
Supplementary Information

Intraspecific and heteroplasmic variations, gene losses and inversions in the chloroplast genome of *Astragalus membranaceus*

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Table S1 Length of introns and exons of the split genes.

Gene Name	Gene Location			Length (bp)			
	Strand	Start	End	Exon I	Intron I	Exon II	Intron II
<i>ycf3</i>	+	16648	18586	117	706	228	735
<i>rpoC1</i>	+	37350	40117	432	713	1623	
<i>atpF</i>	+	47921	49176	168	677	411	
<i>clpP</i>	-	67413	68617	363	615	227	
<i>petB</i>	+	71631	73101	6	823	642	
<i>petD</i>	+	73307	74510	8	712	484	
<i>rpl16</i>	-	78346	79749	9	984	411	
<i>rpl2</i>	-	81189	82728	393	712	435	
<i>ndhB</i>	-	90709	92882	723	689	762	
<i>ndhA</i>	-	111824	114151	552	1236	540	
<i>rps12-5' end</i>	-	67138	67251	114	-	-	
<i>rps12-3' end</i>	-	93682	93939	-	-	258	
<i>trnK-UUU</i>	-	1739	4304	38	2494	36	
<i>trnC-ACA</i>	-	9788	10473	41	589	58	
<i>trnL-UAA</i>	+	13491	14118	36	543	51	
<i>trnT-CGU</i>	-	51154	51931	36	700	44	
<i>trnE-UUC</i>	+	97703	98552	33	778	41	
<i>trnA-UGC</i>	+	98622	99495	38	783	55	

Table S2 Codon usage and codon-anticodon recognition patterns in *A. membranaceus* chloroplast genome.

Codon	Amino acid	Number	tRNA	Codon	Amino acid	Number	tRNA	Codon	Amino acid	Number	tRNA
GCA	A	351	<i>trnA-UGC</i>	AAG	K	263		AGC	S	83	<i>trnS-GCU</i>
GCC	A	173		CTA	L	310	<i>trnL-UAG</i>	AGT	S	361	
GCG	A	120		CTC	L	137		TCA	S	320	<i>trnS-UGA</i>
GCT	A	577		CTG	L	122		TCC	S	234	<i>trnS-GGA</i>
TGC	C	58	<i>trnC-GCA</i>	CTT	L	488		TCG	S	148	
TGT	C	186	<i>trnC-ACA</i>	TTA	L	818	<i>trnL-UAA</i>	TCT	S	476	
GAC	D	160	<i>trnD-GUC</i>	TTG	L	482	<i>trnL-CAA</i>	ACA	T	357	<i>trnT-UGU</i>
GAT	D	698		ATG	M	502	<i>trnM-CAU</i>	ACC	T	191	<i>trnT-GGU</i>
GAA	E	882	<i>trnE-UUC</i>	AAC	N	251	<i>trnN-GUU</i>	ACG	T	102	<i>trnT-CGU</i>
GAG	E	256		AAT	N	854		ACT	T	467	
TTC	F	378	<i>trnF-GAA</i>	CCA	P	279		GTA	V	475	
TTT	F	923		CCC	P	169		GTC	V	140	<i>trnV-GAC</i>
GGA	G	617		CCG	P	99		GTG	V	156	
GGC	G	128	<i>trnG-GCC</i>	CCT	P	346	<i>trnP-UGG</i>	GTT	V	454	
GGG	G	198		CAA	Q	615	<i>trnQ-UUG</i>	TGG	W	372	<i>trnW-CCA</i>
GGT	G	540		CAG	Q	163		TAC	Y	151	<i>trnY-GUA</i>
CAC	H	105	<i>trnH-GUG</i>	AGA	R	369	<i>trnR-UCU</i>	TAT	Y	683	
CAT	H	385		AGG	R	128		TAA	*	43	
ATA	I	644		CGA	R	289		TAG	*	15	
ATC	I	366	<i>trnI-CAU</i> <i>trnM-CAU</i>	CGC	R	92		TGA	*	18	
ATT	I	973		CGG	R	83					
AAA	K	931	<i>trnK-UUU</i>	CGT	R	276	<i>trnR-ACG</i>				

Table S3 Numbers of mono- and dinucleotide SSRs identified in the chloroplast genome of *A. membranaceus*.

Repeat Types	Number of Copies												Total
	4	5	6	7	8	9	10	11	12	13	14	15	
A/T	-	-	-	-	53	33	30	11	8	6	6	1	148
C/G	-	-	-	-	1								1
AC/GT	1												1
AG/CT	10	1											11
AT/AT	49	16	11	1									77

Table S4 Distributions of SSRs in the 14 IRLC chloroplast genomes

Species	Number of SSRs						Total
	1	2	3	4	5	6	
<i>M. truncatula</i>	190	63	9	14	0	2	278
<i>C. arietinum</i>	190	62	5	8	0	1	266
<i>T. subterraneum</i>	241	63	13	11	0	0	328
<i>P. sativum</i>	145	61	6	2	0	1	215
<i>L. sativus</i>	167	44	2	4	0	2	219
<i>T. grandiflorum</i>	174	64	4	5	2	0	249
<i>T. aureum</i>	174	62	5	8	2	0	251
<i>T. repens</i>	204	81	9	9	0	0	303
<i>G. glabra</i>	158	96	7	11	0	0	272
<i>T. meduseum</i>	243	71	8	11	4	1	338
<i>T. boissieri</i>	179	59	1	4	4	0	247
<i>T. glanduliferum</i>	162	66	8	6	3	0	245
<i>T. strictum</i>	156	66	10	11	4	0	247
<i>A. membranaceus</i>	149	89	12	11	1	0	262

Table S5 List of chloroplast genome sequences used in this study.

Tribe	Species Name	Accession	Length (bp)	Protein Number	RNA Number	Created date	Updated date
Cicereae	<i>Cicer arietinum</i> / common:chickpea	NC_011163	125319	75	33	08/26/2008	03/26/2010
Dalbergieae	<i>Arachis hypogaea</i> / common:peanut	NC_026676	156395	81	44	03/20/2015	03/20/2015
Fabeae	<i>Lathyrus sativus</i>	NC_014063	121020	74	34	04/23/2010	12/07/2010
	<i>Pisum sativum</i> / common:pea	NC_014057	122169	74	34	04/23/2010	12/07/2010
Galegeae	<i>Glycyrrhiza glabra</i>	NC_024038	127943	76	34	05/01/2014	09/29/2014
Genisteae	<i>Lupinus albus</i> / common:white lupine	NC_026681	154140	83	45	03/20/2015	03/20/2015
	<i>Lupinus luteus</i> / common:yellow lupine	NC_023090	151894	83	45	12/20/2013	09/17/2014
Indigofereae	<i>Indigofera tinctoria</i>	NC_026680	158367	82	45	03/20/2015	03/20/2015
Loteae	<i>Lotus japonicus</i> / strain:Accession MG-20	NC_002694	150519	82	45	03/01/2001	04/15/2009
Millettieae	<i>Milletia pinnata</i>	NC_016708	152968	83	45	01/27/2012	01/22/2013
Phaseoleae	<i>Apios americana</i>	NC_025909	148772	82	43	12/22/2014	12/22/2014
	<i>Glycine canescens</i> voucher CSIRO:G1232	NC_021647	152518	82	45	07/23/2013	09/06/2013
	<i>Glycine cyrtoloba</i> voucher CSIRO:G1267	NC_021645	152381	81	45	07/23/2013	09/06/2013
	<i>Glycine dolichocarpa</i> voucher CSIRO:G1134	NC_021648	152804	82	45	07/23/2013	09/06/2013
	<i>Glycine falcata</i> voucher CSIRO:G1718	NC_021649	153023	82	45	07/23/2013	09/06/2013
	<i>Glycine max</i> / common:soybean	NC_007942	152218	83	45	04/07/2006	04/15/2009
	<i>Glycine soja</i>	NC_022868	152217	83	46	11/19/2013	11/19/2013
	<i>Glycine stenophita</i> voucher CSIRO:G1974	NC_021646	152618	82	45	07/23/2013	09/06/2013
	<i>Glycine syndetika</i> voucher CSIRO:G1300	NC_021650	152783	82	45	07/23/2013	09/06/2013
	<i>Glycine tomentella</i> voucher CSIRO:G1403 / type:D3	NC_021636	152728	82	45	07/03/2013	07/03/2013

	<i>Pachyrhizus erosus</i>	NC_026682	151947	83	44	03/20/2015	03/20/2015
	<i>Phaseolus vulgaris</i>	NC_009259	150285	83	44	04/03/2007	04/15/2009
	<i>Vigna angularis</i> DNA / common:adzuki bean	NC_021091	151683	81	44	05/17/2013	05/17/2013
	<i>Vigna radiata</i>	NC_013843	151271	82	44	02/18/2010	07/28/2010
	<i>Vigna unguiculata</i> / common:cowpea	NC_018051	152415	84	46	07/02/2012	07/02/2012
Robinieae	<i>Robinia pseudoacacia</i>	NC_026684	154835	80	45	03/20/2015	03/20/2015
Trifolieae	<i>Medicago truncatula</i> / strain:Variety Jema Long A-17; common:barrel medic	NC_003119	124033	76	33	08/31/2001	04/15/2009
	<i>Trifolium aureum</i>	NC_024035	126970	76	34	04/30/2014	09/29/2014
	<i>Trifolium boissieri</i>	NC_025743	125740	74	36	01/13/2015	01/13/2015
	<i>Trifolium glanduliferum</i>	NC_025744	126149	75	35	12/03/2014	12/03/2014
	<i>Trifolium grandiflorum</i>	NC_024034	125628	75	35	05/01/2014	09/29/2014
	<i>Trifolium meduseum</i>	NC_024166	142595	75	34	06/09/2014	09/30/2014
	<i>Trifolium repens</i> / common:white clover	NC_024036	132120	75	35	04/30/2014	09/29/2014
	<i>Trifolium strictum</i>	NC_025745	125834	75	36	01/29/2015	01/29/2015
	<i>Trifolium subterraneum</i>	NC_011828	144763	74	33	01/06/2009	08/06/2014
	<i>Nicotiana tabacum</i> / common:common tobacco	NC_001879	155943	98	45	11/18/1986	04/15/2009
	<i>Arabidopsis thaliana</i> / common:thale cress	NC_000932	154478	85	44	09/15/1999	03/26/201

Table S6 The PCR primers used in this study. The first two letters in each primer's ID represent the names of the adjacent scaffolds, and the gap between the scaffolds was filled by this primer. F and R represent "Forward" and "Reverse," respectively.

Primer ID	Sequence (5'-3')
AB-F	CCTTAGAGCGGACAGATACC
AB-R	CTAAGCGGGCTCACATAACA
BC-F	AAAGGGCAGAGTGAATAGAAATAC
BC-R	AAACCCGTTGCCTTACC
CD-F	AAGGGCTTATTTGATTC
CD-R	TATATGAGATTTGTCCCTACT
DE-F	ACGCAGAGATAAAGAAATAGAGAA
DE-R	TGGATCGGGGACAAACT
EF-F	TGAAAGGTCCAGTAGAGAT
EF-R	GGATGCACCCCAATGAA
FG-F	GATTCTTTCGCTGACCTATGTTGT
FG-R	CTACTTGCTTTTCTTCGGTCCAC
GA-F	CTATAGATTTACCCGTTGTTGATT
GA-R	CTTATTGATATGACCAGGAAAAA

Figure S1 Alignment of multiple *accD* protein sequences from *A. membranaceus* (Asme) and other representative legume species: *G. glabra* (Glg), *R. pseudoacacia* (Rops), *A. americana* (Apam), *T. strictum* (Trst), *L. japonicus* (Loja), *T. glanduliferum* (Trgl), *L. luteus* (Lulu) and *I. tinctoria* (Inti).

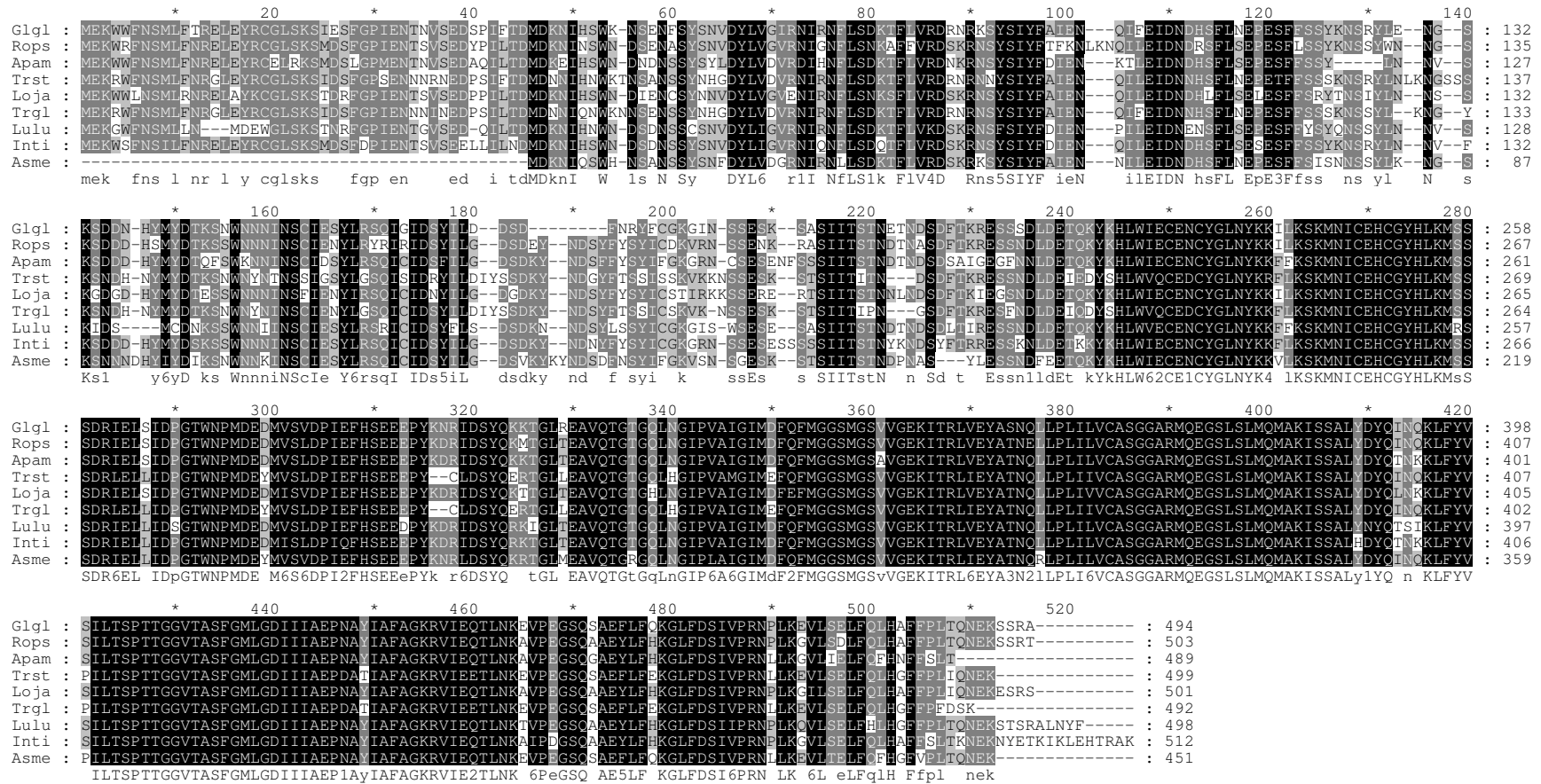


Figure S2 PCR amplification and Sanger sequencing results for the highly polymorphic regions in the *A. membranaceus* genome. Panels A, B, and C shows the three-gap regions between scaffolds, namely, A and B, B and C, and D and E respectively. The left side of each panel shows the agarose gel electrophoresis result, whereas the right side shows the trace files indicating that multiple sequences were obtained from the same PCR bands.

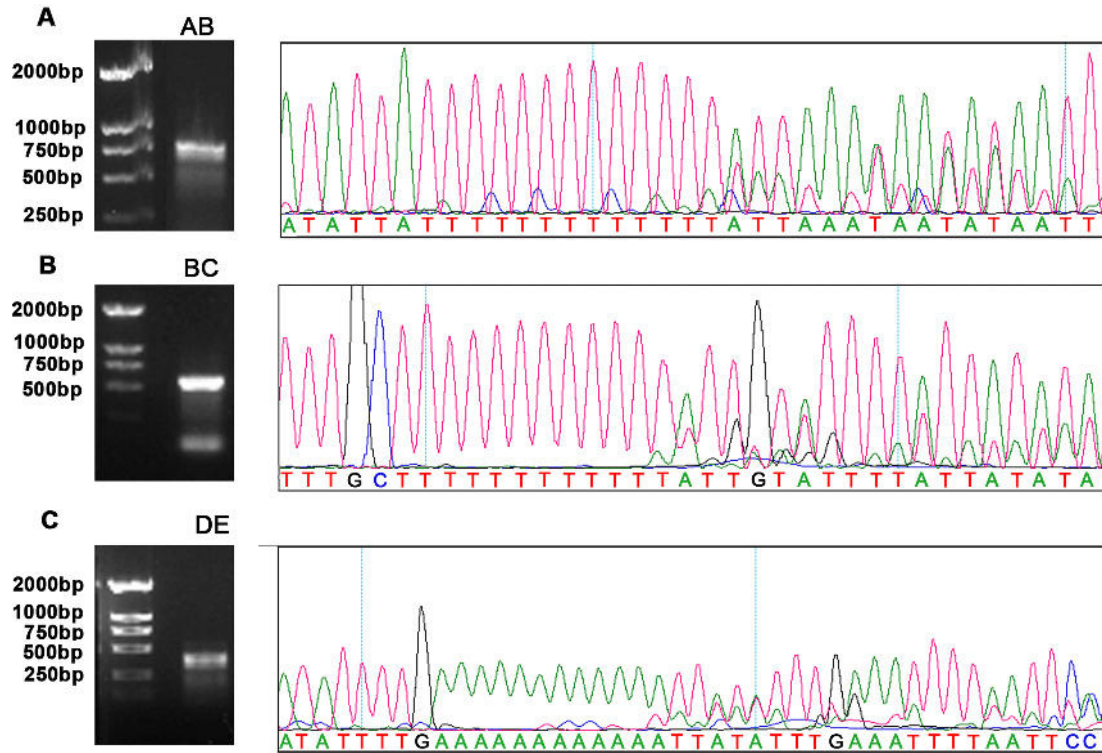


Figure S3 Alignment of the PCR products for the highly polymorphic regions in the *A. membranaceus* genome. All the sequences provided in this file were considered high quality as no overlapping peaks were observed. Panels A, B and C represent the regions AB, BC and DE respectively.

(A) Region AB

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                *      20      *      40      *      60      *      80      *      100      *
AB      : CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 110
AB-i1-c4-F : CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 110
AB-i1-c4-R : CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 110
AB-i1-c11-F : CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 110
AB-i1-c11-R : CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 110
AB-i1-c14-F : CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 110
AB-i1-c14-R : CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 110
AB-i1-c15-F : CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 110
AB-i1-c15-R : CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 110
AB-i1-c16-F : CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 110
AB-i1-c16-R : CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 110
AB-i1-c17-F : CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 110
AB-i1-c17-R : CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 110
AB-i1-c18-F : -CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 109
AB-i1-c18-R : -CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 109
AB-i1-c19-F : CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 110
AB-i1-c19-R : CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 110
AB-i5-c1-F : CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 110
AB-i5-c1-R : CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 110
AB-i5-c2-F : CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 110
AB-i5-c2-R : CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 110
AB-i5-c6-F : CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 110
AB-i5-c6-R : CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 110
AB-i5-c7-F : CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 110
AB-i5-c7-R : CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 110
AB-i5-c11-F : CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 110
AB-i5-c11-R : CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 110
AB-i5-c12-F : CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 110
AB-i5-c12-R : CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 110
AB-i5-c13-F : -CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 109
AB-i5-c13-R : -CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 109
AB-i5-c14-F : CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 110
AB-i5-c14-R : CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 110
AB-i5-c15-F : CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 110
AB-i5-c15-R : CCTTAGAGCGGACAGATACCGACCCATCATTCTAATAATAATATAATAACAAAACAAAAGCAAAAATCATTATTAATTTGCAATTCGATAGAAATATCGAATTTTC : 110

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AB-i5-c2-F : TGAGCCCGCTTAG : 772
AB-i5-c2-R : TGAGCCCGCTTAG : 772
AB-i5-c6-F : TGAGCCCGCTTAG : 772
AB-i5-c6-R : TGAGCCCGCTTAG : 772
AB-i5-c7-F : TGAGCCCGCTTAG : 771
AB-i5-c7-R : TGAGCCCGCTTAG : 771
AB-i5-c11-F : TGAGCCCGCTTAG : 772
AB-i5-c11-R : TGAGCCCGCTTAG : 772
AB-i5-c12-F : TGAGCCCGCTTAG : 772
AB-i5-c12-R : TGAGCCCGCTTAG : 772
AB-i5-c13-F : TGAGCCCGCTTAG : 771
AB-i5-c13-R : TGAGCCCGCTTAG : 771
AB-i5-c14-F : TGAGCCCGCTTAG : 772
AB-i5-c14-R : TGAGCCCGCTTAG : 772
AB-i5-c15-F : TGAGCCCGCTTAG : 772
AB-i5-c15-R : TGAGCCCGCTTAG : 772
AB-i6-c4-F : TGAGCCCGCTTAG : 772
AB-i6-c4-R : TGAGCCCGCTTAG : 772
AB-i6-c7-F : TGAGCCCGCTTAG : 772
AB-i6-c7-R : TGAGCCCGCTTAG : 772
AB-i6-c8-F : TGAGCCCGCTTAG : 773
AB-i6-c8-R : TGAGCCCGCTTAG : 773
AB-i6-c9-F : TGAGCCCGCTTAG : 772
AB-i6-c9-R : TGAGCCCGCTTAG : 772
AB-i6-c11-F : TGAGCCCGCTTAG : 770
AB-i6-c11-R : TGAGCCCGCTTAG : 770
AB-i6-c12-F : TGAGCCCGCTTAG : 772
AB-i6-c12-R : TGAGCCCGCTTAG : 772
AB-i6-c17-F : TGAGCCCGCTTAG : 772
AB-i6-c17-R : TGAGCCCGCTTAG : 772
AB-i7-c11-F : TGAGCCCGCTTAG : 782
AB-i7-c11-R : TGAGCCCGCTTAG : 782
AB-i7-c12-F : TGAGCCCGCTTAG : 782
AB-i7-c12-R : TGAGCCCGCTTAG : 782
AB-i7-c13-F : TGAGCCCGCTTAG : 772
AB-i7-c13-R : TGAGCCCGCTTAG : 772
AB-i7-c14-F : TGAGCCCGCTTAG : 782
AB-i7-c14-R : TGAGCCCGCTTAG : 782
AB-i7-c16-F : TGAGCCCGCTTAG : 781
AB-i7-c16-R : TGAGCCCGCTTAG : 781
AB-i7-c18-F : TGAGCCCGCTTAG : 771
AB-i7-c18-R : TGAGCCCGCTTAG : 771
AB-i7-c19-F : TGAGCCCGCTTAG : 781
AB-i7-c19-R : TGAGCCCGCTTAG : 781
AB-i7-c20-F : TGAGCCCGCTTAG : 782
AB-i7-c20-R : TGAGCCCGCTTAG : 782

tGAGCCCGCTAg

