

Supporting Informations

Röschenbleck et al., – Genus-wide screening reveals four distinct types of structural plastid genome organization in *Pelargonium* (Geraniaceae)

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Supplemental Figures

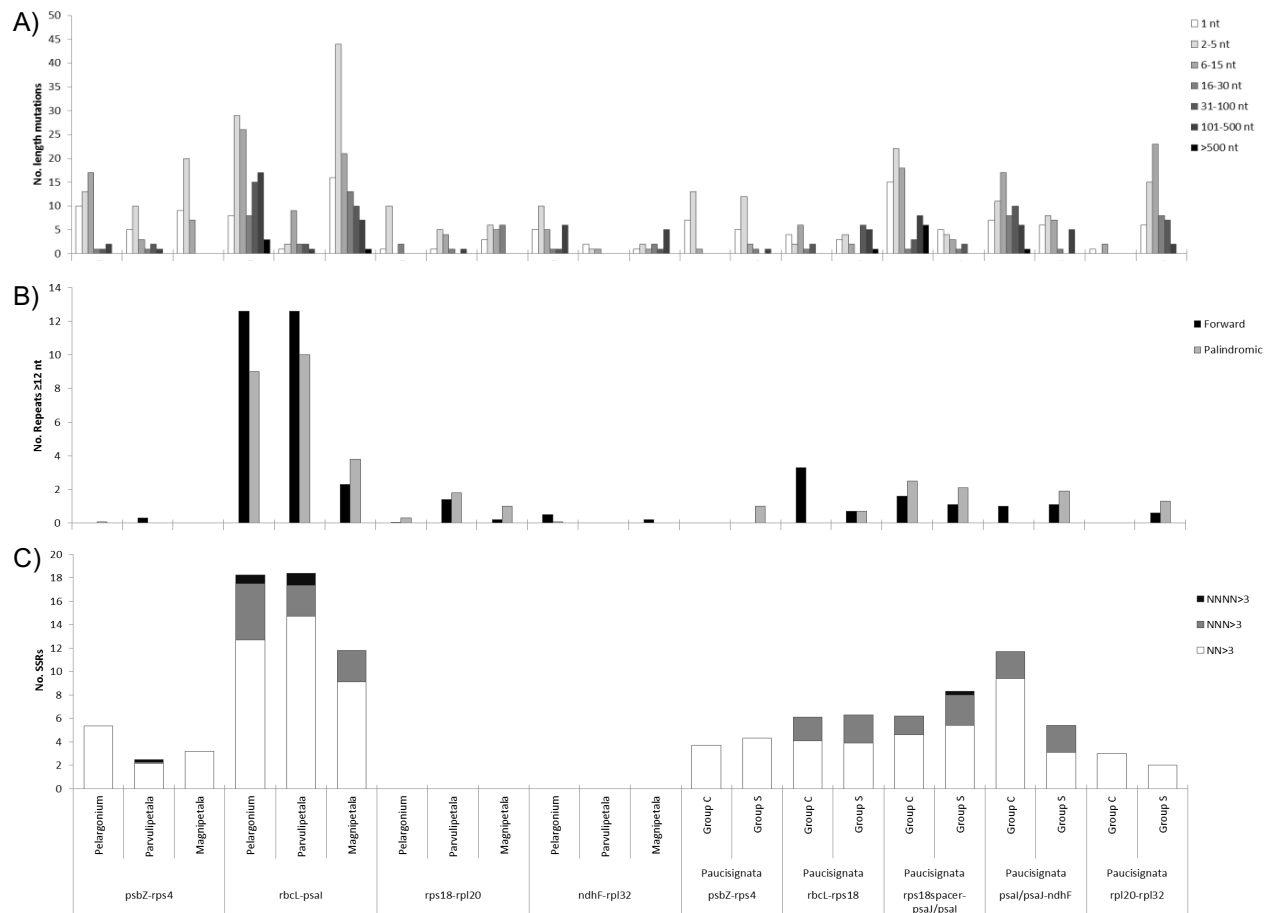


Fig. S1 Microstructural variation in selected plastome regions in *Pelargonium*. (A) Number of length mutations in seven different size classes. (B) Average number of forward and palindromic repeats of at least 12nt length. (C) di-, tri- and tetramer SSRs of at least three repetitions. Note that original data including more detailed metrics (e.g. standard deviations) are deposited in datadryad.org (doi: XXXXXX).

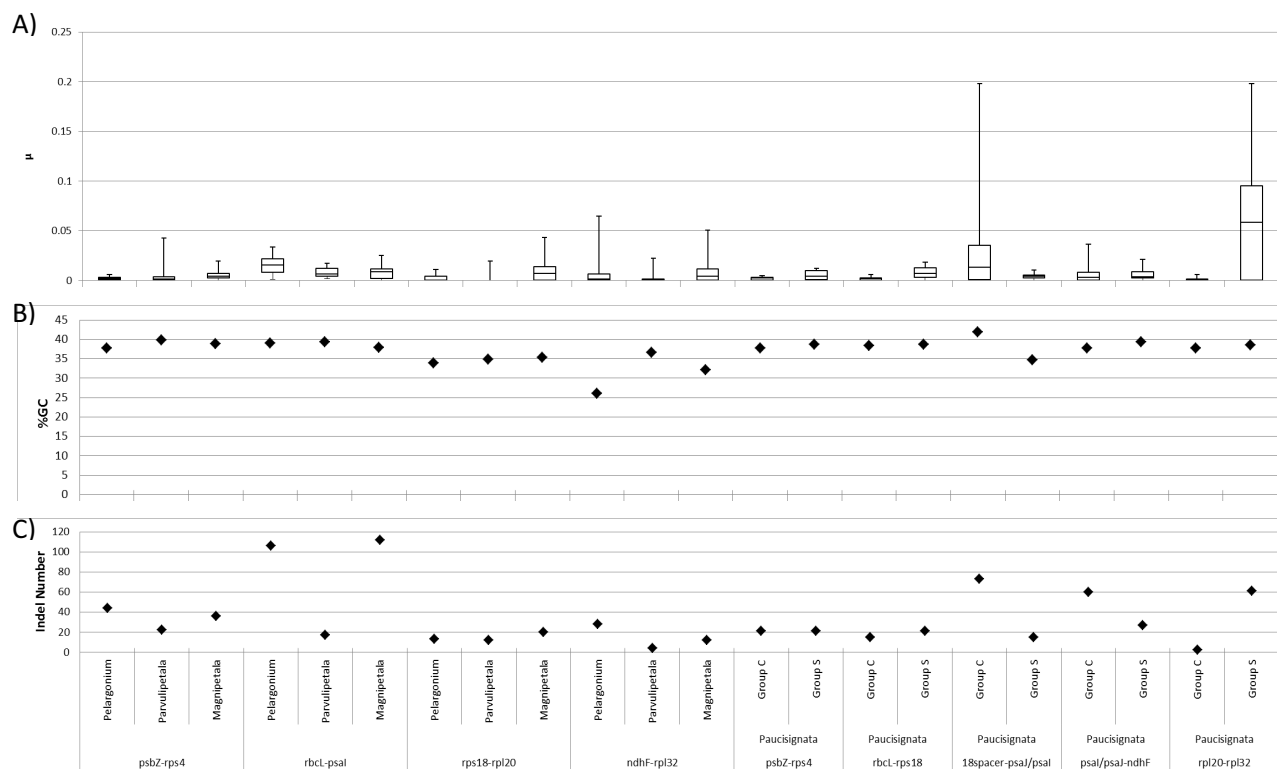


Fig. S2 Data set summary statistics. (a) Nucleotide substitution rates, **(b)** GC content, and **(c)** indel number are given for all regions of the subgenera *Pelargonium*, *Parvulipetala*, *Magnipetala*, and subgroups C and S of *Paucisignata*.

<i>P. trifidum</i> Jacq., MSUN 3179	GB-Nr.	GB-Nr.	GB-Nr.	GB-Nr.	-	-	-	-	-	-	-	-	-	-	-	
<i>P. worcesterae</i> R. Knuth, MSUN 2746	GB-Nr.	GB-Nr.	GB-Nr.	GB-Nr.	-	-	-	-	-	-	-	-	-	-	-	
<i>Paucisignata, Grp C</i>																
<i>P. acetosum</i> (L.) L'Hér., MSUN 526	GB-Nr.	-	-	-	-	GB-Nr.	GB-Nr.	GB-Nr.	-	-	GB-Nr.	GB-Nr.	GB-Nr.	-	-	
<i>P. barklyi</i> Scott-Elliot, MSUN 4037	GB-Nr.	-	-	-	-	GB-Nr.	GB-Nr.	GB-Nr.	-	-	GB-Nr.	GB-Nr.	GB-Nr.	-	-	
<i>P. inquinans</i> (L.) L'Hér., MSUN 530	GB-Nr.	-	-	-	-	GB-Nr.	GB-Nr.	GB-Nr.	-	-	GB-Nr.	GB-Nr.	GB-Nr.	-	-	
<i>P. multibracteatum</i> Hochstetter ex A. Rich., MSUN 2727	GB-Nr.	-	-	-	-	GB-Nr.	GB-Nr.	GB-Nr.	-	-	GB-Nr.	GB-Nr.	GB-Nr.	-	-	
<i>P. peltatum</i> (L.) L'Hér., MSUN 534	GB-Nr.	-	-	-	-	GB-Nr.	GB-Nr.	GB-Nr.	-	-	GB-Nr.	GB-Nr.	GB-Nr.	-	-	
<i>P. ranunculophyllum</i> (Eckl. & Zeyh.) Bak., MSUN 2375	GB-Nr.	-	-	-	-	GB-Nr.	GB-Nr.	GB-Nr.	-	-	GB-Nr.	GB-Nr.	GB-Nr.	-	-	
<i>P. tongaense</i> Vorster, MSUN 4031	GB-Nr.	-	-	-	-	GB-Nr.	GB-Nr.	GB-Nr.	-	-	GB-Nr.	GB-Nr.	GB-Nr.	-	-	
<i>Paucisignata, Grp S</i>																
<i>P. caylae</i> Humbert, MSUN 477	GB-Nr.	-	-	-	-	GB-Nr.	GB-Nr.	-	GB-Nr.	GB-Nr.	-	GB-Nr.	GB-Nr.	-	-	
<i>P. endlicherianum</i> Fenzl, no voucher, s.loc., cult. BG MS	GB-Nr.	-	-	-	-	GB-Nr.	GB-Nr.	-	GB-Nr.	GB-Nr.	-	GB-Nr.	GB-Nr.	-	-	
<i>P. grandicalcaratum</i> R. Knuth, STEU 758	GB-Nr.	-	-	-	-	GB-Nr.	GB-Nr.	-	GB-Nr.	GB-Nr.	-	GB-Nr.	GB-Nr.	-	-	
<i>P. karooicum</i> Compton, MSUN 4040	GB-Nr.	-	-	-	-	GB-Nr.	GB-Nr.	-	GB-Nr.	GB-Nr.	-	GB-Nr.	GB-Nr.	-	-	
<i>P. otaviense</i> R. Knuth, STEU 943	GB-Nr.	-	-	-	-	GB-Nr.	GB-Nr.	-	GB-Nr.	GB-Nr.	-	GB-Nr.	GB-Nr.	-	-	

<i>P. spinosum</i> Willd., STEU 619	GB-Nr.	-	-	-	-	GB-Nr.	GB-Nr.	-	GB-Nr.	GB-Nr.	-	GB-Nr.	GB-Nr.	-	-
<i>P. transvaalense</i> R.Knuth, STEU 1972	GB-Nr.	-	-	-	-	GB-Nr.	GB-Nr.	-	GB-Nr.	GB-Nr.	-	GB-Nr.	GB-Nr.	-	-
<i>Parvulipetala</i>															
<i>P. anceps</i> subsp. nov. ined. ' <i>geniculatum</i> ', MSUN 430	GB-Nr.	GB-Nr.	GB-Nr.	-	GB-Nr.	-	-	-	-	-	-	-	-	-	-
<i>P. album</i> J.J.A. v.d. Walt, MSUN 669	GB-Nr.	n.a.	GB-Nr.	-	GB-Nr.	-	-	-	-	-	-	-	-	-	-
<i>P. australe</i> Willd., MSUN 375	GB-Nr.	n.a.	GB-Nr.	-	GB-Nr.	-	-	-	-	-	-	-	-	-	-
<i>P. cotyledonis</i> (L.) L'Hér., MSUN 4030	GB-Nr.	n.a.	GB-Nr.	-	GB-Nr.	-	-	-	-	-	-	-	-	-	-
<i>P. exstipulatum</i> (Cav.) L'Hér., MSUN 656	GB-Nr.	GB-Nr.	GB-Nr.	-	GB-Nr.	-	-	-	-	-	-	-	-	-	-
<i>P. filicaule</i> R. Knuth, MSUN 310	GB-Nr.	GB-Nr.	GB-Nr.	-	GB-Nr.	-	-	-	-	-	-	-	-	-	-
<i>P. ionidiflorum</i> (Eckl. & Zeyh.) Steud., MSUN 673	GB-Nr.	n.a.	GB-Nr.	-	GB-Nr.	-	-	-	-	-	-	-	-	-	-
<i>P. littorale</i> Hügel, MSUN 249	GB-Nr.	n.a.	GB-Nr.	-	GB-Nr.	-	-	-	-	-	-	-	-	-	-
<i>P. minimum</i> (Cav.) Willd., STEU 4361	GB-Nr.	n.a.	GB-Nr.	-	GB-Nr.	-	-	-	-	-	-	-	-	-	-
<i>P. pseudofumaroides</i> R.Knuth, STEU 2981	GB-Nr.	n.a.	GB-Nr.	-	n.a.	-	-	-	-	-	-	-	-	-	-
<i>P. reniforme</i> subsp. <i>velutinum</i> (Eckl. & Zeyh.) Dreyer, MSUN 2721	GB-Nr.	n.a.	GB-Nr.	-	GB-Nr.	-	-	-	-	-	-	-	-	-	-
<i>P. sidoides</i> DC., MSUN 2666	GB-Nr.	n.a.	GB-Nr.	-	GB-Nr.	-	-	-	-	-	-	-	-	-	-
<i>Pelargonium</i>															

<i>P. magenteum</i> J.J.A. v.d. Walt, MSUN 631	GB-Nr.	GB-Nr.	GB-Nr.	GB-Nr.	-	-	-	-	-	-	-	-	-	GB-Nr.	GB-Nr.
<i>P. nanum</i> L'Hér., MSUN 345	GB-Nr.	n.a.	GB-Nr.	GB-Nr.	-	-	-	-	-	-	-	-	-	GB-Nr.	GB-Nr.
<i>P. oblongatum</i> E.Mey. ex. Harv., MSUN 4027	GB-Nr.	n.a.	GB-Nr.	GB-Nr.	-	-	-	-	-	-	-	-	-	GB-Nr.	GB-Nr.
<i>P. ovale</i> subsp. <i>ovale</i> (Burm.f.) L'Hér., BG Muenster, s.n.	GB-Nr.	GB-Nr.	GB-Nr.	GB-Nr.	-	-	-	-	-	-	-	-	-	GB-Nr.	GB-Nr.
<i>P. radulifolium</i> (Eckl. & Zeyh.) Steud., MSUN 874	GB-Nr.	n.a.	GB-Nr.	GB-Nr.	-	-	-	-	-	-	-	-	-	GB-Nr.	GB-Nr.
<i>P. schizopetalum</i> Sweet, STEU 1873	GB-Nr.	n.a.	GB-Nr.	GB-Nr.	-	-	-	-	-	-	-	-	-	GB-Nr.	GB-Nr.
<i>P. sericifolium</i> J.J.A. v.d. Walt, STEU 1554	GB-Nr.	n.a.	GB-Nr.	GB-Nr.	-	-	-	-	-	-	-	-	-	GB-Nr.	GB-Nr.
<i>P. triandrum</i> E.M. Marais, MSUN 751	GB-Nr.	n.a.	GB-Nr.	GB-Nr.	-	-	-	-	-	-	-	-	-	GB-Nr.	GB-Nr.
<i>P. triste</i> (L.) L'Hér., STEU 1103	GB-Nr.	GB-Nr.	GB-Nr.	GB-Nr.	-	-	-	-	-	-	-	-	-	GB-Nr.	GB-Nr.
<i>P. xerophyton</i> Schltr. ex R. Knuth, MSUN 636	GB-Nr.	n.a.	GB-Nr.	GB-Nr.	-	-	-	-	-	-	-	-	-	GB-Nr.	GB-Nr.

‘-’: not detected; ‘n. a.’: positive result in PCR screening, but not sequenced; MSUN – Herbarium of the University of Muenster; STEU – Herbarium of the University of Stellenbosch; BG – Botanical Garden

Table S2. PCR screening and sequencing details for clades and regions.

Amplicon	No. species	Additional amplicons of flanking genes	Negative control (amplification region)
<i>Pelargonium</i>			
<i>rpoA-petB</i>	24 (24)	-	<i>rbcL</i> (IRa) rev - <i>psbA</i> for, <i>rbcL</i> (IRa) rev- <i>trnI</i> _{CAU} rev
<i>ycf2-rpoA</i>	24 (24)	-	<i>rbcL</i> (IRa) rev - <i>psbA</i> for, <i>rbcL</i> (IRa) rev- <i>trnI</i> _{CAU} rev
<i>ycf3</i> rev- <i>trnE</i> _{UUC} rev	10 (5)	<i>ycf3</i> rev- <i>trnG</i> _{GCC} rev, <i>trnG</i> _{GCC} for- <i>trnE</i> _{UUC} rev	-
<i>psbZ</i> for- <i>rps4</i> for	31 (24)	-	<i>rps4</i> for- <i>ycf3</i> rev
<i>rbcL</i> for- <i>psaI</i> rev	31 (9)	-	<i>rbcL</i> for - <i>psaJ</i> rev
<i>rps18</i> for- <i>rpl20</i> for	28 (24)	<i>psaJ</i> for- <i>rpl20</i> for, <i>psaJ</i> for- <i>rps18</i> rev	-
<i>ndhF</i> rev- <i>rpl32</i> rev	29 (24)	-	<i>ndhF</i> for- <i>rpl32</i> rev
<i>Parvulipetala</i> -			
-	-	-	<i>rbcL</i> (IRa) rev - <i>psbA</i> for, <i>rbcL</i> (IRa) rev- <i>trnI</i> _{CAU} rev
<i>psbZ</i> for- <i>rps4</i> for	12 (12)	-	<i>rps4</i> for- <i>ycf3</i> rev
<i>rbcL</i> for- <i>psaI</i> rev	15 (3)	-	<i>rbcL</i> for- <i>rps18</i> rev, <i>rbcL</i> for- <i>psaJ</i> for
<i>rps18</i> for- <i>rpl20</i> for	16 (12)	<i>psaJ</i> for- <i>rpl20</i> for, <i>psaJ</i> for- <i>rps18</i> rev	<i>rpl20</i> for- <i>rpl32</i> for
<i>ndhF</i> for- <i>rpl32</i> rev	13 (11)	-	<i>ndhF</i> rev- <i>rpl32</i> rev
<i>Magnipetala</i>			
<i>ycf3</i> rev- <i>trnE</i> _{UUC} rev	9 (2)	<i>ycf3</i> rev- <i>trnG</i> _{GCC} rev, <i>ycf3</i> rev- <i>trnY</i> _{GUA} rev	-
<i>psbZ</i> for- <i>rps4</i> for	15 (10)	-	<i>rps4</i> for- <i>ycf3</i> rev
<i>rbcL</i> for- <i>psaI</i> rev	15 (10)	-	<i>rbcL</i> for- <i>rps18</i> rev, <i>trnN</i> _{GUU} for- <i>psaI</i> rev
<i>rps18</i> for- <i>rpl20</i> for	16 (10)	<i>psaJ</i> for- <i>rpl20</i> for, <i>psaJ</i> for- <i>rps18</i> rev	<i>rpl20</i> for- <i>rpl32</i> for
<i>ndhF</i> rev- <i>rpl32</i> rev	16 (10)	<i>ndhF</i> for- <i>trnN</i> _{GUU} rev	<i>ndhF</i> for- <i>trnN</i> _{GUU} for, <i>ndhF</i> rev- <i>rpl32</i> rev
<i>Paucisignata</i>			
<i>rbcL</i> (IRa) rev- <i>psbA</i> for	14 (0)	<i>rbcL</i> (IRa) rev- <i>trnI</i> _{CAU} rev	-
<i>ycf3</i> rev- <i>trnE</i> _{UUC} rev	11 (8)	<i>ycf3</i> rev- <i>trnG</i> _{GCC} rev, <i>trnG</i> _{GCC} for- <i>trnE</i> _{UUC} rev	-
<i>psbZ</i> for- <i>rps4</i> for	14 (14)	-	<i>rps4</i> for- <i>ycf3</i> rev
<i>rbcL</i> for (IRb)- <i>rps18</i> rev	17 (14)	-	-
<i>rpl20</i> rev- <i>rpl32</i> rev	15 (14)	-	<i>ndhF</i> rev- <i>rpl32</i> rev
<i>Paucisignata – Grp. C</i>			
<i>rps18</i> for- <i>psaJ</i> for	9 (7)	<i>rbcL</i> for - <i>psaJ</i> for	<i>rps18</i> for- <i>psaI</i> rev
<i>ndhF</i> for- <i>psaI</i> rev	7 (7)	<i>ndhF</i> for- <i>trnN</i> _{GUU} rev, <i>trnN</i> _{GUU} for- <i>psaI</i> rev	<i>ndhF</i> for- <i>psaJ</i> for, <i>ndhF</i> for- <i>rps18</i> for, <i>rps18</i> rev- <i>psaJ</i> for
<i>Paucisignata – Grp. S</i>			
<i>rps18</i> for - <i>psaI</i> rev	7 (7)	<i>rbcL</i> for- <i>psaI</i> rev	<i>rps18</i> for- <i>psaJ</i> for
<i>ndhF</i> for- <i>psaJ</i> for	7 (7)	<i>ndhF</i> for- <i>trnN</i> _{GUU} rev, <i>trnN</i> _{GUU} for- <i>psaJ</i> for, <i>ndhF</i> for- <i>psaI</i> rev, <i>trnN</i> _{GUU} for- <i>psaI</i> rev	<i>trnN</i> _{GUU} for- <i>rps18</i> for, <i>rps18</i> rev- <i>psaJ</i> for

Numbers in brackets in column 2 are the number of species sequences. for – forward primer, rev – reverse primer;

Table S3. PCR settings including primer primers used in PCR screening and sequencing.

Region	Primer ¹	Primer sequence 5'→3'	Orientation	Cycles, Annealing, Elongation conditions
<i>rbcL-psbA</i>	rbcL-A	GTATCCTTGGTTTCATAATCAGG	Rev	43 x, A 60°C 30sec, E 72°C 1m
	psbA	CCTTGGTATGGAAGTTATGCATG	Rev	
	rbcL-A	GTATCCTTGGTTTCATAATCAGG	Rev	43 x, A 60°C 30sec, E 72°C 1m
	trnI	GTTGGGTGCTTTAACCATTTCAGC	Rev	
<i>psbZ-rps4</i>	rps4	CATAGAATATTATTCTCGTCAGATTTACCC	For	40x, A 54°C 0,45m, E 72°C 1,5m
	psbZ	GTACCCGTTGTATTTGCTTCTC	For	45x, A 54°C 0,45m, E 72°C 1,5m
	rps4	CATAGAATATTATTCTCGTCAGATTTACCC	For	40x, A 54°C 0,45m, E 72°C 1,5m
	ycf3	GGTCTTATCAATAAAAATTTCCATT	Rev	
<i>trnE-ycf3</i>	ycf3	GGTCTTATCAATAAAAATTTCCATT	Rev	45x, A 54°C 1m, E 72°C 2m
	trnE	GAGAGATGTCTGAACCACTAGACGATG	Rev	45x, A 56°C 1m, E 72°C 2,15m
	ycf3-2	GCCTCCCTTTCTCCTGAAGTTGTAGG	Rev	45x, A 54°C 1m, E 72°C 2,15m
	trnG	TCTCTTTGCCAAGGAGAAGACG	For	40x, A 54°C 0.45m, E 72°C 2,15m
	ycf3	GGTCTTATCAATAAAAATTTCCATT	Rev	38x, A54°C 0,45m, E 72°C 1,15m
	trnG	CGTCTTCTCCTTGGCAAAGAGA	Rev	
<i>rbcL-psaI</i>	rbcL-B	GAAGCATGTGTACAAGCTCGTAATG	For	38 x, A 54°C 1,5m, E 72°C 3,5m; 4m; 4,5m
	psaI	TGAAGAAATAAAGAAGCCATTGC	Rev	45 x, A 54°C 1,5m, E 72°C 3,5m; 4m; 4,5m
	rbcL-B2	GTGCCGTAGCTAATCGAGTAGC	For	45x, A 54°C 1m, E 72°C 2m
	IR-1	TCAATTACTCTTTTACCTGCAAATGCAAT	Rev	45x, A 56°C 1m, E 72°C 2m
	IR-2.1	GATTGAAATCGGGATCACCGCTTAC	Rev	
	IR-2.2	CTCTGAAGAGTCTTCAGATTTGT	Rev	
	psaI2	GCAAATGCCGAAATACTAGGC	Rev	
	RI-1.1	ACGTCCTTTTCGTTTCGTGTTGC	For	45x, A 58°C 1m, E 72°C 2m
	RI-2	CATAATCCTAGTGATTGGATTTATATAC	For	45x, A 58°C 1m, E 72°C 1,5m
	RI-1.2	TGTTTCCCCTTTGATTTCAAGTTGA	For	
	RI-3	GTTGACAAAGGTCTCTATATTCACTATAC	For	
	RI-4	GACTTCTACTTATGAAGAACTCAATTGTG	For	
	IR-2.2	CTCTGAAGAGTCTTCAGATTTGT	Rev	
	RI-8	ACAAATCTGAAGACTCTTCAGAG	For	45x, A 58°C 1m, E 72°C 2m
	RI-4	GACTTCTACTTATGAAGAACTCAATTGTG	For	
	psaI	TGAAGAAATAAAGAAGCCATTGC	Rev	
	RI-1.1	ACGTCCTTTTCGTTTCGTGTTGC	For	45x, A58°C 0,45m, E 72°C 1,5m
	RI-1.2	TGTTTCCCCTTTGATTTCAAGTTGA	For	
	RI-3	GTTGACAAAGGTCTCTATATTCACTATAC	For	
	RI-4	GACTTCTACTTATGAAGAACTCAATTGTG	For	

	RI-5	GATCCCATTGAGTGGGATTCAGA	For	
	IR-3	GAATAGCTTCAGGCAATCCCGTG	Rev	
	RI-9	ATTGCATTTGCAGGTAAAAGAGTAATTGA	For	45x, A58°C 0,45m, E 72°C 1,5m
	RI-7	GATTGCCTGAAGCTATTCAAACAGG	For	
	psaI	TGAAGAAATAAAGAAGCCATTGC	Rev	
	rbcL-B	GAAGCATGTGTACAAGCTCGTAATG	For	45x, A58°C 0,45m, E 72°C 1,5m
	RI-1	ACGTCCTTTTCGTTTCGTGTTGC	For	
	IR-5	GATGTCATCAACGAAAAGAGGCAATTC	Rev	
	RI-6	CTCTACTTAGCACTCCTTGCATCTC	For	45x, A58°C 0,45m, E 72°C 1,5m
	psaI	TGAAGAAATAAAGAAGCCATTGC	Rev	
	RI-5	GATCCCATTGAGTGGGATTCAGA	For	45x, A58°C 1m, E 72°C 2m
	IR-4	CTATCCAGATACGCAAGACAAAGAGC	Rev	
<i>rbcL-rps18</i>	rbcL-B,	GAAGCATGTGTACAAGCTCGTAATG	For	45x, A 56°C 0,45m, E 72°C 1,5m
	rps18	GTTTTAAAGTCACTCTATTCACCCGTCTAG	Rev	45x, A 54°C 0,45m, E 72°C 1,5m
	rbcL-B2	GTGCCGTAGCTAATCGAGTAGC	For	40x, A 54°C 0,45m, E 72°C 1,5m
	RI-1.1	ACGTCCTTTTCGTTTCGTGTTGC	For	40x, A 54°C 1m, E 72°C 1,45m
	R18-1	GAGTTCATAATCCTAGTGATTAGTCCT	For	40x, A 54°C 1m, E 72°C 1,5m
	18R-1	GTTGAATGAATATCTGCTGCCGCGTC	Rev	38x, A 54°C 1m, E 72°C 1,5m
<i>rpoA-ycf2</i>	rpoA-f	ATAGATGCCGTCTTCACACCC	Rev	40x, A 54°C 1,5m, E 72°C 3,5m
	ycf2-f	GATGGATTTTCCCGGATGAAATGAAA	For	
<i>rpoA-petB</i>	petB_f	CTTAGCGCTGTATTTATGTTAATCC	For	40x, A 54°C 1,5m, E 72°C 3,5m
	rpoA-f	ATAGATGCCGTCTTCACACCC	Rev	
<i>rps18-psaJ</i>	rbcL-B	GAAGCATGTGTACAAGCTCGTAATG	For	38x, A 54°C 1,5m, E 72°C 4m
	psaJ	GCAGGTCTATTGATAGAGATTAA	For	
	rps18	CTAGACGGGTGAATAGAGTGACTTTAAAAC	For	45x, A 56°C 1,5, E 72°C 4m
	psaJ	GCAGGTCTATTGATAGAGATTAA	For	40x, A 54°C 1,5m, E 72°C 3,5m
				38x, A 54°C 1,5m, E 72°C 3,5m
	RJ-1	CTACCTACTATCCACACGAGGATAATC	For	45x, A 56°C 1m, E 72°C 2m
	psaJ	GCAGGTCTATTGATAGAGATTAA	For	45x, A 54°C 1m, E 72°C 2m
	J18-1	CGTTGTATATCTTCCCAATTGTCTAC	For	45x, A 54°C 1m, E 72°C 2,5m
	J18-2.1	TGTATTCATTGGTTGTACAGATAGAGG	For	45x, A 54°C 1m, E 72°C 2,5m
	J18-2.2	CGGCTGTGCTATCATTCTTCTACC	For	45x, A 54°C 0,45m, E 72°C 1,5m
				43x, A 54°C 0,45m, E 72°C 2,5m
				40x, A 54°C 0,45m, E 72°C 2,5m
	R18-1	GAGTTCATAATCCTAGTGATTAGTCCT	For	45x, A 54°C 1m, E 72°C 2,5m
	psaJ	GCAGGTCTATTGATAGAGATTAA	For	
	rps18V	CGCACTGAATTTATCGCAACCAAG	For	40x, A 54°C 0,45m, E 72°C 2,5m

	rps18H	CGAGCTAGCAATTATCCGACACGCC	Rev	
	rps18V2	GGCTACGCACTGAATTTATCGC	For	
<i>rps18-psaI</i>	rps18	GTTTTAAAGTCACTCTATTCACCCGTCTAG	For	45x, A 54°C 0,45m, E 72°C 1,5m
	psaI-2	GCAAATGCCGGAATACTAGGC	Rev	40x, A54°C 0,45m, E 72°C 2,5m
	rps18V	CGCACTGAATTTATCGCAACCAAG	For	
	R18-1	GAGTTCATAATCCTAGTGATTAGTCCT	For	45x, A 54°C 1m, E 72°C 2,5m
	psaI,	TGAAGAAATAAAGAAGCCATTGC	Rev	40x, A 54°C 0,45m, E 72°C 1,5m
	psaI-2	GCAAATGCCGGAATACTAGGC	Rev	
	rps18-1.1	CGCACTGAATTTATCGCAACCAAG	For	40x, A 54°C 0,45m, E 72°C 2,5m
	R0	CGAGCTAGCAATTATCCGACACGCC	Rev	
	rps18-1.2	GGCTACGCACTGAATTTATCGC	For	
	rps18	GTTTTAAAGTCACTCTATTCACCCGTCTAG	For	45x, A 58°C 1m, E 72°C 2,5m
	psaI	TGAAGAAATAAAGAAGCCATTGC	Rev	45x, A 56°C 0,45m, E 72°C 2m
	rps18-1.1	CGCACTGAATTTATCGCAACCAAG	For	45x, A 56°C 0,45m, E 72°C 1,5m
	rps18-1.2	GGCTACGCACTGAATTTATCGC	For	45x, A 54°C 0,45m, E 72°C 2,5m
	rps18-1.3	CTAGACGGGTGAATAGAGTGAC	For	45x, A 54°C 1m, E 72°C 2,5m
	18I-1	CCG GTAGAAAGSGATTTCCCTAATG	For	45x, A 54°C 1m, E 72°C 2,5m
	18I-2	GTAGAAAGSGATTTCCCTAATG	For	45x, A 54°C 0,45m, E 72°C 1,5m
	18I-3	GTTCAAGGTACGCGATCCA	For	45x, A 54°C 0,45m, E 72°C 2m
	psaI-2	GCAAATGCCGGAATACTAGGC	Rev	43x, A 54°C 0,45m, E 72°C 2,5m
	I18-1	CATTTCTCCGAAGGTCGCTTTCTC	Rev	40x, A 54°C 0,45m, E 72°C 2m
	I18-2	AGATACGCAAGACAAAGAGCAT	Rev	
	I18-3	CTTCCAGTCTTCTGCCATTATACCCAG	Rev	
	I18-4	GAAATTGAAATTCACAAGACTGTCCG	Rev	
	I18-5	GACTTGCCAATCCAATTCTTTG	Rev	
	I18-6	TGGTAAGGGTATTGAGGCTAA	Rev	
<i>rps18-rpl20</i>	rps18	CTAGACGGGTGAATAGAGTGACTTTAAAAC	For	38x, A 54°C 0,45m, E 72°C 3m
	rpl20	CTTGCACAAATAGCTATCTCAAATAGG	For	38x, A 54°C 0,45m, E 72°C 2m
				45x, A 56°C 0,45m, E 72°C 1,5m
				45x, A 58°C 0,45m, E 72°C 1,5m
	rps18	GTTTTAAAGTCACTCTATTCACCCGTCTAG	Rev	38x, A 54°C 0,45m, E 72°C 3m
	psaJ	GCAGGTCTATTGATAGAGATTAA	For	45x, A56°C 1m, E 72°C 1,5m
				40x, A 54°C 0,45m, E 72°C 1m
	psaJ	GCAGGTCTATTGATAGAGATTAA	For	38x, A 54°C 0,45m, E 72°C 3m
	rpl20	CTTGCACAAATAGCTATCTCAAATAGG	For	38x, A 54°C 0,45m, E 72°C 2m
				45x, A 56°C 0,45m, E 72°C 1,5m
<i>psaI-ndhF</i>	ndhF	GATGCGCGAGTTATTGATGGAATTAC	For	38x, A54°C 1,5m, E 72°C 3,5m

	psaI	TGAAGAAATAAAGAAGCCATTGC	Rev	
	ndhF-2	GAGTTACGAAGGAACTTCGAGC	For	45x, A 56°C 0,45m, E 72°C 2m
	psaI	TGAAGAAATAAAGAAGCCATTGC	Rev	45x, A 56°C 1m, E 72°C 1,5m
	trnN	GACTGGTCGTAGGTTCGAATC	For	45x, A 54°C 1m, E 72°C 1,5m
	trnN-2	CCTCAGTAGCTCAGTGGTAGAGC	For	45x, A 54°C 0,45m, E 72°C 1,5m
	trnN-3	TTCGGCTGTTAACCGACTG	For	40x, A 54°C 0,40m, E 72°C 1m
	psaI-2	GCAAATGCCGGAATACTAGGC	Rev	40x, A 54°C 0,45m, E 72°C 1m
	IN-1	GTTGAATTTCGAAAGGCTCTCATCACTG	Rev	40x, A 54°C 0,45m, E 72°C 1,2m
<i>psaJ-ndhF</i>	ndhF	GATGCGCGAGTTATTGATGGAATTAC	For	45x, A 56°C 1m, E 72°C 1,5m
	psaJ	GCAGGTCTATTGATAGAGATTAA	For	45x, A 54°C 1m, E 72°C 1,5m
	ndhF-22	GAGTTACGAAGGAACTTCGAGC	For	
	ndhF	GATGCGCGAGTTATTGATGGAATTAC	For	45x, A 54°C 0,45m, E 72°C 1,5m
	psaJ	GCAGGTCTATTGATAGAGATTAA	For	45x, A 56°C 0,45m, E 72°C 1,5m
	ndhF-2	GAGTTACGAAGGAACTTCGAGC	For	40x, A 54°C 0,45m, E 72°C 1,5m
	trnN	GACTGGTCGTAGGTTCGAATC	For	40x, A 54°C 0,45m, E 72°C 1,2m
	trnN-2	CCTCAGTAGCTCAGTGGTAGAGC	For	40x, A 54°C 1m, E 72°C 2,5m
	trnN-3	TTCGGCTGTTAACCGACTG	For	40x, A 54°C 0,45m, E 72°C 2,5m
	rps18	CTAGACGGGTGAATAGAGTGACTTTAAAAC	For	
	JN-1	CATGTCAGACTATATCTTCGATCTTTAAGTG		
	JN-2	CGTCACCTATGGCGTTCTTCCGAACG		
<i>trnN-ndhF</i>	ndhF	GATGCGCGAGTTATTGATGGAATTAC	For	38x, A 54°C 0,45m, E 72°C 1,5m
	trnN	GCTCTACCACTGAGCTACTGAGG	Rev	45x, A 56°C 0,45m, E 72°C 1,5m
<i>rpl20-rpl32</i>	rpl20	CTTGACAAATAGCTATCTCAAATAGG	For	40x, A 54°C 0,45m, E 72°C 1,5m
	rpl32	CAAAGCTTTCAACGCCGCCCAATG	Rev	45x, A 56°C 0,45m, E 72°C 1,5m
	rpl20-2	GATTTTCGTCGTTTGTGGATCACTC	For	45x, A 60°C 0,45m, E 72°C 1,5m
	rpl32	CAAAGCTTTCAACGCCGCCCAATG	Rev	45x, A 54°C 0,45m, E 72°C 1,5m
<i>ndhF-rpl32</i>	ndhF	GCATAATCCATGAATATTGATATATATGTTCCAT	Rev	40x, A 54°C 0,45m, E 72°C 1,5m
	rpl32	CAAAGCTTTCAACGCCGCCCAATG	Rev	45x, A 56°C 0,45m, E 72°C 1,5m
				45x, A 54°C 0,45m, E 72°C 1,5m
	ndhF	GATGCGCGAGTTATTGATGGAATTAC	For	40x, A 54°C 0,45m, E 72°C 1,5m
	rpl32	CAAAGCTTTCAACGCCGCCCAATG	Rev	45x, A 56°C 0,45m, E 72°C 1,5m
				45x, A 54°C 0,45m, E 72°C 1,5m
	ndhF	GATGCGCGAGTTATTGATGGAATTAC	Rev	38x, A 54°C 0,45m, E 72°C 1,5m
	trnN-2	CCTCAGTAGCTCAGTGGTAGAGC	For	

Primers under the primary primer pair are internal primers used additionally for sequencing.

Table S4. Reconstructed gene order of 111 unique plastid genes in *Pelargonium*.

Subgroup	Genome arrangement
Subg. <i>Magnipetala</i> and <i>Pelargonium</i>	86 -85 84 -1 -2 -3 -4 -5 -6 7 8 -9 10 11 -12 -13 -14 -15 -16 -17 -18 -19 20 21 -22 -23 <u>-24 -25 -30 35 34 33 32 31 26 27 -28 29 36 -37</u> -38 39 40 -41 - 42 -43 -44 45 -46 -47 <u>48 49</u> 50 51 52 -53 -54 -55 -56 57 58 -59 -60 <u>61 62</u> <u>63 -64 -65 -66 67 68 -69 70 71 72 -73 -74 -75 -76 -77 -78 -79 -80 -81 -82</u> -83 -87 -88 -89 90 91 92 93 94 95 96 97 <u>-98 -99 100 111 110 109 108</u> 107 106 105 104 103 -102 -101
Subg. <i>Paucisignata</i>	84 -1 -2 -3 -4 -5 -6 7 8 -9 10 11 -12 -13 -14 -15 -16 -17 -18 -19 20 21 -22 -23 -24 -25 -30 35 34 33 32 62 31 26 27 -28 29 36 -37 -38 39 40 -41 -42 - 43 -44 45 -46 -47 <u>48 63 -61 60 59 -58 -57 56 55 54 53 -52 -51 -50 -49 -</u> <u>98 -99 -97 -96 -95 -94 -93 -92 -91 -90 89 88 87 83 82 81 80 79 78 77 76</u> <u>75 74 73 86 85 -72 -71 -70 69 -68 -67 66 65 64 100 111 110 109</u> 101 102 -103 -104 -105 -106 -107 -108
Group C	84 -1 -2 -3 -4 -5 -6 7 8 -9 10 11 -12 -13 -14 -15 -16 -17 -18 -19 20 21 -22 -23 -24 -25 -30 35 34 33 32 62 31 26 27 -28 29 36 -37 -38 39 40 -41 -42 - 43 -44 45 -46 -47 <u>48 63 -61 60 59 -58 -57 55 56 54 53 -52 -51 -50 -49 -</u> <u>98 -99 -97 -96 -95 -94 -93 -92 -91 -90 89 88 87 83 82 81 80 79 78 77 76</u> <u>75 74 73 86 85 -72 -71 -70 69 -68 -67 66 65 64 100 111 110 109</u> 101 102 -103 -104 -105 -106 -107 -108
Group S	84 -1 -2 -3 -4 -5 -6 7 8 -9 10 11 -12 -13 -14 -15 -16 -17 -18 -19 20 21 -22 -23 -24 -25 -30 35 34 33 32 62 31 26 27 -28 29 36 -37 -38 39 40 -41 -42 - 43 -44 45 -46 -47 <u>48 63 49 50 51 52 -53 -54 -55 -56 57 58 -59 -60 61 -98</u> <u>-99 -97 -96 -95 -94 -93 -92 -91 -90 89 88 87 83 82 81 80 79 78 77 76 75</u> <u>74 73 86 85 -72 -71 -70 69 -68 -67 66 65 64 100 111 110 109</u> 101 102 - 103 -104 -105 -106 -107 -108
Subg. <i>Parvulipetala</i>	86 -85 84 -1 -2 -3 -4 -5 -6 7 8 -9 10 11 -12 -13 -14 -15 -16 -17 -18 -19 20 21 -22 -23 -24 -25 -30 35 34 33 32 31 26 27 -28 29 36 -37 -38 39 40 -41 - 42 -43 -44 45 -46 -47 <u>48 49</u> 50 51 52 -53 -54 -55 -56 57 58 -59 -60 <u>61 62</u> <u>63 -64 -65 -66 67 68 -69 70 71 72 -73 -74 -75 -76 -77 -78 -79 -80 -81 -82</u> -83 -87 -88 -89 90 91 92 93 94 95 96 97 <u>99 98 100 111 110 109 108 107</u> 106 105 104 103 -102 -101

Bold numbers – genes with an altered position compared to *Melianthus*; +/- – strand orientation; underlined numbers – regions covered in PCR-screening; Gene codes: 1=*trnH*_{GUG}; 2=*psbA*; 3=*matK*; 4=*trnK*_{UUU}; 5=*rps16*; 6=*trnQ*_{UUG}; 7=*psbK*; 8=*psbI*; 9=*trnS*_{GCU}; 10=*trnG*_{UCC}; 11=*trnR*_{UCU}; 12=*atpA*; 13=*atpF*; 14=*atpH*; 15=*atpI*; 16=*rps2*; 17=*rpoC2*; 18=*rpoC1*; 19=*rpoB*; 20=*trnC*_{GCA}; 21=*petN*; 22=*psbM*; 23=*trnD*_{GUC}; 24=*trnY*_{GUA}; 25=*trnE*_{UUC}; 26=*psbD*; 27=*psbC*; 28=*trnS*_{UGA}; 29=*psbZ*; 30=*trnG*_{GCC}; 31=*trnM*_{CAU}; 32=*rps14*; 33=*psaB*; 34=*psaA*; 35=*ycf3*; 36=*trnS*_{GGA}; 37=*rps4*; 38=*trnT*_{UGU}; 39=*trnL*_{UAA}; 40=*trnF*_{GAA}; 41=*ndhJ*; 42=*ndhK*; 43=*ndhC*; 44=*trnV*_{UAC}; 45=*trnM*_{CAU}; 46=*atpE*; 47=*atpB*; 48=*rbcL*; 49=*psaI*; 50=*ycf4*; 51=*cemA*; 52=*petA*; 53=*psbJ*; 54=*psbL*; 55=*psbF*; 56=*psbE*; 57=*petL*; 58=*petG*; 59=*trnW*_{CCA}; 60=*trnP*_{UGG}; 61=*psaJ*; 62=*rpl33*; 63=*rps18*; 64=*rpl20*; 65=*rps12* '5; 66=*clpP*; 67=*psbB*; 68=*psbT*; 69=*psbN*; 70=*psbH*; 71=*petB*; 72=*petD*; 73=*rpoA*; 74=*rps11*; 75=*rpl36*; 76=*rps8*; 77=*rpl14*; 78=*rpl16*; 79=*rps3*; 80=*rpl22*; 81=*rps19*; 82=*rpl2*; 83=*rpl23*; 84=*trnI*_{CAU}; 85=*ycf2*; 86=*trnL*_{CAA}; 87=*ndhB*; 88=*rps7*; 89=*rps12* '3; 90=*trnV*_{GAC}; 91=*rrn16*; 92=*trnI*_{GAU}; 93=*trnA*_{UGC}; 94=*rrn23*; 95=*rrn4.5*; 96=*rrn5*; 97=*trnR*_{ACG}; 98=*trnN*_{GUU}; 99=*ndhF*; 100=*rpl32*; 101=*trnL*_{UAG}; 102=*ccsA*; 103=*ndhD*; 104=*psaC*; 105=*ndhE*; 106=*ndhG*; 107=*ndhI*; 108=*ndhA*; 109=*ndhH*; 110=*rps15*; 111=*ycf1*.

Table S5. Length range and mean (brackets) for 10 plastome regions of *Pelargonium*.

Subgenus	<i>psbZ-rps4</i>	<i>rbcL-psaI</i>	<i>rbcL-rps18</i>	18S spacer- <i>psaJ</i>	18s spacer- <i>psaI</i>	<i>rps18-rpl20</i>	<i>psaI-ndhF</i>	<i>psaJ-ndhF</i>	<i>ndhF-rpl32</i>	<i>rpl20-rpl32</i>
<i>Pelargonium</i>	1037-1262 (1222)	4382-5258 (4780)	-	-	-	456-475 (459)	-	-	520-897 (602)	-
<i>Parvulipetala</i>	870-1029 (915)	3770-4013 (3922)	-	-	-	475-653 (505)	-	-	869-881 (876)	-
<i>Magnipetala</i>	1106-1139 (1119)	1219-3849 (3129)	-	-	-	420-475 (453)	-	-	408-869 (508)	-
<i>Paucisignata</i> , Group C	1256-1271 (1261)	-	2261-2425 (2236)	1329-3222 (2345)	-	-	2684-3477 (2911)	-	-	701-713 (703)
<i>Paucisignata</i> , Group S	668-1271 (1005)	-	1674-2163 (1937)	-	1786-2460 (2142)	-	-	1836-5111 (2441)	-	702-1312 (929)

Table S6. Substitution rate, nucleotide divergence, and GC-content in unaltered and rearranged plastome regions.

	Conserved regions	Rearranged regions	<i>Pelargonium</i>	<i>Parvulipetala</i>	<i>Magnipetala</i>	<i>Paucisignata</i> , Group C	<i>Paucisignata</i> , Group S
GC content [%]	34.8	38.3	34.2	37.6	36.1	38.7	38.0
Divergence [%]	2.9	3.3	2.2	1.4	4.1	2.5	5.2
Substitution rate [μ]	0.0078	0.0098	0.0064	0.0041	0.0086	0.0082	0.016

Table S7. Average length, number, and proportion [%] of repeat-rich segments of accD-like regions.

	Total Length	Sum <i>accD</i>	<i>accD1</i> (range)	<i>accD2</i> (range)	<i>accD3</i> (range)	Interspersed region 1 (%length repeats)	Interspersed region 2 (% length repeats)
<i>Pelargonium</i>	3285	1167	322 (318-324)	211 (211-211)	634 (634-634)	728 (34)	1390 (53)
<i>Parvulipetala</i>	2827	887	42 (42-42)	211 (211-211)	634 (634-634)	806 (37)	1134 (43)
<i>Magnipetala</i>	2304	1089	277 (88-317)	182 (153-211)	630 (595-654)	314 (0)	901 (12)
Subclade of <i>Magnipetala</i>	234	234	-	-	234 (234-234)	-	-

Table S8. Summary statistics of sequence comparison for accD segments from blastN analysis.

Subgenus	<i>accD1</i>			<i>accD2</i>			<i>accD3</i>		
	E value	Identity [%]	Length [nt]	E value	Identity [%]	Length [nt]	E value	Identity [%]	Length [nt]
<i>Pelargonium</i>	3.E-09	69	319	0.8	67	185	5.E-135	79	599
<i>Parvulipetala</i>	-	-	-	1.4	67	199	4.E-140	78	604
<i>Magnipetala</i>	2.E-04	70	196	0.1	68	154	8.E-119	76	587