

Suppl. Table S1. Optical map statistics

	Chromosome arm	1AS	1BS	6BS	5DS
Input	No. of arms sorted (million)	4	4	3.6	4.5
	DNA amount (µg)	2.2	2.6	3	1.2
Molecules	Labelling chemistry	NLRS	NLRS	NLRS	DLS
	Raw data > 150 kb (Gb)	75	320	124	81
	Filtered molecules N50 (kb)	209	197	207	227
	Arm coverage	273x	1019x	298x	315x
Assembly	No. of contigs	300	369	566	35
	Assembly length (Mb)	222	294	360	208
	Contig N50 (Mb)	0.9	1.49	0.93	21
	Average contig length (Mb)	0.74	0.8	0.64	5.85

Suppl. Table S2. Frequency of allelic variants in diagnostic SNPs in Illumina reads of flow-sorted chromosome arms

	SNP (position#)	6BS-1 (5090)		6BS-2 (5348)		1BS-1 (5533)		1BS-2 (8010)		5DS-1 (5300)		5DS-2 (8000)	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1AS	A	2	0,7	17	7,8	0	0,0	0	0,0	0	0,0	53	25,7
	C	1	0,4	12	5,5	199	85,4	169	77,5	189	93,1	153	74,3
	T	18	6,6	17	7,8	24	10,3	48	22,0	14	6,9	0	0,0
	G	251	92,3	172	78,9	10	4,3	1	0,5	0	0,0	0	0,0
	-	0	0,0	0	0,0	0	0,0	0	0,0	0	0,0	0	0,0
	Total	272		218		233		218		203		206	
1BS	A	12	0,1	31	0,3	13	0,1	11	0,1	3	0,0	17467	99,3
	C	14	0,1	1	0,0	747	5,5	123	0,7	6424	99,8	106	0,6
	T	69	0,4	45	0,4	12749	94,1	18256	99,0	3	0,0	6	0,0
	G	16379	99,3	10075	99,2	25	0,2	41	0,2	3	0,0	9	0,1
	-	17	0,1	4	0,0	10	0,1	6	0,0	5	0,1	8	0,0
	Total	16491		10156		13544		18437		6438		17596	
6BS	A	2	0,3	0	0,0	2	0,1	2	0,3	0	0,0	676	98,7
	C	2	0,3	0	0,0	2982	99,5	738	97,7	55	100,0	6	0,9
	T	714	94,3	346	98,6	11	0,4	14	1,9	0	0,0	2	0,3
	G	39	5,2	5	1,4	1	0,0	1	0,1	0	0,0	0	0,0
	-	0	0,0	0	0,0	1	0,0	0	0,0	0	0,0	1	0,1
	Total	757		351		2997		755		55		685	
5DS	A	2	0,2	3	0,5	0	0,0	0	0,0	0	0,0	74	13,3
	C	0	0,0	0	0,0	763	95,9	540	97,1	47	13,9	484	86,7
	T	110	9,0	45	7,7	32	4,0	16	2,9	290	86,1	0	0,0
	G	1117	90,9	533	91,7	1	0,1	0	0,0	0	0,0	0	0,0
	-	0	0,0	0	0,0	0	0,0	0	0,0	0	0,0	0	0,0
	Total	1229		581		796		556		337		558	

6BS variant

1BS variant

5DS variant

Non-B variant

SNP position in the wheat 45S rDNA consensus
Chromosomal Illumina reads obtained from IWGSC, 2014

Suppl. Table S3. Frequency of allelic variants at diagnostic SNPs in Illumina reads from three wheat 'Chinese Spring' genome projects

	SNP (position [#])	6BS-1 (5090)		6BS-2 (5348)		1BS-1 (5533)		1BS-2 (8010)		5DS-1 (5300)		5DS-2 (8000)	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
RefSeq v1.0*	A	16	0,0	74	0,2	19	0,1	31	0,1	13	0,0	33196	95,1
	C	10	0,0	92	0,3	23647	64,5	21183	62,7	31952	95,7	1676	4,8
	T	19583	55,9	18822	57,3	12951	35,3	12538	37,1	1385	4,2	10	0,0
	G	15438	44,0	13886	42,2	48	0,1	27	0,1	23	0,1	23	0,1
	-	0	0,0	1	0,0	0	0,0	1	0,0	0	0,0	0	0,0
	Total		35047		32875		36665		33780		33373		34905
Triticum 3.1**	A	16	0,0	73	0,2	12	0,0	27	0,1	4	0,0	49039	95,3
	C	12	0,0	74	0,2	31233	60,0	30399	58,9	44175	95,8	2354	4,6
	T	28604	53,0	24443	53,9	20747	39,8	21135	41,0	1915	4,2	27	0,1
	G	25301	46,9	20796	45,8	70	0,1	28	0,1	24	0,1	30	0,1
	-	0	0,0	0	0,0	1	0,0	0	0,0	0	0,0	0	0,0
	Total		53933		45386		52063		51589		46118		51450
TGACv1 ***	A	1	0,0	13	0,2	3	0,0	1	0,0	2	0,0	6263	94,9
	C	1	0,0	0	0,0	5565	67,7	3894	59,5	6960	96,0	328	5,0
	T	4083	56,2	4548	63,1	2638	32,1	2642	40,4	286	3,9	5	0,1
	G	3180	43,8	2643	36,7	11	0,1	8	0,1	1	0,0	6	0,1
	-	0	0,0	0	0,0	0	0,0	0	0,0	0	0,0	0	0,0
	Total		7265		7204		8217		6545		7249		6602
		6BS variant				1BS variant				5DS variant		Non-B variant	
#	SNP position in the wheat 45S rDNA consensus												
*	IWGSC, 2018												
**	Zimin et al., 2017												
***	Clavijo et al., 2017												

Suppl. Table S4. RNA-seq data

Tissue	Sample	Read length (bp)	Trimmed raw data		Reads after cp/mt rDNA removal		Input (MSeq)	No. of reads mapped to full rDNA unit		No. of reads mapped to ETS/IGS subregion	
			(MSeq)		(MSeq)	Percentage		Counts	Percentage	Counts	Percentage
Embryo 1	98219	148	14.8		14.6	98.6	2	1924225	96.21125	2466	0.1233
Embryo 2	98220	148	15.2		15	98.7	2	1919571	95.97855	2221	0.11105
Root tip 1	81007	122	33.0		32.5	98.5	2	1912845	95.64225	1499	0.07495
Root tip 2	81008	124	32.7		32.2	98.5	2	1910285	95.51425	1464	0.0732
Coleoptile 1	98221	148	15.0		14.3	95.3	2	1926102	96.3051	2191	0.10955
Coleoptile 2	98222	148	14.8		12.4	83.8	2	1931022	96.5511	2181	0.10905
Primary leaf 1	81009	123	33.6		15.7	46.7	2	1852931	92.64655	106	0.0053
Primary leaf 2	81010	127	35.6		19	53.4	2	1866803	93.34015	101	0.00505
Mature leaf 1	98226	148	14.1		8.1	57.4	2	1802848	90.1424	392	0.0196
Mature leaf 2	98227	148	12.7		7.3	57.5	2	1803440	90.172	448	0.0224

Suppl. Table S5. Variant analysis of 26S rRNA in different tissues. The four informative SNPs were used to discriminate between transcription from 1BS and 6BS rDNA loci.

Frequency of diagnostic nucleotides in mapped rRNA-seq Illumina reads

Tissue			embryo		coleoptile		root tip		primary leaf		mature leaf	
Position	SNP	Allele	98219	98220	98221	98222	81007	81008	81009	81010	98226	98227
5090	6BS-1	T	32.50	33.19	35.20	34.70	31.51	31.29	27.02	29.69	26.53	26.18
5090		G	66.81	66.20	64.13	64.70	68.14	68.45	72.59	69.97	72.82	73.18
5348	6BS-2	T	35.64	34.38	37.98	37.19	35.39	36.51	32.69	34.80	29.11	27.10
5348		G	63.69	64.68	61.12	62.27	63.51	62.58	66.14	64.59	70.02	72.01
5533	1BS-1	T	64.78	64.22	62.48	63.01	66.41	66.01	70.74	67.36	71.64	72.20
5533		C	34.33	34.77	36.61	36.00	33.33	33.67	28.96	32.36	27.36	26.73
8010	1BS-2	T	67.46	68.17	65.59	65.91	72.84	72.88	76.51	72.82	74.78	75.46
8010		C	32.54	31.83	34.41	34.09	27.15	27.11	23.49	27.18	25.25	24.54

Statistical evaluation of SNP frequencies between the tissues

Statistical evaluation of differences in variant representation at the 5090 position of the 26S rRNA gene

		81007	81008	81009	81010	98219	98220	98221	98222	98226	98227
Root tip	81007	-	0.456	<u>171.461</u>	<u>27.923</u>	<u>9.086</u>	<u>26.58</u>	<u>121.058</u>	<u>90.851</u>	<u>255.332</u>	<u>303.8</u>
Root tip	81008	0.5141	-	<u>149.552</u>	<u>20.632</u>	<u>48.906</u>	<u>32.638</u>	<u>129.99</u>	<u>99.387</u>	<u>223.376</u>	<u>267.137</u>
Primary leaf	81009	<u>0.0001</u>	<u>0.0001</u>	-	<u>149.552</u>	<u>59.946</u>	<u>268.307</u>	<u>344.801</u>	<u>570.133</u>	<u>506.047</u>	2.444
Primary leaf	81010	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	-	<u>70.534</u>	<u>111.205</u>	<u>259.628</u>	<u>1004.195</u>	<u>33370.88</u>	<u>3621.379</u>
Embryo	98219	<u>0.0026</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	-	<u>4.805</u>	<u>70.15</u>	<u>46.667</u>	<u>391.546</u>	<u>456.878</u>
Embryo	98220	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0286</u>	-	<u>40.305</u>	<u>22.787</u>	<u>497.852</u>	<u>575.016</u>
Coleoptile	98221	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	-	2.373	<u>793.182</u>	<u>894.841</u>
Coleoptile	98222	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	0.1234	-	<u>708.192</u>	<u>802.6001</u>
Old leaf	98226	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	-	1.553
Old leaf	98227	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	0.2128	-

Statistical evaluation of differences in variant representation at 5348 position within the 26S rRNA gene

		81007	81008	81009	81010	98219	98220	98221	98222	98226	98227
Root tip	81007	-	0.659	1.032	0.476	1.843	0.123	<u>10.032</u>	<u>6.736</u>	<u>15.604</u>	<u>32.850</u>
Root tip	81008	0.4170	-	1.032	0.476	1.843	0.247	<u>5.320</u>	<u>2.972</u>	<u>22.957</u>	<u>43.241</u>
Primary leaf	81009	0.3096	0.4170	-	3.356	<u>6.453</u>	2.068	<u>20.149</u>	<u>15.142</u>	<u>15.142</u>	<u>9.853</u>
Primary leaf	81010	0.4901	0.4901	0.067	-	3.356	0.121	<u>7.219</u>	<u>4.248</u>	<u>24.328</u>	<u>46.584</u>
Embryo	98219	0.1746	0.1746	<u>0.011</u>	0.4683	-	1.106	3.791	1.730	<u>31.372</u>	<u>56.040</u>
Embryo	98220	0.1746	0.6194	0.150	0.7275	0.2929	-	<u>8.745</u>	<u>5.535</u>	<u>20.088</u>	<u>40.121</u>
Coleoptile	98221	<u>0.0015</u>	<u>0.0211</u>	<u>0.000</u>	<u>0.0072</u>	0.0515	<u>0.0031</u>	-	0.4368	<u>56.046</u>	<u>87.943</u>
Coleoptile	98222	<u>0.0095</u>	<u>0.0847</u>	<u>0.000</u>	<u>0.0393</u>	0.1884	<u>0.0186</u>	0.5087	-	<u>48.189</u>	<u>78.348</u>
Old leaf	98226	<u>0.0001</u>	<u>0.0001</u>	<u>0.000</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	-	3.330
Old leaf	98227	<u>0.0001</u>	<u>0.0001</u>	<u>0.002</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	0.06800	-

Statistical evaluation of differences in variant representation at the 5533 position within the 26S rRNA gene

		81007	81008	81009	81010	98219	98220	98221	98222	98226	98227
Root tip	81007	-	0.4388	47.64	2.414	7.048	12.377	<u>41.453</u>	<u>30.720</u>	<u>62.95</u>	<u>77.25</u>
Root tip	81008	0.5077	-	<u>58.679</u>	<u>5.083</u>	<u>4.091</u>	<u>8.402</u>	<u>34.256</u>	<u>24.479</u>	<u>75.129</u>	<u>90.889</u>
Primary leaf	81009	0.0001	0.5077	-	<u>30.579</u>	<u>92.091</u>	107.173	<u>179.570</u>	<u>155.826</u>	1.862	<u>4.877</u>
Primary leaf	81010	0.1203	<u>0.0241</u>	<u>0.0001</u>	-	<u>18.344</u>	<u>26.364</u>	<u>66.614</u>	<u>57.548</u>	<u>44.193</u>	<u>56.562</u>
Embryo	98219	0.0079	<u>0.0430</u>	<u>0.0002</u>	<u>0.0001</u>	-	0.8040	<u>14.408</u>	<u>8.428</u>	<u>110.509</u>	<u>129.344</u>
Embryo	98220	0.0005	0.0037	<u>0.0003</u>	<u>0.0001</u>	0.370	-	<u>8.103</u>	<u>3.874</u>	<u>126.088</u>	<u>145.887</u>
Coleoptile	98221	<u>0.0001</u>	<u>0.0001</u>	<u>0.0004</u>	<u>0.0001</u>	0.0015	<u>0.0044</u>	-	0.782	<u>200.250</u>	<u>225.567</u>
Coleoptile	98222	<u>0.0001</u>	<u>0.0001</u>	<u>0.0005</u>	<u>0.0001</u>	0.0037	0.0490	0.3765	-	<u>176.156</u>	<u>199.842</u>
Old leaf	98226	<u>0.0001</u>	<u>0.0001</u>	0.1724	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	-	0.6363
Old leaf	98227	<u>0.0001</u>	<u>0.0001</u>	<u>0.0272</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	0.4251	-

Statistical evaluation of differences in variant representation at the 8010 position within the 26S rRNA gene

		81007	81008	81009	81010	98219	98220	98221	98222	98226	98227
Root tip	81007	-	0.035	<u>83.078</u>	0.001	<u>112.462</u>	<u>88.274</u>	<u>225.104</u>	<u>202.672</u>	<u>232,230</u>	<u>113.008</u>
Root tip	81008	0.8525	-	<u>75.811</u>	0.023	<u>108.731</u>	<u>85.981</u>	<u>224.876</u>	<u>193.754</u>	<u>64.751</u>	<u>101.413</u>
Primary leaf	81009	<u>0.0001</u>	<u>0.0001</u>	-	<u>85.920</u>	<u>425.466</u>	<u>372.387</u>	<u>617.018</u>	<u>581.7703</u>	4.197	0.003
Primary leaf	81010	0.9692	0.8788	<u>0.0001</u>	-	<u>22.860</u>	<u>120.837</u>	<u>94.670</u>	<u>241.430</u>	<u>76.631</u>	<u>6.503</u>
Embryo	98219	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	-	1.538	<u>31.041</u>	<u>20.957</u>	<u>542.940</u>	<u>653.752</u>
Embryo	98220	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	0.2147	-	<u>43.925</u>	<u>32.148</u>	<u>459.757</u>	<u>561.219</u>
Coleoptile	98221	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	-	1.0212	<u>891.659</u>	<u>951.220</u>
Coleoptile	98222	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	0.3121	-	<u>771.025</u>	<u>897.922</u>
Old leaf	98226	<u>0.0001</u>	<u>0.0001</u>	<u>0.0405</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	-	1.8186
Old leaf	98227	<u>0.0001</u>	<u>0.0001</u>	0.9559	<u>0.0108</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	0.1775	-

Footnote:

Above the diagonal - Pearson's chi square values

Below the diagonal - p values

In blue - moderately significant (P<0.05)

In blue and underlined - highly significant (P<0.001)

In red - differences between biological replicas

Suppl. Table S6. FPKM values for nuclear, chloroplast and mitochondrial 16S/18S and 23/26S rRNAs across five tissues.

averaged FPKM	embryo	coleoptile	root tip	primary leaf	mature leaf
nuclear 18S	97491	132376	183671	384204	150118
nuclear 26S	89297	121003	137503	290130	130916
chloroplast 16S	189	2710	219	170745	73903
chloroplast 23S	229	3165	173	177475	86062
mitochondrial 18S	559	642	463	737	657
mitochondrial 26S	552	577	588	860	580

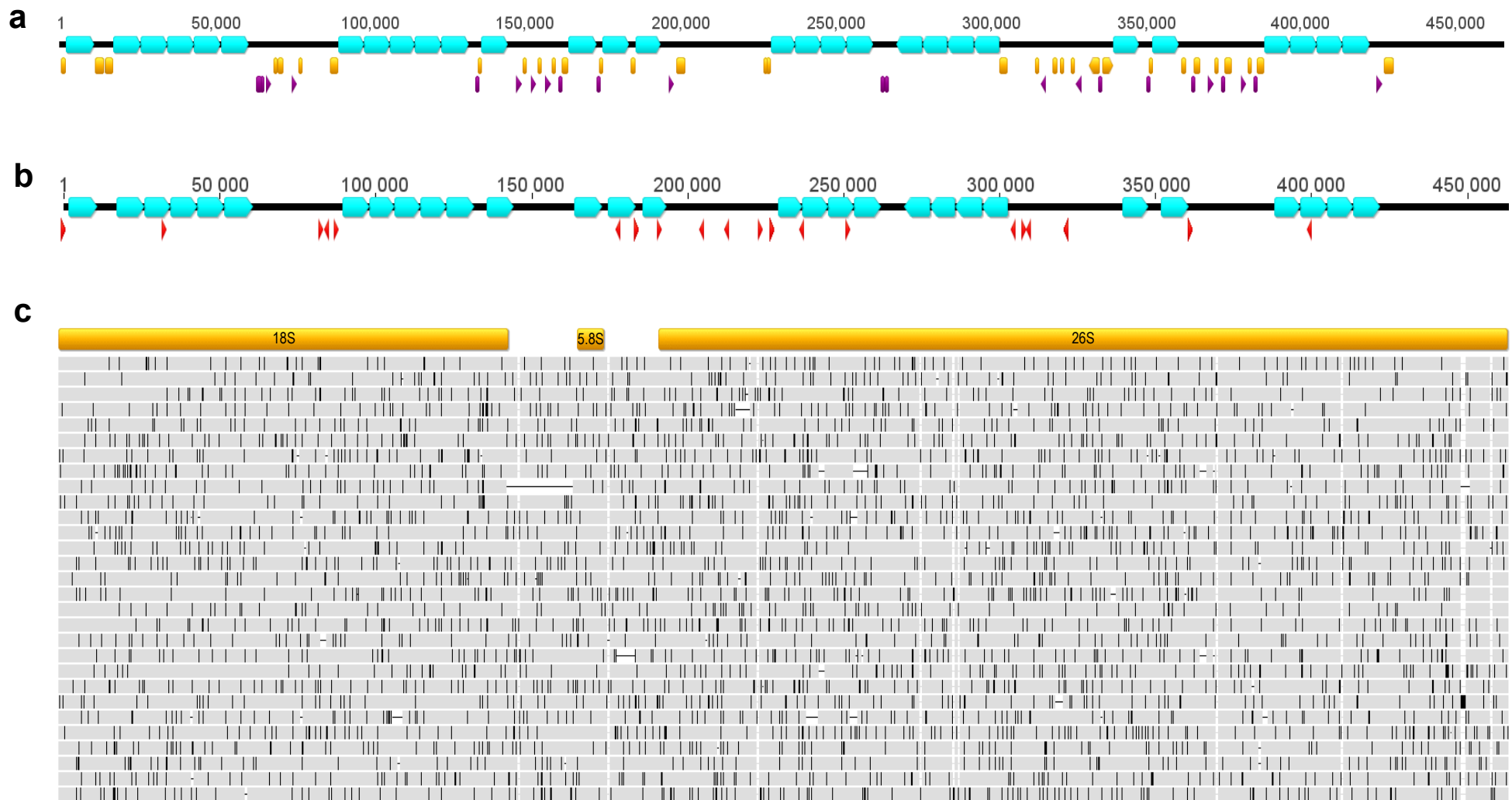
FPKM - Fragments Per Kilobase of exon per Million reads

Suppl. Table S7. DeSeq2 results table with A: log2 fold changes and B: adjusted p values behind the heatmap and its color-code

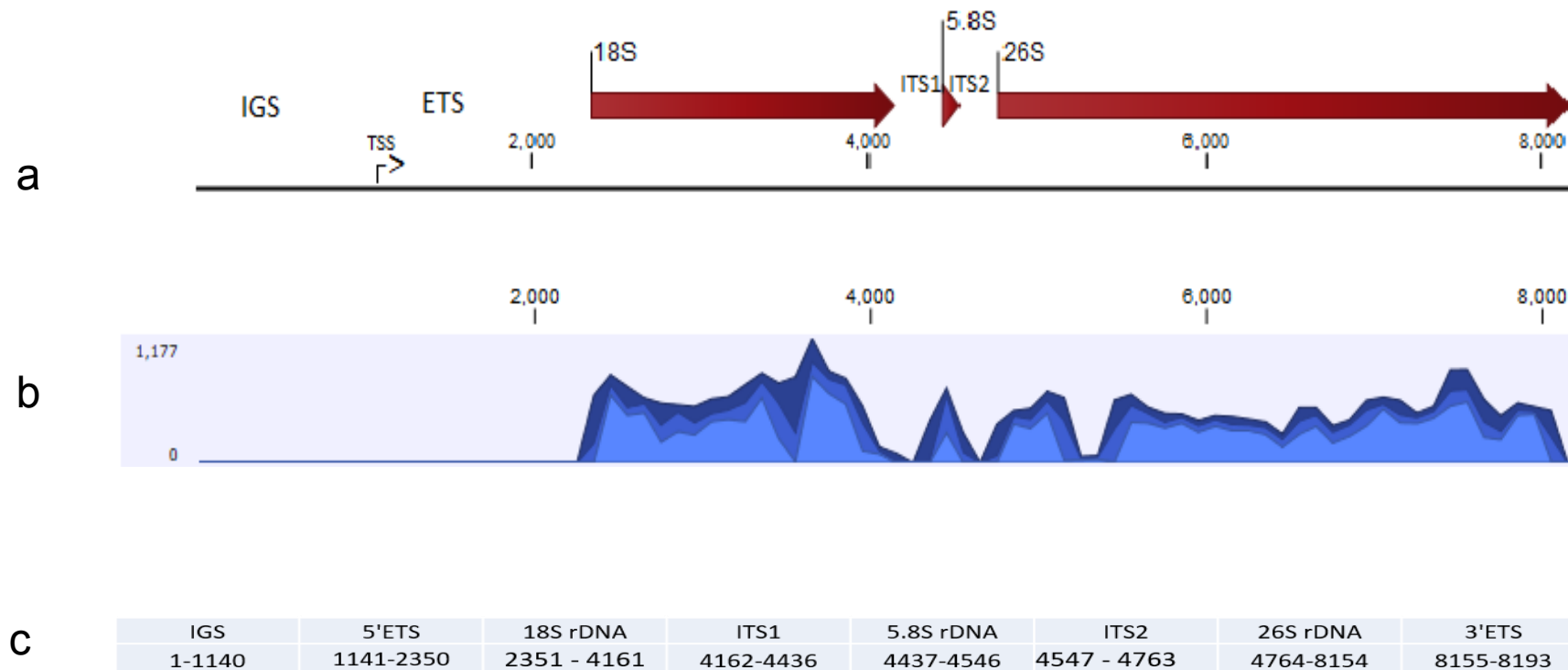
A

log2fold change	coleoptile	root tip	primary leaf	mature leaf
nuclear 18S	0,441310212	0,913788019	1,978536511	0,622763214
nuclear 26S	0,438360179	0,622776541	1,700016039	0,551954843
chloroplast 16S	3,840917255	0,21057889	9,818565181	8,610426266
chloroplast 23S	3,785877305	-0,407878955	9,595116148	8,550945427
mitochondrial 18S	0,200205379	-0,273384147	0,399766864	0,234100439
mitochondrial 26S	0,064613786	0,089848912	0,63898758	0,07071563
B				
padj	coleoptile	root tip	primary leaf	mature leaf
nuclear 18S	0,057516924	4,58E-07	4,89E-37	0,000517994
nuclear 26S	0,089432246	0,004991001	4,09E-24	0,005251552
chloroplast 16S	1,40E-50	0,906501206	0	1,69E-262
chloroplast 23S	6,68E-41	0,631579214	9,39E-272	1,10E-215
mitochondrial 18S	0,788016196	0,622825014	0,099563837	0,377639271
mitochondrial 26S	0,993735334	0,972848182	0,000840141	0,804892936

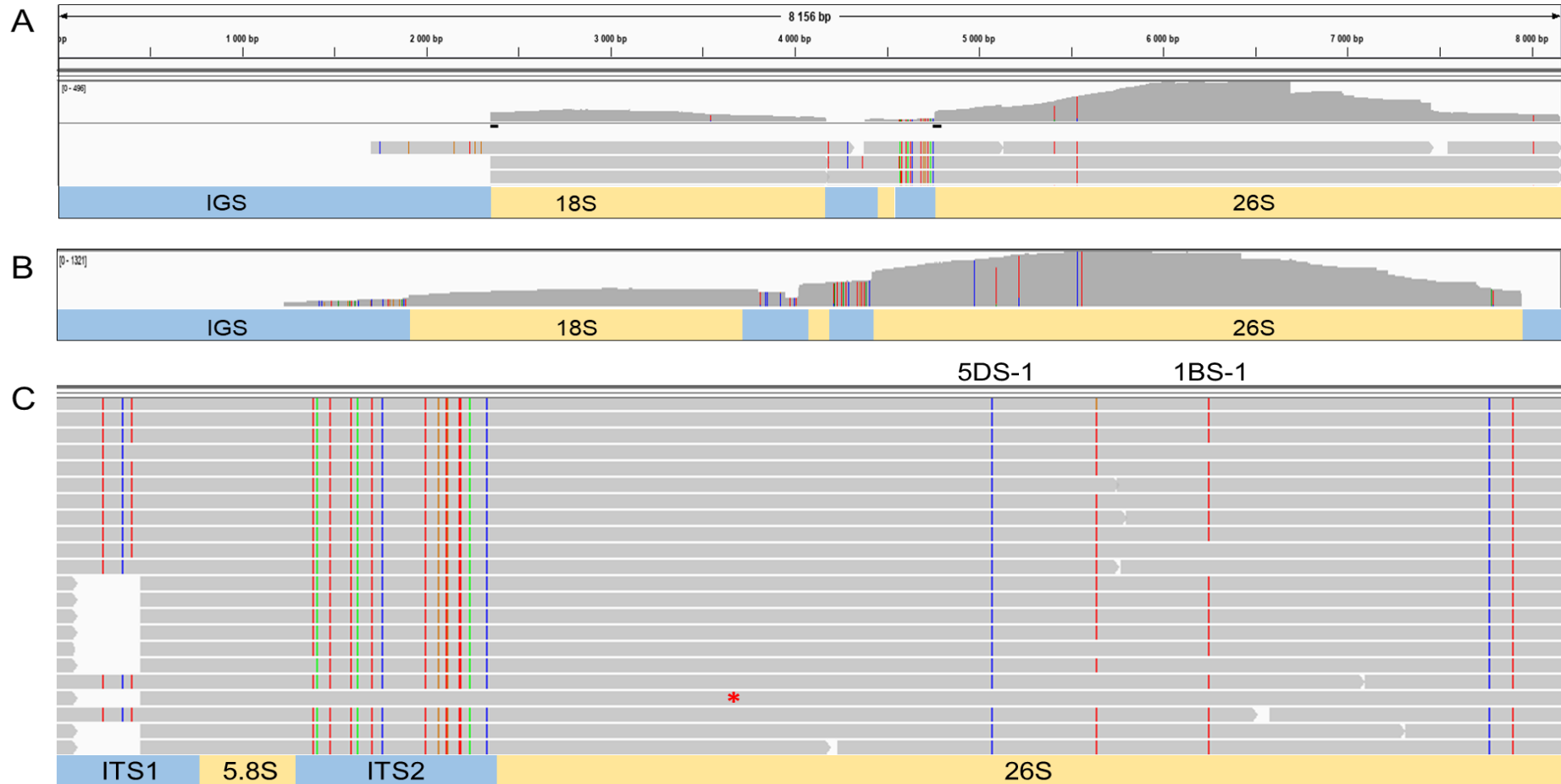
fold change	coleoptile	root tip	primary leaf	mature leaf
nuclear 18S	1,36	1,88	3,94	1,54
nuclear 26S	1,36	1,54	3,25	1,47
chloroplast 16S	14,33	1,16	902,99	390,84
chloroplast 23S	13,79	0,75	773,42	375,05
mitochondrial 18S	1,15	0,83	1,32	1,18
mitochondrial 26S	1,05	1,06	1,56	1,05



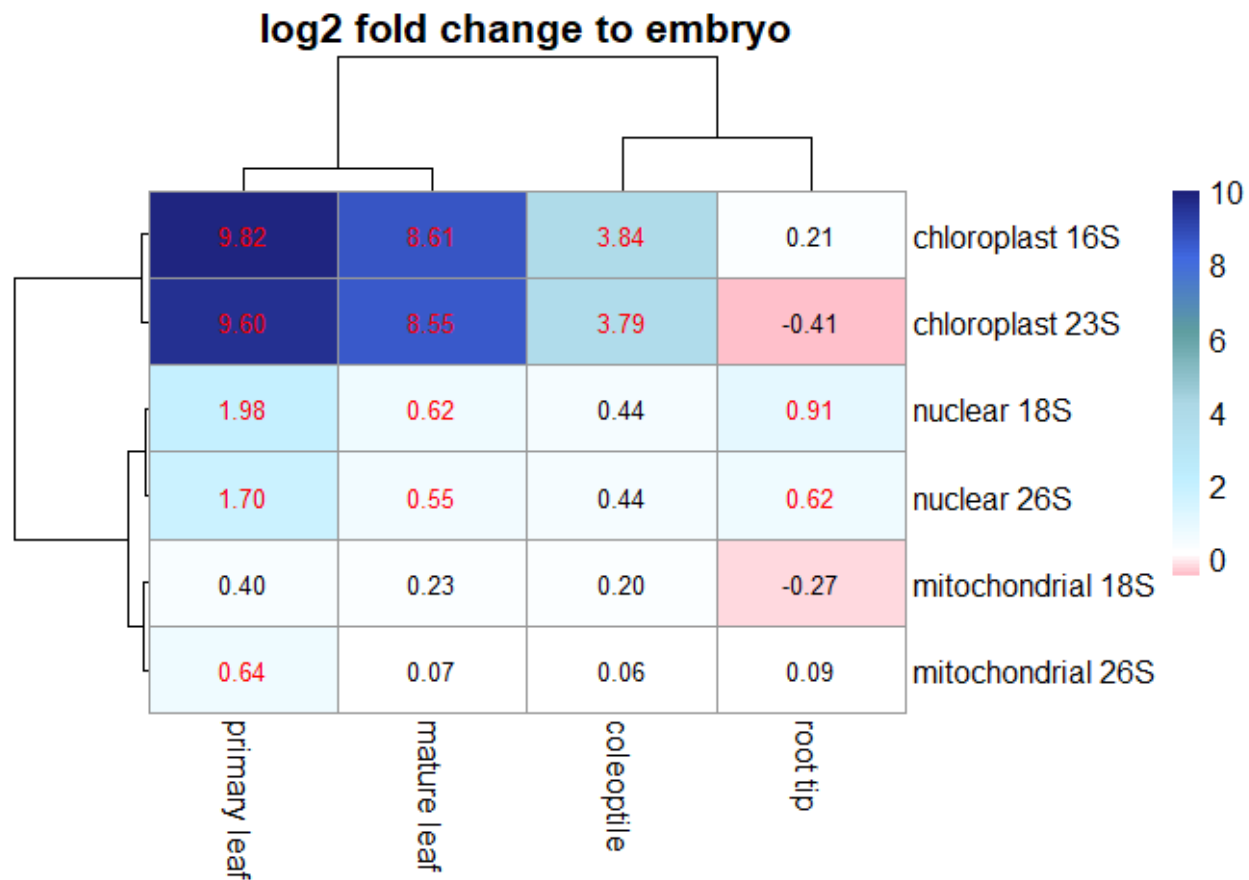
Suppl. Figure S1. Degenerated ribosomal DNA array in the terminal part of chromosome arm 1AS. (a) The 1AS rDNA array is composed of both complete (comprising all of 18S, 5.8S and 26S rRNA genes; blue boxes), and incomplete units. Separated 18S and 26S rRNA genes and gene fragments are marked purple and yellow, respectively. (b) Ribosomal DNA units (blue boxes) are interspersed by telomeric repeats (red triangles) of various orientation. (c) Comparison of 29 complete 45S rDNA units building the 1AS array shows a high degree of variability. Grey bars represent individual rDNA units, disagreements are highlighted by black lines. Gene annotation is shown above the alignment.



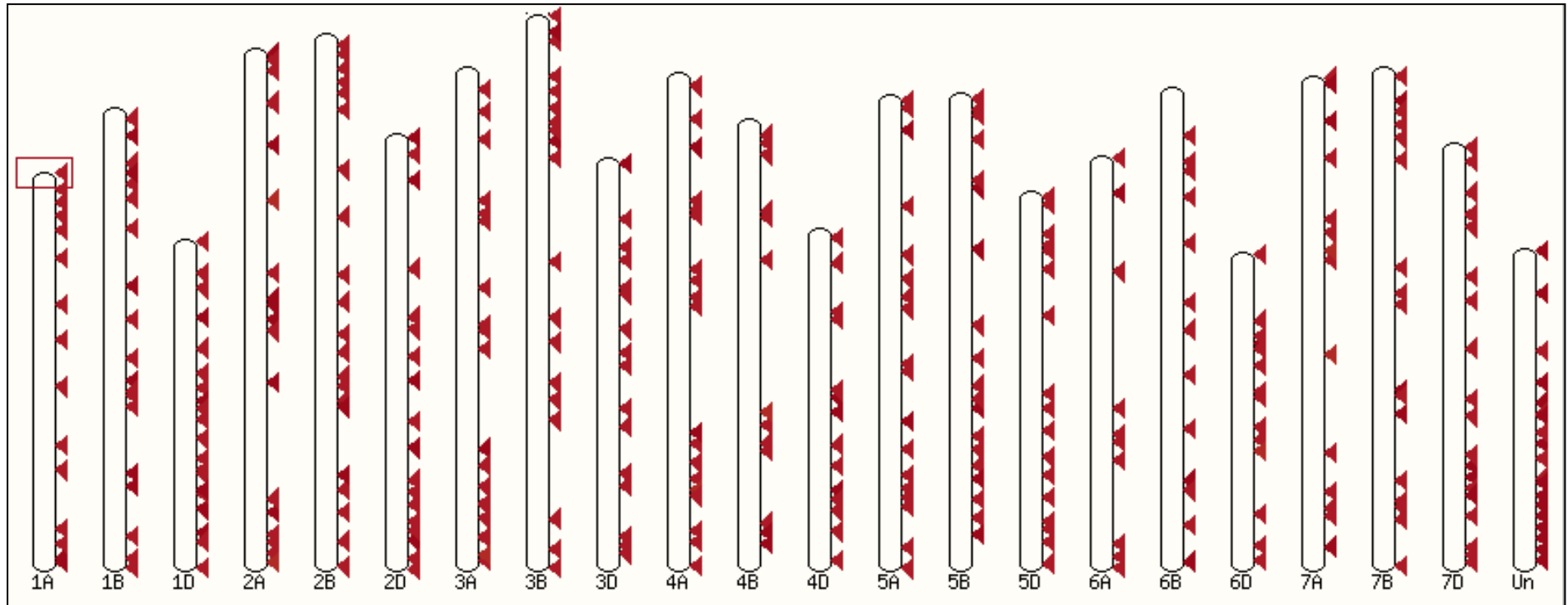
Suppl Figure S2. Wheat consensus 45S rDNA unit and its coverage by RNA-seq reads (a) Scheme of the consensus rDNA unit. Coding regions are depicted as brown arrows. Putative transcription start site is indicated in the position 1141 bp upstream from the 18S rRNA gene. (b) RNA-seq read coverage plot along the rDNA unit. Note, near absence of the ETS/IGS and ITS1/ITS2 transcripts. Both 18S and 26S rRNA genes were relatively equally covered except of a GC-rich subregion close to the 26S rRNA 5'-terminus. (c) Annotation of the rDNA unit



Suppl. Figure S3. Mapping of IsoSeq reads to wheat consensus and 5DS rDNA units. (a) Mapping of 801 CCS IsoSeq reads from PRJEB15048 (Clavijo et al. 2017), sample „Leaf“, to the wheat 45S rDNA consensus. Coverage of the unit by reads is shown in the top, example of reads in the middle and rDNA unit annotation in the bottom of the figure. (b) Total of 1951 CCS IsoSeq reads, sample „Seed“, mapped to the 5DS consensual 45S rDNA unit. (c) In five out of 1079 „Seed“ reads mapping to 5DS-1 position, we could unambiguously recognize the 5DS-specific haplotype. A read showing complete match with the 5DS rDNA consensus, both in ITS2 and 26S rRNA, is marked by red asterisk. The colored lines indicate allelic variants with respect to the applied reference.



Suppl. Figure S4. Nuclear, chloroplast and mitochondrial rRNA - Log2 fold change heatmap across five tissues. The DESeq2 dataset clustered heatmap shows log2 fold transformed differences in amounts of 18/16S and 26/23S rRNA in each of the tissues related to the embryo. Color code of the values indicates padj values where red values correspond to $p < 0.05$ that is considered as statistically significant. Blue shades mark increased expression whereas pink marks decreased expression related to the embryonic rRNA as indicated by the scale.



Suppl. Figure S5. Graphical outcome of a BLAST search for sequences homologous to wheat consensus rDNA unit in the IWGSC RefSeq v1.0 genome. The search was carried out in Ensembl Plants (https://plants.ensembl.org/Triticum_aestivum/Tools/Blast). Position of the irregular rDNA array in 1A is marked by a rectangle.