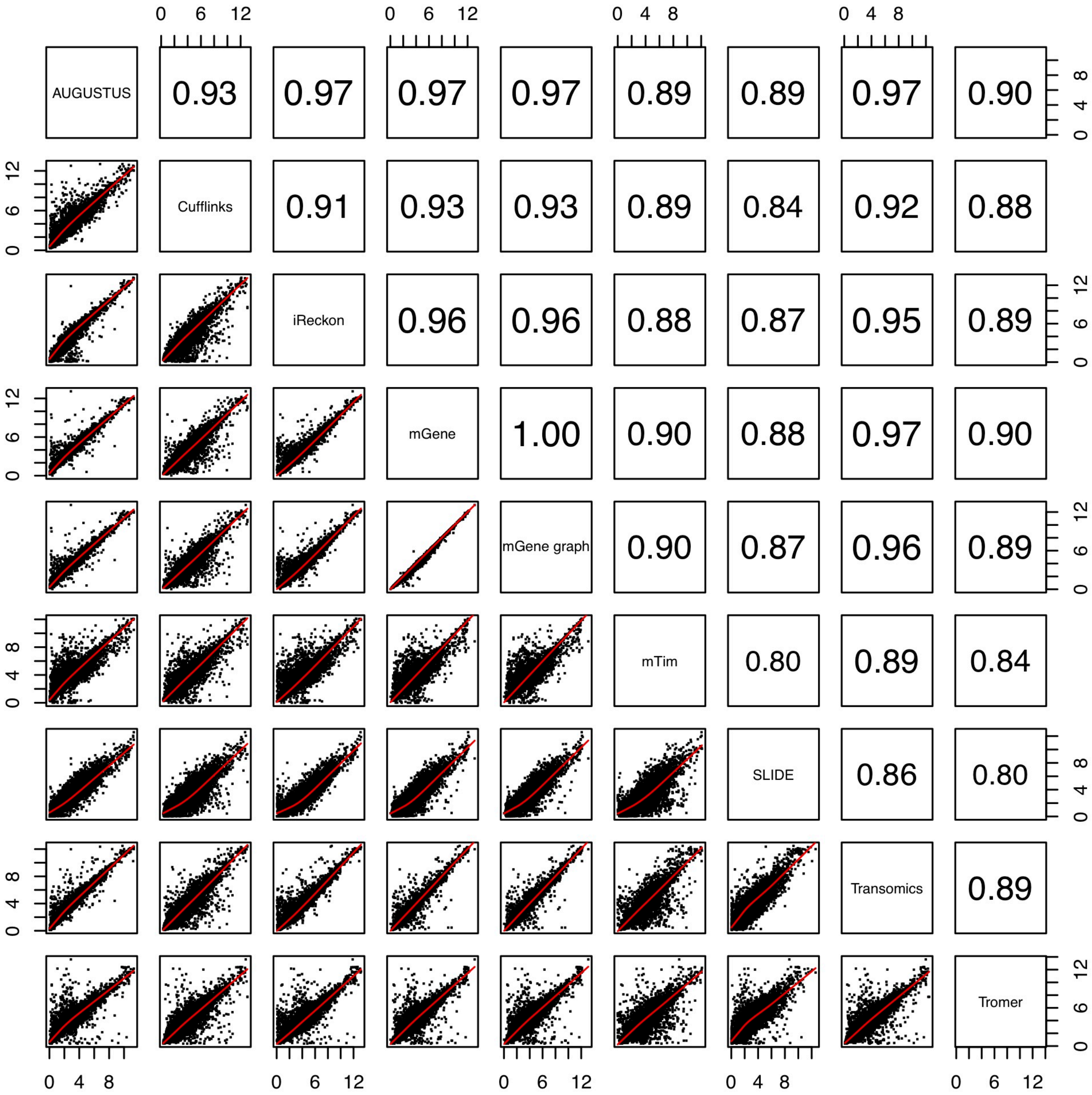
Supplementary Figure 19. Pairwise agreement between methods. Lower triangles show expression correlation (Pearson *r* of log RPKM) for the set of genes identified by all methods. Upper triangles depict the proportion of genes shared between each method pair, i.e. the number of genes identified as expressed in both divided by number of genes identified as expressed in either. Methods were ordered by hierarchical clustering using 1–*r* as the distance metric.



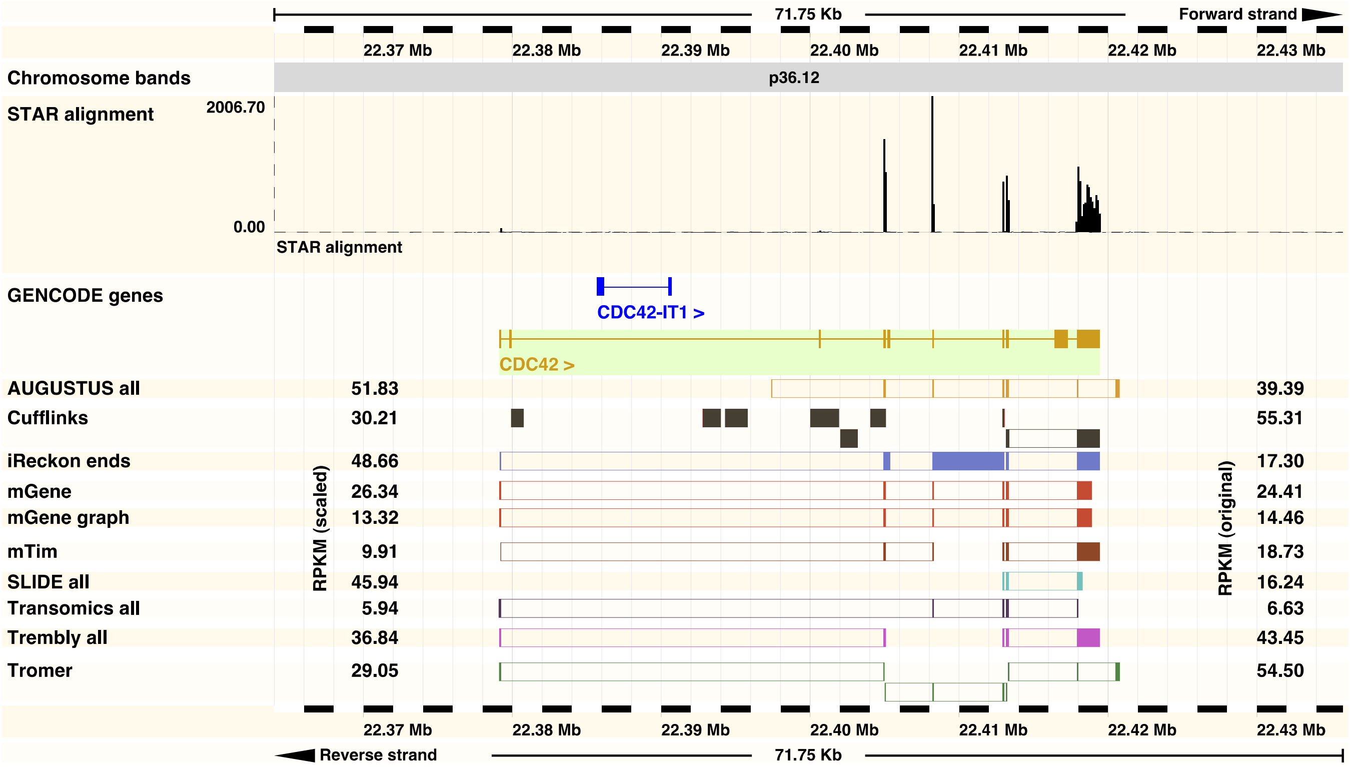
Supplementary Figure 20. Comparison of quantification methods for *H. sapiens*. For each pair of methods, scatter plots relate $\log_2$ RPKM values for the genes identified by all methods. The corresponding correlation coefficients (Pearson *r*) are shown opposite. Where multiple transcripts were reported for the same gene, the highest RPKM value was used, corresponding to the predominant transcript identified by each method. RPKM values for AUGUSTUS, iReckon, SLIDE, Transomics and Trembly correspond to the values reported by their 'all', and 'full' protocols.



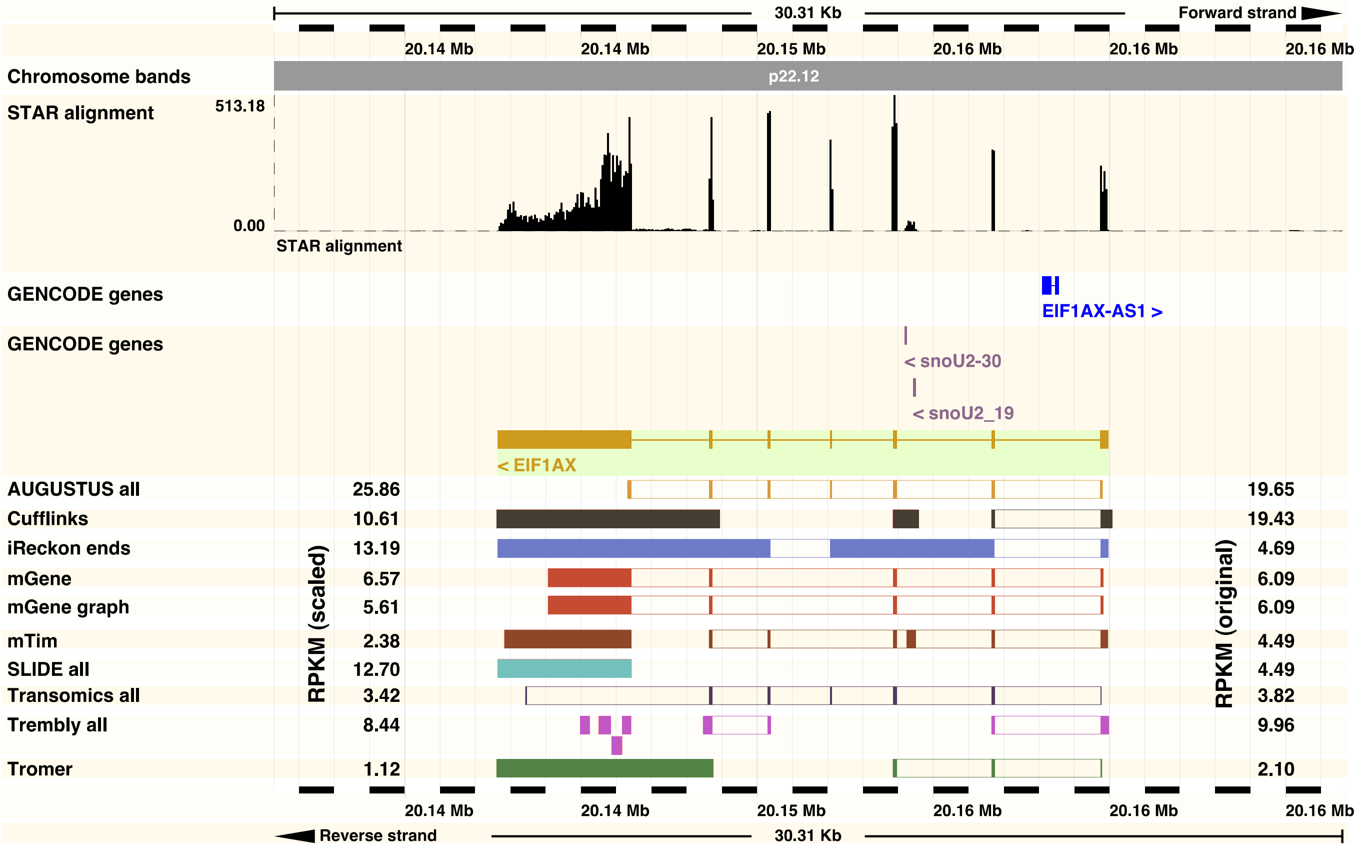
Supplementary Figure 21. Comparison of quantification methods for *D. melanogaster*. See Supplementary Figure 20 for details.



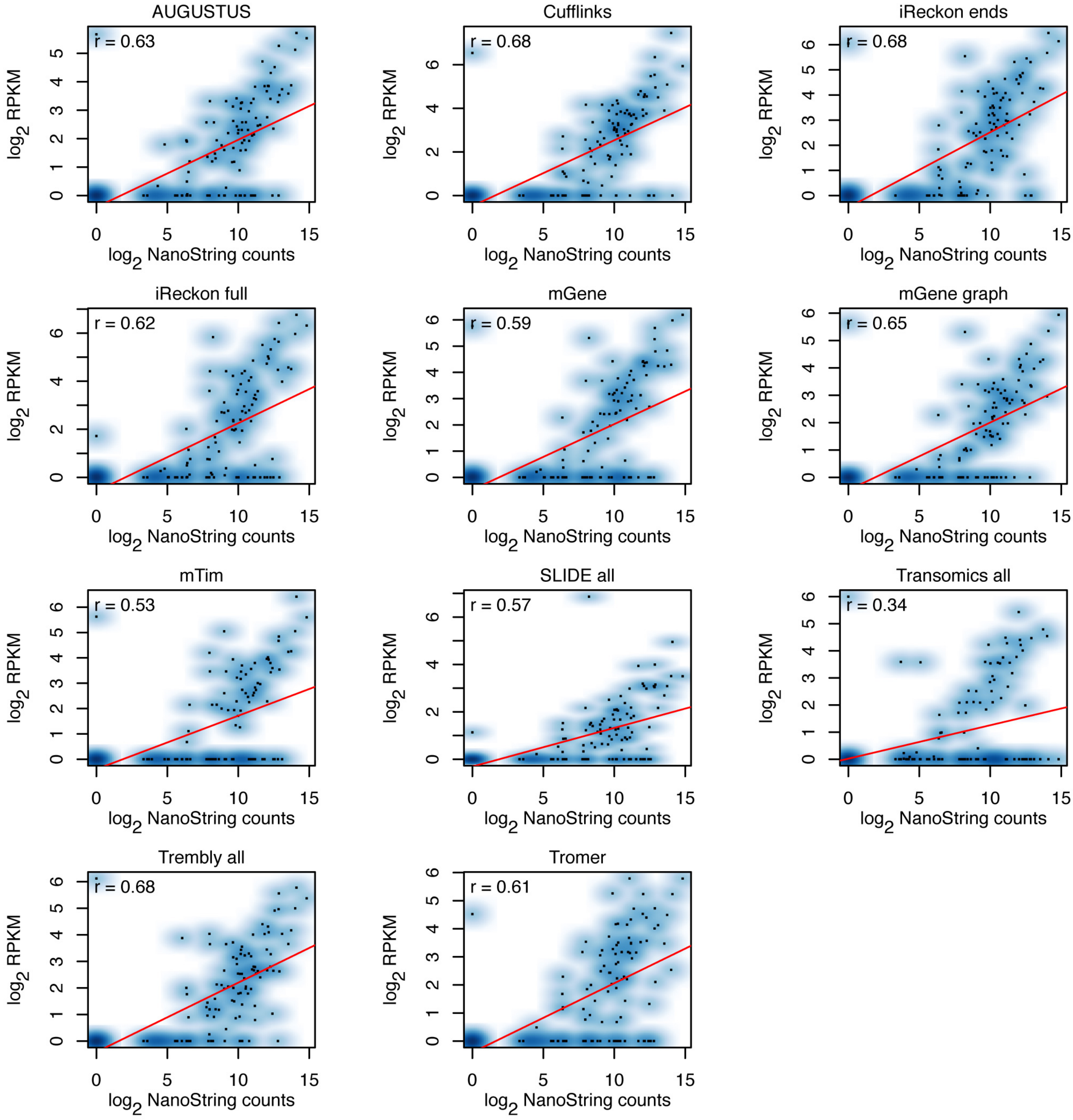
Supplementary Figure 22. Comparison of quantification methods for *C. elegans*. See Supplementary Figure 20 for details.



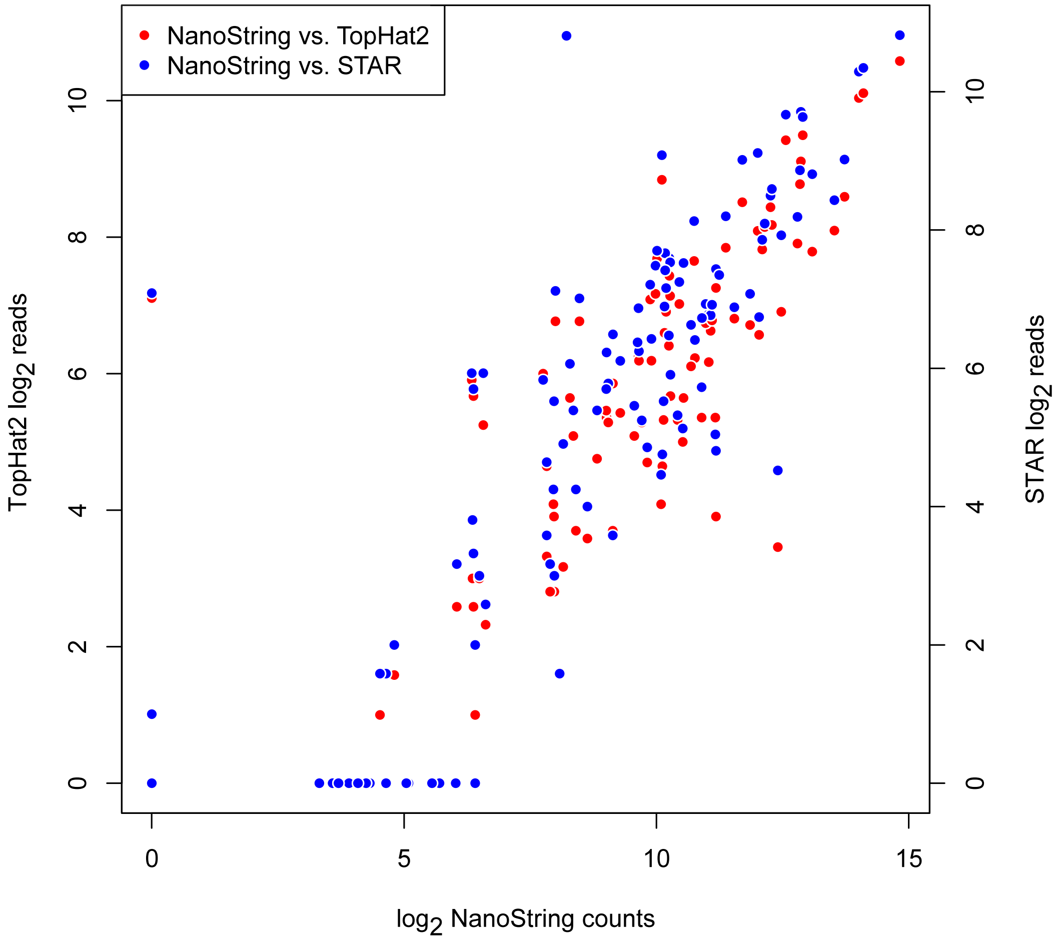
Supplementary Figure 23. Transcript predictions and expression level estimates (in RPKM) at the *CDC42* locus. Upper tracks depict RNA-seq read coverage (from STAR alignments; see Methods) and annotated genes. Exon predictions from the 10 methods that provided RPKM values are illustrated below the annotated gene by colored boxes. Exons reported as part the same transcript isoform are connected. iReckon full does not predict retained introns for this gene. Original and median-scaled RPKMs are presented to the right and left, respectively.



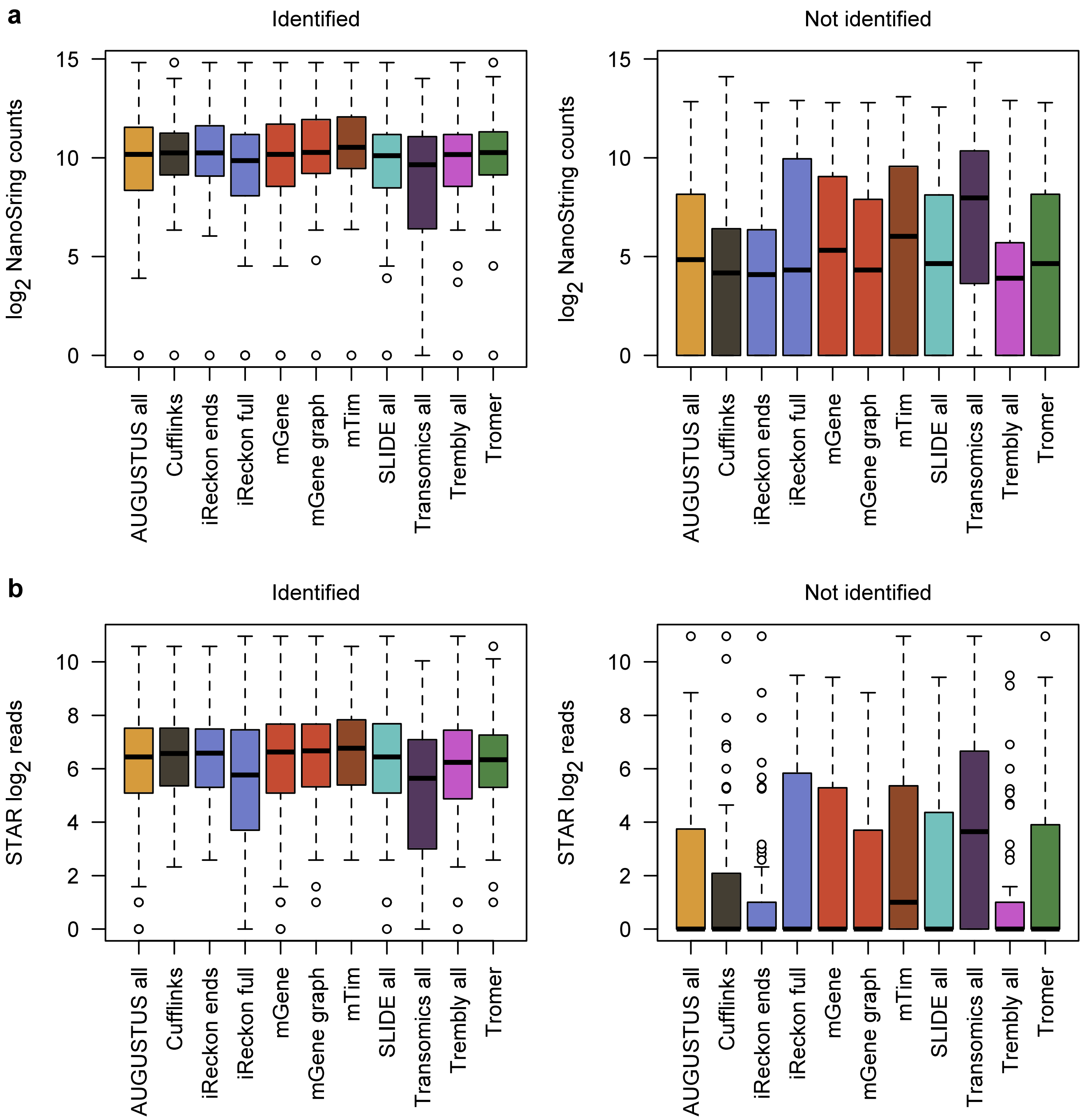
Supplementary Figure 24. Transcript predictions and expression level estimates (in RPKM) at the *EIF1AX* locus. See Supplementary Fig. 23 for details. iReckon full does not predict retained introns for this gene.



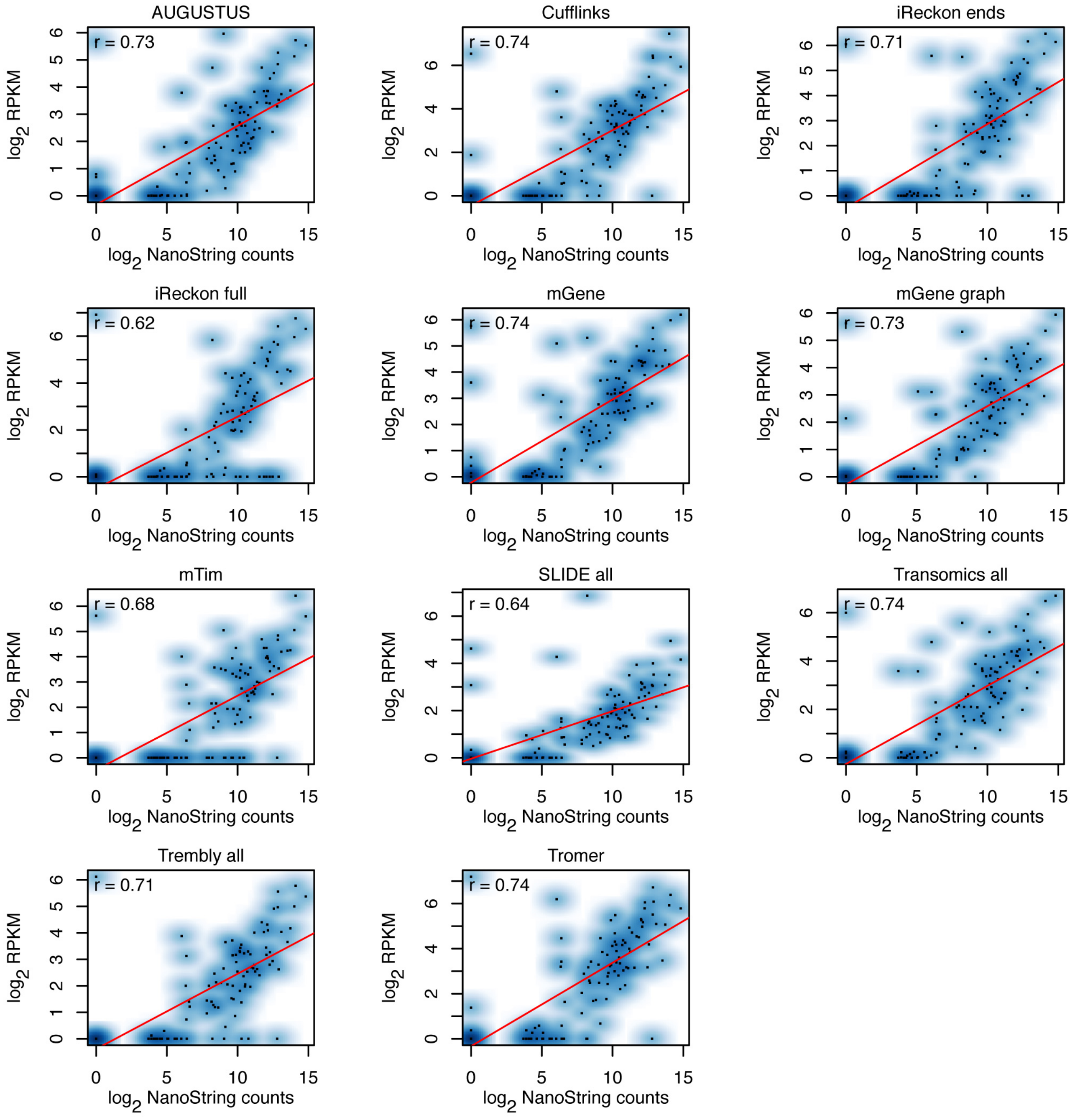
Supplementary Figure 25. Correlation between NanoString counts and transcript RPKMs. Scatter plots show individual data points in black, with color intensity indicating the density of data points. Predicted transcripts were required to contain the exon or junction targeted by the NanoString probe. Where multiple such transcripts were reported for the same gene, the highest RPKM value was used. Where no such transcript was reported, an RPKM of zero was assigned. Correlation coefficients (Pearson *r*) are given for each comparison. Expression values were incremented by 1 prior to log transformation to avoid infinite numbers. Notably, the protocol iReckon ends identifies more genes than iReckon full. When provided with complete gene annotation, iReckon often fails to resolve transcripts in complex loci with many annotated isoforms. This occurs less frequently when the program is given only transcript boundaries.



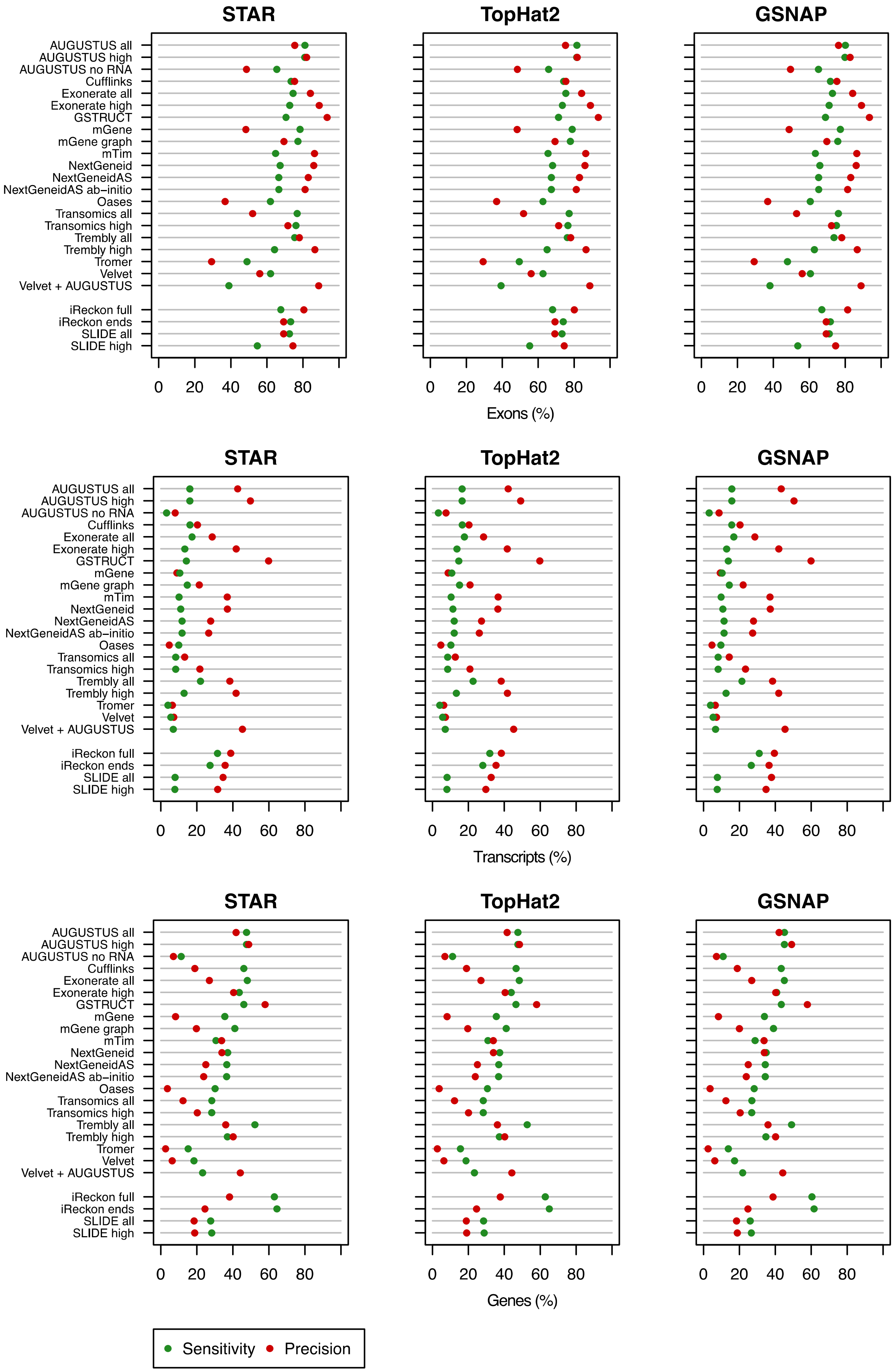
Supplementary Figure 26. Correlation between NanoString counts and numbers of mapped reads for targeted exons and junctions. Scatter plots show individual data points in red (Tophat) and blue (STAR). Count values were incremented by 1 prior to log transformation to avoid infinite numbers.



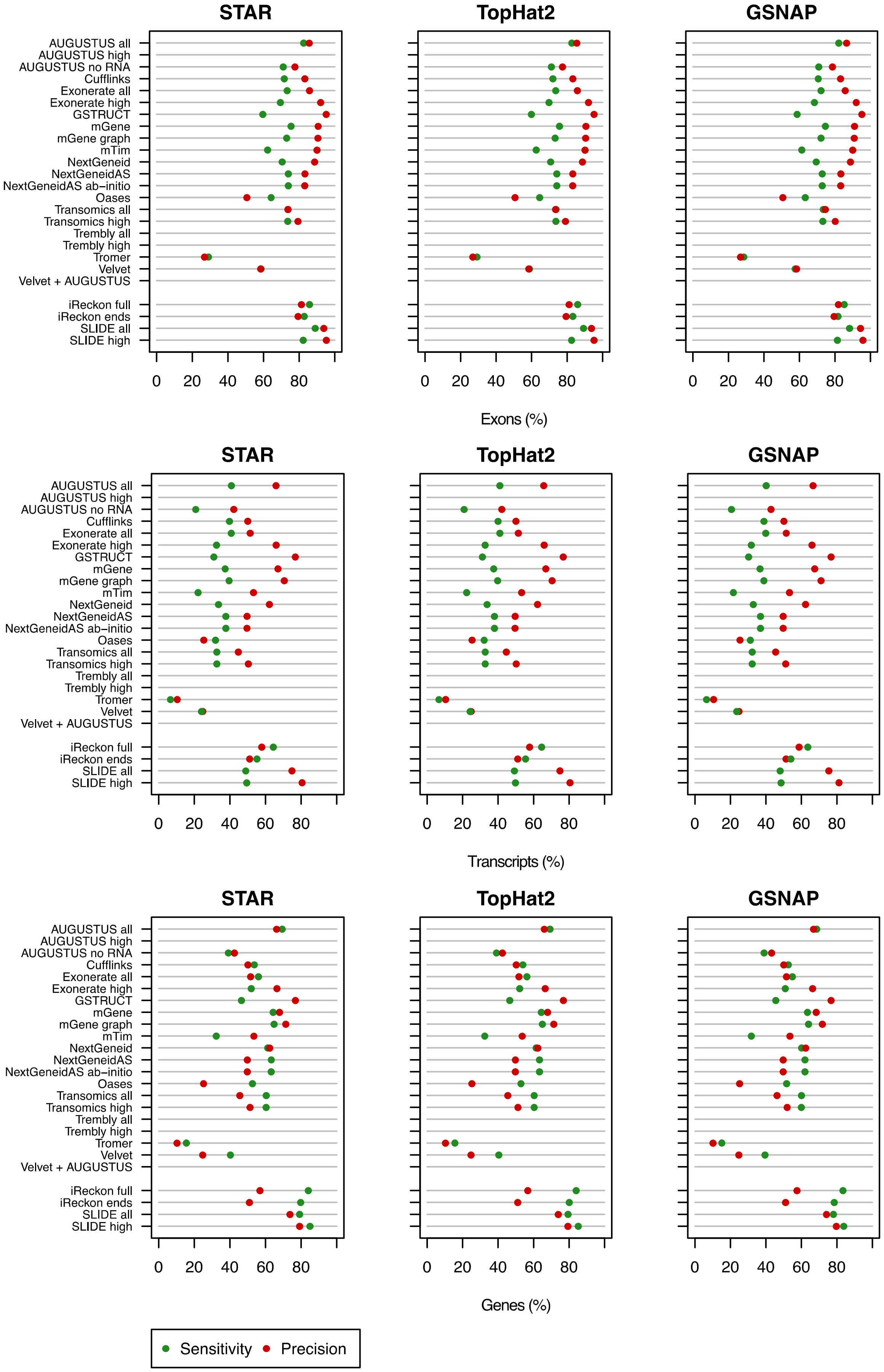
Supplementary Figure 27. Distribution of NanoString counts (a) and mapped reads by the STAR aligner (b) for probes depending on whether a method identified an isoform consistent with a probe (left) or not (right). Both mTim and Transomics failed to identify many exons or junctions targeted by NanoString probes with RNA-seq read support. Count values were incremented by 1 prior to log transformation to avoid infinite numbers.



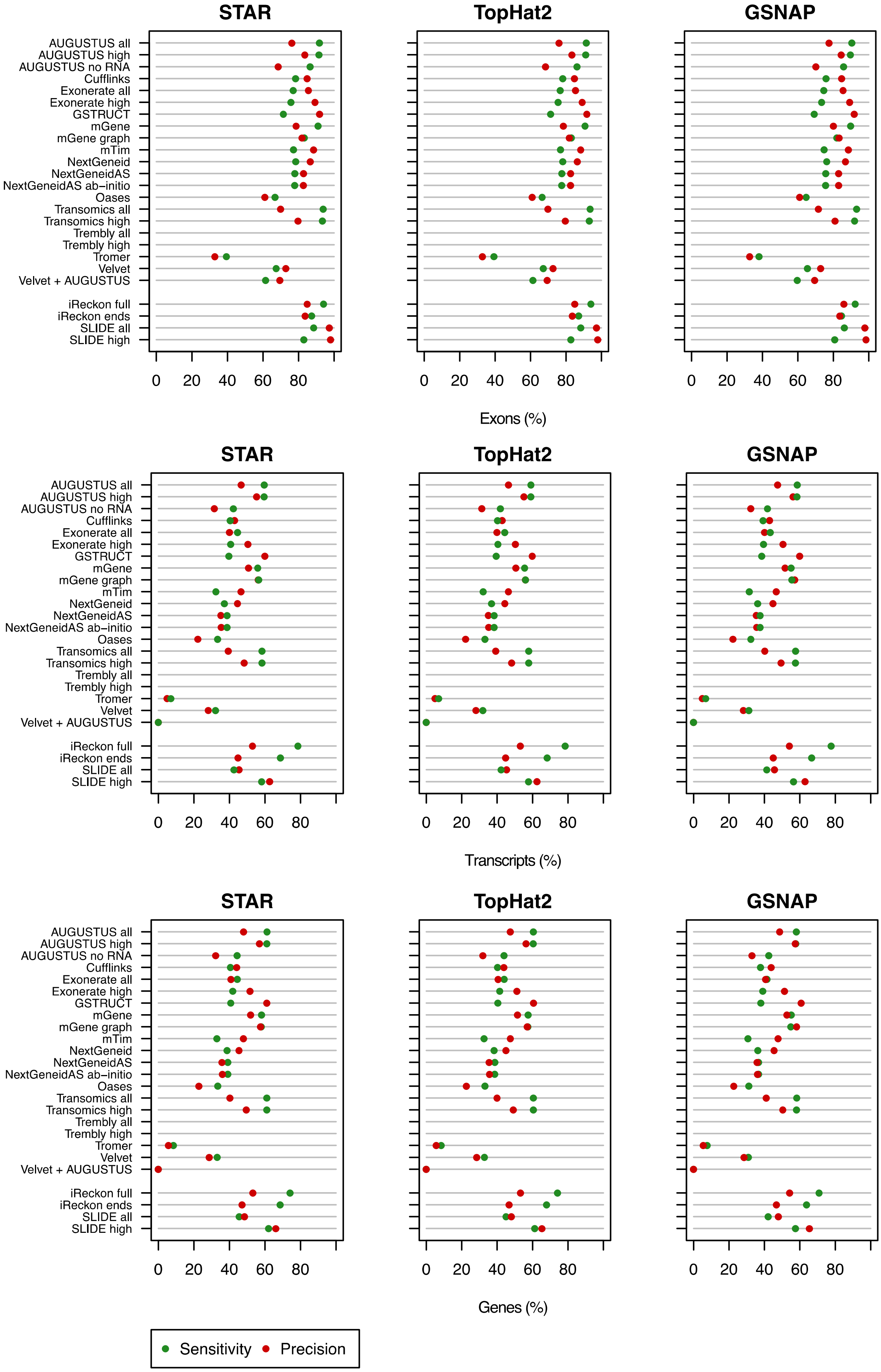
Supplementary Figure 28. Correlation between NanoString counts and gene RPKMs. Scatter plots show individual data points in black, with color intensity indicating the density of data points. Where multiple transcripts were reported for the same gene, the highest RPKM value was used (irrespective of whether that transcript contained the exon or junction targeted by the NanoString probe). Correlation coefficients (Pearson *r*) are given for each comparison.



Supplementary Figure 29. Influence of different aligners on annotation usage (*H. sapiens*). Exon, transcript and gene level performance relative to filtered annotation based on the aligners STAR, TopHat2 and GSNAP.



Supplementary Figure 30. Influence of different aligners on annotattion usage (*D. melanogaster*). See Supplementary Fig. 29 for details.



Supplementary Figure 31. Influence of different aligners on annotation usage (*C. elegans*). See Supplementary Fig. 29 for details.

Supplementary Table 1. Developer team submission details

Developer team	Protocol designation	Underlying alignment programs	Coding sequence predicted	Use of reference annotation	Quantified features	Multiple transcripts reported per gene
H. sapiens						
Iseli	Tromer	fetchGWI, megablast, SIBsim4	yes	no	transcript	yes
Gerstein	Trembly all	TopHat	no	no	transcript	yes
	Trembly high	TopHat	no	no	transcript	yes
Rätsch	mGene	PALMapper	yes	no	transcript	yes
	mGene graph	PALMapper	yes	no	transcript	yes
	mTim	PALMapper	no	no	transcript	yes
Richard	Oases	BLAT	no	no	no	yes
n.a.	Cufflinks	TopHat	no	no	transcript	yes
Stanke	AUGUSTUS high	BLAT	yes	no	transcript	yes
	AUGUSTUS all	BLAT	yes	no	transcript	yes
	AUGUSTUS de-novo	n.a	yes	no	transcript	no
Searle	Exonerate SM all	Exonerate	yes	no	no	no
	Exonerate SM high	Exonerate	yes	no	no	no
Wu	GSTRUCT	GSNAP	no	no	no	no
Guigo	Nextgeneid	GEM	yes	no	no	no
	NextgeneidAS	GEM	yes	no	no	no
	NextgeneidAS de-novo	GEM	yes	no	no	no
Solovyev	Transomics all		yes	no	transcript	no
	Transomics high		yes	no	transcript	no
Wold	Velvet	BLAT	no	no	exon	yes
	Velvet AUGUSTUS	BLAT	no	no	exon	yes
n.a	iReckon full	TopHat	no	yes	transcript	yes
	iReckon ends	TopHat	no	yes	transcript	yes
n.a	SLIDE all	TopHat	no	yes	transcript	yes
	SLIDE high	TopHat	no	yes	transcript	yes
D. melanogaster						
Iseli	Tromer	fetchGWI, megablast, SIBsim4	yes	no	transcript	yes
Rätsch	mGene	PALMapper	yes	no	transcript	yes
	mGene graph	PALMapper	yes	no	transcript	yes
	mTim	PALMapper	no	no	transcript	yes
Richard	Oases	BLAT	no	no	no	yes
n.a.	Cufflinks	TopHat	no	no	transcript	yes
Stanke	AUGUSTUS all	BLAT	yes	no	transcript	yes
	AUGUSTUS de-novo	n.a.	yes	no	transcript	no
Wu	GSTRUCT	GSNAP	no	no	no	no
Guigo	Nextgeneid	GEM	yes	no	no	no
	NextgeneidAS	GEM	yes	no	no	no
	NextgeneidAS de-novo	GEM	yes	no	no	no
Solovyev	Transomics all		yes	no	transcript	no
	Transomics high		yes	no	transcript	no
Wold	Velvet	BLAT	no	no	exon	yes
n.a.	iReckon full	TopHat	no	yes	transcript	yes
	iReckon ends	TopHat	no	yes	transcript	yes
n.a.	SLIDE all	TopHat	no	yes	transcript	yes
	SLIDE high	TopHat	no	yes	transcript	yes
C. elegans						
Iseli	Tromer	fetchGWI, megablast, SIBsim4	yes	no	transcript	yes
Rätsch	mGene	PALMapper	yes	no	transcript	yes
	mGene graph	PALMapper	yes	no	transcript	yes
	mTim	PALMapper	no	no	transcript	yes
Richard	Oases	BLAT	no	no	no	yes
n.a.	Cufflinks	TopHat	no	no	transcript	yes
Stanke	AUGUSTUS high	BLAT	yes	no	transcript	yes
	AUGUSTUS all	BLAT	yes	no	transcript	yes
	AUGUSTUS de-novo	n.a.	yes	no	transcript	no
Searle	Exonerate SM all	Exonerate	yes	no	no	no
	Exonerate SM high	Exonerate	yes	no	no	no
Wu	GSTRUCT	GSNAP	no	no	no	no
Guigo	Nextgeneid	GEM	yes	no	no	no
	NextgeneidAS	GEM	yes	no	no	no
	NextgeneidAS de-novo	GEM	yes	no	no	no
Solovyev	Transomics all		yes	no	transcript	no
	Transomics high		yes	no	transcript	no
Wold	Velvet	BLAT	no	no	exon	yes
	Velvet AUGUSTUS	BLAT	no	no	exon	yes
n.a.	iReckon full	TopHat	no	yes	transcript	yes
	iReckon ends	TopHat	no	yes	transcript	yes
n.a.	SLIDE all	TopHat	no	yes	transcript	yes
	SLIDE high	TopHat	no	yes	transcript	yes

Supplementary Table 2. Nucleotide-level performance

	H. sapiens		*D. melanogaster*		*C. elegans*	
	Sensitivity	**Precision**	**Sensitivity**	**Precision**	**Sensitivity**	**Precision**
AUGUSTUS all	63.77%	77.14%	87.17%	89.26%	96.88%	65.28%
AUGUSTUS high	63.70%	84.75%			96.65%	72.32%
AUGUSTUS no RNA	55.46%	44.91%	83.93%	83.63%	95.68%	60.17%
Cufflinks	79.31%	59.98%	84.98%	90.87%	88.61%	75.81%
Exonerate all	66.89%	87.21%	77.19%	92.87%	84.82%	78.73%
Exonerate high	65.01%	90.33%	75.22%	94.54%	83.78%	80.12%
GSTRUCT	60.59%	86.08%	58.13%	88.91%	76.67%	69.75%
iReckon full	78.33%	31.29%	89.72%	77.56%	96.08%	83.42%
iReckon ends	83.44%	10.01%	92.40%	69.20%	97.17%	73.29%
mGene	71.38%	42.86%	75.33%	87.87%	95.28%	72.73%
mGene graph	67.56%	55.51%	71.98%	90.68%	85.81%	75.70%
mTim	54.06%	90.24%	61.97%	93.06%	78.08%	81.31%
NextGeneid	64.73%	80.59%	80.36%	94.22%	84.51%	79.16%
NextGeneidAS	63.48%	81.01%	79.51%	94.44%	83.32%	79.13%
NextGeneidAS ab-initio	62.95%	79.72%	79.32%	94.37%	83.26%	79.32%
Oases	68.28%	68.53%	75.98%	91.65%	76.58%	80.52%
SLIDE all	89.28%	88.33%	96.67%	96.15%	93.36%	96.90%
SLIDE high	76.48%	87.87%	91.94%	96.64%	88.41%	97.07%
Transomics all	45.65%	53.57%	68.35%	83.70%	98.36%	72.00%
Transomics high	45.29%	75.06%	68.30%	88.99%	98.12%	82.61%
Trembly all	70.54%	85.99%				
Trembly high	53.24%	91.41%				
Tromer	84.67%	39.50%	92.06%	76.70%	87.74%	63.15%
Velvet	65.79%	78.69%	72.54%	88.07%	77.73%	82.18%
Velvet + AUGUSTUS	37.66%	75.51%			65.72%	76.78%

Supplementary Table 3. Exon-, transcript- and gene-level performance for CDS reconstruction

	Exon		Transcript		Gene	
	Sensitivity	**Precision**	**Sensitivity**	**Precision**	**Sensitivity**	**Precision**
H. sapiens						
AUGUSTUS all	66.18%	75.03%	19.51%	43.70%	61.47%	45.64%
AUGUSTUS high	66.09%	81.46%	19.50%	49.45%	61.46%	53.23%
AUGUSTUS no RNA	54.96%	48.88%	5.34%	9.28%	17.61%	9.28%
Exonerate all	57.36%	85.11%	19.77%	31.88%	58.12%	31.88%
Exonerate high	56.04%	89.39%	16.24%	42.65%	54.29%	42.65%
mGene	63.13%	50.32%	14.62%	10.01%	50.01%	10.02%
mGene graph	53.49%	82.44%	16.03%	34.44%	49.33%	46.01%
mTim	28.76%	92.55%	8.82%	46.66%	27.52%	52.53%
NextGeneid	50.47%	85.22%	11.29%	38.01%	40.96%	38.01%
NextGeneidAS	50.11%	82.48%	11.77%	31.47%	39.84%	31.47%
NextGeneidAS ab-initio	50.14%	80.49%	11.76%	29.20%	39.82%	29.20%
Transomics all	66.23%	50.68%	11.10%	14.59%	39.52%	14.59%
Transomics high	65.58%	69.73%	11.10%	23.89%	39.51%	23.89%
Tromer	31.58%	29.65%	2.23%	0.93%	6.30%	1.66%
D. melanogaster						
AUGUSTUS all	73.74%	77.11%	24.47%	39.36%	48.53%	44.03%
AUGUSTUS no RNA	64.97%	70.15%	16.60%	34.09%	33.18%	34.09%
Exonerate all	62.39%	77.96%	17.31%	28.01%	33.88%	28.01%
Exonerate high	60.48%	81.96%	16.34%	39.36%	32.63%	39.36%
mGene	70.73%	81.30%	22.00%	44.02%	43.99%	44.02%
mGene graph	62.54%	82.58%	19.34%	41.26%	38.43%	47.14%
mTim	35.55%	82.59%	8.90%	34.06%	17.66%	40.08%
NextGeneid	59.77%	76.11%	18.69%	38.84%	37.37%	38.84%
NextGeneidAS	61.43%	73.08%	19.20%	32.29%	37.92%	32.29%
NextGeneidAS ab-initio	61.44%	72.99%	19.24%	32.21%	37.99%	32.21%
Transomics all	73.62%	66.12%	23.48%	33.54%	46.95%	33.54%
Transomics high	73.56%	71.22%	23.48%	37.72%	46.93%	37.72%
Tromer	13.85%	18.64%	3.26%	2.81%	6.46%	5.75%
C. elegans						
AUGUSTUS all	84.38%	72.19%	48.20%	36.02%	60.15%	38.76%
AUGUSTUS high	84.21%	79.22%	48.13%	42.44%	60.06%	45.98%
AUGUSTUS no RNA	78.94%	64.66%	36.28%	27.20%	45.52%	27.20%
Exonerate all	67.67%	82.06%	34.18%	32.66%	42.46%	32.66%
Exonerate high	66.55%	84.82%	32.55%	41.25%	40.84%	41.25%
mGene	83.62%	74.15%	45.48%	41.94%	57.05%	41.94%
mGene graph	72.62%	77.61%	45.02%	45.46%	56.38%	47.01%
mTim	45.34%	85.51%	20.24%	36.66%	25.24%	43.02%
NextGeneid	70.15%	81.18%	30.28%	39.80%	37.98%	39.80%
NextGeneidAS	69.78%	79.51%	30.39%	33.37%	37.96%	33.37%
NextGeneidAS ab-initio	69.78%	79.43%	30.39%	33.26%	37.97%	33.26%
Transomics all	86.37%	65.43%	48.30%	32.82%	60.56%	32.82%
Transomics high	86.10%	74.75%	48.28%	40.33%	60.54%	40.33%
Tromer	20.85%	26.55%	1.20%	0.51%	1.50%	1.11%

Supplementary Table 4. Exon-, transcript- and gene-level performance (fixed evaluation mode)

	Exon		Transcript		Gene	
	Sensitivity	**Precision**	**Sensitivity**	**Precision**	**Sensitivity**	**Precision**
H. sapiens						
AUGUSTUS all	49.07%	64.29%	0.00%	0.00%	0.01%	0.01%
AUGUSTUS high	49.00%	69.85%	0.00%	0.01%	0.01%	0.01%
AUGUSTUS no RNA	41.27%	42.41%	0.00%	0.00%	0.00%	0.00%
Cufflinks	42.63%	62.90%	0.00%	0.00%	0.01%	0.00%
Exonerate all	43.32%	71.18%	0.00%	0.00%	0.01%	0.00%
Exonerate high	42.75%	75.85%	0.00%	0.01%	0.01%	0.01%
GSTRUCT	41.58%	79.67%	0.00%	0.00%	0.00%	0.00%
iReckon full	48.48%	68.40%	0.06%	0.06%	0.24%	0.06%
iReckon ends	50.26%	64.32%	0.05%	0.03%	0.18%	0.03%
mGene	47.14%	41.63%	0.00%	0.00%	0.01%	0.00%
mGene graph	46.52%	59.98%	0.00%	0.01%	0.02%	0.01%
mTim	38.28%	73.72%	0.00%	0.00%	0.01%	0.01%
NextGeneid	39.79%	73.26%	0.01%	0.12%	0.02%	0.12%
NextGeneidAS	38.94%	69.02%	0.00%	0.09%	0.02%	0.09%
NextGeneidAS ab-initio	38.97%	67.58%	0.00%	0.14%	0.02%	0.14%
Oases	36.00%	29.91%	0.00%	0.00%	0.01%	0.00%
SLIDE all	52.62%	79.66%	3.13%	4.75%	12.03%	18.18%
SLIDE high	38.56%	84.06%	3.82%	11.53%	14.75%	18.76%
Transomics all	48.52%	45.72%	0.05%	0.63%	0.19%	0.63%
Transomics high	48.01%	62.85%	0.05%	1.02%	0.19%	1.02%
Trembly all	43.14%	65.35%	0.01%	0.01%	0.02%	0.01%
Trembly high	38.76%	74.96%	0.00%	0.00%	0.01%	0.01%
Tromer	23.87%	21.04%	0.00%	0.00%	0.00%	0.00%
Velvet	36.28%	46.45%	0.00%	0.00%	0.00%	0.00%
Velvet + AUGUSTUS	22.57%	73.75%	0.00%	0.00%	0.00%	0.00%
D. melanogaster						
AUGUSTUS all	45.45%	51.16%	0.01%	0.02%	0.03%	0.02%
AUGUSTUS no RNA	40.06%	47.22%	0.00%	0.01%	0.01%	0.01%
Cufflinks	39.06%	49.65%	0.00%	0.01%	0.01%	0.01%
Exonerate all	40.32%	51.70%	0.02%	0.03%	0.04%	0.03%
Exonerate high	39.52%	57.66%	0.02%	0.04%	0.03%	0.04%
GSTRUCT	33.34%	59.03%	0.00%	0.01%	0.01%	0.01%
iReckon full	61.82%	61.71%	0.03%	0.03%	0.05%	0.03%
iReckon ends	58.56%	59.42%	0.02%	0.02%	0.04%	0.02%
mGene	42.61%	55.60%	0.03%	0.05%	0.05%	0.05%
mGene graph	40.36%	54.37%	0.03%	0.05%	0.05%	0.05%
mTim	33.87%	55.67%	0.01%	0.02%	0.02%	0.03%
NextGeneid	37.49%	51.53%	0.01%	0.02%	0.03%	0.02%
NextGeneidAS	38.88%	47.37%	0.02%	0.02%	0.03%	0.02%
NextGeneidAS ab-initio	38.88%	47.33%	0.03%	0.03%	0.04%	0.03%
Oases	33.61%	28.54%	0.01%	0.01%	0.03%	0.01%
SLIDE all	84.78%	94.14%	44.19%	25.52%	76.99%	70.36%
SLIDE high	77.01%	95.78%	44.75%	50.81%	82.73%	75.38%
Transomics all	41.16%	44.40%	0.44%	0.65%	0.88%	0.65%
Transomics high	41.09%	47.77%	0.44%	0.73%	0.88%	0.73%
Tromer	10.46%	10.56%	0.00%	0.00%	0.00%	0.00%
Velvet	30.27%	32.91%	0.02%	0.02%	0.03%	0.02%
C. elegans						
AUGUSTUS all	62.92%	53.19%	0.00%	0.00%	0.00%	0.00%
AUGUSTUS high	62.80%	58.34%	0.00%	0.00%	0.00%	0.00%
AUGUSTUS no RNA	59.53%	47.94%	0.00%	0.00%	0.00%	0.00%
Cufflinks	53.55%	59.41%	0.00%	0.00%	0.00%	0.00%
Exonerate all	53.51%	60.84%	0.00%	0.00%	0.00%	0.00%
Exonerate high	53.01%	63.98%	0.00%	0.00%	0.00%	0.00%
GSTRUCT	49.19%	64.98%	0.00%	0.00%	0.00%	0.00%
iReckon full	78.70%	71.81%	0.00%	0.00%	0.00%	0.00%
iReckon ends	71.85%	69.63%	0.00%	0.00%	0.00%	0.00%
mGene	62.93%	55.35%	0.00%	0.00%	0.00%	0.00%
mGene graph	56.22%	56.33%	0.00%	0.00%	0.00%	0.00%
mTim	52.80%	62.77%	0.00%	0.00%	0.00%	0.00%
NextGeneid	59.65%	67.18%	4.16%	5.03%	5.22%	5.03%
NextGeneidAS	59.67%	64.82%	4.58%	4.21%	5.66%	4.21%
NextGeneidAS ab-initio	59.74%	64.92%	4.63%	4.28%	5.73%	4.28%
Oases	45.33%	42.17%	0.00%	0.00%	0.00%	0.00%
SLIDE all	87.50%	97.24%	40.85%	12.66%	50.26%	43.98%
SLIDE high	81.65%	97.94%	56.51%	50.52%	70.19%	61.42%
Transomics all	88.87%	67.32%	54.36%	36.94%	68.16%	36.94%
Transomics high	88.45%	76.78%	54.35%	45.40%	68.14%	45.40%
Tromer	22.72%	19.22%	0.00%	0.00%	0.00%	0.00%
Velvet	45.87%	50.48%	0.00%	0.00%	0.00%	0.00%
Velvet + AUGUSTUS	58.49%	67.24%	0.00%	0.00%	0.00%	0.00%

Supplementary Table 5. Exon-, transcript- and gene-level performance (flexible evalutation mode)

	Exon		Transcript		Gene	
	Sensitivity	**Precision**	**Sensitivity**	**Precision**	**Sensitivity**	**Precision**
H. sapiens						
AUGUSTUS all	81.16%	77.47%	16.27%	39.26%	56.16%	42.71%
AUGUSTUS high	81.05%	84.15%	16.27%	44.41%	56.16%	49.78%
AUGUSTUS no RNA	65.55%	50.73%	3.52%	8.03%	13.38%	8.03%
Cufflinks	73.45%	79.57%	16.03%	19.29%	53.55%	20.36%
Exonerate all	74.59%	88.34%	17.22%	28.60%	55.53%	28.60%
Exonerate high	72.72%	92.46%	13.23%	41.92%	50.28%	41.92%
GSTRUCT	70.63%	96.42%	14.10%	59.85%	54.02%	59.85%
iReckon full	67.78%	83.05%	31.80%	38.87%	73.63%	38.87%
iReckon ends	73.24%	83.26%	27.78%	35.77%	71.61%	35.77%
mGene	78.40%	50.96%	10.83%	8.98%	41.35%	9.00%
mGene graph	77.27%	73.14%	14.90%	14.89%	48.17%	21.42%
mTim	64.86%	90.42%	10.14%	22.66%	35.78%	36.95%
NextGeneid	67.36%	89.58%	11.30%	36.99%	43.27%	36.99%
NextGeneidAS	66.65%	87.32%	11.96%	27.73%	42.73%	27.73%
NextGeneidAS ab-initio	66.68%	85.56%	11.94%	26.60%	42.63%	26.60%
Oases	62.02%	40.55%	10.04%	5.11%	34.05%	4.74%
SLIDE all	72.58%	84.10%	8.78%	12.68%	29.08%	34.60%
SLIDE high	54.75%	88.22%	8.65%	22.66%	31.09%	31.62%
Transomics all	76.84%	54.35%	8.41%	13.28%	32.16%	13.28%
Transomics high	76.14%	74.64%	8.41%	21.74%	32.16%	21.74%
Trembly all	75.45%	83.44%	21.82%	22.73%	60.53%	38.37%
Trembly high	64.30%	90.24%	12.84%	30.28%	43.60%	41.80%
Tromer	49.02%	37.22%	4.27%	3.70%	13.92%	6.48%
Velvet	62.05%	59.56%	5.57%	6.81%	21.35%	7.26%
Velvet + AUGUSTUS	38.97%	90.68%	7.17%	45.22%	27.44%	45.35%
D. melanogaster						
AUGUSTUS all	82.61%	86.03%	42.66%	61.14%	73.03%	65.92%
AUGUSTUS no RNA	71.12%	77.83%	23.30%	42.22%	41.21%	42.22%
Cufflinks	71.73%	83.70%	36.18%	46.32%	56.37%	50.06%
Exonerate all	73.32%	86.35%	38.51%	51.44%	58.83%	51.44%
Exonerate high	69.54%	92.50%	30.98%	66.02%	54.54%	66.02%
GSTRUCT	59.66%	95.60%	28.21%	76.77%	49.10%	76.77%
iReckon full	85.85%	82.43%	65.22%	57.94%	89.07%	57.94%
iReckon ends	82.93%	80.44%	57.05%	51.13%	83.09%	51.13%
mGene	75.49%	91.06%	38.02%	67.02%	67.17%	67.05%
mGene graph	73.03%	90.92%	40.00%	64.82%	67.78%	70.56%
mTim	62.35%	90.40%	20.59%	40.70%	34.09%	53.27%
NextGeneid	70.53%	89.19%	36.30%	62.23%	64.27%	62.23%
NextGeneidAS	73.97%	83.76%	40.05%	49.64%	66.30%	49.64%
NextGeneidAS ab-initio	73.95%	83.66%	40.05%	49.61%	66.25%	49.61%
Oases	64.31%	51.05%	33.99%	18.61%	55.47%	25.39%
SLIDE all	89.08%	95.36%	52.80%	31.26%	82.02%	74.88%
SLIDE high	82.31%	96.83%	53.22%	58.60%	88.50%	80.58%
Transomics all	73.74%	74.03%	35.11%	44.78%	62.71%	44.78%
Transomics high	73.63%	79.67%	35.11%	50.38%	62.69%	50.38%
Tromer	29.15%	27.41%	9.37%	4.79%	15.69%	10.47%
Velvet	58.59%	58.75%	24.09%	22.63%	42.39%	24.81%
C. elegans						
AUGUSTUS all	91.76%	76.26%	59.28%	44.09%	72.35%	46.62%
AUGUSTUS high	91.48%	83.55%	59.21%	51.96%	72.26%	55.33%
AUGUSTUS no RNA	86.51%	68.56%	42.25%	31.53%	52.77%	31.53%
Cufflinks	78.38%	84.84%	39.75%	37.53%	48.04%	42.88%
Exonerate all	76.99%	85.55%	43.82%	40.02%	52.90%	40.02%
Exonerate high	75.75%	89.27%	39.98%	50.42%	49.83%	50.42%
GSTRUCT	71.48%	91.85%	39.03%	59.92%	48.72%	59.92%
iReckon full	94.09%	84.88%	78.10%	52.98%	89.87%	52.98%
iReckon ends	87.39%	83.71%	68.45%	44.85%	79.25%	44.85%
mGene	90.99%	78.64%	55.27%	50.73%	69.01%	50.73%
mGene graph	83.26%	81.96%	55.78%	54.68%	68.88%	56.18%
mTim	77.16%	88.48%	31.86%	37.72%	38.96%	46.54%
NextGeneid	78.45%	86.60%	37.04%	44.55%	46.19%	44.55%
NextGeneidAS	77.91%	82.80%	38.45%	35.17%	46.71%	35.17%
NextGeneidAS ab-initio	77.85%	82.76%	38.45%	35.41%	46.71%	35.41%
Oases	66.77%	61.04%	32.98%	17.39%	39.70%	22.24%
SLIDE all	88.52%	97.34%	42.85%	13.34%	51.92%	45.44%
SLIDE high	82.97%	98.04%	58.13%	51.78%	71.61%	62.66%
Transomics all	93.90%	69.92%	58.10%	39.32%	72.52%	39.32%
Transomics high	93.43%	79.73%	58.08%	48.32%	72.50%	48.32%
Tromer	39.43%	32.92%	7.24%	1.96%	8.97%	4.96%
Velvet	67.44%	72.86%	31.77%	27.15%	39.54%	28.09%
Velvet + AUGUSTUS	61.49%	69.46%	0.00%	0.00%	0.00%	0.00%

Supplementary Table 6. Alternative splicing and transcript diversity

	Minimum	**First quartile**	**Median**	**Mean**	**Third quartile**	**Maximum**
H, sapiens						
CDS length (bp)	1	79	115	143.7	159	17330
Exon length (bp)	1	87	126	224.9	186	91670
Intron length (bp)	3	498	1569	6410	4481	4251000
Exons per transcript	1	3	5	6.646	8	118
Transcripts per gene	1	1	1	5.225	7	80
D. melanogaster						
CDS length (bp)	1	124	197	372.6	402	27710
Exon length (bp)	1	144	246	476.2	544	28070
Intron length (bp)	4	65	104	1540	733	139300
Exons per transcript	1	2	4	5.496	7	78
Transcripts per gene	1	1	1	1.943	2	31
C. elegans						
CDS length (bp)	1	99	146	205.3	234	14980
Exon length (bp)	1	99	146	205.3	234	14980
Intron length (bp)	3	50	71	330.6	345	21230
Exons per transcript	1	4	6	6.819	9	66
Transcripts per gene	1	1	1	1.255	1	15

Supplementary Table 7. NanoString probes and targeted transcript isoforms

Probe ID	Targeted trancripts (Ensembl IDs)	Probe sequence
adar1_sp1	ENST00000292205, ENST00000494866, ENST00000368471	ACTGGCAGTCTCCGGGTGTCCGGCCGTGTCCCGAGGAAGTGCAAGACCCGGGGTATTCCCTCAGCGGATACTACACCCATCCATTTCAAGGCTATGAGCA
adar1_sp2	ENST00000368474	CGGCGGGTCGGGCCGGGCAATGCCTCGCGGGCGCAATGAATCCGCGGCAGGGGTATTCCCTCAGCGGATACTACACCCATCCATTTCAAGGCTATGAGCA
atf2_common	ENST00000264110, ENST00000345739, ENST00000392543, ENST00000392544, ENST00000409499, ENST00000409833, ENST00000413123, ENST00000415955, ENST00000417080, ENST00000421438, ENST00000426833, ENST00000428760, ENST00000429579, ENST00000435231, ENST00000437522, ENST00000445349, ENST00000456655, ENST00000487334, ENST00000538946, ENST00000542046, ENST00000409635	GGTACTAGATGGAAACTTGAGAAAGGACTGCTTATTGATAACAGCTAAGGTATTCCTGGAAGCAGAGTAAATAAAGCTCATGGCCCACCAGCTAGAAAG
atf2_sp1	ENST00000345739, ENST00000437522, ENST00000435231, ENST00000415955, ENST00000409635, ENST00000456655	ATATGAGTGATGACAAACCCTTTCTATGTACTGCGCCTGGATGTGGCCAGATCAGACCCCAACACCAACAAGATTCTTGAAAAACTGTGAAGAAGTGGGT
atf2_sp2	ENST00000429579, ENST00000538946, ENST00000428760, ENST00000417080, ENST00000421438, ENST00000409437, ENST00000392544, ENST00000264110, ENST00000435004, ENST00000542046, ENST00000426833, ENST00000409833, ENST00000487334	ATATGAGTGATGACAAACCCTTTCTATGTACTGCGCCTGGATGTGGCCAGCGTTTTACCAACGAGGATCATTTGGCTGTCCATAAACATAAACATGAGAT
ATP5J_common	ENST00000284971, ENST00000400087, ENST00000400090, ENST00000400093, ENST00000400094, ENST00000457143, ENST00000486002, ENST00000400099	CAGAGTATCAGCAAGAGCTGGAGAGGGAGCTTTTTAAGCTCAAGCAAATGTTTGGTAATGCAGACATGAATACATTTCCCACCTTCAAATTTGAA
Bcl11a_sp1	ENST00000335712, ENST00000356842, ENST00000359629, ENST00000489516	TTTATCAACGTCATCTAGAGGAATTTGCCCCAAACAGGAACACATAGCAGATAAACTTCTGCACTGGAGGGGCCTCTCCTCCCCTCGTTCTGCACATGGA
Bcl11a_sp2	ENST00000409351	TATCAACGTCATCTAGAGGAATTTGCCCCAAACAGGAACACATAGCAGCTCAGACTGAACTGGAGGATGTATTTGTGTACCTTATGGTGT
BCL3	ENST00000164227, ENST00000403534	CGGAGCCTTACTGCCTTTGTACCCCACTCGGGCCATGGGCTCCCCGTTTCCTCTGGTGAACCTGCCTACACCCCTATACCCCATGATGTGCCCCATGGAA
BHLHB2	ENST00000256495	AAAAGCTTCAAAGTCTTGGTCTGTGAGTCACTCTTCAGTTTGGGAGCTGGGTCTGTGGCTTTGATCAGAAGGTACTTTCAAAAGAGGGCTTTCCAGGGCT
Blnk_sp1_T	ENST00000371176	TGAAAACTATATTCATCCCACAGAAAGCAGTTCACCTCCACCTGAAAAAGGTCGAAACAGTGGGGCCTGGGAAACCAAGTCACCTCCACCAGCTGCACCA
Blnk_sp2	ENST00000413476, ENST00000224337, ENST00000427367, ENST00000467799	TGAAAACTATATTCATCCCACAGAAAGCAGTTCACCTCCACCTGAAAAAGCTCCCATGGTGAATAGATCAACCAAGCCAAATTCCTCAACGCCCGCCTCT
CARM1_sp1_T	ENST00000592516, ENST00000344150	TGTTATTGCCAGTGGCTCCAGCGTGGGCCACAACAACCTGATTCCTTTAGGGTCCTCCGGCGCCCAGGGCAGTGGTGGTGGCAGCACGAGTGCCCACTAT
CD19	ENST00000324662, ENST00000538922, ENST00000565089, ENST00000567541	GAAGGTCTCAGCTGTGACTTTGGCTTATCTGATCTTCTGCCTGTGTTCCCTTGTGGGCATTCTTCATCTTCAAAGAGCCCTGGTCCTGAGGAGGAAAAGA
Cd79b_sp1_T	ENST00000349817	TGAGCCAGTACCAGCAGCCAGATCGGAGGACCGGTACCGGAATCCCAAAGGATTCAGCACCTTGGCACAGCTGAAGCAGAGGAACACGCTGAAGGATGGT
Cd79b_sp2_T	ENST00000559358, ENST00000006750, ENST00000392795	CAACACCTCGGAGGTCTACCAGGGCTGCGGCACAGAGCTGCGAGTCATGGGATTCAGCACCTTGGCACAGCTGAAGCAGAGGAACACGCTGAAGGATGGT
cdkn1a_sp1_T	ENST00000244741	GAGCCGGAGCTGGGCGCGGATTCGCCGAGGCACCGAGGCACTCAGAGGAGGCGCCATGTCAGAACCGGCTGGGGATGTCCGTCAGAACCCATGCGGCAGC
cdkn1a_sp2_T	ENST00000405375, ENST00000478800	GGATGCGTGTTCGCGGGTGTGTGCTGCGTTCACAGGTGTTTCTGCGGCAGGCGCCATGTCAGAACCGGCTGGGGATGTCCGTCAGAACCCATGCGGCAGC
CEBPA	ENST00000425420, ENST00000498907	CTAGTATTTAGGATAACCTTGTGCCTTGGAAATGCAAACTCACCGCTCCAATGCCTACTGAGTAGGGGGAGCAAATCGTGCCTTGTCATTTTATTTGGAG
CTCF_common	ENST00000264010, ENST00000401394	CCCAACGGAGACCTCACGCCCGAGATGATCCTCAGCATGATGGACCGGTGATGGCGGAGCCTTGTGCGTCGCCAGGACTTCTCTGGGCTGTGTTTAAACG
CTCF_sp1	ENST00000566078, ENST00000264010	GAGCTGGGTTCTATTTTCCCTCCTCAAACTGACTTTGCAGCCACGGAGAGGCAGGGGAAATGGAAGGTGATGCAGTCGAAGCCATTGTGGAGGAGTCCGA
CTCFL_sp1	ENST00000433949, ENST00000243914, ENST00000539382, ENST00000371196, ENST00000426658, ENST00000423479, ENST00000422869, ENST00000502686	TGCCAGCAGAGATACCTACAAGCTGAAACGCCACATGAGAACGCACTCAGGTGAGAAGCCTTACGAATGCCACATCTGCCACACCCGCTTCACCCAGAGC
CTCFL_sp2	ENST00000429804	TGCCAGCAGAGATACCTACAAGCTGAAACGCCACATGAGAACGCACTCAGGTGTGCATATGCGCAACTTGCATGCTTACAGCGCTGCAGAGCTGAAATGC
CTDSL_sp2_T	ENST00000273179, ENST00000443503, ENST00000486978	GCCCCAGTGTGCTTCCGCCACTGGTGGAGGAGAATGGTGGGCTTCAGAAGGGTGACCAGAGGCAGGTCATTCCCATACCAAGTCCACCAGCTAAGTACCT
CTDSP1_common_T	ENST00000273062, ENST00000428361, ENST00000443891, ENST00000452977, ENST00000464255, ENST00000473420, ENST00000482272, ENST00000488627, ENST00000491064, ENST00000497677, ENST00000498160	AGCTGACCTGCTGGACAAATGGGGGGCCTTCCGGGCCCGGCTGTTTCGAGAGTCCTGCGTCTTCCACCGGGGGAACTACGTGAAGGACCTGAGCCGGTT
CTDSP2	ENST00000398073	CCTGTCCTGTACCGAGCTCTGTCTGTTCCAGCCTTCATCCTTCCTGGCTGTTGCTTTTCCTCTTAAGGGCCTCAGAACTCTTGCTCTTCCTGGGCTGAGG
CTDSPL_sp1	ENST00000443503	GCCCCAGTGTGCTTCCGCCACTGGTGGAGGAGAATGGTGGGCTTCAGAAGCCACCAGCTAAGTACCTTCTTCCAGAGGTGACGGTGCTTGACTATGGAAA
DES	ENST00000492726, ENST00000477226, ENST00000373960	GAGAACAATTTGGCTGCCTTCCGAGCGGACGTGGATGCAGCTACTCTAGCTCGCATTGACCTGGAGCGCAGAATTGAATCTCTCAACGAGGAGATCGCGT
DNMT1_common_T	ENST00000340748, ENST00000359526, ENST00000540357, ENST00000586588, ENST00000587197, ENST00000588913, ENST00000589294, ENST00000592705	CCTTTCCGGACCATCACGGTGCGAGACACGATGTCCGACCTGCCGGAGGTGCGGAATGGAGCCTCGGCACTGGAGATCTCCTACAACGGGGAGCCTCAGT
E2F4	ENST00000379378, ENST00000567007	GTCAGAAATCTTTGATCCCACACGAGAGTGCATGAGCTCGGAGCTGCTGGAGGAGTTGATGTCCTCAGAAGTGTTTGCCCCTCTGCTTCGTCTTTCTCCA
E2F6_common	ENST00000307236, ENST00000362009, ENST00000381525, ENST00000421117, ENST00000428221, ENST00000437573, ENST00000444832, ENST00000455198, ENST00000468775, ENST00000542100, ENST00000546212	AGGTTGCAACGAAACTGGGAGTCCGAAAGCGGAGAGTGTATGACATCACCAATGTCTTAGATGGAATCGACCTCGTTGAAAAGAAATCCAAGAACCATAT
ebf1_sp1	ENST00000519890, ENST00000380654, ENST00000518836, ENST00000519739, ENST00000313708, ENST00000522192	TGGACAACTGGCCGTGAATGTCTCCGAGGCATCACAAGCCACCAATCAGGGTTTCACCCGCAACTCAAGCAGCGTATCACCACACGGGTACGTGCCGAGC
EGR1	ENST00000239938	CTTCAATGCTAGAAAATCGAGTTGGCAAAATGGGGTTTGGGCCCCTCAGAGCCCTGCCCTGCACCCTTGTACAGTGTCTGTGCCATGGATTTCGTTTTTC
EOMES_common	ENST00000295743, ENST00000449599, ENST00000537516	CAACAAACTAGACATCAGTTCCTATGAATCTGAATATACTTCTAGCACATTGCTCCCATATGGCATTAAATCCTTGCCCCTTCAGACATCCCATGCCCTG
EP300	ENST00000263253	ACAAATATCCCTTTGGCTCCGTCCAGCGGTCAAGCTCCAGTGTCTCAAGCACAAATGTCTAGTTCTTCCTGCCCGGTGAACTCTCCTATAATGCCTCCAG
esr1_common	ENST00000206249, ENST00000338799, ENST00000406599, ENST00000427531, ENST00000440973, ENST00000443427, ENST00000456483, ENST00000544394	GTAGAGGGCATGGTGGAGATCTTCGACATGCTGCTGGCTACATCATCTCGGTTCCGCATGATGAATCTGCAGGGAGAGGAGTTTGTGTGCCTCAAATCTA
esr2_sp1	ENST00000353772, ENST00000554572, ENST00000344288, ENST00000358599	ATGCGCCTGGCTAACCTCCTGATGCTCCTGTCCCACGTCAGGCATGCGAGGGCAGAAAAGGCCTCTCAAACACTCACCTCATTTGGAATGAAGATGGAGA
esr2_sp2	ENST00000554520, ENST00000341099, ENST00000267525, ENST00000555483	ATGCGCCTGGCTAACCTCCTGATGCTCCTGTCCCACGTCAGGCATGCGAGTAACAAGGGCATGGAACATCTGCTCAACATGAAGTGCAAAAATGTGGTCC
ets1_common	ENST00000319397, ENST00000345075, ENST00000526145, ENST00000530924, ENST00000531611, ENST00000535549, ENST00000392668	CAGGAGATGGGGAAAGAGGAAAAACAAACCTAAGATGAATTATGAGAAACTGAGCCGTGGCCTACGCTACTATTACGACAAAAACATCATCCACAAGACA
FBXO15	ENST00000269500, ENST00000581214, ENST00000419743, ENST00000585174, ENST00000583443	CTAGCTGACATTCTCAAACCTGTCAACCCTTACACAGGCCTTCCAGTTAAGACCAAAGAGGCCCTCAGAATATTTGGTTTAGGTTGGGCAATTATACTGA
FOS	ENST00000303562	TCAAGTCCTTACCTCTTCCGGAGATGTAGCAAAACGCATGGAGTGTGTATTGTTCCCAGTGACACTTCAGAGAGCTGGTAGTTAGTAGCATGTTGAGCCA
foxa2_body	ENST00000319993, ENST00000377115, ENST00000419308	CGTTCCGTCCCAAACAGAGGGCCACACAGATACCCCACGTTCTATATAAGGAGGAAAACGGGAAAGAATATAAAGTTAAAAAAAAGCCTCCGGTTTCCAC

Probe ID	Targeted trancripts (Ensembl IDs)	Probe sequence
foxa2_sp2_T	ENST00000377115	CGGGTCCCTGGCGGCCGGTGTCTGAGGAGTCGGAGAGCCGAGGCGGCCAGACCGTGCGCCCCGCGCTTCTCCCGAGGCCGTTCCGGGTCTGAACTGTAAC
FOXA3	ENST00000302177	CCCCGTGTTGGCCATGTCGTCACCATTCTCTCTGGCATGGGTTGGGTAGGGGATGGAGGTGAGAATACTCCTTGGTTTTCTCTGAAGCCCACCCTTTCCC
gabpa_sp1_T	ENST00000354828	CCGGACGGGTCTAGGTGAGACAGAAGCCAAACAGGAGGAGGAAGTGGAGGGACTGATCCTTTGAAATACTCCAGCCATGACTAAAAGAGAAGCAGAGGAG
gabpa_sp2_T	ENST00000400075	CAGCCGGCTCTGGAGTGCGGGCGGGGGCGACAGGGCCGATTCCGGAGTGGGACTGATCCTTTGAAATACTCCAGCCATGACTAAAAGAGAAGCAGAGGAG
GATA1_T	ENST00000376665, ENST00000376670	GTGTCCCACCCGCGAGGACTCTCCTCCCCAGGCCGTGGAAGATCTGGATGGAAAAGGCAGCACCAGCTTCCTGGAGACTTTGAAGACAGAGCGGCTGAGC
HDAC1	ENST00000373548, ENST00000476391	CTGTTTTCGTACCTTCCCACTGGCCTCAAGTGAGCCAAGAAACACTGCCTGCCCTCTGTCTGTCTTCTCCTAATTCTGCAGGTGGAGGTTGCTAGTCTAG
HDAC3_sp1	ENST00000305264, ENST00000495485, ENST00000523353	CATTGACCCATAGCCTGGTCCTGCATTACGGTCTCTATAAGAAGATGATCGTCTTCAAGCCATACCAGGCCTCCCAACATGACATGTGCCGCTTCCACTC
HDAC4	ENST00000345617	TGTCAGCTCACTCCAGCTTCACAAATGTGCTGAGAGCATTACTGTGTAGCCTTTTCTTTGAAGACACACTCGGCTCTTCTCCACAGCAAGCGTCCAGGGC
HDAC5	ENST00000225983, ENST00000336057, ENST00000393622	CAGGGGAGGATCTGGAGGATCCACTACTGTCTTTAAGATGCAGAGTGGAGGGGAGGTGGGCACCCACCCTGCGATTCTCCACCCTTTCCCCTTCTTTCGT
Hif1a_common	ENST00000323441, ENST00000337138, ENST00000394997, ENST00000555014, ENST00000539097, ENST00000557538	TCCAGCAGACTCAAATACAAGAACCTACTGCTAATGCCACCACTACCACTGCCACCACTGATGAATTAAAAACAGTGACAAAAGACCGTATGGAAGACAT
HNF1A	ENST00000257555, ENST00000400024, ENST00000402929, ENST00000538646, ENST00000540108, ENST00000541395, ENST00000541924, ENST00000543427, ENST00000544413	GTGCGCTATGGACAGCCTGCGACCAGTGAGACTGCAGAAGTACCCTCAAGCAGCGGCGGTCCCTTAGTGACAGTGTCTACACCCCTCCACCAAGTGTCCC
HNF1B	ENST00000561193, ENST00000225893	GTTTCCATCTGCAATGGTGGTCACAGATACCAGCAGCATCAGTACACTCACCAACATGTCTTCAAGTAAACAGTGTCCTCTACAAGCCTGGTGATGCCCA
Hnf4g_common_T	ENST00000396423	AGAAAATAGTTATCCATTGACTAGAAATTAGTACATGCCCACAGCTGGCTCCCACGGTAGCCAGGAGAATTATCTATAGGTGGAAAGTCTGTGTCAGCCA
HSF1	ENST00000400780, ENST00000528838, ENST00000528988, ENST00000533240	TGTTCGACCAGGGCCAGTTTGCCAAGGAGGTGCTGCCCAAGTACTTCAAGCACAACAACATGGCCAGCTTCGTGCGGCAGCTCAACATGTATGGCTTCCG
IGF1R_common_T	ENST00000268035, ENST00000558762	GCGATTGCTGGGTGTGGTGTCCCAAGGCCAGCCAACACTGGTCATCATGGAACTGATGACACGGGGCGATCTCAAAAGTTATCTCCGGTCTCTGAGGCCA
IKZF1_common	ENST00000331340, ENST00000343574, ENST00000346667, ENST00000349824, ENST00000357364, ENST00000359197, ENST00000438033, ENST00000439701, ENST00000440768, ENST00000471793	CCTGCTGCGCGCCGCCTCCGAGAACTCGCAGGACGCGCTCCGCGTGGTCAGCACCAGCGGGGAGCAGATGAAGGTGTACAAGTGCGAACACTGCCGGGTG
IKZF1_sp1_T	ENST00000357364, ENST00000331340, ENST00000343574, ENST00000413698, ENST00000346667, ENST00000349824, ENST00000440768, ENST00000359197	CGAGGATCAGTCTTGGCCCCAAAGCGCGACGCACAAATCCACATAACCTGAGGACCATGGATGCTGATGAGGGTCAAGACATGTCCCAAGTT
IKZF1_sp2	ENST00000438033, ENST00000492782, ENST00000462201, ENST00000439701	GTGTGGAAAAGGCAGCTCTCACTTGGCCTTGGCGAGGCCTCGGTTGGTTGATAACCTGAGGACCATGGATGCTGATGAGGGTCAAGACATGTCCCAAGTT
IKZF3_sp1_T	ENST00000377958, ENST00000377944, ENST00000346872, ENST00000535189, ENST00000467757	CAAGGAGCGCTGCCGTACATTTCTTCAGAGCACTGACCCAGGGGACACTGCAAGTGCGGAGGCAAGACACATCAAAGCAGAGATGGGAAGTGAAAGAGCT
IKZF3_sp2	ENST00000583368, ENST00000293068, ENST00000348427	TCACTGACCACAGCAGGTACCCCAGGCAAGAATCTGAGCAGTTATAACAGCAAGTGCGGAGGCAAGACACATCAAAGCAGAGATGGGAAGTGAAAGAGCT
IL6	ENST00000258743, ENST00000401630, ENST00000404625, ENST00000407492, ENST00000485300	GCTCTTCGGCAAATGTAGCATGGGCACCTCAGATTGTTGTTGTTAATGGGCATTCCTTCTTCTGGTCAGAAACCTGTCCACTGGGCACAGAACTTATGTT
IL6receptor_common	ENST00000344086, ENST00000368485	TCCAATATTCGCTGTGTCAGCATAGAAGTAACTTACTTAGGTGTGGGGGAAGCACCATAACTTTGTTTAGCCCAAAACCAAGTCAAGTGAAAAAGGAGGA
IL8	ENST00000307407, ENST00000483500, ENST00000401931	GGAAGGAACCATCTCACTGTGTGTAAACATGACTTCCAAGCTGGCCGTGGCTCTCTTGGCAGCCTTCCTGATTTCTGCAGCTCTGTGTGAAGGTGCAGTT
IL8RA	ENST00000295683	GTCCATTGGGCAGGCAGATGTTCCTAATAAAGCTTCTGTTCCGTGCTTGTCCCTGTGGAAGTATCTTGGTTGTGACAGAGTCAAGGGTGTGTGCAGCATT
IL8RB	ENST00000318507	GATAGACAAATCTCCACCTTCAGACTGGTAGGCTCCTCCAGAAGCCATCAGACAGGAAGATGTGAAAATCCCCAGCACTCATCCCAGAATCACTAAGTGG
IRF8	ENST00000268638, ENST00000566369	CCCTCTGTCTGGGGTGGGATGCCTTACTTTGCACTTAATTTAATAAGGGCATTCTCGGAGGAGTAGACGTTTAATACGAAGTGGCGGCATAGCCCTGCCG
JUND	ENST00000252818	TGCTACGAGTCCACATTCCTGTTTGTAATCCTTGGTTCGCCCGGTTTTCTGTTTTCAGTAAAGTCTCGTTACGCCAGCTCGGCAAAAAAAAAAAAAAAAA
KAISO_sp1_T	ENST00000326624	CCAGCCTTCCGCGCGTCCGGAGGAGGAGAAGCGGCGGCGCCGGGAAGCAGGCATGGAGAGTAGAAAACTGATTTCTGCTACAGACATTCAGTACTCTGGC
KAP1_common_T	ENST00000253024, ENST00000341753	CAGGCCGAGTGCAAACAGGGCAGCAGGCGGGGCTCCCTCTCGGGTCGCAGGCGCTCTCTGCACACGCCGCAGTGCTCCAGCAGCTCCAGCGCCTCGGCGC
KLF4	ENST00000374672, ENST00000493306, ENST00000497048	CCGAGCATTTTCCAGGTCGGACCACCTCGCCTTACACATGAAGAGGCATTTTTAAATCCCAGACAGTGGATATGACCCACACTGCCAGAAGAGAATTCAG
LEF1_sp1	ENST00000509428, ENST00000438313, ENST00000505379, ENST00000510624, ENST00000504775, ENST00000510135, ENST00000379951	TATCCCTTGTCTCCGGGTGGTGTTGGACAGATCACCCCACCTCTTGGCTGGTTTTCCCATCATATGATTCCCGGTCCTCCTGGTCCCCACACAACTGGCA
LEF1_sp2	ENST00000265165, ENST00000506680, ENST00000510717, ENST00000504950, ENST00000515500	TCCCTTGTCTCCGGGTGGTGTTGGACAGATCACCCCACCTCTTGGCTGGCAAGGTCAGCCTGTATATCCCATCACGGGTGGATTCAGGCAACCCTACC
LIN28_common	ENST00000254231, ENST00000326279	GTCTGGAATCCATCCGTGTCACCGGACCTGGTGGAGTATTCTGTATTGGGAGTGAGAGGCGGCCAAAAGGAAAGAGCATGCAGAAGCGCAGATCAAAAGG
MAX_sp1	ENST00000556443, ENST00000358664, ENST00000358402, ENST00000553928, ENST00000555667, ENST00000394606, ENST00000284165, ENST00000553951, ENST00000556979, ENST00000556892, ENST00000557746, ENST00000557277	ACAGCTTTCACAGTTTGCGGGACTCAGTCCCATCACTCCAAGGAGAGAAGGCATCCCGGGCCCAAATCCTAGACAAAGCCACAGAATATATCCAGTATAT
mef2a_common_T	ENST00000354410, ENST00000449277, ENST00000557785, ENST00000557942, ENST00000558812, ENST00000561125, ENST00000338042, ENST00000453228	CCCGCAGCCCCAGCCCCGACAGGAAATGGGGCGCTCCCCTGTGGACAGTCTGAGCAGCTCTAGTAGCTCCTATGATGGCAGTGATCGGGAGGATCCACGG
mef2a_sp3	ENST00000558812, ENST00000557785, ENST00000449277, ENST00000557942, ENST00000453228, ENST00000338042	GATTCAGCAAACTAAATGAAGATAGTGATTTTATTTTCAAACGAGGCCCTCCTGGTCTGCCACCTCAGAACTTTTCAATGTCTGTCACAGTTCCAGTGAC
mef2a_sp4	ENST00000354410	AAAAAATTAATGAGGAATTTGATAATATGATGCGGAATCATAAAATCGCACCTGGTCTGCCACCTCAGAACTTTTCAATGTCTGTCACAGTTCCAGTGAC
mef2b_sp10_T	ENST00000477565	CCCCACTGCCACTCCCAGCTGCAAGGACCGTCTCTCAGCTGCGCTGGGAACCGCTGCTTCTCGCTTATTAGAAAACTGTCTCTTTCCTTTTGTCCTGGT
mef2b_sp11_T	ENST00000585679, ENST00000514819, ENST00000462498, ENST00000444486	GTCGCTATGGAGGAGCCGGAGATGCAGCTCAAGGGGAAGAAAGCGCCGTGAAGAACCTGGTGGACAGCAGCGTCTACTTCCGCAGCGTGGAGG
mef2b_sp12	ENST00000591398, ENST00000162023, ENST00000494489, ENST00000462790, ENST00000588208, ENST00000488252, ENST00000354191, ENST00000477565	TGGGAGGAGCAGAGCCAGGGAGCCATCTACACTGTGGAGTACGCCTGCAGCGCCGTGAAGAACCTGGTGGACAGCAGCGTCTACTTCCGCAGCGTGGAGG
mef2b_sp7_T	ENST00000410050, ENST00000424583, ENST00000409224	CAGCCGCCGCGGGTCCGTGCGCCCAGCGTCCCAGGGCCCAGGCCGAGCAGACAAAGATCATTCCACTCAGCCTGGGACGATGGGGAGGAAAAAAATCCAG
mef2c_sp1	ENST00000508569, ENST00000514015, ENST00000510942, ENST00000514028, ENST00000437473, ENST00000504921, ENST00000503554, ENST00000506554	GGAAAATTAACGAAGATATTGATCTAATGATCAGCAGGCAAAGATTGTGTGCTGTTCCACCTCCCAACTTCGAGATGCCAGTCTCCATCCCAGTGTCCAG
mef2c_sp3	ENST00000424173, ENST00000340208	GAAGTAAAGAACGGAAGGCAAATGATTGTGGCAGTAAAGAAGTGTATGTGCAGGAACGAATGCAGGAATTTGGGAACTGAGCTGTGCAAGTGCTGAAGAA
mef2c_sp4	ENST00000514015, ENST00000510942, ENST00000514028, ENST00000504921, ENST00000513252, ENST00000506554	TTAAGAAAGGAAAATATCCCAAGGACTAATCTGATCGGGTCTTCCTTCATCAGGAACGAATGCAGGAATTTGGGAACTGAGCTGTGCAAGTGCTGAAGAA
mef2d_sp4	ENST00000454816, ENST00000360595, ENST00000368240	CCTGCGAGTCATCACTTCCCAGGCAGGAAAGGGGTTAATGCATCACTTGACTGAGGACCATTTAGATCTGAACAATGCCCAGCGCCTTGGGGTCTCCCA
MYC	ENST00000377970, ENST00000524013	AGGAGCAAAAGCTCATTTCTGAAGAGGACTTGTTGCGGAAACGACGAGAACAGTTGAAACACAAACTTGAACAGCTACGGAACTCTTGTGCGTAAGGAAA
MYF5	ENST00000228644	TGGATTGCTTATCCAACATAGTGGACCGGATCACCTCCTCAGAGCAACCTGGGTTGCCTCTCCAGGATCTGGCTTCTCTCTCTCCAGTTGCCAGCACCGA

Probe ID	Targeted trancripts (Ensembl IDs)	Probe sequence
MYF6	ENST00000228641	GGAGGAGCAAGTATTGATTCGTCAGCCTCGAGTAGCCTTCGATGCCTTTCTTCCATCGTGGACAGTATTTCCTCGGAGGAACGCAAACTCCCCTGCGTGG
MYOD1	ENST00000250003	GCATGGTGTGTGGTGCTACAGGGAATTTGTACGTTTATACCGCAGGCGGGCGAGCCGCGGGCGCTCGCTCAGGTGATCAAAATAAAGGCGCTAATTTATA
NANOG	ENST00000229307, ENST00000526286, ENST00000526434, ENST00000541267	CTTCACCTATGCCTGTGATTTGTGGGCCTGAAGAAAACTATCCATCCTTGCAAATGTCTTCTGCTGAGATGCCTCACACGGAGACTGTCTCTCCTCTTCC
ncor2_sp1_T	ENST00000404621, ENST00000429285, ENST00000397355, ENST00000404121, ENST00000448614	GGCGTCGGGCGTGAGCGGAAATGAGGAGGAGATGGTGGAGGAGGCTGAAGCCACTGTCAACAACAGCTCAGACACCGAGAGCATCCCCTCTCCTCACACT
NFKB1_common	ENST00000226574, ENST00000394820, ENST00000504044, ENST00000505458, ENST00000600343	ACTGAATCTAAAAAGGACCCTGAAGGTTGTGACAAAAGTGATGACAAAAACACTGTAAACCTCTTTGGGAAAGTTATTGAAACCACAGAGCAAGATCAGG
NOTCH1	ENST00000277541	GAGGGCTTCAGCGGTCCCAACTGCCAGACCAACATCAACGAGTGTGCGTCCAACCCATGTCTGAACCAGGGCACGTGTATTGACGACGTTGCCGGGTACA
NR2F2_sp2_T	ENST00000394166	CCAGTACTGCCGCCTCAAAAAGTGCCTCAAAGTGGGCATGAGACGGGAAGCGGTGCAGAGGGGCAGGATGCCGCCGACCCAGCCGACCCACGGGCAGTTC
OCT4_common	ENST00000259915, ENST00000441888, ENST00000471529, ENST00000512818, ENST00000513407	ATTCAGCCAAACGACCATCTGCCGCTTTGAGGCTCTGCAGCTTAGCTTCAAGAACATGTGTAAGCTGCGGCCCTTGCTGCAGAAGTGGGTGGAGGAAGCT
ONECUT1	ENST00000305901	CTTGGCAAGACAAATGATGAGCAGGAAAACACCACTGGATCTCACACCTTCAATCCATGACCATCCTCGCTGTGCTTGGCTGTTTAGTGGTTTGGAGCAT
ONECUT2	ENST00000262095, ENST00000491143	CCCAAACCAAAATGCTTGACATAAAGCCAAATCAACTGCCAAGCACACTTTATTTTGCATAGGAGTATGCAGCCTAGGGAACCTTGGTTGAAAAGCAGCA
pbx1_common	ENST00000367897, ENST00000420696, ENST00000465089, ENST00000468104, ENST00000496120, ENST00000560469, ENST00000560641	TTTCTCTCCCAACGCTGAAGCGGTCAGACTGGAGGTCGAAGCAATCAGCAAACACAATAAGAGTCTCCTTCTCTTCTCTTCTTTGGGATGCTATTTCAGC
pbx3_sp1	ENST00000373483, ENST00000373482, ENST00000373492	AATGAAACCAGCGCTCTTCAGCGTCCTGTGTGAGATCAAAGAGAAAACAGGCATGTAATGAATTTACTACACATGTGATGAACCTTCTCCGAGAACAGAG
pbx3_sp2_T	ENST00000491787, ENST00000447726, ENST00000342287, ENST00000373489, ENST00000373487	AATGAAACCAGCGCTCTTCAGCGTCCTGTGTGAGATCAAAGAGAAAACAGGTCTCAGCATCAGAGGAGCCCAGGAGGAGGACCCTCCCGATCCCCAGCTA
PER1	ENST00000581395, ENST00000579065, ENST00000354903, ENST00000582719, ENST00000317276	AGCACATCACGTCTGAGTACACACTTCAGAACCAGGATACCTTCTCAGTGGCTGTCTCCTTCCTGACGGGCCGAATCGTCTACATTTCGGAGCAGGCAGC
POLR2A	ENST00000322644	TTCTACTCCAACATTCAGACTGTCATTAACAACTGGCTCCTCATCGAGGGTCATACTATTGGCATTGGGGACTCCATTGCTGATTCTAAGACTTACCAGG
POU5F1_T	ENST00000259915, ENST00000441888, ENST00000471529, ENST00000512818, ENST00000513407	GAGGCTGCTGGGTCTCCTTTCTCAGGGGGACCAGTGTCCTTTCCTCTGGCCCCAGGGCCCCATTTTGGTACCCCAGGCTATGGGAGCCCTCACTTCACTG
PTEN	ENST00000371953	TTGGATGTGCAGCAGCTTACATGTCTGAAGTTACTTGAAGGCATCACTTTTAAGAAAGCTTACAGTTGGGCCCTGTACCATCCCAAGTCCTTTGTAGCTC
rbpj_sp2	ENST00000506956, ENST00000514807, ENST00000355476, ENST00000504907, ENST00000511546, ENST00000342320, ENST00000509158, ENST00000511451, ENST00000505958, ENST00000511401, ENST00000514730, ENST00000512351, ENST00000514675	CTGTGACTTACCTTAACATGTTCTTGAAGTACCATGGCGTGGATTAAAAGGAAATTTGGTGAGCGGCCTCCACCTAAACGACTTACTAGGGAAGCTATGC
RCOR1	ENST00000262241, ENST00000570597	GAAGGAACCACACCCCAGTTGTGCCGATTACATTAGTGTTGGCACACAGTCGGGTGCTAGTGTAACACAAATGCCGCGTTGTCTGGGTGTACAGTGTTTG
RELL2_common_T	ENST00000297164, ENST00000521367, ENST00000518856, ENST00000517794, ENST00000444782	GAATGAGGACACAGTAGAGAGGATTGTTCGCTGCATCATCCAGAATGAAGCCAATGCTGAGGCCTTGAAGGAGATGCTGGGGGACAGTGAAGGAGAAGGG
rrad_common	ENST00000299759, ENST00000420652, ENST00000566577, ENST00000568915	ACTCAGACGAGAGCGTTTACAAGGTGCTGCTGCTGGGGGCGCCCGGCGTGGGCAAGAGCGCCCTGGCGCGCATCTTCGGCGGTGTGGAGGACGGGCCTGA
Runx1_sp1	ENST00000300305, ENST00000437180, ENST00000475045, ENST00000416754, ENST00000486278	AGACAGCATATTTGAGTCATTTCCTTCGTACCCACAGTGCTTCATGAGAGAATGCATACTTGGAATGAATCCTTCTAGAGACGTCCACGATGCCAGCACG
Runx1_sp2	ENST00000344691, ENST00000358356, ENST00000399240	CCCTGTCGCCGTCTGGTAGGAGCTGTTTGCAGGGTCCTAACTCAATCGGCTTGTTGTGATGCGTATCCCCGTAGATGCCAGCACGAGCCGCC
SIN3A	ENST00000394949, ENST00000360439, ENST00000394947	CTTCTATGGCAGATGCCAGCAAACATGGTGGTGGAACAGAATCGTTATTTTTTGATAAGGTCCGAAAGGCTCTTCGGAGTGCAGAAGCCTACGAAAATTT
SOX2	ENST00000325404, ENST00000431565	GCCTTTCCAAAAAATAATAATAACAATCATCGGCGGCGGCAGGATCGGCCAGAGGAGGAGGGAAGCGCTTTTTTTGATCCTGATTCCAGTTTGCCTCTCT
SOX4	ENST00000244745, ENST00000543472	GCATGCAGGCTTTTTGGCTTCCTACCTTGCAACAAAATAATTGCACCAACTCCTTAGTGCCGATTCCGCCCACAGAGAGTCCTGGAGCCACAGTCTTTTT
SP1	ENST00000426431	AGCCCTGGTGCTACTTGCTTGAAGTTTTCAGTGTAAGTACCCTGATGCCTTTTGGACCTTGGGATCAGATCAAGAGTTTTGGAGATCAGGTACCAAGGAA
SREBF2	ENST00000361204, ENST00000424354, ENST00000491541	CTGAGTTGCTGTAGCGTCTTGATTCTCTCCCTGGGTCTGCGTTCCCTCCCCTGGGCCTGACTGAGCCTGCTCATTGTTTTTCCCTTTATTACACAGGACA
SRF	ENST00000265354	AGAGCCTACCTTCACCACCTATATCCAGAAGGGGAGCTTTTTCAGAAACAGGGCAGCAGTGGGGTGAAATTTTCTTAACCCCTAAGACTGCCTTCAGTAG
stat1_common	ENST00000361099, ENST00000392322, ENST00000409465, ENST00000452281, ENST00000540176, ENST00000392323	TGCTGAATGTCACTGAACTTACCCAGAATGCCCTGATTAATGATGAACTAGTGGAGTGGAAGCGGAGACAGCAGAGCGCCTGTATTGGGGGGCCGCCCAA
STAT2	ENST00000314128, ENST00000555665, ENST00000556539, ENST00000557235	CAACATTTTAATAGTTGGTTAGGCTAAACTGGTGCATACTGGCATTGGCCCTTGGTGGGGAGCACAGACACAGGATAGGACTCCATTTCTTTCTTCCATT
stat3_common	ENST00000264657, ENST00000389272, ENST00000404395, ENST00000585517, ENST00000588969	GCTGAAATCATCATGGGCTATAAGATCATGGATGCTACCAATATCCTGGTGTCTCCACTGGTCTATCTCTATCCTGACATTCCCAAGGAGGAGGCATTCG
STAT5A	ENST00000588868, ENST00000345506, ENST00000452307	GCCGCCGGTTTGAGTGAGGGTTTCTGAGCTGCTCTGAATTAGTCCTTGCTTGGCTGCTTGGCCTTGGGCTTCATTCAAGTCTATGATGCTGTTGCCCACG
STAT5B	ENST00000293328	GCCTAGAGAGTGGAGATTTTTGATGAAAGGTGTGCTCGCTCTCTGCGTTCTATCTTCTCTCTCCTCCTTGTTCCTGCAAACCACAAGATAAAGGTAGTGG
TAF1_sp1	ENST00000449580, ENST00000373790	CCAATGAAGAAGGATAAGGACCAGGATTCTATTACTGGTGAGAAAGTGGACTTCAGTAGTTCCTCTGACTCAGAATCTGAGATGGGACCT
TAF1_sp2	ENST00000276072, ENST00000423759	CCAATGAAGAAGGATAAGGACCAGGATTCTATTACTGGTGTGTCTGAAAATGGAGAAGGCATCATCTTGCCCTCCATCATTGCCCCTTCC
TCF12_common_T	ENST00000267811, ENST00000333725, ENST00000438423, ENST00000452095, ENST00000557843, ENST00000559609, ENST00000560190, ENST00000561449, ENST00000343827, ENST00000537840, ENST00000543579, ENST00000559703, ENST00000559710, ENST00000561420	CCTGAACAGAAGATAGAAAGGGAGAAGGAGAGGCGGATGGCTAACAATGCCAGAGAACGCTTACGCGTGCGGGATATTAATGAAGCATTCAAAGAGCTTG
TCF3_sp2_T	ENST00000593064, ENST00000588136	CCCACCCAGGCCTGAGCGAAGCCCACAACCCCGCCGGGCACATGTGAAAGTAAACAAAACCTGAAAGCAAGCAACAAAACATACACTTTGTCAGAGAAGA
TCF3_sp3_T	ENST00000262965, ENST00000344749, ENST00000395423, ENST00000585731, ENST00000590684	CCCACCCAGGCCTGAGCGAAGCCCACAACCCCGCCGGGCACATGTGAAAGGTATGCCTCCGTGGGACGAGCCACCCGCTTTCAGCCCTGTGCTCTGGCCC
TCF3_sp4	ENST00000586164, ENST00000593064, ENST00000587425, ENST00000395423, ENST00000592628, ENST00000262965	CACCAGGCTGTCTCGGTCATCCTGAACTTGGAGCAGCAAGTGCGAGAGCGGAACCTGAATCCCAAAGCAGCCTGTTTGAAACGGCGAGAAGAGGAA
TCF3_sp5_T	ENST00000588136, ENST00000453954, ENST00000344749, ENST00000585731, ENST00000585855, ENST00000590684, ENST00000592395	CCTGCAGCAGGCCGTGCAGGTCATCCTGGGGCTGGAGCAGCAGGTGCGAGAGCGGAACCTGAATCCCAAAGCAGCCTGTTTGAAACGGCGAGAAGAGGAA
TCF3_sp6_T	ENST00000395423	CGGGAAGGGCCGGCCCGCCTCCCTGGCCGGGGCGCAGTTCGGAGGTTCAGGCAAGAGCGGTGAGCGGGGCGCCTATGCCTCCTTCGGGAGAGACGCAGGC
TCF3_sp7_T	ENST00000588136, ENST00000344749, ENST00000262965	GTCGCACAGCAGCCTCTCTTCATCCACATTCCTGGGACCGGGACTCGGAGGCAAGAGCGGTGAGCGGGGCGCCTATGCCTCCTTCGGGAGAGACGCAGGC
TNFRSF13B	ENST00000261652, ENST00000437538, ENST00000579315, ENST00000581616, ENST00000582931, ENST00000583789, ENST00000584950	AGGAGCAAGGCAAGTTCTATGACCATCTCCTGAGGGACTGCATCAGCTGTGCCTCCATCTGTGGACAGCACCCTAAGCAATGTGCATACTTCTGTGAGAA
TNFRSF13C	ENST00000291232	GTTTGGTGTGCTTGCCTTTGGCTTCAGACCTCACCATCTTTGACAGCCCTTGAAGGTGGTAGCCCAGCTCCTGTTCCTGTGCCTTCAAAAGGCTGGGGCA
TNFRSF17_sp1	ENST00000053243	GCTAAGGAAGATAAACTCTGAACCATTAAAGGACGAGTTTAAAAACACAGGATCAGGTCTCCTGGGCATGGCTAACATTGACCTGGAAAAGAGCAGGACT
TNFSF13B	ENST00000375887, ENST00000430559	AACAGGAAATGATCCATTCCCTGTGGTCACTTATTCTAAAGGCCCCAACCTTCAAAGTTCA

Probe ID	Targeted trancripts (Ensembl IDs)	Probe sequence
		AGTAGTGATATGGATGACTCCACAGAAAGGGAGCAGTCA
USF1_common_T	ENST00000368019, ENST00000368021, ENST00000435396, ENST00000472217, ENST00000473969, ENST00000528768, ENST00000531842, ENST00000368020	AAGCTTGTGATTATATCCAGGAGCTTCGGCAGAGTAACCACCGCTTGTCTGAAGAACTGCAGGGACTTGACCAACTGCAGCTGGACAATGACGTGCTTCG
YY1	ENST00000262238, ENST00000554579, ENST00000554804, ENST00000555735	ACATGCTAAGGCCAAAAACAACCAGTGAAAAGAAGAGAGAAGACCCTTCTCGACCACGGGAAGCATCTTCCAGAAGTGTGATTGGGAATAAATATGCCTC
ZIC3	ENST00000287538	TCAGTTAGTGGCCATGACATCTCAATCTTGTACTTCAAAGACTGAGAAGCTGGATTTAATCATCCCTGCCCTACATATATAAACATAAGGTAACCTACTG
ZNF217_sp3_T	ENST00000371471	AGTCCCGGCCGCCGCCGCGCGAGGAAATGGCCGAGGAGCCGGAGCCGCAGGGTTTGGAAATCCCTTGTCTCCAGGTTGCTGGGATTGACTTCTTGCTCAA

Supplementary Table 8. NanoString counts and RPKMs for predominant compatible isoforms

	Nanocounts	mGene graph	Tromer	SLIDE all	mGene	mTim	AUGUSTUS all	Cufflinks	iReckon full	iReckon ends	Trembly all	Transomics all
adar1_sp1	2101	8.019	11.800	0.000	0.000	4.737	0.000	12.261	27.993	23.975	15.097	0.000
adar1_sp2	2321	6.343	10.100	3.529	15.642	0.000	13.182	11.639	0.422	1.950	0.732	18.420
atf2_common	4911	6.016	20.100	6.902	13.868	12.906	9.553	12.499	0.000	15.860	5.974	0.000
atf2_sp1	2299	6.016	19.100	0.000	0.000	12.906	0.000	2.711	0.000	4.636	1.503	0.000
atf2_sp2	2156	4.211	54.300	6.902	13.868	0.000	9.553	12.499	0.000	15.860	5.974	21.380
ATP5J_common	16504	6.764	4.800	10.377	18.577	32.224	33.923	174.528	61.534	50.080	30.978	22.280
Bcl11a_sp1	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Bcl11a_sp2	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
BCL3	1737	0.000	1.700	0.884	0.557	3.767	2.791	6.189	5.926	2.468	0.000	3.780
BHLHB2	17578	39.789	36.600	29.947	62.179	84.314	51.583	0.000	107.521	87.483	53.854	0.000
Blnk_sp1_T	33	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.000	10.930
Blnk_sp2	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CARM1_sp1_T	1168	4.848	3.700	0.000	0.000	9.981	8.519	19.494	0.000	18.037	9.809	0.000
CD19	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.067	0.071	0.000	0.000
Cd79b_sp1_T	24	0.000	0.000	0.000	0.043	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Cd79b_sp2_T	0	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000
cdkn1a_sp1_T	6057	21.884	0.000	0.000	0.000	0.000	21.888	43.565	53.015	42.893	29.102	0.000
cdkn1a_sp2_T	5429	5.581	0.000	0.000	0.000	11.081	0.000	0.000	0.702	0.793	1.044	0.000
CEBPA	7109	0.000	0.000	7.464	0.000	0.000	8.776	0.000	21.206	8.371	0.000	0.000
CTCF_common	255	9.574	10.600	1.337	11.822	10.033	8.977	16.957	20.358	16.725	11.564	0.000
CTCF_sp1	563	9.574	8.400	2.043	11.822	10.033	8.977	16.957	20.358	16.725	11.564	0.000
CTCFL_sp1	19	0.000	0.000	0.000	0.000	0.000	0.261	0.000	0.000	0.000	0.000	0.060
CTCFL_sp2	11	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CTDSL_sp2_T	940	18.993	37.300	0.000	20.476	3.870	9.696	16.728	0.000	22.162	7.568	17.710
CTDSP1_common_T	4117	10.701	22.100	8.134	20.135	14.558	11.711	14.311	25.545	20.837	9.889	42.120
CTDSP2	3330	0.000	0.000	14.322	0.000	0.000	25.306	23.741	44.528	38.755	20.104	0.000
CTDSPL_sp1	4193	18.993	36.700	0.000	20.476	3.870	5.744	11.590	0.000	6.027	5.331	0.000
DES	0	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000
DNMT1_common_T	1011	2.208	0.000	2.193	6.619	2.270	3.566	7.063	11.401	6.460	4.820	6.190
E2F4	4533	16.233	0.000	0.000	19.528	14.254	13.402	23.889	29.670	25.409	16.069	17.580
E2F6_common	5693	3.922	1.500	1.555	5.451	0.000	4.099	0.000	0.000	0.000	5.260	2.960
ebf1_sp1	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
EGR1	956	7.684	8.700	2.694	7.895	8.305	4.390	8.910	10.040	8.180	6.463	0.000
EOMES_common	14	0.000	0.000	0.007	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.170
EP300	1914	11.002	16.700	2.775	11.107	10.824	8.552	14.063	16.915	13.800	8.142	12.510
esr1_common	12	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
esr2_sp1	33	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
esr2_sp2	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020
ets1_common	561	0.000	0.600	0.566	0.307	0.000	0.210	0.376	0.303	0.153	0.368	0.320
FBXO15	14	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010
FOS	755	1.783	2.400	0.542	1.783	0.000	1.255	2.589	2.962	2.395	1.888	0.000
foxa2_body	8700	14.687	16.500	3.488	17.953	0.000	12.329	21.703	14.811	12.235	15.405	0.000
foxa2_sp2_T	250	0.000	0.000	3.488	0.000	17.363	0.000	4.916	11.101	8.888	15.405	0.000
FOXA3	1223	9.923	17.100	3.160	12.663	11.999	8.652	17.657	19.049	15.646	10.745	0.000
gabpa_sp1_T	251	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.681	0.000	1.288	0.000
gabpa_sp2_T	237	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.386	0.256	0.000	0.000
GATA1_T	12	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	11.040
HDAC1	7349	9.321	15.100	7.233	37.448	24.484	0.000	45.483	48.939	38.679	30.272	0.000
HDAC3_sp1	3703	7.760	10.500	0.000	7.760	0.000	13.394	26.599	0.000	23.591	15.355	12.670
HDAC4	902	0.000	3.100	0.786	0.000	1.532	0.000	0.825	3.016	0.093	0.813	0.000
HDAC5	65	0.000	0.000	0.445	0.000	0.000	0.000	0.000	0.000	1.188	13.694	0.000
Hif1a_common	13561	18.760	21.500	7.487	27.504	18.168	13.669	33.054	21.754	18.023	17.016	26.690
HNF1A	1485	8.247	3.600	0.754	8.251	9.176	4.968	2.700	10.798	7.026	3.155	10.810
HNF1B	836	2.287	4.400	1.487	2.903	2.837	1.275	2.439	2.896	2.302	1.823	2.570
Hnf4g_common_T	1906	2.897	3.600	0.793	3.550	0.000	0.000	3.802	4.060	1.977	2.896	0.000
HSF1	4997	13.666	10.900	0.348	19.782	9.781	18.878	22.358	39.006	28.277	8.647	15.140
IGF1R_common_T	2654	5.520	25.300	2.545	12.103	7.032	4.483	12.120	10.999	12.999	5.215	10.780
IKZF1_common	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IKZF1_sp1_T	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IKZF1_sp2	16	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IKZF3_sp1_T	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.060
IKZF3_sp2	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IL6	0	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000
IL6receptor_common	2319	6.038	10.300	2.339	6.584	6.117	0.000	8.476	9.792	8.532	5.920	0.000
IL8	84	0.298	0.000	0.817	0.298	0.000	0.031	0.000	0.516	0.231	0.000	0.000
IL8RA	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IL8RB	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IRF8	18	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
JUND	297	38.720	0.000	115.202	38.720	0.000	0.000	0.000	56.163	45.642	0.000	0.000
KAISO_sp1_T	1368	4.853	0.000	2.190	4.853	0.000	2.848	4.740	6.764	5.589	4.147	0.000
KAP1_common_T	0	48.402	22.000	1.195	53.394	48.369	49.948	91.944	2.299	65.311	68.574	62.880
KLF4	226	0.536	1.200	0.504	0.536	0.000	0.000	0.598	0.692	0.466	0.944	0.000
LEF1_sp1	0	0.000	0.000	0.000	0.102	0.000	0.000	0.000	0.046	0.000	0.000	0.000
LEF1_sp2	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
LIN28_common	0	0.000	0.000	0.000	0.043	0.000	0.011	0.000	0.000	0.000	0.000	0.010
MAX_sp1	2984	6.122	7.900	0.000	5.156	6.801	4.591	10.640	0.000	3.786	4.277	8.040
mef2a_common_T	515	1.998	0.000	1.386	4.385	5.082	2.034	4.035	5.451	4.205	2.928	4.700
mef2a_sp3	1470	1.998	8.000	0.309	0.000	5.082	2.034	4.035	5.451	4.205	2.928	0.000
mef2a_sp4	1090	1.266	7.600	1.386	4.385	0.000	0.851	1.627	1.744	1.473	0.892	4.700
mef2b_sp10_T	81	0.000	8.000	0.000	0.000	0.000	4.953	0.000	0.000	1.182	0.000	2.280
mef2b_sp11_T	248	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.074	0.193	0.000

	Nanocounts	mGene graph	Tromer	SLIDE all	mGene	mTim	AUGUSTUS all	Cufflinks	iReckon full	iReckon ends	Trembly all	Transomics all
mef2b_sp12	311	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.182	0.882	0.000
mef2b_sp7_T	215	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.424	0.000
mef2c_sp1	9	3.852	2.200	1.395	3.852	0.000	2.842	3.425	0.000	0.000	0.000	0.000
mef2c_sp3	18	60.369	54.200	10.330	72.155	47.513	45.253	60.126	0.000	0.000	0.000	0.000
mef2c_sp4	16	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
mef2d_sp4	80	0.000	0.000	0.000	0.000	0.000	0.000	0.000	3.045	2.576	3.004	0.000
MYC	28966	0.000	0.000	0.000	0.000	0.000	0.000	0.000	78.566	69.472	40.507	0.000
MYF5	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.070
MYF6	51	3.416	9.700	1.049	0.000	2.773	1.998	6.599	0.000	0.000	0.000	7.360
MYOD1	32	5.066	0.000	1.638	3.331	3.444	2.847	9.295	0.000	0.000	0.000	4.760
NANOG	64	0.226	0.000	0.000	0.226	0.000	2.475	0.000	0.000	0.011	0.000	0.190
ncor2_sp1_T	1143	8.053	0.000	0.000	8.053	0.000	5.163	9.870	0.000	1.034	2.510	11.050
NFKB1_common	355	1.011	0.000	0.000	1.011	3.434	1.299	0.504	4.062	4.897	3.380	0.980
NOTCH1	27	6.427	8.000	1.518	6.427	0.000	3.314	6.747	0.284	0.032	0.000	0.000
NR2F2_sp2_T	800	4.323	4.400	0.963	4.323	0.000	2.183	2.163	4.223	3.783	7.895	0.000
OCT4_common	284	0.000	0.000	0.023	0.000	0.000	0.000	0.000	0.084	1.796	0.000	0.000
ONECUT1	2013	0.000	0.000	0.000	0.000	0.000	0.000	0.823	7.115	5.992	5.487	0.000
ONECUT2	805	0.752	0.000	0.000	1.089	1.154	0.774	1.008	4.046	3.806	3.005	0.960
pbx1_common	0	2.905	0.000	1.231	2.905	2.990	2.404	4.568	0.001	0.000	0.000	3.320
pbx3_sp1	97	15.249	0.000	7.541	17.974	15.275	13.497	22.026	0.702	0.592	0.000	20.140
pbx3_sp2_T	89	0.000	0.000	0.828	0.000	3.434	1.299	0.000	1.215	1.032	0.860	0.980
PER1	452	3.611	2.700	0.000	4.498	0.000	0.000	0.000	5.530	4.592	3.320	0.000
POLR2A	4377	0.000	3.900	0.000	0.000	0.000	2.728	5.559	31.669	26.517	18.840	0.000
POU5F1_T	94	0.000	7.800	0.888	0.000	5.158	3.436	0.000	0.084	1.796	0.000	0.000
PTEN	1128	0.629	0.000	0.258	2.280	0.000	1.567	1.227	4.292	1.040	0.000	3.240
rbpj_sp2	82	0.577	1.300	1.881	0.577	0.602	0.407	1.140	0.000	5.918	2.401	0.700
RCOR1	2199	0.000	0.000	0.000	0.000	0.000	0.000	0.000	8.782	6.885	5.339	0.000
RELL2_common_T	226	0.000	0.000	0.600	0.000	0.000	1.474	3.147	0.000	0.000	1.725	0.000
rrad_common	82	5.612	10.400	1.445	6.486	5.683	4.965	8.384	0.409	0.740	0.000	6.620
Runx1_sp1	24	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Runx1_sp2	338	0.000	4.600	1.114	0.000	0.000	0.000	0.000	1.734	1.439	1.291	0.000
SIN3A	1241	0.000	7.300	1.759	0.000	32.098	0.000	6.523	5.741	8.155	0.000	0.000
SOX2	0	28.334	25.500	14.937	50.738	27.716	37.410	80.463	0.000	0.034	0.000	0.000
SOX4	528	0.000	12.200	4.700	0.000	14.379	7.759	13.846	0.063	2.135	0.000	0.000
SP1	513	8.426	0.000	3.291	9.046	7.994	7.303	12.281	7.665	6.247	5.301	3.290
SREBF2	7451	3.716	6.000	1.516	6.431	0.000	2.611	8.957	84.859	3.228	46.195	0.000
SRF	790	8.985	3.300	7.878	26.855	10.632	10.560	30.035	17.672	14.304	12.139	21.140
stat1_common	1154	1.007	1.700	0.416	1.491	0.000	0.000	1.743	13.717	10.893	8.726	0.000
STAT2	1235	7.422	0.000	4.175	9.714	0.000	6.814	12.711	2.849	6.198	1.597	0.000
stat3_common	7640	0.000	2.600	0.000	0.000	0.000	0.000	0.000	0.000	0.000	5.170	0.000
STAT5A	396	0.960	3.000	1.889	2.421	0.000	1.776	2.442	1.113	1.393	1.264	3.290
STAT5B	1717	2.932	6.700	1.193	7.708	5.653	5.623	8.754	15.297	12.872	8.823	5.400
TAF1_sp1	270	0.000	0.000	0.000	0.000	0.000	3.949	5.753	0.136	0.000	0.000	0.000
TAF1_sp2	326	0.000	16.900	3.247	0.000	0.000	0.000	7.678	2.182	0.000	2.013	0.000
TCF12_common_T	2427	1.818	0.600	0.000	0.000	0.000	0.000	7.183	8.493	6.724	4.822	0.000
TCF3_sp2_T	1214	1.903	0.000	3.247	8.286	0.000	3.949	7.678	0.000	10.253	4.776	10.600
TCF3_sp3_T	1102	1.818	5.700	0.000	0.000	1.393	0.000	0.000	0.000	5.126	0.000	0.000
TCF3_sp4	1153	1.650	3.900	0.000	0.000	4.494	3.949	7.183	0.000	8.657	4.776	10.600
TCF3_sp5_T	1031	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	10.253	2.450	0.090
TCF3_sp6_T	1111	0.000	0.400	0.161	0.158	0.000	0.000	0.000	0.000	2.463	4.776	0.000
TCF3_sp7_T	1646	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	10.253	2.450	0.000
TNFRSF13B	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	2.110
TNFRSF13C	22	2.951	5.400	2.270	4.313	2.831	3.584	5.177	0.203	0.125	0.000	3.250
TNFRSF17_sp1	0	17.687	10.100	5.461	17.687	17.874	11.013	13.888	0.000	0.000	0.000	0.000
TNFSF13B	84	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.018	0.000	0.000
USF1_common_T	623	0.000	0.800	5.349	0.000	0.000	0.000	13.414	5.824	4.726	3.154	0.000
YY1	11801	8.019	11.800	0.000	0.000	4.737	0.000	12.261	22.801	18.355	11.602	0.000
ZIC3	46	6.343	10.100	3.529	15.642	0.000	13.182	11.639	0.000	0.000	0.000	18.420
ZNF217_sp3_T	1401	6.016	20.100	6.902	13.868	12.906	9.553	12.499	20.481	15.954	8.342	0.000

Supplementary Table 9. NanoString counts and RPKMs for predominant isoforms

	Nanocounts	AUGUSTUS all	Cufflinks	iReckon full	iReckon ends	mGene	mGene graph	mTim	SLIDE all	Transomics all	Trembly all	Tromer
adar1_sp1	2101	13.182	12.261	27.993	23.975	15.642	8.019	4.737	10.275	18.420	18.420	29.500
adar1_sp2	2321	13.182	12.261	27.993	23.975	15.642	8.019	4.737	10.275	18.420	18.420	29.500
atf2_common	4911	9.553	12.499	0.000	15.860	13.868	6.016	12.906	6.902	21.380	21.380	54.300
atf2_sp1	2299	9.553	12.499	0.000	15.860	13.868	6.016	12.906	6.902	21.380	21.380	54.300
atf2_sp2	2156	9.553	12.499	0.000	15.860	13.868	6.016	12.906	6.902	21.380	21.380	54.300
ATP5J_common	16504	33.923	174.528	61.534	50.080	18.577	6.764	32.224	10.377	22.280	22.280	59.500
Bcl11a_sp1	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.140	0.140	0.000
Bcl11a_sp2	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.140	0.140	0.000
BCL3	1737	2.791	6.189	5.926	3.573	4.486	4.486	3.767	1.557	3.780	3.780	7.200
BHLHB2	17578	51.583	82.714	107.521	87.483	62.179	39.789	84.314	29.947	88.550	88.550	82.600
Blnk_sp1_T	33	0.007	0.000	0.008	0.000	7.716	7.716	0.000	0.011	10.930	10.930	0.000
Blnk_sp2	0	0.007	0.000	0.008	0.000	7.716	7.716	0.000	0.011	10.930	10.930	0.000
CARM1_sp1_T	1168	8.519	19.494	0.000	18.037	20.105	9.535	9.981	3.743	9.540	9.540	43.900
CD19	0	0.612	2.669	0.067	0.071	0.681	0.000	0.000	7.420	0.190	0.190	1.600
Cd79b_sp1_T	24	0.018	0.000	0.000	0.000	0.043	0.000	0.000	0.000	0.000	0.000	0.000
Cd79b_sp2_T	0	0.018	0.000	0.000	0.000	0.043	0.000	0.000	0.000	0.000	0.000	0.000
cdkn1a_sp1_T	6057	21.888	43.565	53.015	42.893	27.358	21.884	11.361	5.722	37.470	37.470	17.200
cdkn1a_sp2_T	5429	21.888	43.565	53.015	42.893	27.358	21.884	11.361	5.722	37.470	37.470	17.200
CEBPA	7109	8.776	0.000	21.206	8.371	5.980	5.274	0.000	7.464	6.380	6.380	0.000
CTCF_common	255	8.977	16.957	20.358	16.725	11.822	9.574	10.033	3.169	13.750	13.750	21.100
CTCF_sp1	563	8.977	16.957	20.358	16.725	11.822	9.574	10.033	3.169	13.750	13.750	21.100
CTCFL_sp1	19	0.261	0.000	0.012	0.041	0.099	0.000	0.000	0.721	0.060	0.060	0.200
CTCFL_sp2	11	0.261	0.000	0.012	0.041	0.099	0.000	0.000	0.721	0.060	0.060	0.200
CTDSL_sp2_T	940	9.696	16.728	0.000	22.162	20.476	18.993	19.435	6.404	17.710	17.710	37.300
CTDSP1_common_T	4117	11.711	14.311	25.545	20.837	20.135	10.701	14.558	8.134	42.120	42.120	38.700
CTDSP2	3330	25.306	23.741	44.528	38.755	30.798	25.746	24.823	14.322	20.100	20.100	22.300
CTDSPL_sp1	4193	9.696	16.728	0.000	22.162	20.476	18.993	19.435	6.404	17.710	17.710	37.300
DES	0	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
DNMT1_common_T	1011	3.566	12.116	11.401	6.460	6.619	2.208	2.270	2.888	6.190	6.190	16.400
E2F4	4533	13.402	23.889	29.670	25.409	19.528	16.233	14.254	3.340	17.580	17.580	35.100
E2F6_common	5693	4.099	7.950	0.000	0.000	5.451	3.922	4.733	2.139	2.960	2.960	8.000
ebf1_sp1	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
EGR1	956	4.390	8.910	10.040	8.180	7.895	7.684	8.305	2.694	9.340	9.340	8.700
EOMES_common	14	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.170	0.170	0.000
EP300	1914	8.552	14.063	16.915	13.800	11.107	11.002	10.824	2.775	12.510	12.510	18.200
esr1_common	12	0.000	0.000	0.000	0.001	0.005	0.000	0.000	0.001	0.000	0.000	0.000
esr2_sp1	33	0.000	0.000	0.000	0.081	0.087	0.000	0.000	1.196	0.020	0.020	0.200
esr2_sp2	0	0.000	0.000	0.000	0.081	0.087	0.000	0.000	1.196	0.020	0.020	0.200
ets1_common	561	0.210	0.376	0.303	0.153	0.307	0.000	0.000	0.566	0.320	0.320	0.600
FBXO15	14	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.938	0.010	0.010	0.300
FOS	755	1.255	2.589	2.962	2.395	1.783	1.783	0.000	1.000	2.290	2.290	2.400
foxa2_body	8700	12.329	21.703	14.811	12.235	17.953	14.687	17.363	3.488	18.410	18.410	33.200
foxa2_sp2_T	250	12.329	21.703	14.811	12.235	17.953	14.687	17.363	3.488	18.410	18.410	33.200
FOXA3	1223	8.652	17.657	19.049	15.646	12.663	9.923	11.999	3.160	11.930	11.930	17.100
gabpa_sp1_T	251	2.389	6.092	2.225	0.256	2.723	1.769	0.000	1.391	3.480	3.480	6.400
gabpa_sp2_T	237	2.389	6.092	2.225	0.256	2.723	1.769	0.000	1.391	3.480	3.480	6.400
GATA1_T	12	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	11.040	11.040	0.000
HDAC1	7349	27.696	86.127	48.939	38.679	37.448	9.321	24.484	7.233	27.570	27.570	44.600
HDAC3_sp1	3703	13.394	26.599	0.000	23.591	20.748	20.748	19.399	4.831	12.670	12.670	21.700
HDAC4	902	0.863	1.522	3.016	2.396	2.217	0.943	1.532	0.786	1.720	1.720	8.300
HDAC5	65	12.839	26.933	0.000	47.104	33.155	7.620	15.081	18.303	26.610	26.610	72.300
Hif1a_common	13561	13.669	33.054	21.754	18.023	27.504	18.760	18.168	7.487	26.690	26.690	32.500
HNF1A	1485	4.968	2.700	10.798	7.026	8.251	8.247	9.176	2.089	10.810	10.810	3.600
HNF1B	836	1.275	2.439	2.896	2.302	2.903	2.287	2.837	1.487	2.570	2.570	6.600
Hnf4g_common_T	1906	2.359	3.802	4.060	1.977	3.550	2.897	2.040	0.818	1.220	1.220	4.200
HSF1	4997	18.878	22.358	39.006	28.277	19.782	13.666	9.781	5.110	15.140	15.140	65.500
IGF1R_common_T	2654	9.782	12.120	10.999	12.999	17.784	17.784	7.032	2.545	14.320	14.320	25.300
IKZF1_common	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IKZF1_sp1_T	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IKZF1_sp2	16	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IKZF3_sp1_T	0	0.000	0.000	0.000	0.015	11.141	3.412	0.000	0.000	0.060	0.060	0.000
IKZF3_sp2	0	0.000	0.000	0.000	0.015	11.141	3.412	0.000	0.000	0.060	0.060	0.000
IL6	0	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.010	0.000
IL6receptor_common	2319	4.373	8.476	9.792	8.532	6.584	6.038	6.117	3.045	5.370	5.370	14.900
IL8	84	0.031	0.475	0.516	0.231	0.298	0.298	0.000	0.817	0.160	0.160	0.500
IL8RA	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.030	0.030	0.000
IL8RB	0	0.013	0.000	0.000	0.000	0.029	0.000	0.000	0.262	0.010	0.010	0.000
IRF8	18	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
JUND	297	25.218	0.000	56.163	45.642	38.720	38.720	0.000	115.202	46.830	46.830	0.000
KAISO_sp1_T	1368	2.848	4.740	6.764	5.589	4.853	4.853	0.000	2.190	4.920	4.920	6.000
KAP1_common_T	0	49.948	91.944	119.818	65.311	53.394	48.402	48.369	23.632	62.880	62.880	145.100
KLF4	226	0.137	0.598	0.692	0.466	0.536	0.536	0.000	0.504	0.370	0.370	1.200
LEF1_sp1	0	0.030	0.000	0.046	0.006	0.102	0.000	0.000	0.000	0.040	0.040	0.300
LEF1_sp2	0	0.030	0.000	0.046	0.006	0.102	0.000	0.000	0.000	0.040	0.040	0.300
LIN28_common	0	0.011	0.000	0.000	0.000	0.043	0.000	0.000	0.000	0.010	0.010	0.000
MAX_sp1	2984	4.591	10.640	0.000	3.786	5.156	6.122	6.801	2.226	8.040	8.040	7.900
mef2a_common_T	515	2.034	4.035	5.451	4.205	4.385	1.998	5.082	1.386	4.700	4.700	9.200
mef2a_sp3	1470	2.034	4.035	5.451	4.205	4.385	1.998	5.082	1.386	4.700	4.700	9.200
mef2a_sp4	1090	2.034	4.035	5.451	4.205	4.385	1.998	5.082	1.386	4.700	4.700	9.200
mef2b_sp10_T	81	4.953	3.464	0.000	1.182	1.384	1.088	1.294	1.760	2.280	2.280	8.000
mef2b_sp11_T	248	0.000	0.000	0.000	3.168	0.000	0.000	0.000	0.000	0.000	2.280	0.000

	Nanocounts	AUGUSTUS all	Cufflinks	iReckon full	iReckon ends	mGene	mGene graph	mTim	SLIDE all	Transomics all	Trembly all	Tromer
mef2b_sp12	311	0.000	0.000	0.000	3.168	0.000	0.000	0.000	0.000	0.000	2.280	0.000
mef2b_sp7_T	215	0.000	0.000	0.000	3.168	0.000	0.000	0.000	0.000	0.000	2.280	0.000
mef2c_sp1	9	2.842	3.425	0.002	0.000	3.852	3.852	0.000	1.395	3.900	0.000	8.200
mef2c_sp3	18	45.253	60.126	0.002	0.000	72.155	60.369	47.513	16.867	103.100	0.000	54.200
mef2c_sp4	16	0.733	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
mef2d_sp4	80	0.733	0.000	3.045	2.576	0.000	0.000	0.000	0.000	0.000	3.900	0.000
MYC	28966	0.000	0.000	78.566	69.472	0.000	0.000	0.000	0.000	0.000	103.100	0.000
MYF5	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.070	0.000	0.000
MYF6	51	1.998	6.599	0.000	0.000	8.090	3.416	2.773	5.106	7.360	0.000	24.700
MYOD1	32	2.847	9.295	0.000	0.000	3.331	5.066	3.444	1.638	4.760	0.000	9.700
NANOG	64	2.475	0.496	0.000	0.011	0.226	0.226	0.000	0.310	0.190	0.070	0.500
ncor2_sp1_T	1143	5.163	9.870	0.000	7.648	8.053	8.053	9.022	2.367	11.050	7.360	5.800
NFKB1_common	355	1.299	0.504	4.062	4.897	1.011	1.011	3.434	0.828	0.980	4.760	0.000
NOTCH1	27	3.314	6.747	0.284	0.062	6.427	6.427	4.973	1.518	4.420	0.190	8.000
NR2F2_sp2_T	800	2.183	2.163	6.076	4.056	4.323	4.323	0.000	0.963	1.900	11.050	4.400
OCT4_common	284	0.000	0.000	0.084	1.796	0.000	0.000	0.000	0.023	0.010	0.980	0.000
ONECUT1	2013	0.774	1.008	7.115	5.992	1.089	0.752	1.154	0.476	0.960	4.420	2.100
ONECUT2	805	0.774	1.008	4.046	3.806	1.089	0.752	1.154	0.476	0.960	1.900	2.100
pbx1_common	0	2.404	4.568	0.001	0.000	2.905	2.905	10.530	1.598	3.320	0.010	2.300
pbx3_sp1	97	13.497	22.026	1.215	1.032	17.974	15.249	15.275	7.810	20.140	0.960	30.500
pbx3_sp2_T	89	1.299	0.504	1.215	1.032	1.011	1.011	3.434	0.828	0.980	0.960	0.000
PER1	452	5.492	7.897	5.530	4.592	4.498	3.611	0.000	3.308	35.660	3.320	7.000
POLR2A	4377	2.927	11.227	31.669	26.517	6.303	3.903	6.441	2.160	1.790	20.140	9.700
POU5F1_T	94	3.436	6.237	0.084	1.796	4.716	4.716	5.158	0.888	2.200	0.980	7.800
PTEN	1128	1.567	1.227	4.292	4.046	2.280	0.629	0.000	0.696	3.240	35.660	3.100
rbpj_sp2	82	0.407	1.140	0.000	5.918	0.577	0.577	0.602	1.881	0.700	1.790	21.000
RCOR1	2199	1.474	3.147	8.782	6.885	1.992	1.741	2.388	0.991	1.860	2.200	4.600
RELL2_common_T	226	1.474	3.147	0.000	1.064	1.992	1.741	2.388	0.991	1.860	3.240	4.600
rrad_common	82	4.965	8.384	0.409	0.740	6.486	5.612	5.683	1.445	6.620	0.700	13.800
Runx1_sp1	24	0.008	0.000	1.734	1.439	0.022	0.022	0.000	0.009	0.020	1.860	0.000
Runx1_sp2	338	0.608	0.000	1.734	1.439	1.566	1.566	0.000	1.114	1.950	1.860	4.600
SIN3A	1241	61.269	6.523	5.741	8.155	5.816	5.799	32.098	1.759	6.870	6.620	8.800
SOX2	0	37.410	80.463	0.000	0.034	50.738	28.334	27.716	14.937	60.260	0.020	104.400
SOX4	528	7.759	13.846	0.063	2.135	11.998	11.998	14.379	5.375	11.910	1.950	12.200
SP1	513	7.303	16.601	7.665	6.247	9.046	8.426	7.994	3.291	3.290	6.870	18.900
SREBF2	7451	2.611	8.957	84.859	70.533	6.431	3.716	1.655	2.549	5.450	60.260	9.500
SRF	790	10.560	30.035	17.672	14.304	26.855	8.985	10.632	10.479	21.140	11.910	66.800
stat1_common	1154	0.911	1.743	13.717	10.893	1.491	1.007	1.712	0.416	5.160	3.290	2.200
STAT2	1235	6.814	12.711	2.849	6.198	9.714	7.422	8.864	4.175	8.570	5.450	19.700
stat3_common	7640	1.776	5.084	0.000	0.000	2.421	0.960	10.924	1.889	3.290	21.140	5.000
STAT5A	396	1.776	5.084	1.113	1.393	2.421	0.960	10.924	1.889	3.290	5.160	5.000
STAT5B	1717	5.623	9.242	15.297	12.872	7.708	2.932	5.653	3.924	5.400	8.570	13.600
TAF1_sp1	270	3.949	7.678	3.525	6.240	8.286	1.903	4.494	3.247	10.600	3.290	16.900
TAF1_sp2	326	3.949	7.678	3.525	6.240	8.286	1.903	4.494	3.247	10.600	3.290	16.900
TCF12_common_T	2427	3.949	7.678	8.493	6.724	8.286	1.903	4.494	3.247	10.600	5.400	16.900
TCF3_sp2_T	1214	3.949	7.678	0.000	10.253	8.286	1.903	4.494	3.247	10.600	10.600	16.900
TCF3_sp3_T	1102	3.949	7.678	0.000	10.253	8.286	1.903	4.494	3.247	10.600	10.600	16.900
TCF3_sp4	1153	3.949	7.678	0.000	10.253	8.286	1.903	4.494	3.247	10.600	10.600	16.900
TCF3_sp5_T	1031	0.000	0.000	0.000	10.253	0.338	0.000	0.000	0.031	0.090	10.600	0.000
TCF3_sp6_T	1111	0.000	0.000	0.000	10.253	0.158	0.000	0.000	0.161	0.110	10.600	0.400
TCF3_sp7_T	1646	0.000	0.000	0.000	10.253	0.000	0.000	0.000	0.000	0.000	10.600	0.000
TNFRSF13B	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	2.110	0.090	0.000
TNFRSF13C	22	3.584	5.177	0.203	0.125	4.313	2.951	2.831	2.270	3.250	0.110	13.500
TNFRSF17_sp1	0	11.013	13.888	0.000	0.000	17.687	17.687	17.874	5.461	10.590	0.000	10.100
TNFSF13B	84	0.000	0.000	0.008	0.018	0.000	0.000	0.000	0.000	0.170	2.110	0.000
USF1_common_T	623	7.432	13.414	5.824	4.726	12.480	9.747	0.000	5.349	17.100	3.250	11.200
YY1	11801	13.182	12.261	22.801	18.355	15.642	8.019	4.737	10.275	18.420	10.590	29.500
ZIC3	46	13.182	12.261	0.000	0.000	15.642	8.019	4.737	10.275	18.420	0.170	29.500
ZNF217_sp3_T	1401	9.553	12.499	20.481	15.954	13.868	6.016	12.906	6.902	21.380	17.100	54.300

Supplementary Table 10. Summary of transcript reconstruction tools

Method	URL	Main application	Additional features
AUGUSTUS[1,2]	bioinf.uni-greifswald.de/augustus	Gene prediction, genome annotation	Can incorporate external expression data (e.g. SAGE or CAGE). Can make use of protein homology information.
Cufflinks[3]	cufflinks.cbcb.umd.edu	Transcript assembly and quantification	Can be run without or without gene annotation, or optionally applied to quantify known transcripts. Can correct for fragment bias and improve transcript quantification if provided with estimated pre-mRNA levels.
Exonerate[4]	www.ebi.ac.uk/~guy/exonerate	Transcript assembly and quantification	
GSTRUCT	—	Transcript assembly and quantification	
iReckon[5]	compbio.cs.toronto.edu/ireckon	Transcript assembly and quantification	Can identify pre-mRNAs and retained introns. Can incorporate external expression data (e.g. SAGE or CAGE).
mGene[6,7]	mgene.org	Gene prediction, genome annotation	
mTim	galaxy.raetschlab.org	Transcript assembly and quantification	Can use external gene expression, protein, RepeatMasker or sequence conservation data. Can identify open reading frames and thus predict coding exons.
NextGeneid[8]	—	Gene prediction, genome annotation	
Oases[9]	www.ebi.ac.uk/~zerbino/oases	De-novo transcriptome assembly	
SLIDE[10]	sites.google.com/site/jingyijli/SLIDE.zip	Transcript assembly and quantification	Can be applied to independent transcript quantification. Can incorporate external expression data (e.g. SAGE or CAGE).
Transomics	www.softberry.com	Gene prediction, genome annotation	
Trembly	—	Transcript assembly and quantification	
Tromer[11]	tromer.sourceforge.net	Transcript assembly and quantification	
Velvet[12]	www.ebi.ac.uk/~zerbino/velvet	De-novo genome assembly	

Supplementary Note: Description of transcript reconstruction protocols

This document provides details about specific transcript reconstruction methods. For methods that underlie several protocols, subheadings designate procedural variants. All protocols used the same reference genome sequences: *H. sapiens* assembly GRCh37, *D. melanogaster* release 5 from the Berkeley Drosophila Genome Project, and *C. elegans* assembly WS200. See also Supplementary Table 1, where details of the protocols are tabulated, including the alignment programs used to map RNA-seq reads to the genome sequences.

1. AUGUSTUS

The gene finder AUGUSTUS was initially built to predict gene structures from genomic sequences alone[1]. An extended version of the generalized hidden Markov model used by AUGUSTUS was developed to incorporate evidence from external sources, such as syntenic genomic sequences or expressed sequence tags[2]. Such data is used to inform the detection of start and stop codons, acceptor and donor splice sites, and exonic regions. For each triple of a genomic sequence, a gene structure, and a set of hints, AUGUSTUS assigns a joint probablility and then finds the gene structure that maximizes the posterior probability. The software is available at http://bioinf.uni-greifswald.de/augustus.

1.1. AUGUSTUS all

AUGUSTUS was run using gene expression evidence generated from RNA-seq data. All reconstructed transcripts are reported.

1.2. AUGUSTUS high

AUGUSTUS was run using gene expression evidence generated from RNA-seq data. Only genes with RPKM > 0 are reported.

1.3. AUGUSTUS de-novo

AUGUSTUS was run purely on the genomic sequences. No RNA-seq information is provided.

1.4 Additional features

AUGUSTUS can optionally make use of protein homology information to identify coding genes. Other types of external transcriptomic information can also be incorporated, such as SAGE or CAGE data.

2. Cufflinks

Cufflinks is designed to reconstruct transcripts using RNA-seq reads mapped to the genome with the aligner TopHat[3], but can also process output from other spliced aligners. A overlap graph is generated based on the read alignments, including both spliced and unspliced mappings. Reads that are incompatible, i.e. must have originated from different transcript isoforms, are not connected in the graph, whereas compatbile reads are connected. Paths through the graph correspond to different transcript isoforms. The Cufflinks algorithm aims to find a minimal set of paths that covers all fragments, by searching for the largest set of reads with the property that no two of them could have originated from the same transcript isoform.

Here, Cufflinks version 2.0.2 was used together with TopHat version 2.0.3. Both programs were executed with intron settings tailored to the characteristics of each species: minimum intron lengths were set to 30, 40, and 50 bp for *C. elegans*, *D. melanogaster*, and *H. sapiens*, respectively. The software can be obtained from http://cufflinks.cbcb.umd.edu.

2.1 Additional features

Cufflinks can optionally be provided with genome annotation to improve transcript assembly. Two modes of operation are implemented: the first will use annotation as a guide, but novel transcript isoforms are still predicted; the second mode uses RNA-seq data solely to quantify annotated transcripts. Cufflinks offers additional options to correct for fragment bias and improve transcript assembly by estimating the expected pre-mRNA fraction within the sample.

3. Exonerate SM

This method, which is based on Exonerate[4], was developed by Steve Searle and colleagues at the Wellcome Trust Sanger Institute (http://www.sanger.ac.uk). Briefly, sequencing reads are aligned to the genome and processed to build approximate transcript models. This is followed by a refinement stage, where reads are realigned against the models using a method that takes splicing signals into account. Exonerate SM is currently unreleased.

3.1. Exonerate SM high

This set of transcripts contains the highest scoring model for each locus only.

3.2. Exonerate SM all

This output contains additional alternative isoforms.

4. GSTRUCT

The GSTRUCT pipeline was developed by Thomas Wu and colleagues at Genentech. GSTRUCT uses bounded graph analysis to assemble transcripts based on RNA-seq read mappings produced with the aligner GSNAP[13]. GSTRUCT is currently unreleased, but GSNAP can be obtained from http://research-pub.gene.com/gmap.

5. iReckon

The iReckon algorithm first uses spliced alignments, and if applicable annotated introns, to build a splice graph[5]. All possible transcript isoforms are then identifed by enumerating paths from each of the possible transcription start sites to the end sites. For each putative isoform, the sequence is extracted and reads are realigned using the program BWA[14]. Finally, expressed isoforms and their abundances are predicted by a regularized expectation maximization algorithm, which penalizes low-abundance isoforms. iReckon also reports pre-spliced mRNAs and isoforms with retained introns.

iReckon version 1.0.7 was applied using initial alignments from TopHat version 2.0.3 and BWA version 0.6.2 for realignment. These software components are available from http://compbio.cs.toronto.edu/ireckon, http://tophat.cbcb.umd.edu and http://bio-bwa.sourceforge.net, respectively.

5.1 iReckon full

The program was provided with complete annotation for protein coding genes. Unspliced or retained intron transcripts and were removed from the program output so as not to bias evaluations based on protein-coding annotation.

5.2 iReckon ends

The program was provided with transcript boundary coordinates (start and end coordinates, but not intron information) from the reference annotation used for the evaluation. Unspliced transcripts were removed from the program output so as not to bias evaluations based on protein-coding annotation.

5.3 Additional features

iReckon can predict pre-mRNAs and retained introns from RNA-seq data. The software distribution also includes a plug-in for the Savant Genome Browser to visualize read assignment to transcript isoforms.

6. mGene and mTim

The protocols mGene, mGene graph and mTim are based on combinations of the individual programs PALMapper[15], mGene[6,16], mTim, SplAdder and rQuant[17], all developed by the same group.

PALMapper was used to align the RNA-seq reads by allowing spliced and unspliced alignments. The program considers base call quality scores and computational splice site predictions during alignment. Alignments were filtered with different settings for mGene and mTim (see below).

The basic mGene algorithm uses a two-layered machine learning approach. The first layer employs support vector machine models to scan genomic sequence for transcription start and stop sites, translation start and stop sites, and splice donors and acceptors. The second uses hidden semi-Markov support vector machines to combine those features into valid coding gene predictions. While the fundamental strategy relies only on genomic sequence, mGene can also include information from features tracks in the scoring function. Various tracks were used in this protocol, mostly from RNA-seq alignments. The balance between signal predictions and feature tracks is optimized during training. This extension of mGene has recently been documented.

In contrast to mGene, mTim uses a simpler hidden Markov support vector machine approach, in which states directly correspond to intergenic, exonic and intronic nucleotides with a certain expression level (five submodels were used, each corresponding to an expression quintile). Based on features derived from the RNA-seq alignments, the most likely state is inferred for each nucleotide, taking context-dependencies into account (in the form of a state-transition model). The model does not distinguish between coding and non-coding regions. The parameters of the model are trained on a small subset of the reference gene annotation.

SplAdder builds a splice graph based on initial predictions and RNA-seq evidence. The splice graph is then used to generate possible transcript isoforms. For each transcript SplAdder determines the maximal open reading frame to predict coding regions.

Expression levels of predicted isoforms were estimated using the program rQuant that can take fragment biases into account. Those transcripts that scored low using an SVM classifier were removed from the prediction set. The SVM was trained on a small fraction of the genome annotation using estimated abundance, length, coding sequence length and number of exons as features.

The programs PALMapper, mGene, mTim, SplAdder, and rQuant can be obtained from http://raetschlab.org/suppl/rgasp2.

Based on these algorithms, prediction sets were created as follows:

6.1. mGene

For each organism mGene was trained with multiple features from PALMapper RNA-seq alignments, as well as other genomic features, output from SVM-based signal predictors that were previously trained on a part of the annotation. The RNA-seq based features included exon coverage, intron coverage (number of spliced reads spanning a given position, indicating that this position may be part of an intron) and intron lists including the count of supporting reads. Alignments were filtered by excluding reads with more than one mismatch, fewer than eight aligned nucleotides in any exon flanking a spliced alignment, or a spliced alignment indicating and intron longer than 20 kb (100 kb for human).

For human, repeat elements identified by RepeatMasker were included, in addition to other sequence-based features. Similar to exon and intron coverage tracks, 15 additional tracks were included: "DNA", "LINE", "Low_complexity", "LTR", "Other", "RC", "tRNA", "Satellite", "Simple_repeat", "SINE", "Unknown", "rRNA", "scRNA", "snRNA" and "RNA".

6.2. mGene graph

As the mGene protocol above, with the addition that alternative transcripts were predicted using SplAdder, subsequently quantified using rQuant and filtered using the SVM classifier.

6.3. mTim

RNA-seq alignments generated by PALMapper were filtered specifically for each organism. Spliced alignments for *C. elegans*, *D. melanogaster* and *H. sapiens* were filtered out if the minimal segment length within an alignment was shorter than 15nt, 20nt, or 15nt, respectively. A general threshold of at most one edit operation was applied to all alignments for all organisms.

mTim was trained with multiple features from RNA-seq alignments as well as splice signals from genomic sequence (based on an SVM classifier previously trained on a subset of the annotation). Features derived from the RNA-seq alignments included exon coverage, intron coverage (number of spliced reads spanning a given position), scores for acceptor and donor splice sites deduced from spliced alignments (number of spliced alignments with a given junction and alignment confidence scores) as well as mate-pair coverage (number of read pairs with an insert spanning a given position). SplAdder was applied to the raw mTim transcript predictions to generate alternative transcripts, which were quantified by rQuant and filtered using the SVM approach.

7. NextGeneid

NextGeneid is a modified version of Geneid (version 1.3)[8]. Geneid identifies splice sites and start/stop codons from genomic sequence. These features are then combined to predict exons, which are scored based on supporting features and coding potential. From the set of predicted exons, gene structures are assembled. NextGeneid additionally incorporates RNA-seq read alignments to the genome, produced with the GEM mapper[18], including spliced alignments from the GEM component gem-split-mapper. Read alignments were used to modify the scores of potential exons, determine transcript start and end coordinates, and constrain the exon-chaining algorithm in Geneid based on spliced alignments. NextGeneid has not been released.

7.1. NextGeneid

Geneid was previously trained on several species, including the three used in this study. No modifications to the signal or coding potential position weight arrays were performed. Transcripts reported by NextGeneid with RPKM > 1 were retained.

7.2. NextGeneidAS

As NextGeneid, but iterated to increase intron detection sensitivity.

7.3. NextGeneidAS de-novo

As NextGeneid, with the addition of *ab initio* Geneid predictions falling within intergenic space to the final set of transcripts.

8. Oases

Oases assembles transcripts from RNA-seq data without using genomic sequence[9]. It is based on the short-read assembler Velvet[12], adapting several steps to the different characteristics of RNA-seq data, such as uneven coverage across transcripts and the expression of alternative isoforms. Reads of low quality were first removed from the data and low-quality bases were trimmed from from both ends of reads. Velvet was then used to build a de Bruijn graph from the RNA-seq data using a *k*-mer size of 33. Mate pair and coverage information was used to predict and assemble transcripts using Oases v0.1. The resulting transcripts were then aligned to each genome using BLAT[19]. Oases is available at http://www.ebi.ac.uk/~zerbino/oases.

9. SLIDE

SLIDE is a stochastic method based on a linear model with a design matrix that computes the sampling probability of RNA-seq reads from different transcript isoforms[10]. It utilizes exon boundary information from annotations to enumerate all possible isoforms. Discovery of expressed isoforms is implemented as a sparse estimation problem, related to the number of isoforms that are expected to be expressed. Sparse estimation is achieved by a modified lasso method[20]. SLIDE is available at https://sites.google.com/site/jingyijli.

9.1. SLIDE all

All transcript isoforms reported by SLIDE.

9.2. SLIDE high

The subset of isoforms identified as "high confidence" by SLIDE.

10. Transomics

The Transomics pipeline is based on the gene finding pipeline Fgenesh++[21], extended to incoporate splice site information from RNA-seq read mappings to the genome. The relative abundance of alternative transcripts generated from the same gene locus is estimated using a solution of a system of linear equations. Further details are available at http://linux5.softberry.com/cgi-bin/berry/programs/Transomics.

10.1. Transomics all

All predicted genes are reported.

10.2. Transomics high

Only transcripts with RPKM > 0.02 are reported.

11. Trembly

Trembly is an unpublished software package for transcript reconstruction from RNA-seq data developed in Mark Gerstein's group at Yale University (http://www.gersteinlab.org). Trembly was applied to RNA-seq reads aligned with TopHat. A signal track of mapped reads is generated and a set of transcriptionally active regions (TARs) is identified. Splice junctions are then inferred from adjacent TARs. Both the predicted splice junctions and TARs are provided as input for transcript assembly, which generates all possible transcript isoforms compatible with the data. Expression levels of predicted transcripts were estimated using the program IQSeq developed by the same group[22].

11.1. Trembly all

The full output from Trembly.

11.2. Trembly high

The subset of transcripts with RPKM above 0.1.

12. Tromer

The Tromer pipeline first maps reads to the genome using fetchGWI to identify unique exact matches[11]. MegaBLAST is used to recover unmapped reads. In a third step, spliced alignment is carried out with SIBsim4, taking mate pair information into account. The output of these three steps is combined to create graphs representing all possible alternative splice variants of a gene. A greedy algorithm is applied to the graphs, designed to output a set of transcripts such that each edge is covered at least once. The algorithm proceeds in three steps: 1) select a seed edge, 2) extend toward the 5´ end, and 3) extend toward the 3´ end. The seed edge is first selected among unused 5´-most exons, and then among remaining unused edges. The extension process attempts to include unused edges derived from the same read pair as the seed edge. Further details are available at http://tromer.sourceforge.net.

13. Velvet

These protocols are based on the genome assembly program Velvet[12]. Transcripts assembled by Velvet were mapped to the respective genomes using BLAT[19]. Exons were quantified using ERANGE[23]. These programs are available from http://www.ebi.ac.uk/~zerbino/velvet, http://genome.ucsc.edu (BLAT) and http://woldlab.caltech.edu (ERANGE).

13.1. Velvet

This protocol corresponds to the Velvet pipeline outlined above.

13.2. Velvet + Augustus

Transcripts structures assembled by the Velvet pipeline were provided to AUGUSTUS as evidence. The final set of transcripts consisted of AUGUSTUS models that agreed with the original isoforms from Velvet over more than 25% of their length.

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