

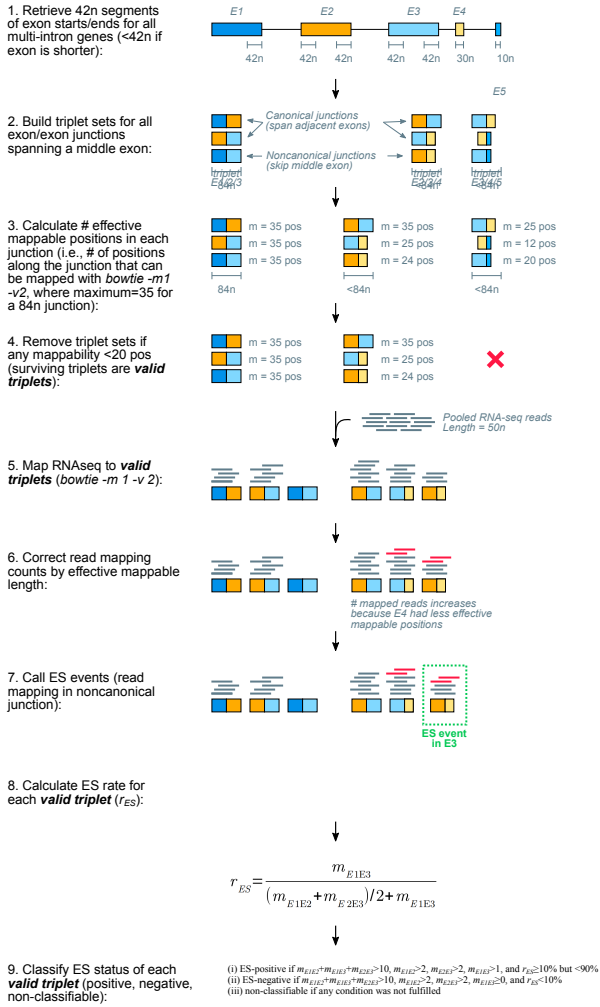
Species	Abbr.	Genome sequence and annotation	Transcriptomes	Taxonomy				
<i>Homo sapiens</i>	Hsap	Ensembl 78	SRP007412 / Brawand 2012 SRP056969 / Duff 2015	Vertebrata (chordate)	Metazoa	Holozoa	Opisthokonta	Amorphea
<i>Mus musculus</i>	Mmus	Ensembl 78	SRP007412 / Brawand 2012 SRP015997 / Barbosa-Morais 2012	Vertebrata (chordate)	Metazoa	Holozoa	Opisthokonta	Amorphea
<i>Monodelphis domestica</i>	Mdom	Ensembl 78	SRP007412 / Brawand 2012	Vertebrata (chordate)	Metazoa	Holozoa	Opisthokonta	Amorphea
<i>Ornithorhynchus anatinus</i>	Oana	Ensembl 78	SRP007412 / Brawand 2012	Vertebrata (chordate)	Metazoa	Holozoa	Opisthokonta	Amorphea
<i>Gallus gallus</i>	Ggal	Ensembl 78	SRP007412 / Brawand 2012	Vertebrata (chordate)	Metazoa	Holozoa	Opisthokonta	Amorphea
<i>Danio rerio</i>	Drex	Ensembl 78	SRP048807	Vertebrata (chordate)	Metazoa	Holozoa	Opisthokonta	Amorphea
<i>Xenopus tropicalis</i>	Xtro	Ensembl 78	SRP012375 / Tan 2012 SRP015997 / Barbosa-Morais 2012	Vertebrata (chordate)	Metazoa	Holozoa	Opisthokonta	Amorphea
<i>Ciona intestinalis</i>	Cint	Ensembl 78	SRP042651	Tunicata (chordate)	Metazoa	Holozoa	Opisthokonta	Amorphea
<i>Branchiostoma belcheri</i>	Brabel	PRJNA214454	SRP025148	Urochordata (chordate)	Metazoa	Holozoa	Opisthokonta	Amorphea
<i>Strongylocentrotus purpuratus</i>	Spur2	Ensembl Metazoa 27	GSE97267 / Burguera 2017	Echinodermata	Metazoa	Holozoa	Opisthokonta	Amorphea
<i>Drosophila melanogaster</i>	Dmel	Ensembl Metazoa 27	SRP001696 / modENCODE 2011 SRP082392 / Katzenberger 2016	Ecdysozoa	Metazoa	Holozoa	Opisthokonta	Amorphea
<i>Caenorhabditis elegans</i>	Cele	Ensembl 78	SRP000401	Ecdysozoa	Metazoa	Holozoa	Opisthokonta	Amorphea
<i>Crassostrea gigas</i>	Cgig	Ensembl Metazoa 27	SRP014559	Lophotrochozoa	Metazoa	Holozoa	Opisthokonta	Amorphea
<i>Capitella teleta</i>	Ctel	Ensembl Metazoa 27	SRP102138	Lophotrochozoa	Metazoa	Holozoa	Opisthokonta	Amorphea
<i>Schistosoma mansoni</i>	Sman	Ensembl Metazoa 27	ERP000427	Lophotrochozoa	Metazoa	Holozoa	Opisthokonta	Amorphea
<i>Trichoplax adhaerens</i>	Tadh	Ensembl Metazoa 27	<b>This study – PRJEB23829</b>	Placozoa	Metazoa	Holozoa	Opisthokonta	Amorphea
<i>Hydra magnipapillata</i>	Hmag	NCBI PRJNA12876	SRP051110	Cnidaria	Metazoa	Holozoa	Opisthokonta	Amorphea
<i>Nematostella vectensis</i>	Nvec	Ensembl Metazoa 27	SRP021895 / Schwaiger 2014 SRP018739 / Helm 2013	Cnidaria	Metazoa	Holozoa	Opisthokonta	Amorphea
<i>Aiptasia</i> sp.	Aipt	(Baumgarten et al. 2015)	SRP047443	Cnidaria	Metazoa	Holozoa	Opisthokonta	Amorphea
<i>Mnemiopsis leidyi</i>	Mlei	Ensembl Metazoa 27	SRP014828	Ctenophora	Metazoa	Holozoa	Opisthokonta	Amorphea
<i>Amphimedon queenslandica</i>	Aque	Ensembl Metazoa 27	SRR1511618	Porifera	Metazoa	Holozoa	Opisthokonta	Amorphea
<i>Oscarella carmela</i>	Ocar	(Nichols et al. 2012)	SRR1042012	Porifera	Metazoa	Holozoa	Opisthokonta	Amorphea
<i>Sycon ciliatum</i>	Scil	(Fortunato et al. 2014)	ERP005418	Porifera	Metazoa	Holozoa	Opisthokonta	Amorphea
<i>Salpingoeca rosetta</i>	Sros	Ensembl Protist 27	SRP005692	Choanoflagellata	Metazoa	Holozoa	Opisthokonta	Amorphea
<i>Capssapora owczarzakii</i>	Cowc	(Suga et al. 2013)	SRP022579 / Sebé-Pedrós et al. 2013	Filisterea	Metazoa	Holozoa	Opisthokonta	Amorphea
<i>Creolimax fragrantissima</i>	Cfra	(de Mendoza et al. 2015)	SRP058061 / de Mendoza et al. 2015	Ichthyosporaea (Ichthyophonida)	Metazoa	Holozoa	Opisthokonta	Amorphea
<i>Sphaerofarma arctica</i>	Sar3	Broad Institute Multicellularity Initiative	<b>This study – PRJEB23831</b>	Ichthyosporaea (Ichthyophonida)	Metazoa	Holozoa	Opisthokonta	Amorphea
<i>Chromosphaera perkinsii</i>	Cper	(Grau-Bové et al. 2017)	SRP097609	Ichthyosporaea (Dermocystida)	Metazoa	Holozoa	Opisthokonta	Amorphea
<i>Neurospora crassa</i>	Ncrx	Ensembl Fungi 27	SRP016065	Fungi	Holomycota		Opisthokonta	Amorphea
<i>Schizosaccharomyces pombe</i>	Spom	Ensembl Fungi 27	ERP001483	Fungi	Holomycota		Opisthokonta	Amorphea
<i>Aspergillus oryzae</i>	Aory	Ensembl Fungi 27	SRP016952	Fungi	Holomycota		Opisthokonta	Amorphea
<i>Cryptococcus neoformans</i>	Cneo	Broad Institute Fungi Initiative	SRR847297	Fungi	Holomycota		Opisthokonta	Amorphea
<i>Ustilago maydis</i>	Umay	Ensembl Fungi 27	ERP001905	Fungi	Holomycota		Opisthokonta	Amorphea
<i>Tuber melanosporum</i>	Tmel	Ensembl Fungi 27	SRP028655	Fungi	Holomycota		Opisthokonta	Amorphea
<i>Rhizopus oryzae</i>	Rory	Broad Institute Fungi Initiative	SRP031602	Fungi	Holomycota		Opisthokonta	Amorphea
<i>Allomyces macrogynus</i>	Amac	Broad Institute Fungi Initiative	SRP022576	Fungi	Holomycota		Opisthokonta	Amorphea
<i>Spizellomyces punctatus</i>	Spun	Broad Institute Multicellularity Initiative	SRR343043	Fungi	Holomycota		Opisthokonta	Amorphea
<i>Rhizophagus irregularis</i>	Rirr	Ensembl Fungi 27	DRP002784	Fungi	Holomycota		Opisthokonta	Amorphea
<i>Conidiobolus coronatus</i>	Ccor	JGI v1	SRR427173	Fungi	Holomycota		Opisthokonta	Amorphea
<i>Gonapodya prolifera</i>	Gpro	JGI v3	SRR427152	Fungi	Holomycota		Opisthokonta	Amorphea
<i>Fonticula alba</i>	Falb	Broad Institute Multicellularity Initiative	SRP022580	Nucleariida	Holomycota		Opisthokonta	Amorphea
<i>Dictyostelium discoideum</i> AX4	Ddis2	Ensembl Protist 27 / Dictybase	SRP060392	Amoebozoa				Amorphea
<i>Polysphondylium pallidum</i>	Ppal	Ensembl Protist 27	SRP004023	Amoebozoa				Amorphea
<i>Acanthamoeba castellanii</i>	Acas	Ensembl Protist 27	SRP028620	Amoebozoa				Amorphea
<i>Arabidopsis thaliana</i>	Atha	Ensembl Plants 27	SRP052658 / Max Planck MPP 2015 (full nutrition samples only) SRP074840 / Hsu 2016	Embryophyta		Viridiplantae		Diaphorotickes
<i>Vitis vinifera</i>	Vvin	Ensembl Plants 27	SRP065417	Embryophyta		Viridiplantae		Diaphorotickes
<i>Mimulus guttatus</i>	Mgut	JGI GCF_000504015	SRP045683	Embryophyta		Viridiplantae		Diaphorotickes
<i>Oryza sativa</i>	Osat2	Ensembl Plants 27	DRP000315	Embryophyta		Viridiplantae		Diaphorotickes
<i>Physcomitrella patens</i>	Ppat	Ensembl Plants 27	SRP011279	Embryophyta		Viridiplantae		Diaphorotickes
<i>Selaginella moellendorffii</i>	Smoe	Ensembl Plants 27	SRP059539	Embryophyta		Viridiplantae		Diaphorotickes
<i>Klebsormidium netis</i> (formerly <i>flaccidum</i> )	Kfla	(Hori et al. 2014)	SRP048567	Charophyta		Viridiplantae		Diaphorotickes
<i>Volvox carterii</i>	Vcar	JGI 317_v2	SRP066714	Chlorophyta		Viridiplantae		Diaphorotickes
<i>Chlorella variabilis</i>	Cvar	JGI NC64A - 2014-08-18	<b>NOT ANALYSED</b>	Chlorophyta		Viridiplantae		Diaphorotickes
<i>Chlamydomonas reinhardtii</i>	Crei	Ensembl Plants 27	ERP001997	Chlorophyta		Viridiplantae		Diaphorotickes
<i>Micromonas pusilla</i>	Mpus	JGI 20110615	SRR847305	Chlorophyta		Viridiplantae		Diaphorotickes
<i>Cyanophora paradoxa</i>	Cpar	Assembly (Price et al. 2012) annotation in-home	SRR363339	Glaucochyta		Viridiplantae		Diaphorotickes
<i>Ectocarpus siliculosus</i>	Esil	(Cock et al. 2010)	SRP037532	Stramenopile		SAR+H		Diaphorotickes
<i>Phytophthora infestans</i>	Pinf	Ensembl Protist 27	SRR1640225	Stramenopile		SAR+H		Diaphorotickes
<i>Aureococcus anophagefferens</i>	Aano	Ensembl Protist 27	SRP045642	Stramenopile		SAR+H		Diaphorotickes
<i>Plasmodium falciparum</i> 3D7	Pfal2	Ensembl Protist 27	SRP069075	Alveolata		SAR+H		Diaphorotickes
<i>Tetrahymena thermophila</i>	Tthe	Ensembl Protist 27	SRP016619	Alveolata		SAR+H		Diaphorotickes
<i>Bigelowiella natans</i> CCMP2755	BnatMEA	Ensembl Protist 27	MMETSP / SRP042159 (Bnat samples only) SeqBioproject PRJNA47111 / Curtis et al 2012 DRP003230 / Suzuki 2016	Rhizaria		SAR+H		Diaphorotickes
<i>Emiliania huxleyi</i>	Ehux	Ensembl Protist 27	SRR847300	Haptophyta		SAR+H		Diaphorotickes
<i>Guillardia theta</i>	Gthe	Ensembl Protist 27	SRR747855	Cryptophyta				Diaphorotickes
<i>Naegleria gruberi</i>	Ngru	Ensembl Protist 27	<b>This study – PRJEB23822</b>	Excavata				see note

Note: Unikonta is a deprecated name that included all amorphean species here included  
Note: Bikonta is a deprecated name that included all Diaphorotickes species here included EXCEPT for the Excavate *Naegleria gruberi*  
Species with replicates in blue

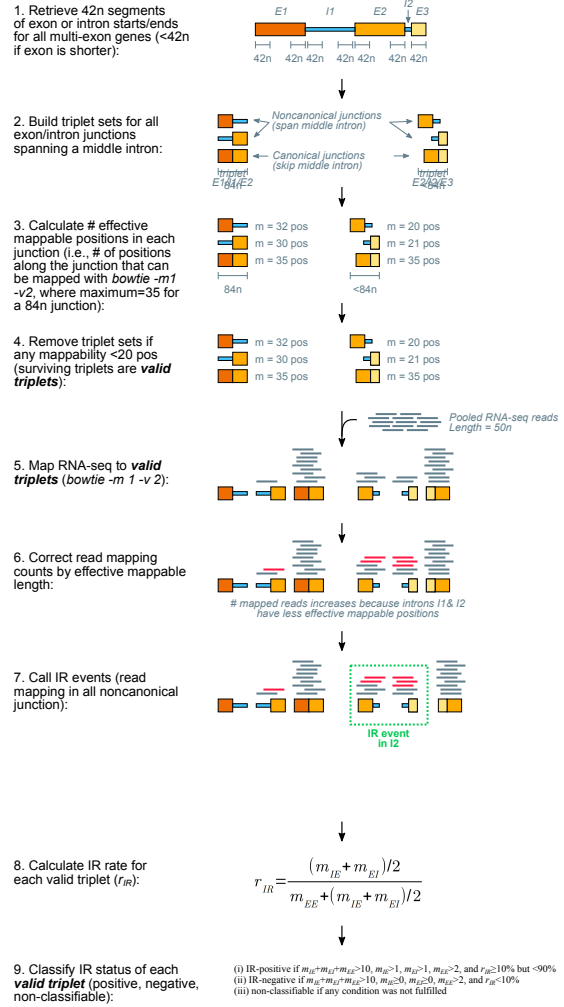
**Fig S1.** Summary of the eukaryotic species used in this study, including its a 4-letter abbreviation, the source of genome assemblies, GFF annotations, and RNA-seq experiments. Dataset accession numbers from SRA unless otherwise stated.

Spe	Introns_per_Alnpct		Introns_per_gene		GCC	Nl_gname	Nv_gname	Nl_gname	Nv_gname	Ql_gname	Qv_gname	Nl_transcript	Nv_transcript	Ql_transcript	Qv_transcript	Nl_exon	Nv_exon	Ql_exon	Qv_exon	Nl_intron	Nv_intron	Ql_intron	Qv_intron	Nl_intronic	Nv_intronic	Ql_intronic	Qv_intronic						
	q1	q3	q1	q3																													
Hhap	4.86	8.40	0.40	20.346.00	49.576.62	108.719.49	4.364.04	16.412.50	48.399.25	20.346.00	1.680.98	1.808.68	760.00	1.253.00	2.021.75	198.096.00	172.68	292.53	86.00	123.00	171.00	170.876.00	5.335.75	18.061.04	490.00	1.493.00	3.888.00	20.306.00	86.896.29	375.804.21	5.551.25	20.204.00	65.875.00
Hsus	4.74	7.71	0.40	22.622.00	49.639.07	12.434.00	10.889.00	32.627.00	22.633.00	1.567.07	1.957.04	708.00	1.129.00	1.896.00	198.777.00	177.64	274.62	89.00	129.00	173.00	173.785.00	4.364.75	15.904.11	443.00	1.279.00	3.102.00	22.492.00	81.306.72	254.072.79	6.348.75	18.901.50	54.922.25	
Mdom	4.63	7.74	0.40	21.327.00	51.246.38	100.271.47	2.352.50	15.435.00	53.702.50	21.327.00	1.577.76	1.507.03	777.00	1.158.00	1.895.00	187.581.00	179.38	264.22	86.00	124.00	174.00	164.984.00	5.960.23	16.152.71	532.00	1.633.00	4.476.00	21.316.00	117.400.00	280.182.60	10.624.00	35.061.50	104.131.00
Oma	5.70	6.58	0.46	21.698.00	20.127.15	41.448.09	2.095.25	12.656.00	18.924.50	21.698.00	1.133.76	1.202.28	441.00	815.00	1.374.00	165.174.00	148.94	193.51	75.00	117.00	166.00	142.860.00	2.758.12	7.162.71	377.00	1.036.00	2.380.00	10.705.00	68.391.55	176.033.30	6.282.00	17.277.00	56.273.00
Sfa	5.74	9.45	0.42	15.508.00	25.223.35	48.351.56	3.018.75	9.050.00	25.064.25	15.508.00	1.057.19	1.728.97	734.75	1.222.00	2.022.00	162.527.00	158.13	214.92	84.00	122.00	168.00	146.625.00	2.411.86	7.494.01	326.00	765.00	1.741.00	14.699.00	41.975.95	117.841.59	3.351.00	9.159.00	29.724.50
Wcax	4.41	9.38	0.40	17.785.00	37.879.18	12.078.09	1.968.00	4.776.50	12.638.00	17.785.00	1.579.71	1.794.99	673.00	1.213.00	1.862.00	191.723.00	155.64	211.76	75.00	117.00	167.00	172.028.00	1.990.27	4.840.28	296.00	813.00	1.788.00	16.275.00	46.457.13	93.861.43	1.743.00	16.212.00	47.381.00
Drex	5.25	8.20	0.36	26.459.00	24.095.14	42.961.53	3.835.00	10.421.00	25.126.00	26.459.00	1.593.47	1.656.86	752.00	1.180.00	1.912.00	245.365.00	171.83	266.71	83.00	122.00	173.00	217.089.00	2.628.71	7.494.20	135.00	963.00	2.528.00	26.001.00	28.438.17	6.672.31	3.946.00	9.259.00	25.814.00
Cint	5.13	5.37	0.36	16.671.00	3.458.44	4.448.11	975.00	2.133.00	4.312.00	16.671.00	0.953.00	1.036.57	427.50	703.00	1.236.00	107.347.00	148.98	160.87	88.00	127.00	162.00	89.508.00	434.76	619.23	322.00	322.00	480.00	15.938.00	5.938.26	5.169.30	758.00	1.776.00	3.935.50
Q06	4.06	6.01	0.41	50.817.00	9.116.95	12.701.34	1.900.00	4.932.00	10.995.00	50.817.00	1.424.26	1.517.25	593.00	1.026.00	1.725.00	356.631.00	202.95	294.13	95.00	136.00	194.00	305.567.00	1.275.04	2.744.69	339.00	531.00	1.030.00	48.842.00	8.420.33	14.514.58	1.620.00	3.756.00	8.842.50
Q07	4.48	6.18	0.34	26.089.00	8.068.40	9.281.77	1.700.50	5.047.00	11.114.00	26.089.00	1.334.78	1.510.57	412.00	943.00	1.467.00	265.004.00	179.56	241.76	75.00	117.00	185.00	163.901.00	1.080.49	1.851.91	208.00	403.00	1.058.00	22.775.00	11.369.58	17.951.16	1.845.00	5.042.00	17.381.00
Q08	3.73	4.33	0.40	30.375.00	2.651.28	3.203.57	814.00	1.632.00	3.368.50	31.375.00	1.050.68	1.068.88	484.00	806.00	1.284.00	171.418.00	199.09	251.17	89.00	134.00	204.00	139.288.00	369.66	1.037.20	52.00	60.00	256.00	25.094.00	6.208.96	7.986.67	1.509.00	3.957.00	7.270.00
Sman	3.85	5.34	0.35	10.772.00	14.644.48	19.071.54	1.505.00	2.870.00	20.163.00	10.772.00	1.430.76	1.620.16	475.00	971.00	1.828.00	68.476.00	225.07	286.29	95.00	159.00	266.00	57.502.00	2.423.04	3.276.50	646.00	1.576.00	2.901.00	10.353.00	18.611.24	25.216.00	4.871.00	11.536.00	22.934.00
Tadh	5.48	7.41	0.33	11.520.00	3.460.18	3.349.96	1.388.00	2.627.00	8.620.00	11.520.00	1.350.78	1.273.52	647.00	1.028.00	1.605.00	96.959.00	120.49	288.43	70.00	101.00	153.00	85.416.00	281.92	667.75	93.00	133.00	256.00	10.952.00	5.438.46	13.402.13	1.486.00	2.804.00	5.602.25
Hmgs	3.61	5.17	0.28	20.947.00	15.036.94	18.415.39	1.457.00	7.656.00	22.497.50	20.947.00	1.406.59	1.212.84	704.00	1.092.00	1.696.00	140.041.00	227.53	322.14	80.00	129.00	231.00	103.671.00	6.210.53	4.110.70	146.00	667.00	3.473.00	11.102.00	13.924.14	15.882.14	2.784.00	9.829.50	19.896.00
Nvex	3.77	4.31	0.41	24.723.00	4.444.29	6.046.06	736.00	2.300.00	5.872.00	24.723.00	998.89	987.34	431.00	750.00	1.215.00	131.666.00	187.84	248.78	72.00	117.00	198.00	108.828.00	785.90	1.466.68	231.00	436.00	818.00	20.875.00	7.665.90	10.212.96	1.602.00	4.107.00	9.412.50
Ajpt	3.80	5.02	0.36	29.271.00	5.548.84	6.546.66	1.644.50	3.463.00	6.960.50	29.271.00	1.543.98	1.662.30	638.00	1.102.00	1.820.00	212.739.00	212.44	388.36	82.00	116.00	207.00	146.958.00	1.210.73	2.062.29	116.00	283.00	558.00	25.895.00	7.339.09	875.00	2.269.00	4.820.50	
Mlei	3.26	4.46	0.39	16.058.00	5.381.68	7.110.61	1.058.25	2.450.00	16.058.00	1.377.51	1.225.39	621.00	1.049.00	1.688.00	88.550.00	249.80	414.55	101.00	138.00	198.00	71.632.00	803.79	1.548.92	172.00	271.00	207.00	14.367.00	3.327.87	6.798.16	793.00	2.020.00	4.992.00	
Aque	3.80	4.05	0.36	40.122.00	2.384.84	4.478.39	477.00	1.080.00	2.441.00	40.122.00	1.054.25	1.300.55	352.00	662.00	1.284.00	204.935.00	206.40	353.89	78.00	122.00	206.00	162.577.00	248.86	708.53	49.00	67.00	702.00	30.899.00	1.441.49	5.263.45	174.00	501.00	1.967.50
Ccar	4.01	0.98	0.40	8.694.00	3.581.62	3.335.94	1.497.25	2.614.00	4.552.75	8.694.00	1.570.28	1.365.71	744.00	1.215.00	1.935.00	65.960.00	208.97	307.77	94.00	134.00	195.00	57.245.00	303.35	171.91	178.00	297.00	387.00	8.942.00	1.953.37	1.436.29	735.25	1.213.00	2.009.00
Scil	2.76	4.22	0.47	26.105.00	6.459.26	9.220.71	635.00	2.596.00	8.744.00	26.105.00	1.247.78	1.611.41	443.00	914.00	1.590.00	140.348.00	237.11	444.73	80.00	125.00	219.00	110.121.00	1.074.71	1.538.08	378.00	642.00	1.201.00	22.679.00	4.655.72	9.653.28	833.00	3.070.00	7.874.00
Sros	4.73	7.44	0.56	11.624.00	3.872.65	3.467.72	1.653.75	2.876.50	4.909.00	11.624.00	1.987.00	2.149.36	819.00	1.400.00	2.418.00	98.139.00	235.35	552.61	70.00	108.00	178.00	86.511.00	251.35	324.28	178.00	222.00	274.00	11.474.00	863.93	1.378.55	3.222.00	5.400.00	8.511.00
Cowc	2.41	3.47	0.54	8.741.00	2.457.38	1.841.11	1.276.00	2.042.00	3.138.00	8.741.00	1.951.30	1.755.08	871.00	1.493.00	2.492.00	42.674.00	399.69	775.58	88.00	156.00	357.00	30.361.00	120.21	272.25	82.00	100.00	132.00	8.665.00	2.447.03	5.238.65	229.50	598.00	3.952.00
Sar3	3.22	4.86	0.38	16.015.00	4.258.88	5.879.84	894.50	2.075.00	5.222.00	16.015.00	1.468.33	1.409.67	566.00	1.068.00	1.909.00	92.075.00	250.53	452.04	88.00	138.00	220.00	77.911.00	571.41	858.70	133.00	376.00	743.00	13.322.51	4.411.29	4.956.00	1.821.00	4.503.50	
Nk52	0.59	0.63	0.43	12.463.00	1.724.22	1.642.00	731.50	1.322.00	12.463.00	1.628.03	1.627.01	656.00	1.286.00	2.006.00	20.267.00	999.91	1.324.24	208.00	537.00	1.352.00	7.804.00	154.81	314.27	85.00	99.00	121.00	9.806.00	1.066.58	1.213.54	277.00	614.00	1.620.00	
Ncrr	2.10	1.74	0.48	9.820.00	1.694.61	2.182.95	895.50	1.422.50	2.142.25	9.820.00	1.456.12	1.198.03	664.00	1.208.50	1.868.25	26.267.00	531.05	746.81	91.00	239.00	691.00	17.104.00	134.92	123.73	64.00	83.00	150.00	9.699.00	2.007.41	2.817.63	797.00	1.350.00	2.339.00
Cncr	1.24	1.36	0.56	5.144.00	1.474.80	1.113.16	765.00	1.216.00	1.843.25	5.144.00	1.392.25	1.114.38	667.75	1.133.00	1.750.00	10.210.00	701.44	963.11	105.00	306.00	986.00	5.060.00	80.82	71.34	45.00	54.00	84.00	5.138.00	966.50	1.676.75	385.00	681.00	1.154.00
Acry	1.83	1.93	0.48	12.074.00	1.574.64	1.143.95	830.00	1.301.50	1.920.75	12.074.00	1.343.44	1.039.10	709.00	1.112.00	1.633.75	35.404.00	458.16	633.20	103.00	249.00	574.00	25.330.00	117.66	223.23	53.00	61.00	82.00	12.063.00	1.567.04	3.158.09	493.50	939.00	1.870.00
Cneo	4.18	5.25	0.48	6.962.00	1.901.06	1.265.25	1.039.25	1.613.00	2.392.75	6.962.00	1.562.49	1.143.37	794.00	1.299.00	1.991.00	43.483.00	252.55	738.00	144.00	248.00	361.518.00	62.54	43.80	50.00	54.00	62.00	6.948.00	806.75	1.897.31	960.79	488.00	906.25	
Urmy	0.76	0.75	0.54	6.522.00	1.931.39	1.386.88	996.00	1.574.00	2.473.00	6.522.00	1.835.27	1.362.25	890.00	1.502.00	2.378.00	11.420.00	1.048.13	1.239.99	149.00	567.00	1.574.00	4.896.00	128.00	109.88	62.00	94.00	144.25	6.258.00	994.33	960.89	388.00	713.00	1.270.75
Uryy	2.84	2.87	0.45																														

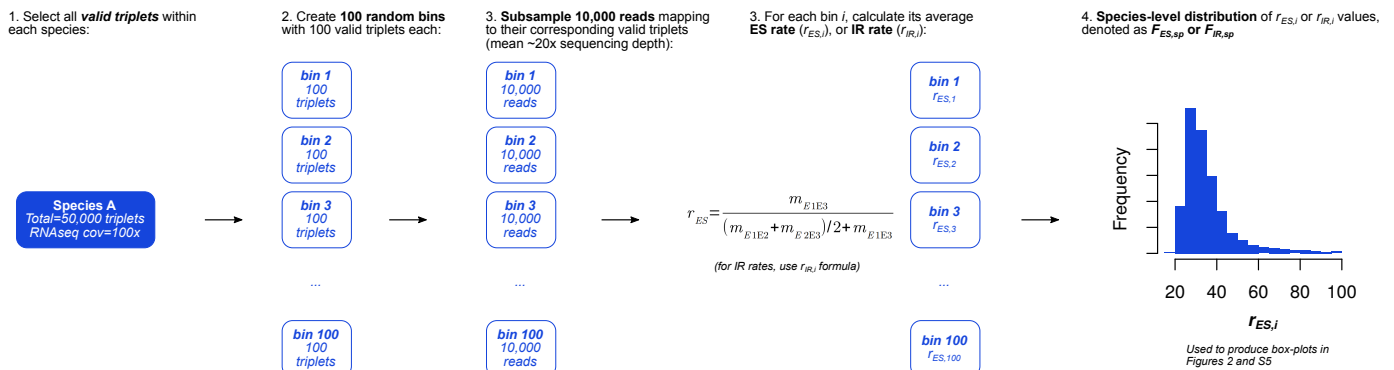
### A) Construction of ES catalog, and ES event calling:



### B) Construction of IR events catalog, and IR event calling:



### C) Quantification procedure (valid for ES and IR):



**Fig S3.** Methods overview.

A-B) Summary of the methodology employed to detect ES (A) and IR events (B), including junction construction, mappability correction and filtering, RNA-seq mapping, AS event calling, and frequency calculations.

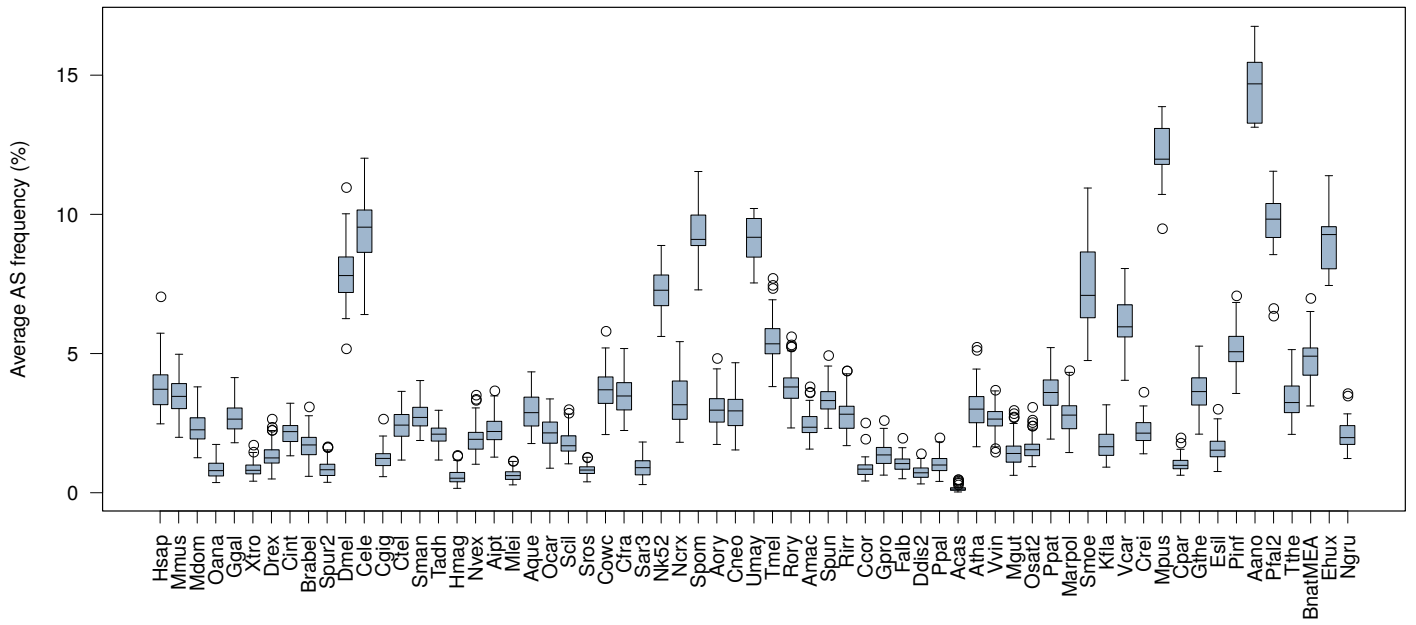
C) Quantification procedure followed to analyse ES and IR frequencies at the species level, using valid triplets.

Sps	num total genes	num total introns	num positive genes ES	num positive tripl ES	num negative genes ES	num negative tripl ES	num positive genes IR	num positive tripl IR	num negative genes IR	num negative tripl IR	% positive genes ES	% positive tripl ES	% positive genes IR	% positive tripl IR
Hsap	20,346	177,720	2,144	2,559	10,463	87,405	2,182	4,519	5,480	39,978	10.538	1.440	10.724	2.543
Hsax	20,346	177,720	2,299	2,733	12,390	104,580	5,682	17,646	6,542	37,045	11.300	1.538	27.927	9.929
Mmus	22,533	176,244	1,693	1,978	13,452	121,566	3,321	6,570	7,493	61,161	7.513	1.122	14.738	3.728
Mmum	22,533	176,244	1,167	1,315	11,707	97,783	1,430	2,188	6,852	50,228	5.179	0.746	6.346	1.241
Mdom	21,327	166,254	1,502	1,786	12,062	98,246	1,778	2,974	6,519	50,004	7.043	1.074	8.337	1.789
Oana	21,698	143,476	922	1,015	11,538	64,925	389	456	7,253	37,296	4.249	0.707	1.793	0.318
Ggal	15,508	147,019	1,029	1,195	10,828	90,527	1,923	3,156	5,708	43,245	6.635	0.813	12.400	2.147
Xtro	18,442	173,281	595	652	11,763	83,821	534	636	6,519	47,559	3.226	0.376	2.896	0.367
Xtrx	18,442	173,281	431	471	10,886	72,310	981	1,303	6,255	37,597	2.337	0.272	5.319	0.752
Drex	26,459	218,906	1,370	1,554	17,505	138,745	1,608	2,056	10,223	74,712	5.178	0.710	6.077	0.939
Drer	26,459	218,906	701	774	15,964	124,743	595	712	8,642	64,939	2.649	0.354	2.249	0.325
Cint	16,671	90,676	592	631	6,997	26,701	353	406	3,607	13,358	3.551	0.696	2.117	0.448
Brabel	30,392	234,612	1,279	1,575	16,211	123,136	1,935	2,664	10,087	72,485	4.208	0.671	6.367	1.135
Spur2	28,842	167,677	1,541	1,875	8,863	40,461	246	299	5,490	25,598	5.343	1.189	0.853	0.190
Dmel	13,917	41,189	342	397	6,658	23,632	2,207	4,198	3,805	12,403	2.457	0.964	15.858	10.192
Dmel2	13,917	41,189	350	402	6,593	22,604	616	856	4,305	14,233	2.515	0.976	4.426	2.078
Cele	20,447	104,192	557	616	13,732	63,476	6,517	13,362	6,608	29,035	2.724	0.591	31.873	12.824
Cgig	26,089	160,801	552	657	11,548	79,503	743	993	7,055	46,293	2.116	0.409	2.848	0.618
Ctel	32,175	139,243	425	482	11,147	65,114	1,669	2,193	6,489	35,746	1.321	0.346	5.187	1.575
Sman	10,772	57,702	462	490	5,340	26,280	718	1,036	2,987	14,393	4.289	0.849	6.665	1.795
Tadh	11,520	85,439	20	20	6,613	43,359	402	500	3,494	19,505	0.174	0.023	3.490	0.585
Hmag	20,047	103,994	329	341	9,360	54,471	204	215	5,698	30,965	1.641	0.328	1.018	0.207
Nvec	24,773	106,893	240	250	8,315	49,134	1,288	1,924	4,643	27,336	0.969	0.234	5.199	1.800
Nvex	24,773	106,893	318	335	9,230	53,440	1,229	1,495	5,976	33,802	1.284	0.313	4.961	1.399
Aipt	29,271	183,468	123	124	9,399	54,748	883	1,200	5,273	26,298	0.420	0.068	3.017	0.654
Mlei	16,058	72,492	153	165	7,037	33,668	118	135	3,843	18,998	0.953	0.228	0.735	0.186
Aque	40,122	164,813	101	103	9,914	63,675	1,856	2,827	6,121	32,300	0.252	0.062	4.626	1.715
Ocar	11,152	62,573	12	12	5,571	30,963	790	1,084	3,309	17,622	0.108	0.019	7.084	1.732
Scil	26,105	114,243	85	85	7,771	47,151	523	599	3,839	20,399	0.326	0.074	2.003	0.524
Sros	11,624	86,515	8	8	6,852	38,486	125	127	3,489	15,726	0.069	0.009	1.075	0.147
Cowc	8,741	32,468	9	9	4,357	15,501	592	804	2,250	7,923	0.103	0.028	6.773	2.476
Cfra	8,694	57,266	78	79	6,228	41,670	1,327	2,098	3,282	20,254	0.897	0.138	15.263	3.664
Sar3	16,015	77,911	292	317	6,069	37,679	291	415	3,492	21,533	1.823	0.407	1.817	0.533
Sarc4	33,969	74,290	322	350	7,147	45,274	681	910	4,246	26,269	0.948	0.471	2.005	1.225
Nk52	12,463	7,804	5	5	1,619	2,824	517	721	1,392	2,305	0.040	0.064	4.148	9.239
Ncra	9,820	17,106	5	5	2,445	3,937	378	425	2,526	4,388	0.051	0.029	3.849	2.485
Spom	5,144	5,066	5	5	1,160	2,230	491	693	845	1,421	0.097	0.099	9.545	13.679
Aory	12,074	23,330	0	0	3,356	6,524	370	461	2,523	5,329	0.000	0.000	3.064	1.976
Cneo	6,962	36,521	6	6	3,694	9,484	394	452	2,225	5,409	0.086	0.016	5.659	1.238
Umay	6,522	4,898	0	0	281	407	193	227	389	530	0.000	0.000	2.959	4.635
Tmel	7,496	21,525	11	11	3,977	10,458	1,013	1,446	2,532	7,114	0.147	0.051	13.514	6.718
Rory	17,459	40,515	4	4	4,865	11,891	826	948	3,468	8,756	0.023	0.010	4.731	2.340
Amac	18,773	43,776	3	3	3,497	5,938	234	245	2,798	4,950	0.016	0.007	1.246	0.560
Spun	8,952	43,038	3	3	4,605	17,308	446	537	2,400	7,261	0.034	0.007	4.982	1.248
Rirr	29,822	72,193	49	49	5,627	21,892	715	908	3,411	12,121	0.164	0.068	2.398	1.258
Rirr2	29,822	72,193	4	4	1,765	4,188	38	41	703	1,506	0.013	0.006	0.127	0.057
Ccor	10,635	18,544	1	1	2,401	6,634	34	37	1,519	3,950	0.009	0.005	0.320	0.200
Gpro	13,902	61,811	4	5	7,501	30,953	411	484	4,259	17,948	0.029	0.008	2.956	0.783
Falb	5,881	21,937	0	0	3,270	9,950	55	57	1,963	5,445	0.000	0.000	0.935	0.260
Ddis2	13,212	16,788	5	5	2,496	3,985	32	32	2,580	4,234	0.038	0.030	0.242	0.191
Ppal	12,367	33,347	0	0	3,651	7,086	86	96	2,871	5,931	0.000	0.000	0.695	0.288
Acas	14,973	90,785	5	5	8,007	40,958	22	23	4,546	24,761	0.033	0.006	0.147	0.025
Atha	27,416	113,157	118	120	13,995	76,060	2,567	3,590	8,243	43,766	0.430	0.106	9.363	3.173
Atha3	27,416	113,157	88	91	10,294	48,586	732	848	5,855	24,626	0.321	0.080	2.670	0.749
Vvin	29,927	112,194	338	351	10,345	56,299	1,529	2,223	5,877	30,872	1.129	0.313	5.109	1.981
Mgut	27,232	116,240	146	150	12,184	67,903	905	1,089	7,310	39,298	0.536	0.129	3.323	0.937
Osat2	35,679	99,011	70	70	9,147	36,854	367	393	4,902	15,525	0.196	0.071	1.029	0.397
Ppat	32,273	117,531	385	396	10,800	56,093	2,022	2,838	5,697	25,914	1.193	0.337	6.265	2.415
Marpol	19,287	73,310	391	423	7,805	50,721	1,386	2,203	4,564	27,604	2.027	0.577	7.186	3.005
Smoe	34,799	162,434	5	5	803	1,574	288	354	788	1,164	0.014	0.003	0.828	0.218
Kfla	16,544	92,208	72	76	8,992	48,544	967	1,214	5,130	28,534	0.435	0.082	5.845	1.317
Vcar	14,247	95,854	421	445	9,018	52,947	2,905	5,380	4,888	24,212	2.955	0.464	20.390	5.613
Crei	14,416	104,675	48	48	8,764	42,403	764	1,001	4,945	25,836	0.333	0.046	5.300	0.956
Mpus	9,886	10,959	13	13	878	1,549	367	532	678	776	0.131	0.119	3.712	4.854
Cpar	11,011	60,591	3	3	1,411	5,681	42	53	731	2,873	0.027	0.005	0.381	0.087
Gthe	24,945	132,687	14	14	11,695	63,250	2,082	4,495	6,453	34,660	0.056	0.011	8.346	3.388
GtheME	24,945	132,687	42	44	10,088	50,474	624	1,044	5,277	24,479	0.168	0.033	2.502	0.787
Esil	16,271	113,633	210	223	11,955	71,691	1,143	1,547	6,813	41,978	1.291	0.196	7.025	1.361
Pinf	17,785	31,359	6	6	2,563	6,125	604	739	1,835	4,140	0.034	0.019	3.396	2.357
Aano	11,520	14,801	2	2	309	684	286	473	274	393	0.017	0.014	2.483	3.196
Pfal2	5,349	8,417	22	24	1,450	4,796	773	1,131	988	2,479	0.411	0.285	14.451	13.437
Tthe	24,725	89,334	48	50	7,841	28,021	969	1,309	4,746	17,065	0.194	0.056	3.919	1.465
BnatMEA	21,706	167,127	258	291	14,630	96,954	4,874	8,715	7,963	49,635	1.189	0.174	22.455	5.215
Bnat	21,706	167,127	114	119	9,994	50,847	1,246	2,076	4,475	19,884	0.525	0.071	5.740	1.242
Bnao	21,706	167,127	501	727	8,456	56,882	2,451	5,623	3,196	10,859	2.308	0.435	11.292	3.365
Ehux	38,544	96,189	5	5	969	1,959	262	365	581	916	0.013	0.005	0.680	0.379
Ngru	15,709	10,688	3	3	1,332	2,091	113	122	1,537	2,534	0.019	0.028	0.719	1.141

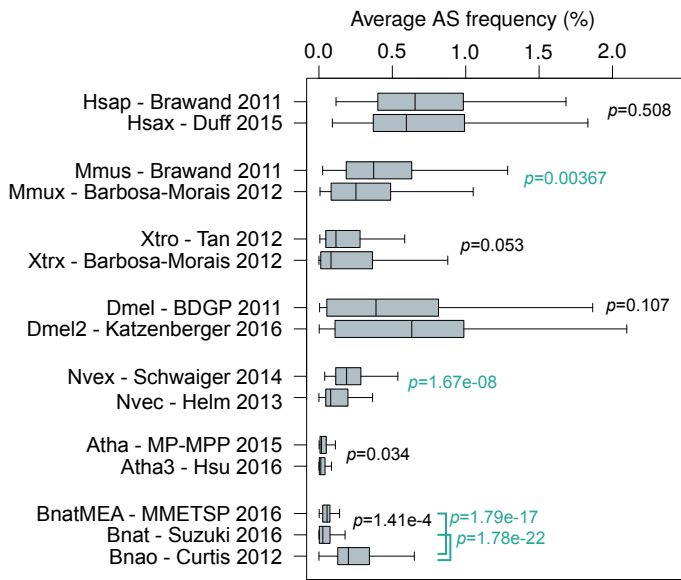
**Fig S4.** Counts of ES and IR events per species, at the gene and exon/intron level.

sp	Median # reads ES	Median # genes ES	# bins ES	Median # reads IR	Median # genes IR	# bins IR
Hsap	10000.0	99.0	100.0	10000	99.00	100
Hsax	10000.0	99.0	100.0	10000	99	100
Mmus	10000.0	99.0	100.0	10000	99	100
Mmux	10000.0	99.0	100.0	10000	99	100
Mdom	10000.0	100.0	100.0	10000	99	100
Oana	10000.0	99.0	100.0	10000	99	100
Ggal	10000.0	99.0	100.0	10000	99	100
Xtro	10000.0	96.0	100.0	10000	99	100
Xtrx	10000.0	99.0	100.0	10000	99	100
Drex	10000.0	99.0	100.0	10000	99	100
Drer	10000.0	96.5	100.0	10000	99	100
Cint	10000.0	99.0	100.0	10000	99	100
Brabel	10000.0	98.0	100.0	10000	99	100
Spur2	10000.0	99.0	100.0	10000	98	100
Dmel	10000.0	98.0	100.0	10000	98	100
Dmel2	10000.0	99.0	100.0	10000	98	100
Cele	10000.0	98.0	100.0	10000	99	100
Cgig	10000.0	99.0	100.0	10000	99	100
Ctel	10000.0	99.0	100.0	10000	99	100
Sman	10000.0	99.0	100.0	10000	98	100
Tadh	7628.0	99.0	100.0	5629	98	100
Hmag	10000.0	99.0	100.0	10000	99	100
Nvec	10000.0	99.0	100.0	9406.5	98	100
Nvex	10000.0	99.0	100.0	10000	99	100
Aipt	10000.0	99.0	100.0	8778.5	98	100
Mlei	10000.0	99.0	100.0	10000	98	100
Aque	10000.0	99.0	100.0	10000	99	100
Ocar	10000.0	98.5	100.0	10000	97.5	100
Scil	10000.0	99.0	100.0	9346.5	98	100
Sros	6653.5	99.0	100.0	4789.5	98	100
Cowc	10000.0	98.5	100.0	10000	98	87
Cfra	10000.0	99.0	100.0	10000	98	100
Sar3	10000.0	99.0	100.0	10000	98	100
Sarc4	10000.0	99.0	100.0	10000	98	100
Nk52	10000.0	97.5	28.0	10000	98	30
Ncrx	10000.0	96.0	39.0	10000	99	48
Spom	10000.0	94.5	22.0	10000	97	21
Aory	10000.0	98.0	65.0	10000	98	57
Cneo	10000.0	98.5	94.0	10000	97	58
Umay	10000.0	87.0	4.0	10000	96	7
Tmel	10000.0	98.0	100.0	10000	98	85
Rory	10000.0	99.0	100.0	10000	98	97
Amac	6187.0	99.0	59.0	5542	99	51
Spun	7202.0	98.0	100.0	5701	97	77
Rirr	10000.0	99.0	100.0	9521	98	100
Rirr2	3141.0	98.0	41.0	2983	94	15
Ccor	10000.0	98.0	66.0	10000	97	39
Gpro	10000.0	99.0	100.0	10000	99	100
Falb	10000.0	98.0	99.0	7628	97	55
Ddis2	10000.0	97.0	39.0	10000	99	42
Ppal	10000.0	99.0	70.0	10000	99	60
Acas	10000.0	99.0	100.0	10000	99	100
Atha	10000.0	99.0	100.0	10000	99	100
Atha3	10000.0	99.0	100.0	10000	99	100
Vvin	10000.0	99.0	100.0	10000	99	100
Mgut	10000.0	99.0	100.0	10000	99	100
Osat2	8999.0	100.0	100.0	6125	99	100
Ppat	9404.0	99.0	100.0	6799	99	100
Marpol	10000.0	99.0	100.0	10000	98	100
Smoe	10000.0	95.0	15.0	10000	96	15
Kfla	10000.0	99.0	100.0	10000	99	100
Vcar	10000.0	98.0	100.0	10000	99	100
Crei	10000.0	99.0	100.0	10000	99	100
Mpus	10000.0	93.0	15.0	10000	92	13
Cpar	10000.0	94.0	56.0	10000	92	29
Gthe	10000.0	100.0	100.0	10000	99	100
GtheME	10000.0	99.0	100.0	7745	99	100
Esil	10000.0	98.0	100.0	10000	99	100
Pinf	10000.0	98.0	61.0	10000	97	48
Aano	10000.0	83.0	6.0	10000	88.5	8
Pfal2	10000.0	93.0	48.0	10000	95	36
Tthe	10000.0	99.0	100.0	10000	99	100
BnatMEA	10000.0	100.0	100.0	10000	99	100
Bnat	6836.0	99.0	100.0	5268.5	99	100
Bnao	4641.5	99.0	100.0	4324.5	98	100
Ehux	9002.0	95.0	19.0	10000	95	12
Ngru	10000.0	96.0	20.0	10000	98	26

**Fig S5.** Number of bins and sub-sampled reads used in the quantification of ES and IR for each species.

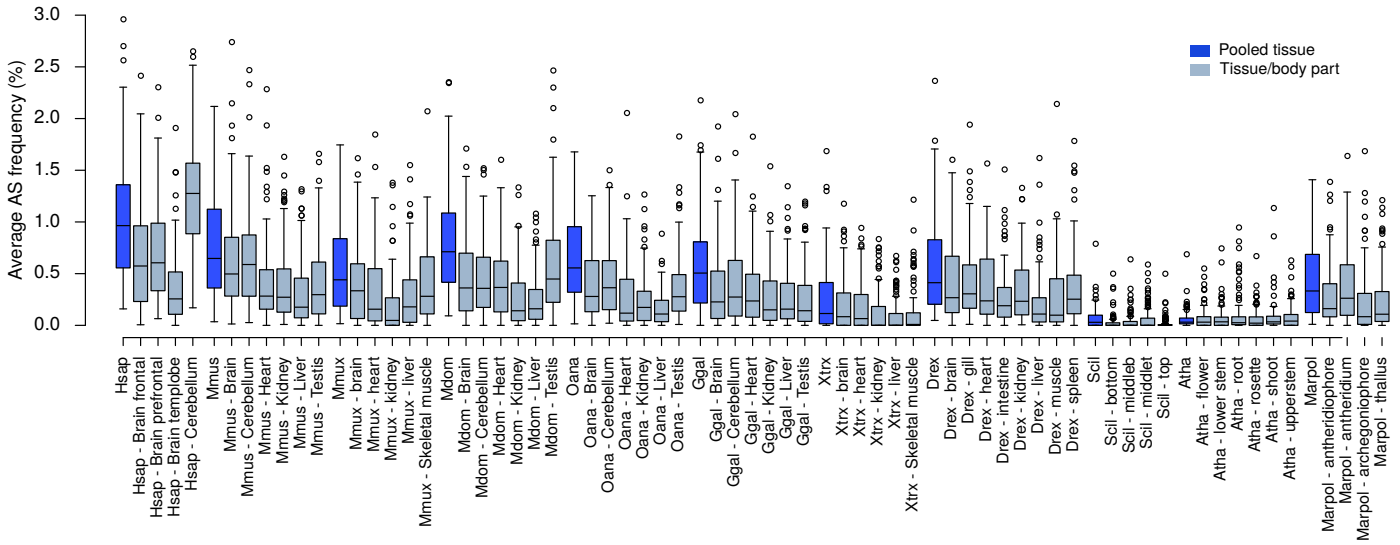


**Fig S6.** Distribution of IR frequencies across 65 eukaryotes (see Supplementary Figure S1 for complete list of transcriptomic datasets). Each box-plot represents the distribution of IR frequencies ( $FIR_{sp}$ ) in a given species (as %), drawn from mapping up to 10,000 RNA-seq reads in 100 exon junctions, in up to 100 independent sub-sets (see Methods and Supplementary Figure S3).

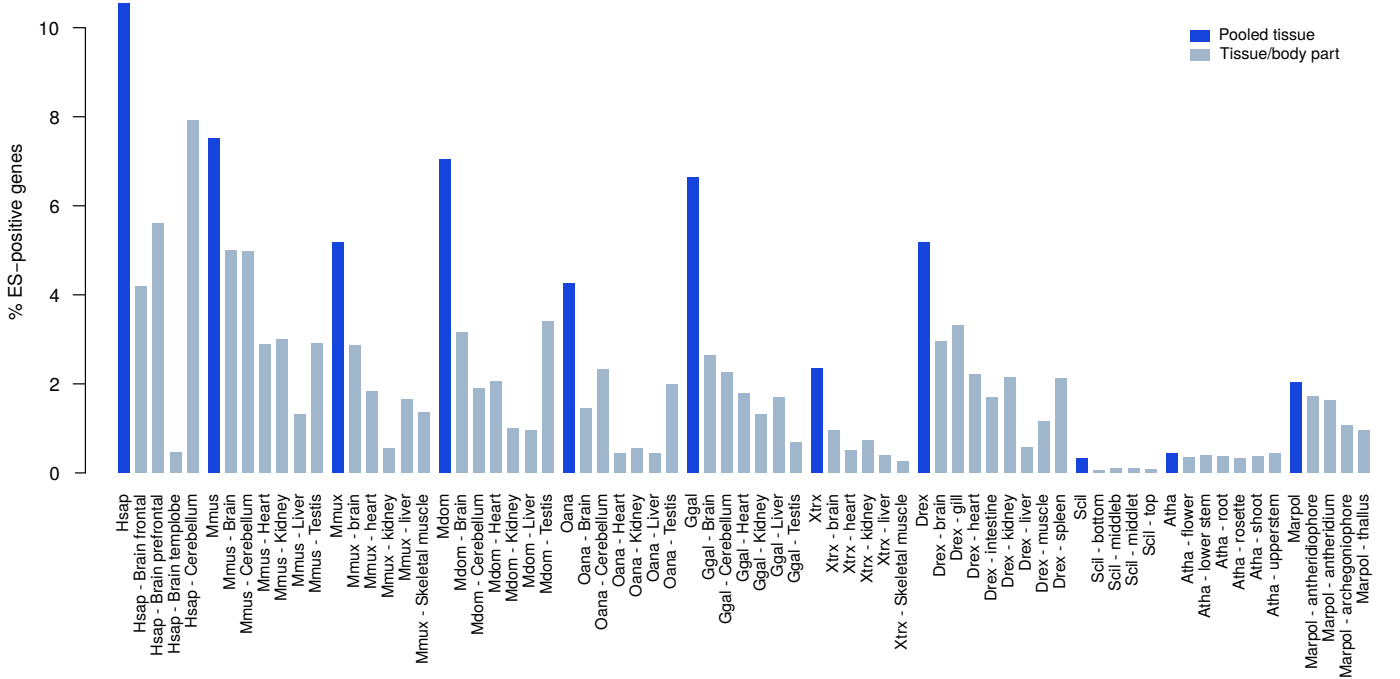


**Fig S7.** Replicate analyses of ES frequency ( $FES,sp$ ) in a selection of species, using independently-generated RNA-seq datasets (see Supplementary Figure S1 for a complete list of transcriptomic dataset sources). Replicate analyses have been performed for *H. sapiens*, *M. musculus*, *X. tropicalis*, *D. melanogaster*, *N. vectensis* (animals), *A. thaliana* (plant) and *B. natans* (chlorarachniophyte).

A) ES frequency, per species and tissue



B) ES-positive genes per species and tissue



**Fig S8.** Tissue-level ES quantification in samples from selected animals (*H. sapiens*, 2x *M. musculus*, *M. domestica*, *O. anatinus*, *G. gallus*, *X. tropicalis*, *D. rerio*, *S. ciliatum*) and plants (*A. thaliana* and *M. polymorpha*).

A) Percentage of ES-positive genes per sample, including multi-tissue pool.

B) Distribution of ES frequencies per sample, including multi-tissue pool.



**Complement to Fig2 C – Animals only**

Group i	Group j	pval	D statistic	Ni	Nj
Verts	Nonvert-bil	1.02E-03	9.61E-02	700	800
Verts	Nonbil	2.73E-114	5.92E-01	700	800
Verts	Unihol	1.56E-95	6.41E-01	700	428
Verts	Uniopi	9.10E-262	8.64E-01	700	948
Nonvert-bil	Verts	1.21E-01	5.32E-02	800	700
Nonvert-bil	Nonbil	5.54E-92	5.13E-01	800	800
Nonvert-bil	Unihol	5.53E-77	5.61E-01	800	428
Nonvert-bil	Uniopi	1.99E-227	7.76E-01	800	948
Nonbil	Verts	1.00E+00	-2.17E-17	800	700
Nonbil	Nonvert-bil	1.00E+00	0.00E+00	800	800
Nonbil	Unihol	4.64E-02	7.42E-02	800	428
Nonbil	Uniopi	6.67E-38	3.14E-01	800	948
Unihol	Verts	1.00E+00	7.37E-17	428	700
Unihol	Nonvert-bil	1.00E+00	9.54E-17	428	800
Unihol	Nonbil	2.40E-02	8.18E-02	428	800
Unihol	Uniopi	9.85E-25	3.06E-01	428	948
Uniopi	Verts	1.00E+00	8.33E-17	948	700
Uniopi	Nonvert-bil	1.00E+00	1.05E-16	948	800
Uniopi	Nonbil	1.00E+00	1.05E-16	948	800
Uniopi	Unihol	1.00E+00	9.54E-18	948	428

**Species groupings.**

Verts= "Hsap","Mmus","Mdom","Oana","Ggal","Xtro","Drex"  
 Nonvert-bil= "Cint","Brabel","Spur2","Dmel","Cele","Cgig","Ctel","Sman"  
 Nonbil= "Tadh","Hmag","Nvec","Aipt","Mlei","Aque","Ocar","Scil"  
 Unihol= "Sar3","Sros","Cowc","Cfra","Nk52"  
 Uniopi= "Ncrx","Spom","Aory","Cneo","Umay","Tmel","Rory",  
 "Amac","Spun","Rir","Ccor","Gpro","Falb"

**Hypothesis:**

Alternative hypothesis: the CDF of *i* lies below that of *j*

*Ni*= num bins in group I

*Nj*= num bins in group J

**Complement to Fig2 D – All holozoans**

Group i	Group j	pval	D statistic	Ni	Nj
Bilateria	Cnidaria	6.81E-35	3.97E-01	1500	300
Bilateria	Trichoplax	6.49E-57	8.31E-01	1500	100
Bilateria	Mnemiopsis	9.30E-18	4.57E-01	1500	100
Bilateria	Porifera	1.94E-122	7.49E-01	1500	300
Bilateria	Unicell-Holozoa	1.20E-102	5.94E-01	1500	428
Cnidaria	Bilateria	1.00E+00	-9.76E-17	300	1500
Cnidaria	Trichoplax	3.93E-27	6.37E-01	300	100
Cnidaria	Mnemiopsis	1.55E-02	1.67E-01	300	100
Cnidaria	Porifera	1.07E-28	4.63E-01	300	300
Cnidaria	Unicell-Holozoa	1.15E-16	3.23E-01	300	428
Trichoplax	Bilateria	1.00E+00	-5.42E-17	100	1500
Trichoplax	Cnidaria	1.00E+00	4.34E-17	100	300
Trichoplax	Mnemiopsis	1.00E+00	0.00E+00	100	100
Trichoplax	Porifera	1.00E+00	4.34E-17	100	300
Trichoplax	Unicell-Holozoa	1.00E+00	-9.54E-17	100	428
Mnemiopsis	Bilateria	1.00E+00	-5.42E-17	100	1500
Mnemiopsis	Cnidaria	4.80E-01	7.00E-02	100	300
Mnemiopsis	Trichoplax	4.48E-19	6.50E-01	100	100
Mnemiopsis	Porifera	2.68E-10	3.83E-01	100	300
Mnemiopsis	Unicell-Holozoa	2.06E-04	2.29E-01	100	428
Porifera	Bilateria	1.00E+00	-9.76E-17	300	1500
Porifera	Cnidaria	1.00E+00	0.00E+00	300	300
Porifera	Trichoplax	2.95E-08	3.40E-01	300	100
Porifera	Mnemiopsis	1.00E+00	-4.34E-17	300	100
Porifera	Unicell-Holozoa	1.00E+00	9.97E-04	300	428
Unicell-Holozoa	Bilateria	1.00E+00	4.12E-17	428	1500
Unicell-Holozoa	Cnidaria	1.00E+00	1.39E-16	428	300
Unicell-Holozoa	Trichoplax	2.34E-20	5.28E-01	428	100
Unicell-Holozoa	Mnemiopsis	1.00E+00	9.54E-17	428	100
Unicell-Holozoa	Porifera	1.13E-07	2.13E-01	428	300

**Species groupings.**

Bilateria= "Hsap","Mmus","Mdom","Oana","Ggal","Xtro","Drex",  
 "Cint","Brabel","Spur2","Dmel","Cele","Cgig","Ctel","Sman"  
 Cnidaria-bil= "Hmag","Nvec","Aipt"  
 Trichoplax= Tadh  
 Mnemiopsis= Mlei  
 Porifera= "Aque","Ocar","Scil"  
 Uc. Holozoa= "Sar3","Sros","Cowc","Cfra","Nk52"

**Hypothesis:**

Alternative hypothesis: the CDF of *i* lies below that of *j*

*Ni*= num bins in group I

*Nj*= num bins in group J

**Complement to Fig2 E – Unicell Holozoa**

Group i	Group j	pval	D statistic	Ni	Nj
Sros	Cowc	1.00E+00	0.00E+00	100	100
Sros	Sarc	1.00E+00	0.00E+00	100	100
Sros	Cfra	1.00E+00	0.00E+00	100	100
Sros	Cper	1.00E+00	-7.63E-17	100	28
Cowc	Sros	1.78E-26	7.70E-01	100	100
Cowc	Sarc	1.00E+00	0.00E+00	100	100
Cowc	Cfra	5.27E-01	8.00E-02	100	100
Cowc	Cper	9.68E-01	2.71E-02	100	28
Sarc	Sros	2.33E-34	8.80E-01	100	100
Sarc	Cowc	3.78E-27	7.80E-01	100	100
Sarc	Cfra	1.63E-18	6.40E-01	100	100
Sarc	Cper	4.49E-10	7.01E-01	100	28
Cfra	Sros	2.10E-21	6.90E-01	100	100
Cfra	Cowc	6.82E-04	2.70E-01	100	100
Cfra	Sarc	1.00E+00	0.00E+00	100	100
Cfra	Cper	5.49E-01	1.17E-01	100	28
Cper	Sros	6.91E-13	8.00E-01	28	100
Cper	Cowc	3.13E-02	2.81E-01	28	100
Cper	Sarc	9.71E-01	2.57E-02	28	100
Cper	Cfra	5.17E-01	1.23E-01	28	100

**Hypothesis:**

Alternative hypothesis: the CDF of *i* lies below that of *j*

*Ni*= num bins in group I

*Nj*= num bins in group J

**Complement to Fig2 F – plants**

Group i	Group j	pval	D statistic	Ni	Nj
Plants	V. carteri	9.28E-01	2.09E-02	615	100
Plants	Unicell chlorophytes	4.13E-08	2.31E-01	615	215
Plants	Unicell Bikonta	3.92E-31	3.64E-01	615	462
V. carteri	Plants	9.86E-14	4.17E-01	100	615
V. carteri	Unicell chlorophytes	8.67E-22	5.96E-01	100	215
V. carteri	Unicell Bikonta	3.91E-37	7.14E-01	100	462
Unicell chlorophytes	Plants	1.00E+00	1.30E-17	215	615
Unicell chlorophytes	V. carteri	1.00E+00	3.47E-17	215	100
Unicell chlorophytes	Unicell Bikonta	2.69E-07	2.27E-01	215	462
Unicell Bikonta	Plants	1.00E+00	5.38E-04	462	615
Unicell Bikonta	V. carteri	9.99E-01	2.16E-03	462	100
Unicell Bikonta	Unicell chlorophytes	9.99E-01	2.16E-03	462	215

**Species groupings.**

Plants= "Atha","Vvin","Mgut","Osat2","Ppat","Marpo","Smoe"  
 V. carteri-bil= Vcar  
 Unicell chlorophyta= "Crei","Mpus","Kfla"  
 Unicell bikonta= "Cpar","Pinf","Aano","Pfal","Tthe","Bnat","Ehux","Gthe","Ngru"

**Hypothesis:**

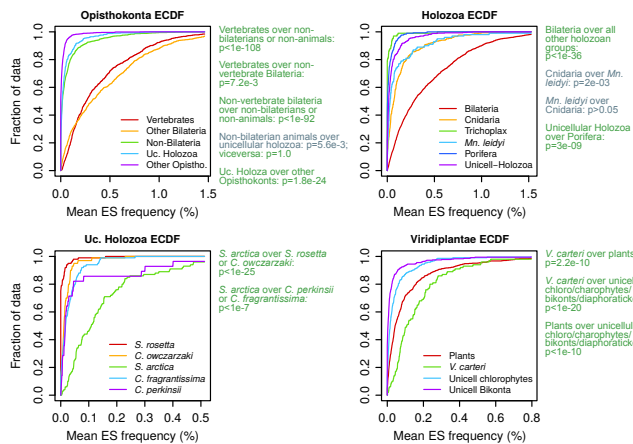
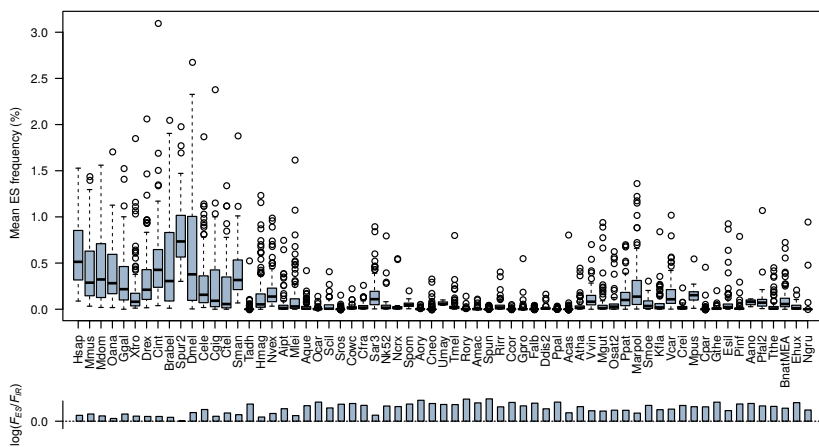
Alternative hypothesis: the CDF of *i* lies below that of *j*

*Ni*= num bins in group I

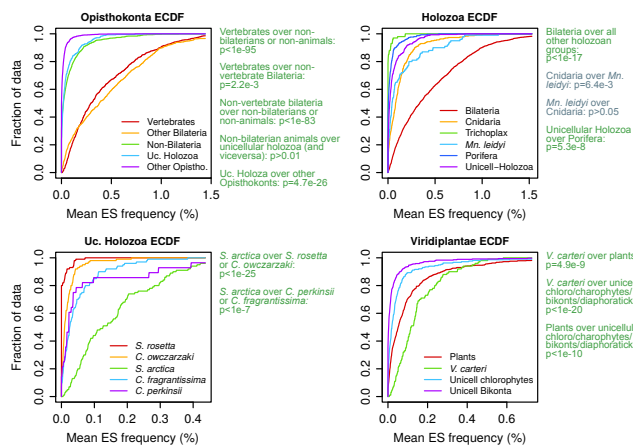
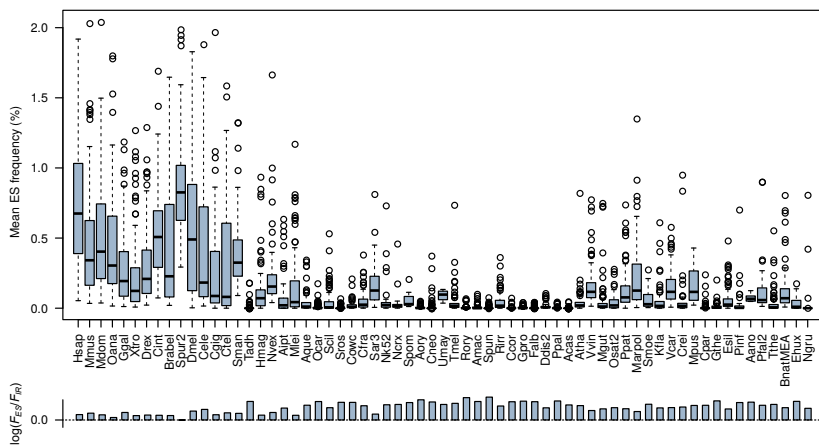
*Nj*= num bins in group J

**Fig S9.** Inter-group comparisons of ES frequencies using one-sided Kolmogorov-Smirnov 2-sample tests. If significant, ES frequencies are higher in species *i* than *j*. For each comparison, p-values, D statistic and number of observations are reported.

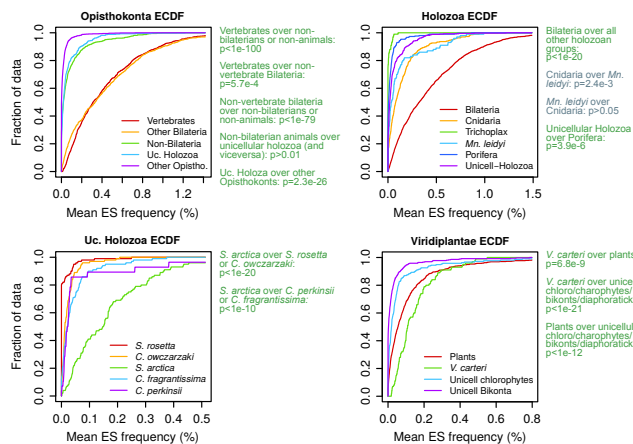
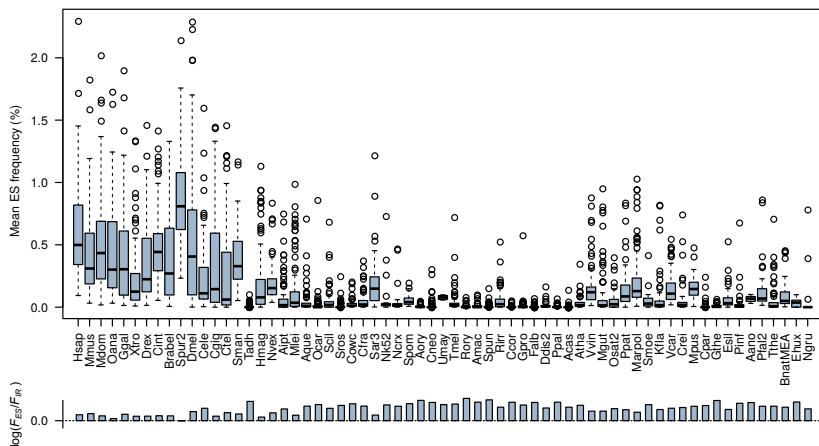
**A) ES frequencies in 65 eukaryotic transcriptomes at 2x sequencing depth**



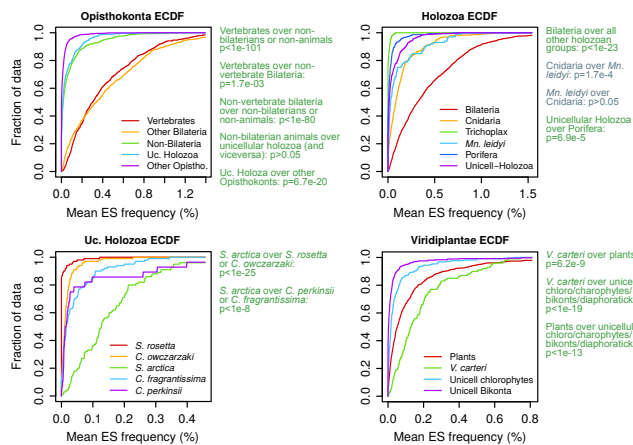
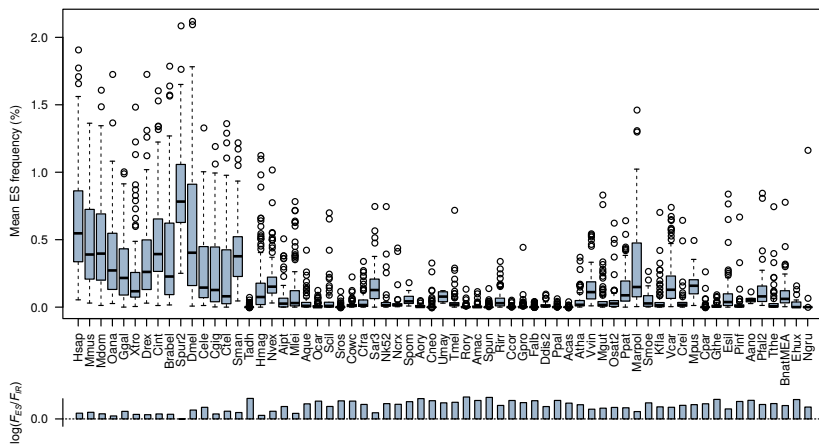
**B) ES frequencies in 65 eukaryotic transcriptomes at 5x sequencing depth**



**C) ES frequencies in 65 eukaryotic transcriptomes at 10x sequencing depth**



**D) ES frequencies in 65 eukaryotic transcriptomes at 15x sequencing depth**



**Fig S10.** Distribution of ES frequencies across 65 eukaryotic transcriptomes (as in Figure 2), downsampling each RNA-seq sample at 2x (A), 5x (B), 10x (C), and 15x (D).

For each downsampling analysis, we report 1) box-plots representing the distribution of ES frequencies ( $FES_{sp}$ ) in a given species (as %; upper left panel); 2) log-ratio of mean  $FES_{sp}$  (for ES) over mean  $FIR_{sp}$  (for IR) in each species (lower left panel); 3) comparisons of ES frequencies among phylogenetically-related eukaryotic groups (right panels) using ECDF plots representing pooled  $FES_{sp}$  values of multiple species.

Comparison plots also report whether the statistical contrasts described in Figure 2 and S7 (one-sided Kolmogorov-Smirnov 2-sample tests) remain significant (in green) or not (in grey) in each RNA-seq downsampling analysis.

spi	IR-neg 3n-neg	IR-neg 3n-pos	% IR-neg 3n-pos	IR-pos 3n-neg	IR-pos 3n-pos	% IR-pos 3n-pos	Fisher pval	Fisher statistic	Is there any bias? P<0.01	% IR positive
Hsap	26,595	13,383	0.335	3,007	1,512	0.335	9.87E-01	0.999	FALSE	10.724
Mmus	40,705	20,456	0.334	4,413	2,157	0.328	3.22E-01	0.973	FALSE	14.738
Mdom	33,246	16,758	0.335	1,982	992	0.334	8.73E-01	0.993	FALSE	8.337
Oana	24,950	12,346	0.331	296	160	0.351	3.68E-01	1.092	FALSE	1.793
Ggal	28,862	14,383	0.333	2,138	1,018	0.323	2.56E-01	0.955	FALSE	12.400
Xtro	31,706	15,853	0.333	417	219	0.344	5.54E-01	1.050	FALSE	2.896
Drex	49,843	24,869	0.333	1,399	657	0.320	2.09E-01	0.941	FALSE	6.077
Cint	8,870	4,488	0.336	273	133	0.328	7.49E-01	0.963	FALSE	2.117
Brabel	48,424	24,061	0.332	1,780	884	0.332	1.00E+00	0.999	FALSE	6.367
Spur2	17,169	8,429	0.329	202	97	0.324	9.02E-01	0.978	FALSE	0.853
Dmel	8,324	4,079	0.329	2,801	1,397	0.333	6.49E-01	1.018	FALSE	15.858
Cele	19,476	9,559	0.329	8,946	4,416	0.330	7.98E-01	1.006	FALSE	31.873
Cgig	30,939	15,354	0.332	667	326	0.328	8.38E-01	0.985	FALSE	2.848
Ctel	24,212	11,534	0.323	1,489	704	0.321	8.88E-01	0.992	FALSE	5.187
Sman	9,668	4,725	0.328	711	325	0.314	3.55E-01	0.935	FALSE	6.665
Tadh	13,090	6,415	0.329	334	166	0.332	8.85E-01	1.014	FALSE	3.490
Hmag	20,628	10,337	0.334	143	72	0.335	1.00E+00	1.005	FALSE	1.018
Nvex	22,681	11,121	0.329	979	516	0.345	1.96E-01	1.075	FALSE	4.961
Aipt	17,460	8,838	0.336	777	423	0.353	2.48E-01	1.075	FALSE	3.017
Mlei	12,689	6,309	0.332	94	41	0.304	5.22E-01	0.877	FALSE	0.735
Aque	21,605	10,695	0.331	1,933	894	0.316	1.08E-01	0.934	FALSE	4.626
Ocar	11,879	5,743	0.326	731	353	0.326	1.00E+00	0.999	FALSE	7.084
Scil	13,580	6,819	0.334	399	200	0.334	1.00E+00	0.998	FALSE	2.003
Sros	10,602	5,124	0.326	81	46	0.362	3.93E-01	1.175	FALSE	1.075
Cowc	5,347	2,576	0.325	552	252	0.313	5.27E-01	0.948	FALSE	6.773
Cfra	13,520	6,734	0.332	1,411	687	0.327	6.61E-01	0.978	FALSE	15.263
Sar3	14,413	7,120	0.331	286	129	0.311	4.29E-01	0.913	FALSE	1.817
Nk52	1,540	765	0.332	502	219	0.304	1.72E-01	0.878	FALSE	4.148
Ncrx	2,966	1,422	0.324	268	157	0.369	5.85E-02	1.222	FALSE	3.849
Spom	961	460	0.324	451	242	0.349	2.58E-01	1.121	FALSE	9.545
Aory	3,683	1,646	0.309	319	142	0.308	1.00E+00	0.996	FALSE	3.064
Cneo	3,670	1,739	0.322	314	138	0.305	4.96E-01	0.928	FALSE	5.659
Umay	368	162	0.306	168	59	0.260	2.22E-01	0.798	FALSE	2.959
Tmel	4,780	2,334	0.328	987	459	0.317	4.42E-01	0.952	FALSE	13.514
Rory	5,924	2,832	0.323	656	292	0.308	3.42E-01	0.931	FALSE	4.731
Amac	3,361	1,589	0.321	179	66	0.269	9.23E-02	0.780	FALSE	1.246
Spun	4,942	2,319	0.319	376	161	0.300	3.62E-01	0.913	FALSE	4.982
Rirr	8,040	4,081	0.337	606	302	0.333	8.27E-01	0.982	FALSE	2.398
Ccor	2,646	1,304	0.330	27	10	0.270	4.88E-01	0.752	FALSE	0.320
Gpro	12,264	5,684	0.317	328	156	0.322	8.05E-01	1.026	FALSE	2.956
Falb	3,670	1,775	0.326	44	13	0.228	1.54E-01	0.611	FALSE	0.935
Ddis2	2,795	1,439	0.340	22	10	0.313	8.52E-01	0.883	FALSE	0.242
Ppal	3,975	1,956	0.330	73	23	0.240	6.31E-02	0.640	FALSE	0.695
Acas	16,658	8,103	0.327	14	9	0.391	5.11E-01	1.322	FALSE	0.147
Atha	29,349	14,417	0.329	2,395	1,195	0.333	6.71E-01	1.016	FALSE	9.363
Vvin	20,767	10,105	0.327	1,460	763	0.343	1.23E-01	1.074	FALSE	5.109
Mgut	26,074	13,224	0.337	738	351	0.322	3.46E-01	0.938	FALSE	3.323
Osat2	10,286	5,239	0.337	262	131	0.333	9.14E-01	0.982	FALSE	1.029
Ppat	17,338	8,576	0.331	1,864	974	0.343	1.93E-01	1.056	FALSE	6.265
Marpol	18,396	9,208	0.334	1,473	730	0.331	8.51E-01	0.990	FALSE	7.186
Smoe	790	374	0.321	238	116	0.328	8.46E-01	1.030	FALSE	0.828
Kfla	19,087	9,447	0.331	801	413	0.340	5.13E-01	1.042	FALSE	5.845
Vcar	16,235	7,977	0.329	3,642	1,738	0.323	3.69E-01	0.971	FALSE	20.390
Crei	17,232	8,604	0.333	640	361	0.361	7.04E-02	1.130	FALSE	5.300
Mpus	518	258	0.332	375	157	0.295	1.64E-01	0.841	FALSE	3.712
Cpar	1,990	883	0.307	35	18	0.340	6.53E-01	1.159	FALSE	0.381
Gthe	23,553	11,107	0.320	3,038	1,457	0.324	6.22E-01	1.017	FALSE	8.346
Esil	28,134	13,844	0.330	1,052	495	0.320	4.25E-01	0.956	FALSE	7.025
Pinf	2,806	1,334	0.322	494	245	0.332	6.39E-01	1.043	FALSE	3.396
Aano	280	113	0.288	329	144	0.304	6.02E-01	1.084	FALSE	2.483
Pfal2	1,625	854	0.344	762	369	0.326	2.89E-01	0.921	FALSE	14.451
Tthe	12,277	4,788	0.281	939	370	0.283	8.73E-01	1.010	FALSE	3.919
BnatMEA	33,268	16,367	0.330	5,911	2,804	0.322	1.45E-01	0.964	FALSE	22.455
Ehux	613	303	0.331	240	125	0.342	6.94E-01	1.054	FALSE	0.680
Ngru	1,752	782	0.309	75	47	0.385	8.85E-02	1.404	FALSE	0.719

**Fig S11.** Enrichment in 3n-divisibility of intron lengths in IR events from whole transcriptomes ('all'), with Fisher's exact test.

A) Whole transcriptome

spi	ES-neg 3n-neg	ES-neg 3n-pos	% ES-neg 3n-pos	ES-pos 3n-neg	ES-pos 3n-pos	% ES-pos 3n-pos	Fisher pval	Fisher statistic	Is there any bias? P<0.01	ES-positive %
Hsap	52,747	34,658	0.397	1,182	1,377	0.538	5.83E-46	1.773	TRUE	10.538
Mmus	73,063	48,503	0.399	650	1,328	0.671	1.09E-129	3.078	TRUE	7.513
Mdom	59,150	39,096	0.398	558	1,228	0.688	2.15E-132	3.330	TRUE	7.043
Oana	38,757	26,168	0.403	352	663	0.653	4.64E-57	2.790	TRUE	4.249
Ggal	54,658	35,869	0.396	427	768	0.643	4.75E-65	2.741	TRUE	6.635
Xtro	50,021	33,800	0.403	198	454	0.696	4.65E-51	3.393	TRUE	3.226
Drex	82,685	56,060	0.404	400	1,154	0.743	1.60E-159	4.255	TRUE	5.178
Cint	16,557	10,144	0.380	386	245	0.388	6.78E-01	1.036	FALSE	3.551
Brabel	72,694	50,442	0.410	280	1,295	0.822	5.81E-245	6.666	TRUE	4.208
Spur2	23,998	16,463	0.407	1,134	741	0.395	3.24E-01	0.953	FALSE	5.343
Dmel	14,973	8,659	0.366	62	335	0.844	3.41E-84	9.340	TRUE	2.457
Cele	38,663	24,813	0.391	191	425	0.690	3.67E-50	3.467	TRUE	2.724
Cgig	47,042	32,461	0.408	161	496	0.755	5.31E-72	4.464	TRUE	2.116
Ctel	39,205	25,909	0.398	116	366	0.759	7.66E-58	4.774	TRUE	1.321
Sman	15,931	10,349	0.394	303	187	0.382	6.08E-01	0.950	FALSE	4.289
Tadh	25,836	17,523	0.404	8	12	0.600	1.08E-01	2.212	FALSE	0.174
Hmag	32,045	22,426	0.412	181	160	0.469	3.58E-02	1.263	FALSE	1.641
Nvex	31,930	21,510	0.403	166	169	0.504	1.76E-04	1.511	TRUE	1.284
Aipt	31,841	22,907	0.418	47	77	0.621	6.41E-06	2.277	TRUE	0.420
Mlei	22,173	13,495	0.378	38	127	0.770	2.81E-24	5.491	TRUE	0.953
Aque	36,229	27,446	0.431	56	47	0.456	6.20E-01	1.108	FALSE	0.252
Ocar	17,558	13,405	0.433	7	5	0.417	1.00E+00	0.936	FALSE	0.108
Scil	26,985	20,166	0.428	44	41	0.482	3.25E-01	1.247	FALSE	0.326
Sros	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.069
Cowc	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.103
Cfra	25,903	15,767	0.378	52	27	0.342	5.62E-01	0.853	FALSE	0.897
Sar3	23,723	13,956	0.370	226	91	0.287	2.33E-03	0.684	TRUE	1.823
Nk52	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.040
Ncrx	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.051
Spom	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.097
Aory	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.000
Cneo	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.086
Umay	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.000
Tmel	6,774	3,684	0.352	7	4	0.364	1.00E+00	1.051	FALSE	0.147
Rory	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.023
Amac	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.016
Spun	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.034
Rirr	13,729	8,163	0.373	38	11	0.224	3.74E-02	0.487	FALSE	0.164
Ccor	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.009
Gpro	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.029
Falb	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.000
Ddis2	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.038
Ppal	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.000
Acas	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.033
Atha	41,473	34,587	0.455	71	49	0.408	3.15E-01	0.828	FALSE	0.430
Vvin	30,632	25,667	0.456	239	112	0.319	2.13E-07	0.559	TRUE	1.129
Mgut	37,281	30,622	0.451	98	52	0.347	1.08E-02	0.646	FALSE	0.536
Osat2	20,132	16,722	0.454	38	32	0.457	1.00E+00	1.014	FALSE	0.196
Ppat	30,423	25,670	0.458	266	130	0.328	2.11E-07	0.579	TRUE	1.193
Marpol	27,268	23,453	0.462	254	169	0.400	1.08E-02	0.774	FALSE	2.027
Smoe	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.014
Kfla	26,808	21,736	0.448	42	34	0.447	1.00E+00	0.998	FALSE	0.435
Vcar	29,686	23,261	0.439	265	180	0.404	1.50E-01	0.867	FALSE	2.955
Crei	23,980	18,423	0.434	25	23	0.479	5.62E-01	1.197	FALSE	0.333
Mpus	836	713	0.460	9	4	0.308	4.03E-01	0.521	FALSE	0.131
Cpar	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.027
Gthe	35,801	27,449	0.434	8	6	0.429	1.00E+00	0.978	FALSE	0.056
Esil	35,599	36,092	0.503	124	99	0.444	8.12E-02	0.787	FALSE	1.291
Pinf	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.034
Aano	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.017
Pfal2	2,946	1,850	0.386	14	10	0.417	8.34E-01	1.137	FALSE	0.411
Tthe	16,232	11,789	0.421	14	36	0.720	2.19E-05	3.540	TRUE	0.194
BnatMEA	59,443	37,511	0.387	199	92	0.316	1.34E-02	0.733	FALSE	1.189
Ehux	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.013
Ngru	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.019

NA=less than 10 exons in any group; discarded

**Fig S12.** Enrichment in 3n-divisibility of exon lengths in ES events, measured with Fisher's exact test. Includes data from:

- (A) whole transcriptomes ('all')
- (B) a sub-set of above-median gene expression and length ('high constraint' or 'HC' dataset)
- (C) a sub-set with high ES frequency.

B) 'High constraint' gene sub-set (above median expression and gene length)

spi	ES-neg 3n-neg	ES-neg 3n-pos	% ES-neg 3n-pos	ES-pos 3n-neg	ES-pos 3n-pos	% ES-pos 3n-pos	Fisher pval	Fisher statistic	Is there any bias? P<0.01	ES-positive %
Hsap	26,873	17,662	0.397	436	750	0.632	1.53E-58	2.617	TRUE	10.538
Mmus	37,139	24,095	0.393	194	703	0.784	9.11E-124	5.585	TRUE	7.513
Mdom	29,868	19,763	0.398	184	628	0.773	2.47E-103	5.158	TRUE	7.043
Oana	19,701	13,174	0.401	132	289	0.686	9.50E-32	3.274	TRUE	4.249
Ggal	27,936	17,920	0.391	165	419	0.717	1.29E-56	3.959	TRUE	6.635
Xtro	25,321	17,075	0.403	58	206	0.780	1.97E-35	5.267	TRUE	3.226
Drex	42,245	27,523	0.394	135	525	0.795	2.30E-97	5.969	TRUE	5.178
Cint	8,696	5,366	0.382	90	71	0.441	1.42E-01	1.278	FALSE	3.551
Brabel	36,632	25,511	0.411	74	776	0.913	2.67E-207	15.057	TRUE	4.208
Spur2	12,338	8,526	0.409	367	276	0.429	3.09E-01	1.088	FALSE	5.343
Dmel	7,456	4,384	0.370	19	204	0.915	3.29E-64	18.257	TRUE	2.457
Cele	19,463	12,570	0.392	62	257	0.806	5.83E-51	6.418	TRUE	2.724
Cgig	23,774	16,250	0.406	64	337	0.840	6.10E-71	7.702	TRUE	2.116
Ctel	19,669	12,992	0.398	61	242	0.799	1.36E-45	6.006	TRUE	1.321
Sman	8,155	5,317	0.395	126	97	0.435	2.40E-01	1.181	FALSE	4.289
Tadh	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.174
Hmag	16,787	11,831	0.413	65	75	0.536	4.37E-03	1.637	TRUE	1.641
Nvex	15,828	11,095	0.412	30	62	0.674	6.03E-07	2.948	TRUE	1.284
Aipt	15,824	11,908	0.429	14	39	0.736	9.14E-06	3.702	TRUE	0.420
Mlei	11,223	6,857	0.379	19	75	0.798	1.93E-16	6.460	TRUE	0.953
Aque	19,012	13,773	0.420	14	10	0.417	1.00E+00	0.986	FALSE	0.252
Ocar	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.108
Scil	13,402	10,479	0.439	28	26	0.481	5.84E-01	1.188	FALSE	0.326
Sros	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.069
Cowc	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.103
Cfra	12,952	8,272	0.390	5	9	0.643	5.94E-02	2.818	FALSE	0.897
Sar3	11,882	7,145	0.376	62	27	0.303	1.88E-01	0.724	FALSE	1.823
Nk52	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.040
Ncix	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.051
Spom	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.097
Aory	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.000
Cneo	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.086
Umay	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.000
Tmel	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.147
Rory	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.023
Amac	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.016
Spun	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.034
Rirr	7,008	4,069	0.367	10	6	0.375	1.00E+00	1.033	FALSE	0.164
Ccor	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.009
Gpro	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.029
Falb	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.000
Ddis2	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.038
Ppal	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.000
Acas	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.033
Atha	20,328	17,810	0.467	16	13	0.448	8.55E-01	0.927	FALSE	0.430
Vvin	15,286	13,034	0.460	69	35	0.337	1.34E-02	0.595	FALSE	1.129
Mgut	18,476	15,699	0.459	18	21	0.538	3.39E-01	1.373	FALSE	0.536
Osat2	10,156	8,556	0.457	8	11	0.579	3.58E-01	1.632	FALSE	0.196
Ppat	15,578	12,986	0.455	121	66	0.353	6.26E-03	0.654	TRUE	1.193
Marpol	13,750	11,719	0.460	66	90	0.577	3.69E-03	1.600	TRUE	2.027
Smoe	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.014
Kfla	13,347	10,987	0.452	13	20	0.606	8.16E-02	1.869	FALSE	0.435
Vcar	14,841	11,731	0.441	124	91	0.423	6.30E-01	0.928	FALSE	2.955
Crei	12,044	9,337	0.437	5	11	0.688	7.41E-02	2.838	FALSE	0.333
Mpus	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.131
Cpar	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.027
Gthe	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.056
Esil	17,890	18,220	0.505	28	25	0.472	6.81E-01	0.877	FALSE	1.291
Pinf	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.034
Aano	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.017
Pfal2	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.411
Tthe	8,446	5,740	0.405	5	7	0.583	2.45E-01	2.060	FALSE	0.194
BnatMEA	29,557	19,009	0.391	122	44	0.265	7.78E-04	0.561	TRUE	1.189
Ehux	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.013
Ngru	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.019

NA=less than 10 exons in any group; discarded

**Fig S12 (continued).** Enrichment in 3n-divisibility of exon lengths in ES events, measured with Fisher's exact test. Includes data from:

(A) whole transcriptomes ('all')

(B) a sub-set of above-median gene expression and length ('high constraint' or 'HC' dataset)

(C) a sub-set with high ES frequency.

C) High ES frequency (rES = 30-70%)

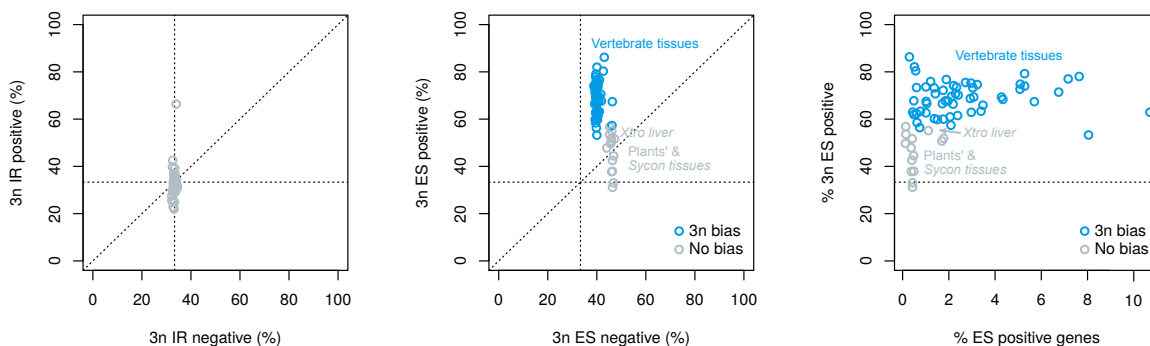
spi	ES-neg 3n-neg	ES-neg 3n-pos	% ES-neg 3n-pos	ES-pos 3n-neg	ES-pos 3n-pos	% ES-pos 3n-pos	Fisher pval	Fisher statistic	Is there any bias? P<0.01	ES-positive %
Hsap	54,914	36,247	0.398	271	500	0.649	2.57E-44	2.795	TRUE	10.538
Mmus	74,149	49,507	0.400	123	531	0.812	1.66E-102	6.467	TRUE	7.513
Mdom	60,525	40,320	0.400	125	497	0.799	2.04E-91	5.968	TRUE	7.043
Oana	39,746	27,059	0.405	92	244	0.726	2.08E-32	3.896	TRUE	4.249
Ggal	56,017	36,834	0.397	91	340	0.789	1.36E-61	5.682	TRUE	6.635
Xtro	50,891	34,469	0.404	44	163	0.787	4.71E-29	5.469	TRUE	3.226
Drex	83,443	56,945	0.406	90	492	0.845	9.05E-106	8.009	TRUE	5.178
Cint	17,822	10,891	0.379	142	91	0.391	7.35E-01	1.049	FALSE	3.551
Brabel	73,944	51,779	0.412	61	545	0.899	1.98E-139	12.759	TRUE	4.208
Spur2	25,658	17,523	0.406	182	173	0.487	1.97E-03	1.392	TRUE	5.343
Dmel	15,175	8,875	0.369	21	126	0.857	4.44E-34	10.257	TRUE	2.457
Cele	39,278	25,282	0.392	46	153	0.769	5.48E-27	5.167	TRUE	2.724
Cgig	47,913	33,256	0.410	32	189	0.855	2.44E-42	8.508	TRUE	2.116
Ctel	39,746	26,339	0.399	27	172	0.864	6.02E-42	9.612	TRUE	1.321
Sman	16,627	10,820	0.394	65	44	0.404	8.45E-01	1.040	FALSE	4.289
Tadh	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.174
Hmag	34,551	24,141	0.411	61	53	0.465	2.54E-01	1.244	FALSE	1.641
Nvex	32,468	21,892	0.403	25	37	0.597	2.61E-03	2.195	TRUE	1.284
Aipt	32,474	23,339	0.418	5	32	0.865	2.70E-08	8.904	TRUE	0.420
Mlei	22,738	13,899	0.379	15	60	0.800	1.35E-13	6.543	TRUE	0.953
Aque	37,387	28,475	0.432	7	19	0.731	2.56E-03	3.564	TRUE	0.252
Ocar	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.108
Scil	27,546	20,598	0.428	8	16	0.667	2.24E-02	2.675	FALSE	0.326
Sros	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.069
Cowc	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.103
Cfra	26,557	16,135	0.378	11	10	0.476	3.74E-01	1.496	FALSE	0.897
Sar3	24,111	14,136	0.370	33	21	0.389	7.79E-01	1.085	FALSE	1.823
Nk52	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.040
Ncix	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.051
Spom	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.097
Aory	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.000
Cneo	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.086
Umay	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.000
Tmel	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.147
Rory	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.023
Amac	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.016
Spun	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.034
Rirr	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.164
Ccor	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.009
Gpro	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.029
Falb	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.000
Ddis2	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.038
Ppal	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.000
Acas	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.033
Atha	41,711	34,760	0.455	12	14	0.538	4.34E-01	1.400	FALSE	0.430
Vvin	30,997	25,861	0.455	40	24	0.375	2.11E-01	0.719	FALSE	1.129
Mgut	37,698	30,905	0.450	26	18	0.409	6.51E-01	0.844	FALSE	0.536
Osat2	20,512	17,011	0.453	9	4	0.308	4.06E-01	0.536	FALSE	0.196
Ppat	31,325	26,355	0.457	66	26	0.283	7.41E-04	0.468	TRUE	1.193
Marpol	27,565	23,600	0.461	57	68	0.544	7.22E-02	1.393	FALSE	2.027
Smoe	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.014
Kfla	27,106	21,987	0.448	11	14	0.560	3.16E-01	1.569	FALSE	0.435
Vcar	30,591	23,880	0.438	41	38	0.481	4.96E-01	1.187	FALSE	2.955
Crei	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.333
Mpus	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.131
Cpar	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.027
Gthe	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.056
Esil	36,113	36,546	0.503	25	26	0.510	1.00E+00	1.028	FALSE	1.291
Pinf	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.034
Aano	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.017
Pfal2	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.411
Tthe	16,539	12,056	0.422	3	12	0.800	3.57E-03	5.488	TRUE	0.194
BnatMEA	60,592	38,185	0.387	41	29	0.414	6.26E-01	1.122	FALSE	1.189
Ehux	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.013
Ngru	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.019

NA=less than 10 exons in any group; discarded

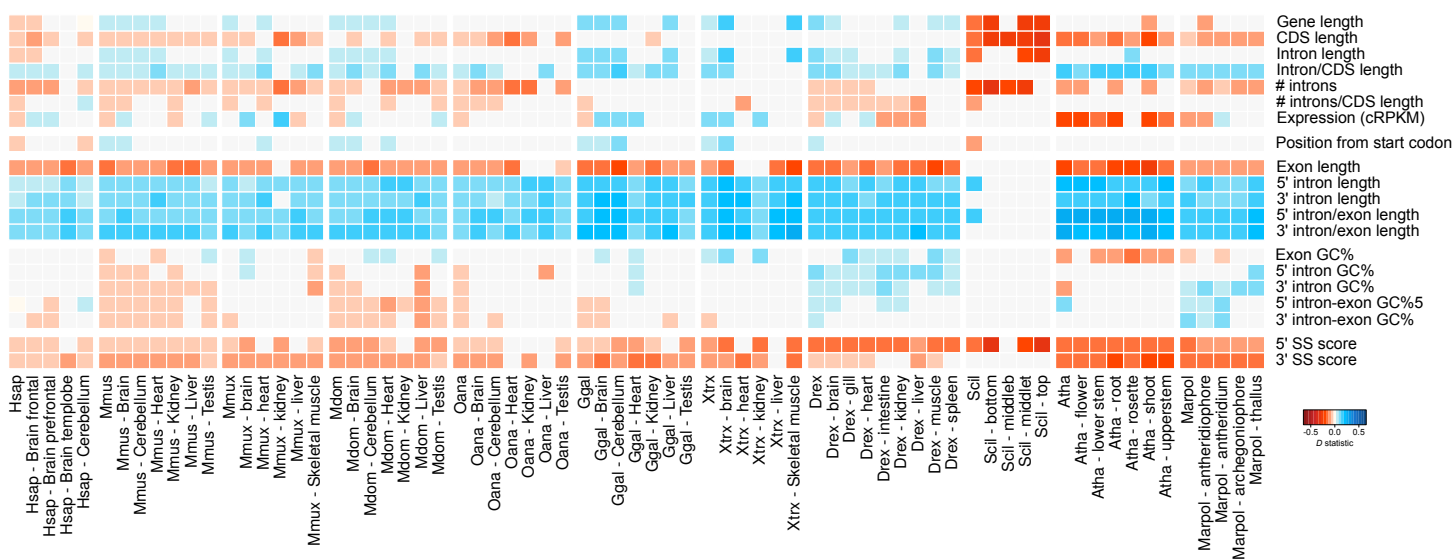
**Fig S12 (continued).** Enrichment in 3n-divisibility of exon lengths in ES events, measured with Fisher's exact test. Includes data from:

- (A) whole transcriptomes ('all')
- (B) a sub-set of above-median gene expression and length ('high constraint' or 'HC' dataset)
- (C) a sub-set with high ES frequency.

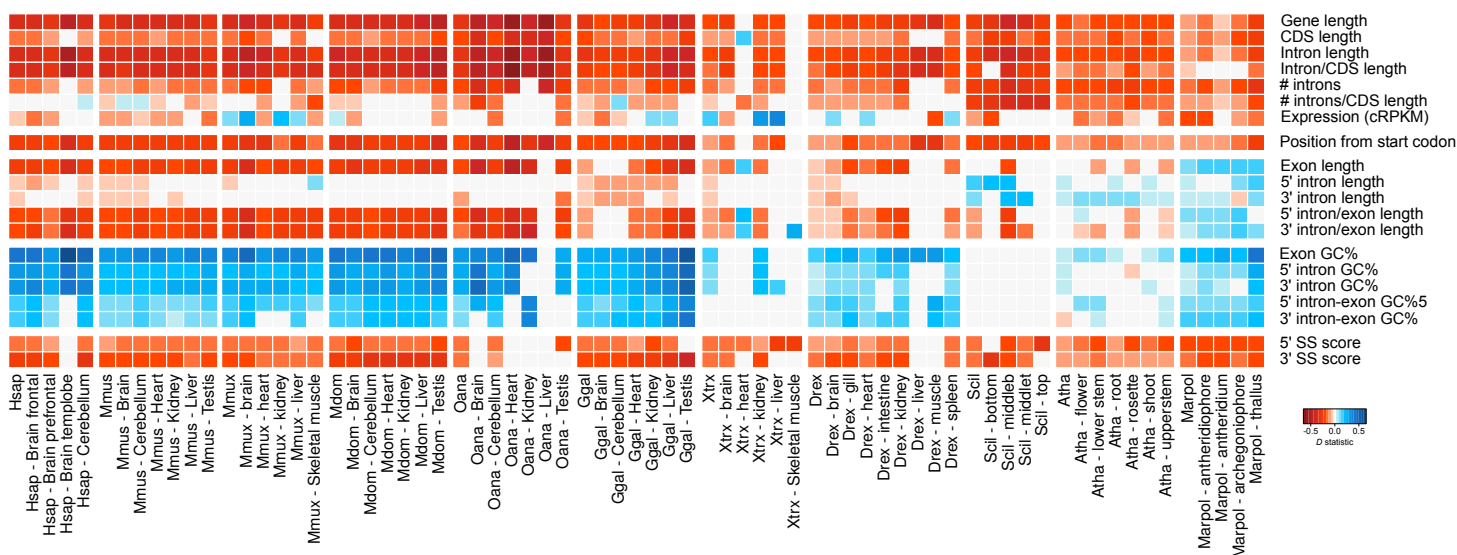
A) Fraction of 3n introns with IR, exons with ES, and exons with ES vs. % ES-positive genes, for selected animal and plant tissues



B) Correlation of ES events and gene architecture, including pooled transcriptomes and tissue-level transcriptomes for selected animals and plants



C) Correlation of IR events and gene architecture, including pooled transcriptomes and tissue-level transcriptomes for selected animals and plants



**Fig S13.** Tissue-level analysis of gene architectural effects on AS, using samples from selected animals (*H. sapiens*, 2x *M. musculus*, *M. domestica*, *O. anatinus*, *G. gallus*, *X. tropicalis*, *D. rerio*, *S. ciliatum*) and plants (*A. thaliana* and *M. polymorpha*).

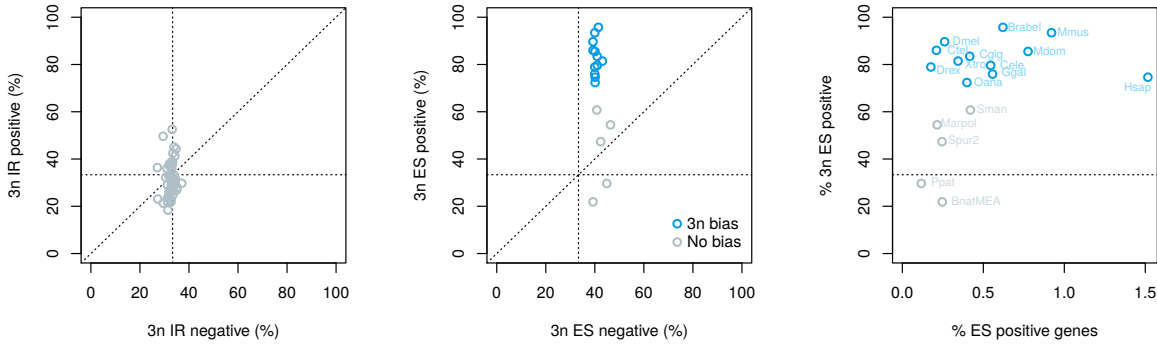
A) Fraction of 3-divisible introns and exons involved in AS events (as in Figure 3).

B) Relationship between ES and gene architectural traits, as in Figure 4.

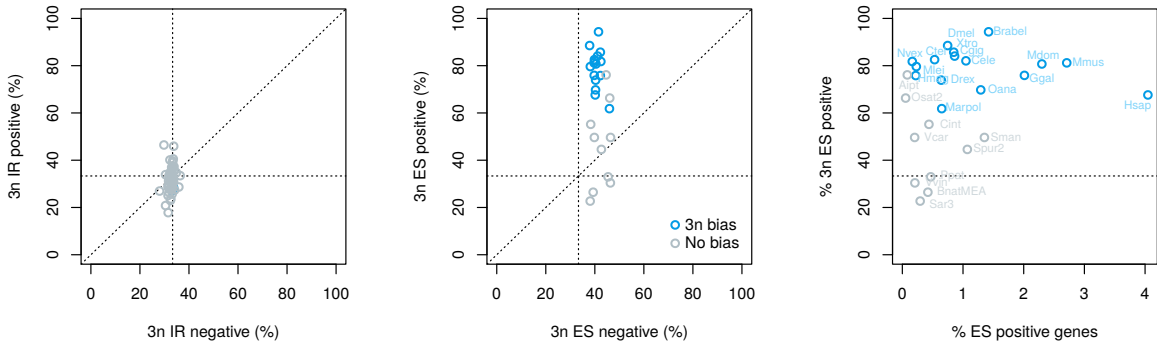
C) Relationship between IR and gene architectural traits, as in Figure 5.

Each heatmap represents the distance between distributions of gene architecture values for ES- or IR-positive and -negative exons or introns, measured with the D statistic of the one-sided Kolmogorov-Smirnov two-sample test (significant if  $p < 0.01$ ; otherwise grey). D values are recorded as positive/negative (blue/red) according to two one-sided Kolmogorov-Smirnov tests with complementary alternative hypotheses: positive/negative D (blue/red) reflects a positive/negative relationship between ES or IR and the given feature.

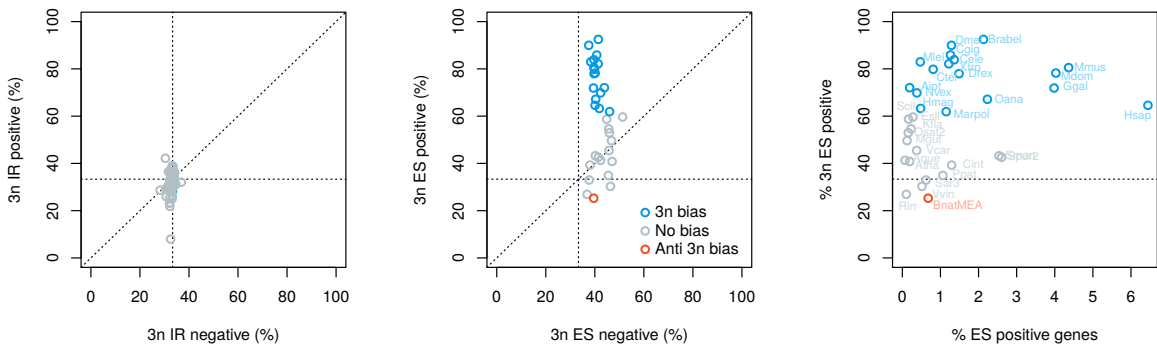
**A)** Fraction of 3n introns with IR, exons with ES, and exons with ES vs. % ES-positive genes, transcriptome at 2x sequencing depth



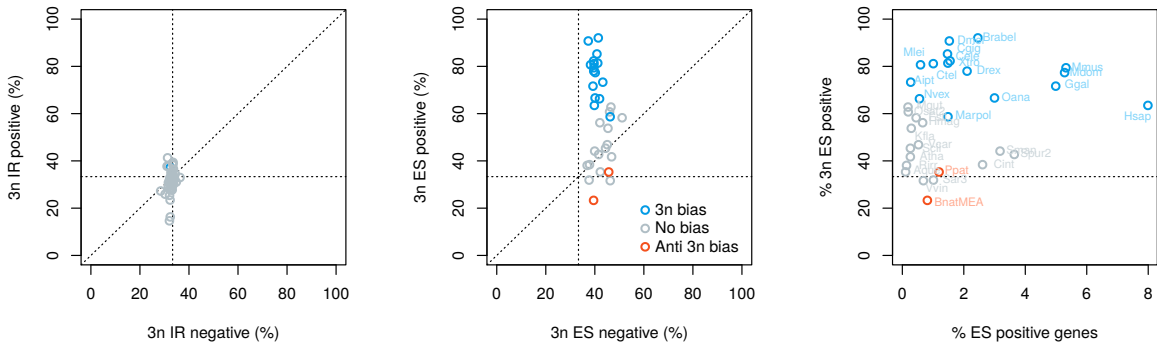
**C)** Fraction of 3n introns with IR, exons with ES, and exons with ES vs. % ES-positive genes, transcriptome at 5x sequencing depth



**C)** Fraction of 3n introns with IR, exons with ES, and exons with ES vs. % ES-positive genes, transcriptome at 10x sequencing depth



**D)** Fraction of 3n introns with IR, exons with ES, and exons with ES vs. % ES-positive genes, transcriptome at 15x sequencing depth



**Fig S14.** Fraction of 3-divisible introns and exons involved in AS events (as in Figure 3), downsampling each RNA-seq sample at 2x (A), 5x (B), 10x (C), and 15x (D).

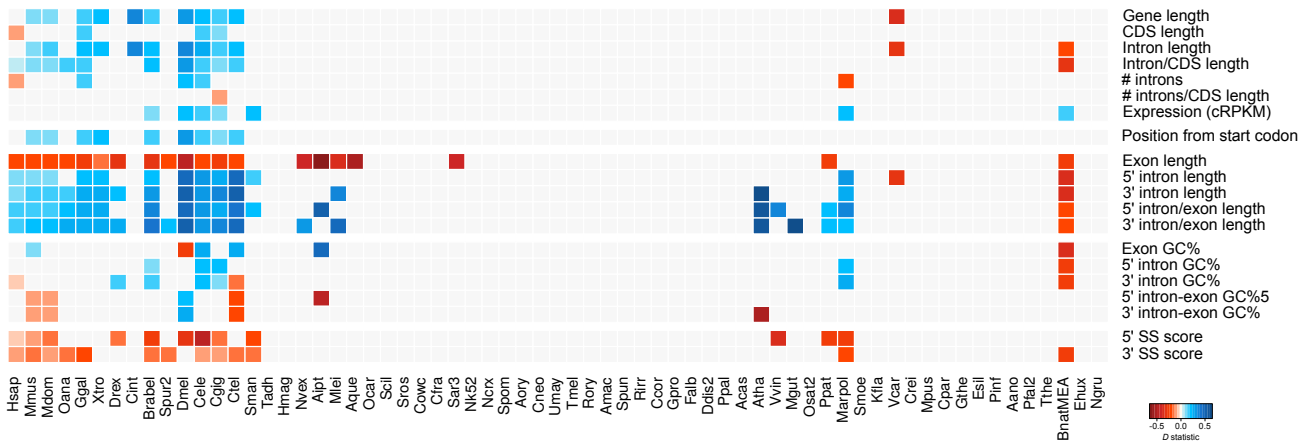
In each case, we report 1) % of introns with 3-divisible lengths that were classified as IR-positive (x-axis) and IR-negative (y-axis), 2) % exons with 3-divisible lengths that were classified as ES-negative (x-axis) and ES-negative (y-axis) in each species with >10 ES-positive exons; 3) ES-positive exons that are 3n (y-axis) with respect to the percentage of genes with ES-positive exons (x-axis), per species.



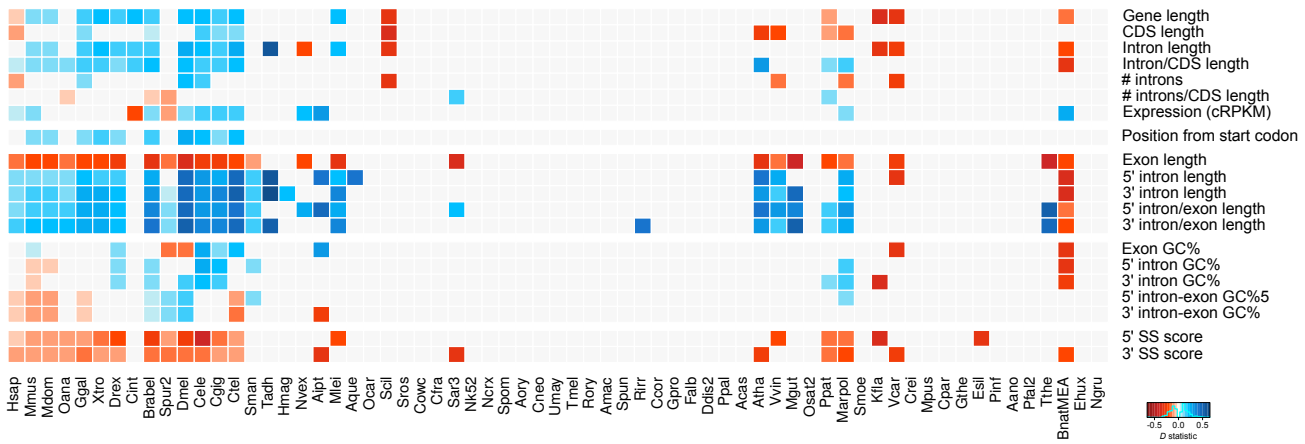




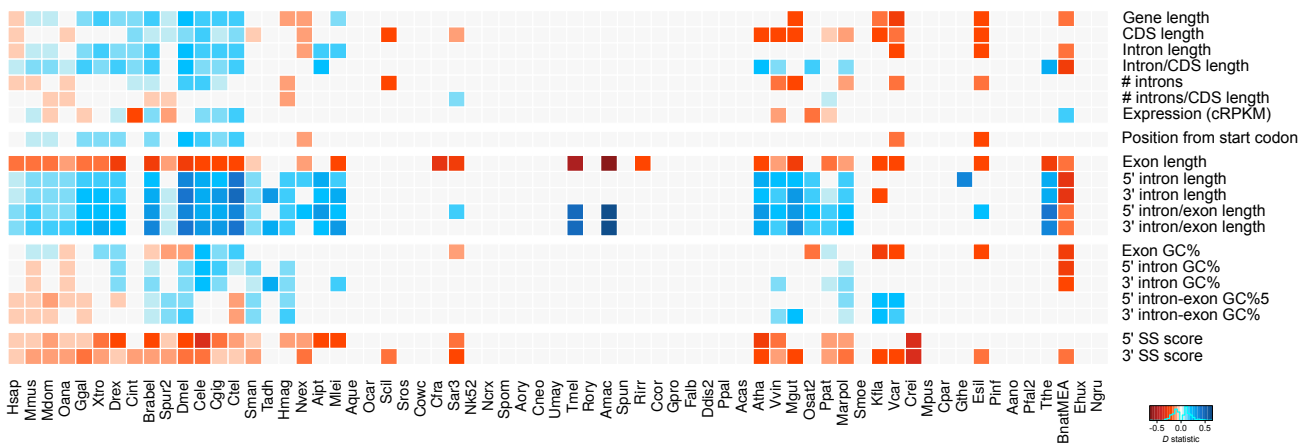
A) Correlation of ES events and gene architecture, transcriptome at 2x sequencing depth



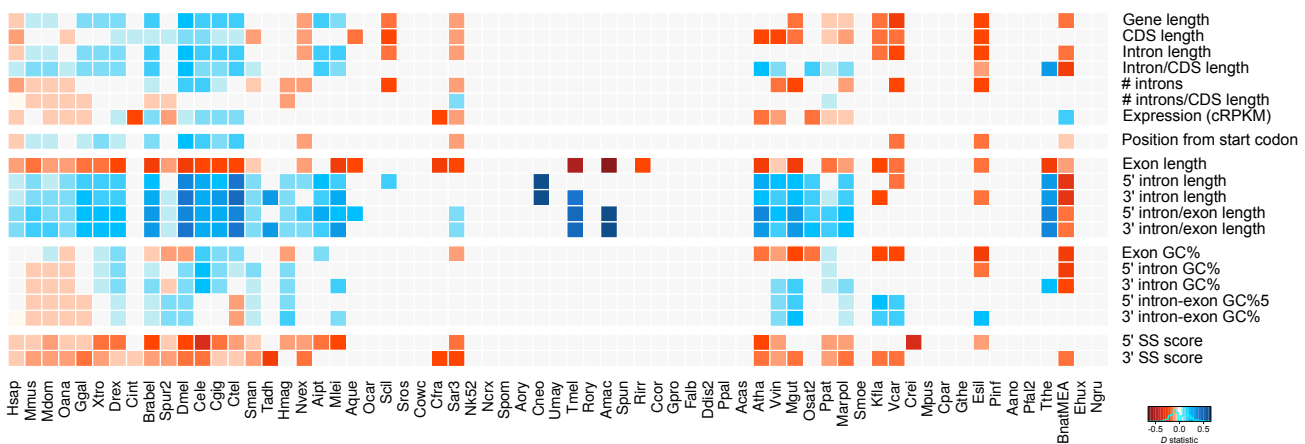
C) Correlation of ES events and gene architecture, transcriptome at 5x sequencing depth



C) Correlation of ES events and gene architecture, transcriptome at 10x sequencing depth



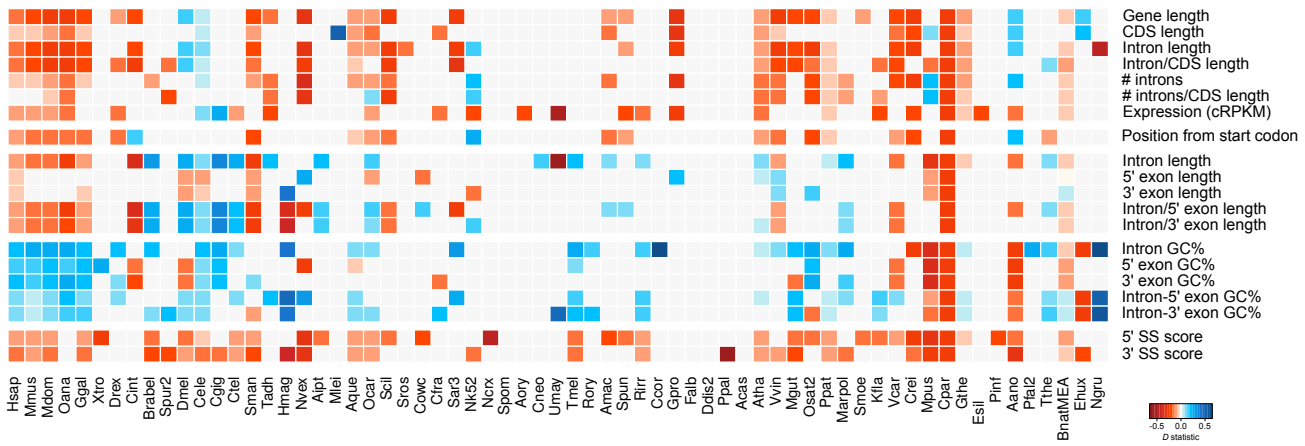
D) Correlation of ES events and gene architecture, transcriptome at 15x sequencing depth



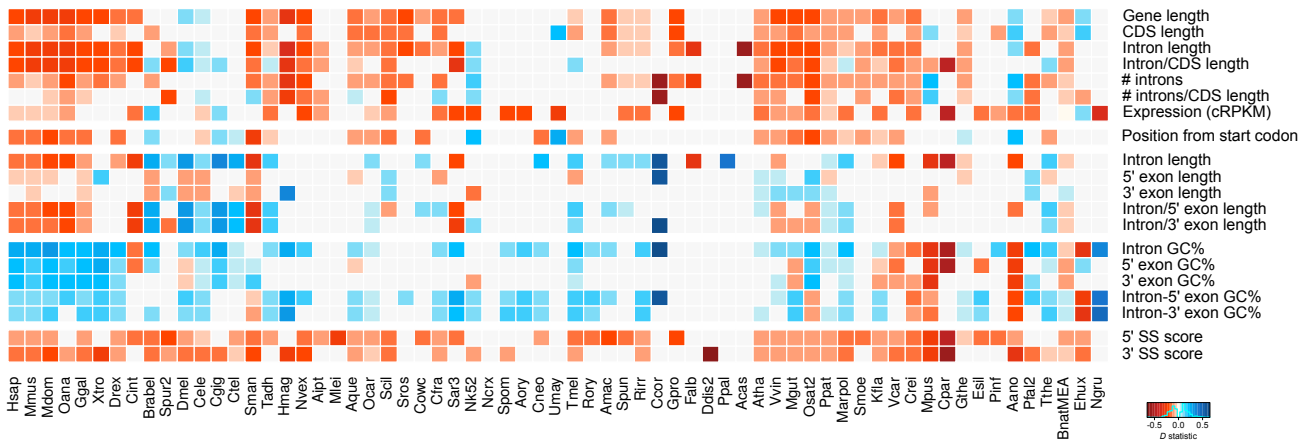
**Fig S17.** Relationship between gene structure and sequence composition and ES (as in Figure 4), downsampling each RNA-seq sample at 2x (A), 5x (B), 10x (C), and 15x (D).

Each heatmap represents the distance between distributions of gene architecture values for ES-positive and ES-negative exons, measured with the D statistic of the one-sided Kolmogorov-Smirnov two-sample test (significant if  $p < 0.01$ ; otherwise grey). D values are recorded as positive/negative (blue/red) according to two one-sided Kolmogorov-Smirnov tests with complementary alternative hypotheses: positive/negative D (blue/red) reflects a positive/negative relationship between ES and the given feature.

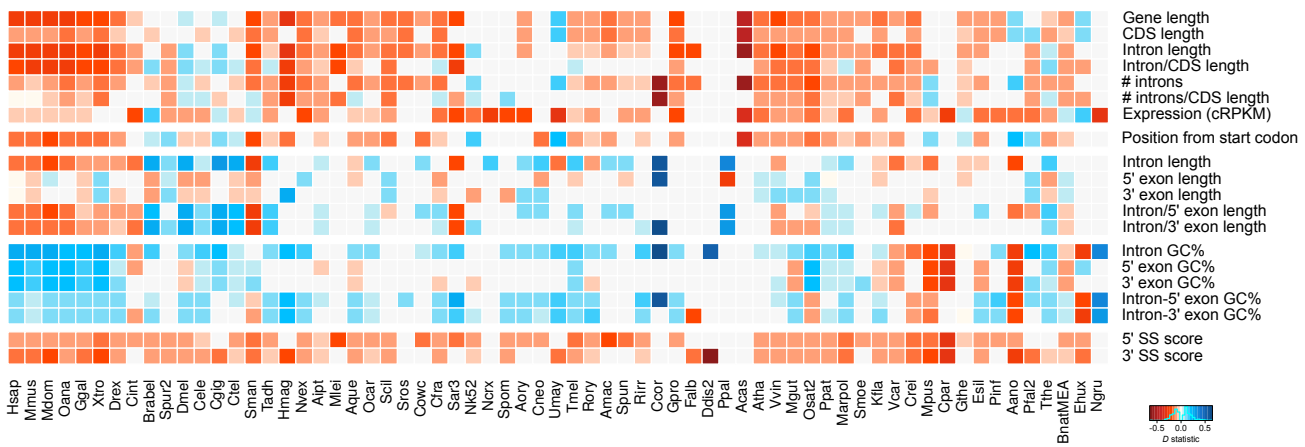
A) Correlation of IR events and gene architecture, transcriptome at 2x sequencing depth



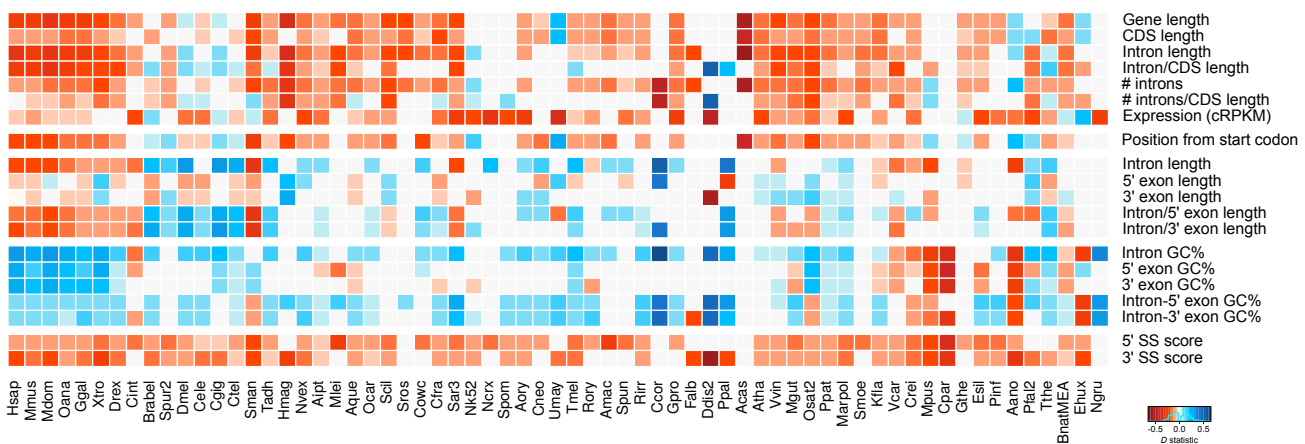
C) Correlation of IR events and gene architecture, transcriptome at 5x sequencing depth



C) Correlation of IR events and gene architecture, transcriptome at 10x sequencing depth

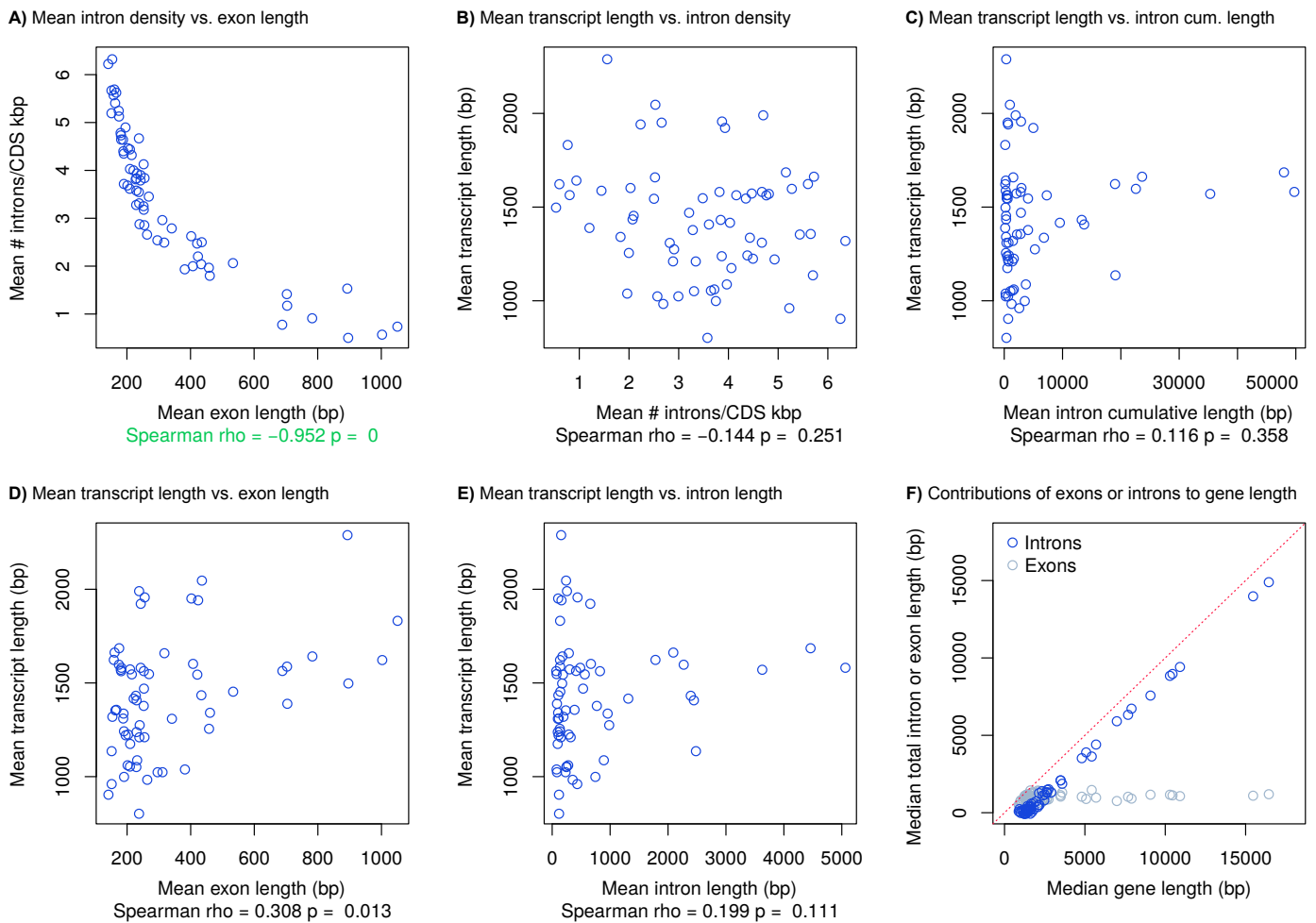


D) Correlation of IR events and gene architecture, transcriptome at 15x sequencing depth



**Fig S18.** Relationship between gene structure and sequence composition and IR (as in Figure 5), downsampling each RNA-seq sample at 2x (A), 5x (B), 10x (C), and 15x (D).

Each heatmap represents the distance between distributions of gene architecture values for IR-positive and IR-negative introns, measured with the D statistic of the one-sided Kolmogorov-Smirnov two-sample test (significant if  $p < 0.01$ ; otherwise grey). D values are recorded as positive/negative (blue/red) according to two one-sided Kolmogorov-Smirnov tests with complementary alternative hypotheses: positive/negative D (blue/red) reflects a positive/negative relationship between IR and the given feature.



**Fig S19.** Signatures of intron gain processes in the genome architecture of 65 eukaryotic species.

- A) Correlation of mean exon length (bp) and mean intron density (measured as introns/CDS kbp).
- B) Correlation of mean intron density (measured as introns/CDS kbp) and mean transcript length (bp).
- C) Correlation of mean intron cumulative length (all introns within gene, in bp) and mean transcript length (bp).
- D) Correlation of mean exon length (bp) and mean transcript length (bp).
- E) Correlation of mean intron length (bp) and mean transcript length (bp).
- F) Contribution of cumulative exon and intron lengths to gene length (using median values).

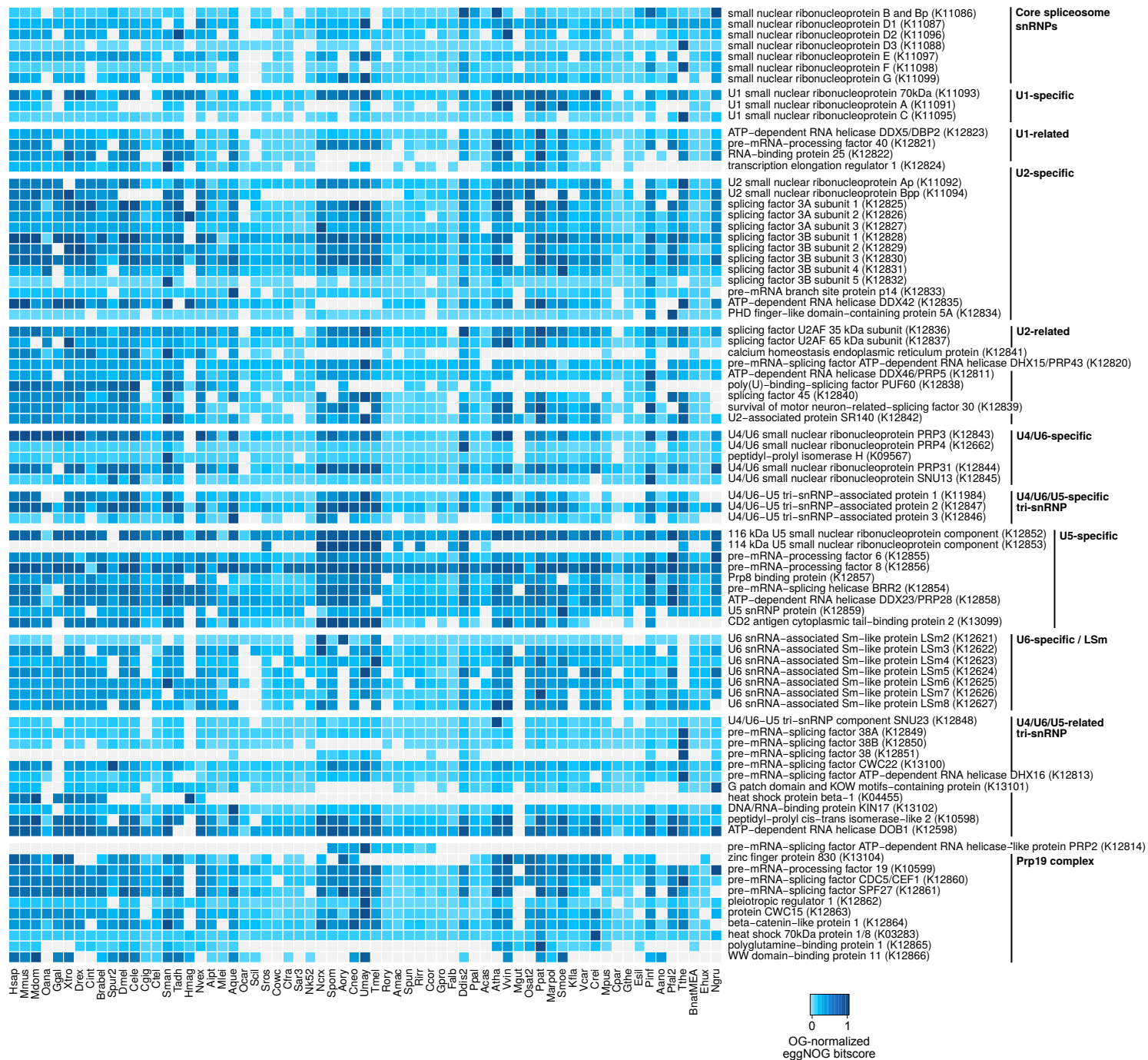
For plots A-E, rho parameter and p-value of Spearman's rank correlation test are indicated. Note that all correlations are non-significant (at the  $p < 0.01$  level) except for exon length and intron density (panel A). This plot indicates that transcript size remains relatively constant across eukaryotes and does not increase in organisms that have undergone more intron gains (panel B). On the other hand, as new introns are inserted in transcripts of quasi-constant mean length, exons become distinctly shorter (panel A).

species	Architectural feature	Spearman correlation pval	Rho statistic
Hsap	crpkm	5.36E-84	-0.2413
Hsax	crpkm	2.53E-188	-0.1878
Mmus	crpkm	9.28E-32	-0.1138
Mmux	crpkm	2.60E-36	-0.2209
Mdom	crpkm	3.63E-30	-0.1670
Oana	crpkm	4.69E-02	-0.0755
Ggal	crpkm	1.81E-55	-0.2257
Xtro	crpkm	8.07E-01	0.0074
Xtrx	crpkm	2.09E-25	-0.2413
Drex	crpkm	6.13E-01	0.0088
Drer	crpkm	1.83E-08	-0.1736
Cint	crpkm	2.09E-12	-0.3077
Bflo	crpkm	4.36E-03	-0.1309
Spur2	crpkm	4.56E-07	-0.2382
Dmel	crpkm	2.43E-85	-0.2300
Dmel2	crpkm	2.58E-03	-0.0878
Cele	crpkm	0.00E+00	-0.2796
Cgig	crpkm	9.62E-02	0.0432
Ctel	crpkm	1.15E-03	-0.0544
Sman	crpkm	9.18E-22	-0.2332
Tadh	crpkm	2.52E-29	-0.3873
Hmag	crpkm	3.67E-01	-0.0547
Nvec	crpkm	3.24E-131	-0.4115
Nvex	crpkm	7.43E-05	0.0812
Aipt	crpkm	4.56E-67	-0.3963
Mlei	crpkm	8.11E-01	-0.0181
Aque	crpkm	1.11E-86	-0.2950
Ocar	crpkm	5.26E-18	-0.2139
Scil	crpkm	3.44E-37	-0.4087
Sros	crpkm	6.27E-06	-0.3244
Cowc	crpkm	4.51E-02	-0.0562
Cfra	crpkm	2.06E-60	-0.2939
Sar3	crpkm	1.09E-20	-0.3590
Nk52	crpkm	6.20E-21	-0.2852
Ncrx	crpkm	3.99E-06	-0.1911
Spom	crpkm	3.34E-12	-0.2235
Aory	crpkm	3.12E-28	-0.4089
Cneo	crpkm	2.07E-16	-0.3309
Umay	crpkm	1.99E-11	-0.3438
Tmel	crpkm	4.77E-02	-0.0396
Rory	crpkm	7.76E-17	-0.2076
Amac	crpkm	2.39E-05	-0.2320
Spun	crpkm	1.71E-33	-0.4273
Rirr	crpkm	1.44E-41	-0.3523
Rirr2	crpkm	1.92E-05	-0.5855
Ccor	crpkm	9.48E-01	-0.0096
Gpro	crpkm	6.01E-15	-0.2849
Falb	crpkm	1.14E-03	-0.3323
Ddis2	crpkm	1.12E-01	-0.2519
Ppal	crpkm	3.57E-02	-0.1777
Acas	crpkm	8.00E-02	-0.2838
Atha	crpkm	7.51E-20	0.1186
Atha3	crpkm	8.25E-08	-0.1607
Vvin	crpkm	1.80E-52	-0.2406
Mgut	crpkm	4.90E-07	-0.1147
Osat2	crpkm	2.43E-19	-0.3509
Ppat	crpkm	1.38E-134	-0.3727
Smoe	crpkm	4.58E-01	-0.0379
Kfla	crpkm	3.37E-05	-0.0951
Vcar	crpkm	1.60E-01	-0.0159
Crei	crpkm	7.25E-13	-0.1689
Mpus	crpkm	1.25E-04	-0.1552
Cpar	crpkm	5.05E-03	-0.3014
Gthe	crpkm	6.34E-27	-0.1286
GtheME	crpkm	7.74E-55	-0.3847
Esil	crpkm	3.70E-20	-0.1812
Pinf	crpkm	7.40E-17	-0.2557
Aano	crpkm	2.55E-10	-0.2615
Pfal2	crpkm	3.90E-04	-0.0925
Tthe	crpkm	2.41E-57	-0.3247
BnatMEA	crpkm	2.89E-14	-0.0652
Bnat	crpkm	8.10E-103	-0.3806
Bnao	crpkm	4.37E-67	-0.2128
Ehux	crpkm	5.14E-01	0.0336
Ngru	crpkm	1.15E-06	-0.3426
Marpol	crpkm	1.96E-19	-0.1559
Brabel	crpkm	1.51E-01	0.0230

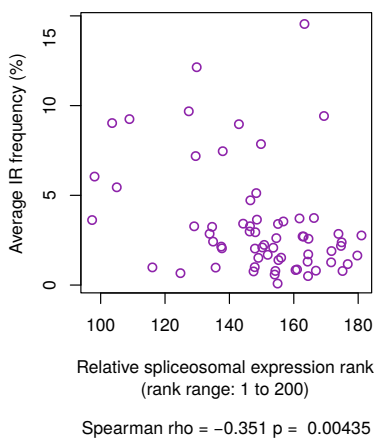
Spearman correlation between IR levels in IR-positive introns and crpkm  
Negative rho=negative association  
Significant if p<0.01

**Fig S20.** Correlation analysis of IR and expression rates among IR-positive events, with Spearman correlation test.

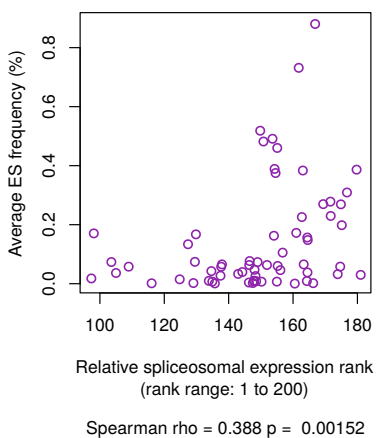
A) Conservation of the core spliceosomal toolkit across eukaryotes



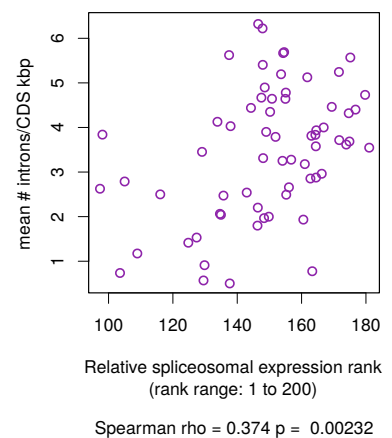
B) IR frequency and spliceosome relative expression



C) ES frequency and spliceosome relative expression



D) Intron density and spliceosome relative expression



**Fig S21.** Evolutionary conservation of the core spliceosome.

A) Heatmap representing presence/absence of core spliceosomal genes across eukaryotes, as identified by eggNOG mapper and KEGG orthologous groups (KEGG codes in brackets). Blue shadings indicate ortholog presence, and a color-scale has been added to indicate the OG-normalized bitscore value as obtained in eggNOG.

B) Correlation between average IR frequencies (as in Supplementary Figure S6) and the relative rank of expression of the spliceosomal genes, per species.

C) Correlation between average ES frequencies (as in Figure 2) and the relative rank of expression of the spliceosomal genes, per species.

D) Correlation between intron content (introns/CDS kbp) and the relative rank of expression of the spliceosomal genes, per species.

See Methods for details on the calculation of relative rank of expression.

**Coefficients summary and significance (Wald test)**

Variable	Estimate	Std. Error	z statistic (Wald test)	P-value (Wald test)
(Intercept)	6.00E+00	2.63E-01	22.80	4.56E-115
lengene	2.46E-06	6.16E-07	3.99	6.60E-05
lencds	2.81E-05	1.04E-05	2.71	6.79E-03
ralncd	8.14E-03	1.86E-03	4.36	1.28E-05
numin	-8.92E-03	2.35E-03	-3.80	1.43E-04
ranincd	-2.88E-02	8.63E-03	-3.33	8.60E-04
lenex	-6.90E-05	6.55E-05	-1.05	2.92E-01
lenin1	-5.44E-06	8.57E-06	-0.64	5.25E-01
lenin2	-5.18E-05	1.03E-05	-5.02	5.20E-07
ralinex1	6.30E-03	1.00E-03	6.28	3.40E-10
ralinex2	1.41E-02	1.21E-03	11.69	1.51E-31
ss5score	-3.98E-01	1.94E-02	-20.50	2.27E-93
ss3score	-5.91E-01	3.45E-02	-17.12	1.12E-65
is3n	8.86E-01	3.23E-02	27.41	2.36E-165

**ANOVA deviance table (variables added sequentially)**

Variable	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)
NULL	NA	NA	18635	25834.98	NA
lengene	1	898.05	18634	24936.93	2.60E-197
lencds	1	18.93	18633	24918.00	1.35E-05
lenints	0	0.00	18633	24918.00	NA
ralncd	1	157.51	18632	24760.49	3.97E-36
numin	1	26.33	18631	24734.16	2.87E-07
ranincd	1	7.27	18630	24726.89	7.03E-03
lenex	1	38.66	18629	24688.24	5.05E-10
lenin1	1	54.61	18628	24633.63	1.47E-13
lenin2	1	81.37	18627	24552.26	1.87E-19
ralinex1	1	249.31	18626	24302.95	3.67E-56
ralinex2	1	183.87	18625	24119.08	6.91E-42
ss5score	1	475.83	18624	23643.25	1.73E-105
ss3score	1	334.52	18623	23308.73	9.95E-75
is3n	1	761.47	18622	22547.26	1.29E-167

**NULL deviance** 25834.98171

**Model deviance** 22547.2616

**Gene features**

lengene	gene length
lencds	CDS length
ralncd	ration intron length/CDS length
ranincd	number of introns/CDS length
lenex	exon length
lenin1	5' intron length
lenin2	3' intron length
ralinex1	5' intron length/exon length
ralinex2	3' intron length/exon length
ss5score	5' SS heterogeneity score
ss3score	3' SS heterogeneity score
is3n	boolean: is exon length 3-divisible?

**Species list Total=24**

Mmus  
Mdom  
Ggal  
Xtro  
Drex  
Brabel  
Dmel  
Cele  
Cgig  
Ctel  
Hmag  
Nvex  
Aipt  
Mlei  
Aque  
Scil  
Cfra  
Sar3  
Atha  
Vvin  
Mgut  
Marpol  
Vcar  
Crei

**Fig S22.** Coefficients of the logistic binomial regression model of ES prediction, plus their significance according to Wald test and its corresponding ANOVA deviance table with sequential Chi-square tests.



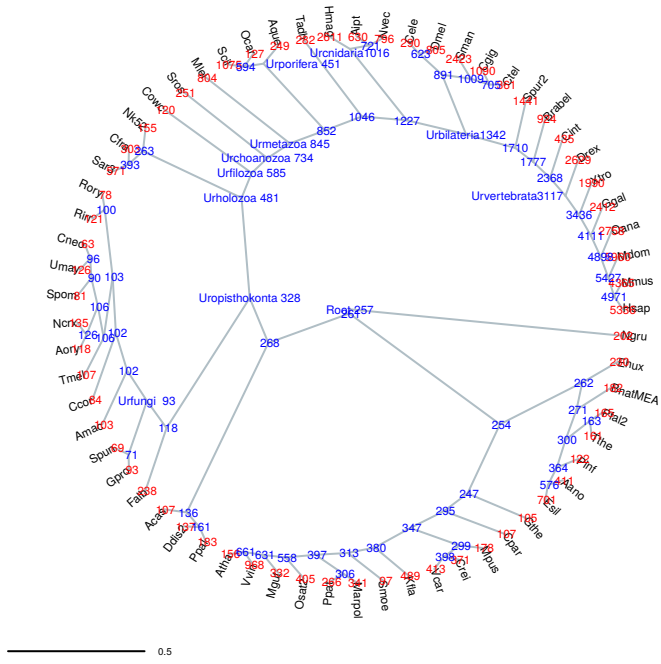
Variable	Mean	Standard deviation	Distribution type
Gene length	9.29036105	1.09385885	Log-normal
CDS length	7.575259591	0.638614526	Log-normal
Exon length	4.750703651	0.475946827	Log-normal
Intron length	Input-dependent	1.21862551	Log-normal
5' SS heterogeneity score	9.267095764	0.850434144	Normal
3' SS heterogeneity score	5.146840410	0.479275742	Normal

**Species list** **Total=24**

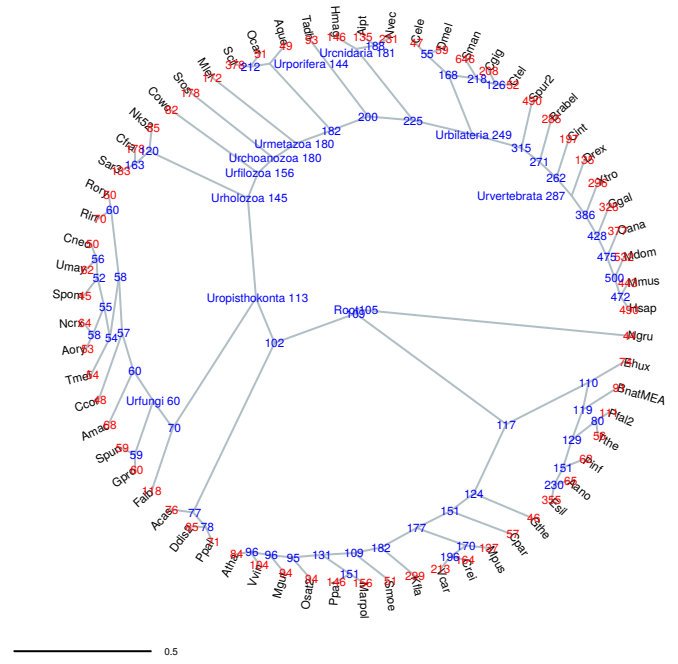
Mmus  
Mdom  
Ggal  
Xtro  
Drex  
Brabel  
Dmel  
Cele  
Cgig  
Ctel  
Hmag  
Nvex  
Aipt  
Mlei  
Aque  
Scil  
Cfra  
Sar3  
Atha  
Vvin  
Mgut  
Marpol  
Vcar  
Crei

**Fig S23.** Summary of input parameters for the simulation of genome architectures, including the distribution shape, means and standard deviations for each variable (gene, CDS, exon and intron lengths, and 5' and 3' SS heterogeneity). Includes the list of species employed in this analysis.

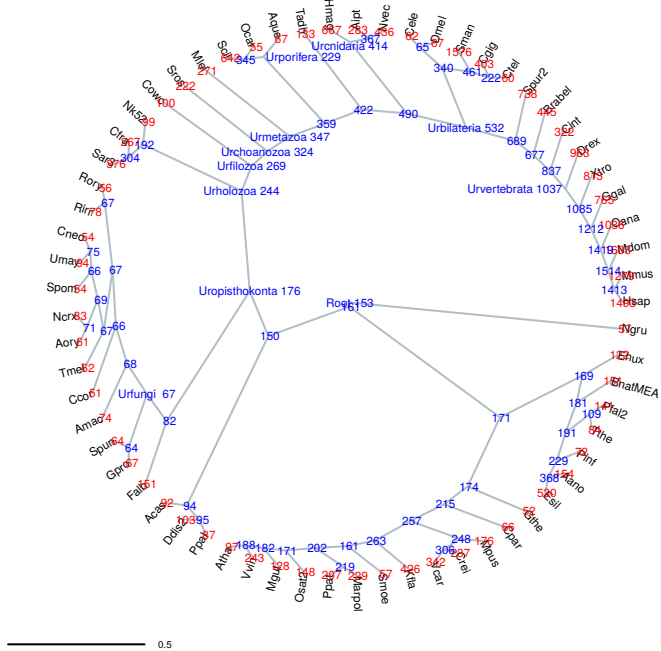
A) Mean intron length (bp) from PIC



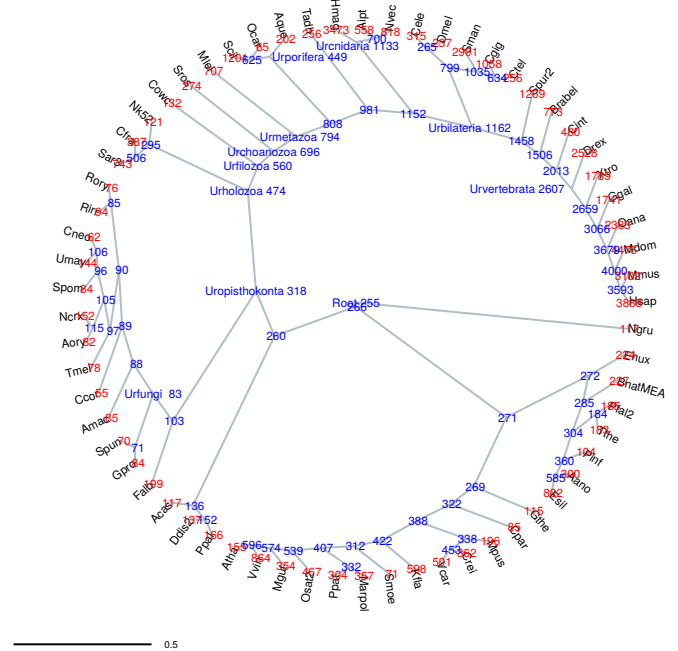
B) Q1 intron length (bp) from PIC



C) Q2 intron length (bp) from PIC

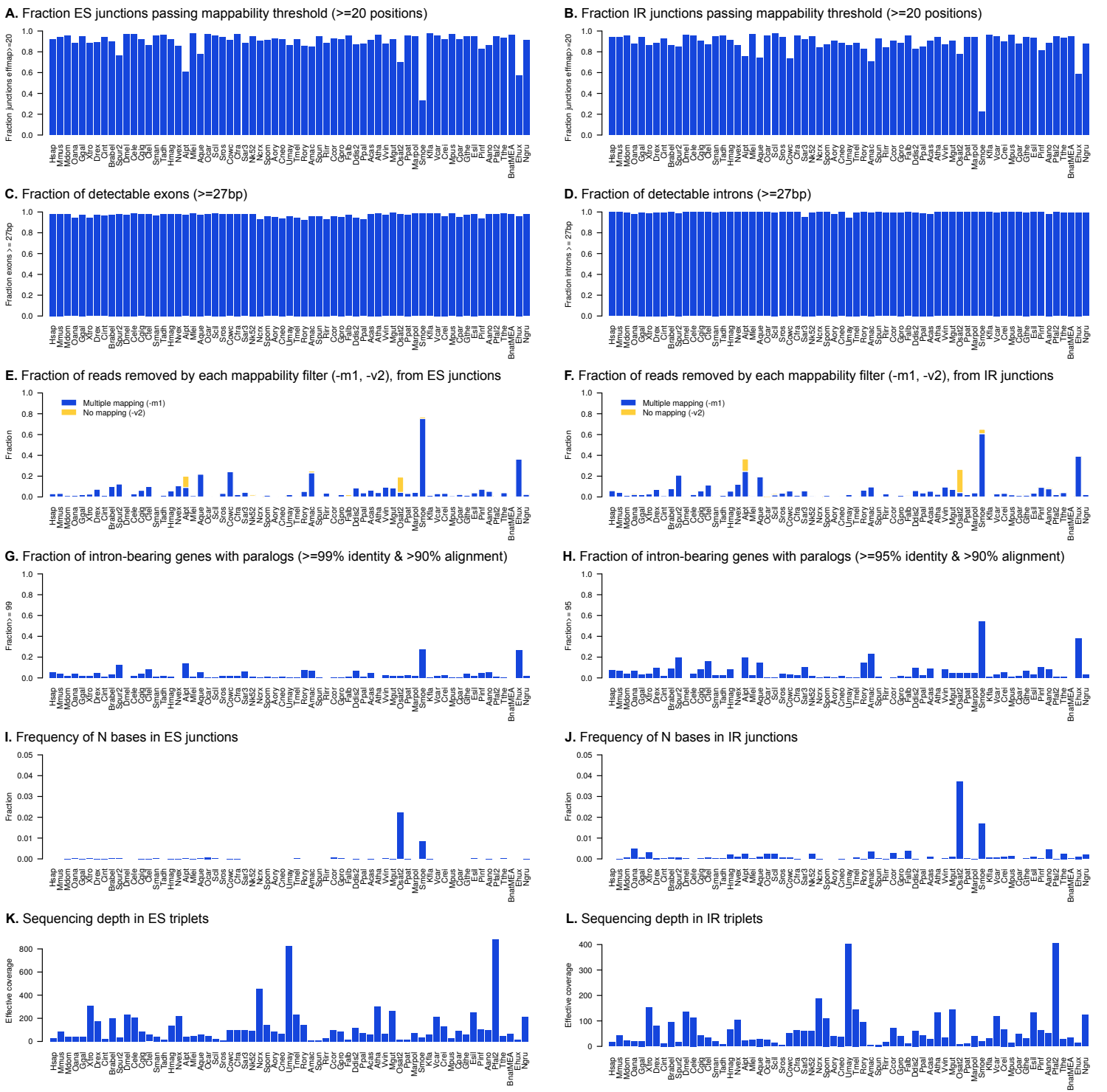


D) Q3 intron length (bp) from PIC



**Fig S24.** Estimations of ancestral intron length distributions using phylogenetically independent contrasts (PIC) under a 65-species phylogenetic tree obtained from the concatenation of 429 single-copy orthologs, using a constrained topology (see Methods).

Each tree displays the following statistics in ancestors (blue) and extant (red) organisms: A) mean, B) first quartile, C) second quartile and D) third quartile.



**Fig S25.** Quality control of the ES and IR detection procedures, analysing possible effects of genome architecture.

A-B) Fraction of exons and introns that pass the mappability filtering step ( $\geq 20$  mappable positions) in the ES and IR analyses, respectively.

C-D) Fraction of exons and introns with lengths above the minimal detection length imposed by the mappability filter ( $\geq 27$  bp for  $\geq 20$  mappable positions in a 84 bp junction).

E-F) Fraction of reads discarded in the RNA-seq mapping filters due to multiple mapping (-m1) or excess of mismatches (-v2), for ES and IR junctions respectively.

G-H) Fraction of intron-bearing genes with at least one close paralog (which can hinder RNA-seq mapping by multiple mapping problems), as measured using two thresholds:  $> 99\%$  or  $> 95\%$  amino acidic sequence identity, and  $> 90\%$  reciprocal sequence alignment.

I-J) Fraction of uncalled bases (N) in ES and IR junctions of each species, respectively (not scaled from 0-1).

K-L) Sequencing depths for the triplets of ES and IR junctions of each species, respectively (using mappability-corrected number of reads).