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Article: Family-specific vs. universal PCR primers for the study of mitochondrial DNA in plants

Supplementary Material Table S1. The outcomes of *in silico* PCR amplification of various mitochondrial loci in six *Fabaceae* species with 39 published mitochondrial primer pairs

Lokus 1: <i>atp6-2</i> (reference: 3)									
Species	F primer	5'-3' position and orientation	Com (%)	T _m (°C)	R primer	5'-3' position and orientation	Com (%)	T _m (°C)	Fragm length
<i>Vicia faba</i>	atp6-2	GCATCATTC AAGTAAATACA			atp6-2	GTGAAGCTGTCTGGAGGG			
		2185 → 2204	100	45.3					No product
		454990 → 455009	100	45.3					
		242392 → 242411	75	12.0					
<i>Vigna angularis</i>	atp6-2	478894 → 478913	75	12.0	atp6-2	381153 → 381170 GTGAAGCTGTCTGGAGGG	78	43.3	No product
		GCATCATTC AAGTAAATACA							
		288364 → 288383	100	45.3					
		18896 → 18915	65	19.3					
<i>Vigna radiata</i>	atp6-2	GCATCATTC AAGTAAATACA			atp6-2	16956 → 16973 GTGAAGCTGTCTGGAGGG	78	38.3	No product
		231401 → 231420	100	45.3					
		216559 → 216578	65	19.3					
		230069 → 230088	75	18.1					
<i>Milletia pinnata</i>	atp6-2	GCATCATTC AAGTAAATACA			atp6-2	161917 → 161934 214619 → 214636 GTGAAGCTGTCTGGAGGG	78	38.3	No product
		335457 → 335476	100	46.6			78	38.3	
		GCATCATTC AAGTAAATACA							
		121207 ← 121226	100	45.3			65	4.0	
<i>Lotus japonicus</i>	atp6-2	GCATCATTC AAGTAAATACA			atp6-2	361790 ← 361809 GTGAAGCTGTCTGGAGGG			No product
		361751 → 361770	95	41.3			70	4.0	
		63867 ← 63886	95	41.3					
		399791 ← 399810	65	8.2					
<i>Glycine max</i>	atp6-2	GCATCATTC AAGTAAATACA			atp6-2	346527 → 346546 GTGAAGCTGTCTGGAGGG			No product
		159839 ← 159856					78	38.3	

Locus 2: *atp9* (reference: 3)

Species	F primer	5'-3' position and orientation	Com (%)	T _m (°C)	R primer	5'-3' position and orientation	Com (%)	T _m (°C)	Fragm length	T _a (°C)
<i>Vicia faba</i>	atp9	CCAAGTGAGATGTCCAAGAT			atp9	CTTCGGTTAGAGCAAAGCC				
		128978 → 128997	85	42.6		129228 ← 129246	95	51.2	269	58
		253103 → 253122	85	42.6		253353 ← 253371	95	51.2	269	58
<i>Vigna angularis</i>	atp9				atp9	427899 → 427917	68	33.3		
						385964 ← 385982	68	33.3		
		CCAAGTGAGATGTCCAAGAT				CTTCGGTTAGAGCAAAGCC				
		114172 ← 114191	85	42.6		113923 → 113941	95	51.2	269	58
		377165 ← 377184	75	24.2						
		402478 → 402497	90	45.6						
<i>Vigna radiata</i>	atp9	262835 → 262854	70	31.3	atp9					
						355936 ← 355954	95	51.2		
						151952 ← 151970	74	21.1		
						41243 → 41261	68	33.3		
		CCAAGTGAGATGTCCAAGAT				CTTCGGTTAGAGCAAAGCC				
		111647 ← 111666	85	42.6		111398 → 111416	95	51.2	269	58
<i>Milletia pinnata</i>	atp9	368289 → 368308	75	24.2	atp9					
		293856 → 293875	90	45.6						
		144529 ← 144548	70	31.3						
						343310 ← 343328	95	51.2		
						189591 → 189609	74	21.1		
						185461 → 185479	68	33.3		
<i>Lotus japonicus</i>	atp9	CCAAGTGAGATGTCCAAGAT			atp9	CTTCGGTTAGAGCAAAGCC				
		248352 ← 248371	85	40.7		248103 → 248121	95	51.2	269	58
		105713 → 105732	75	24.2						
						393622 ← 393640	68	33.3		
						205494 ← 205512	68	33.3		
		CCAAGTGAGATGTCCAAGAT				CTTCGGTTAGAGCAAAGCC				
<i>Glycine max</i>	atp9	229514 ← 229533	85	40.7	atp9	228934 → 228952	95	51.2	600	58
		299144 → 299163	75	24.2						
						262677 ← 262695	68	33.3		
						219243 → 219261	89	35.6		
		CCAAGTGAGATGTCCAAGAT				CTTCGGTTAGAGCAAAGCC				
		11583 ← 11602	75	24.2		3906 → 3924	68	33.3	7697	61
		200860 → 200879	90	45.6						
						328811 ← 328829	95	51.2		
						383196 ← 383214	79	33.0		
						44338 ← 44356	74	17.0		

Locus 3: ccb203 (reference: 3)

Species	F primer	5'-3' position and orientation	Com (%)	T _m (°C)	R primer	5'-3' position and orientation	Com (%)	T _m (°C)	Fragm length	T _a (°C)
<i>Vicia faba</i>	ccb203	ASGTTCTACGGACCGATGCC 78225 → 78244	95	56.9	ccb203	CACGGGGAGGGAGCRGGCGA 78717 ← 78736	98	64.0	512	65
<i>Vigna angularis</i>	ccb203	ASGTTCTACGGACCGATGCC 194984 → 195003	95	58.0	ccb203	CACGGGGAGGGAGCRGGCGA 195476 ← 195495 384170 ← 384189	92 72	60.1 20.3	512	65
<i>Vigna radiata</i>	ccb203	ASGTTCTACGGACCGATGCC 313752 ← 313771	95	58.0	ccb203	CACGGGGAGGGAGCRGGCGA 313260 → 313279 361296 → 361315	92 72	60.1 20.3	512	65
<i>Millettia pinnata</i>	ccb203	ASGTTCTACGGACCGATGCC 46211 → 46230	95	56.9	ccb203	CACGGGGAGGGAGCRGGCGA 46703 ← 46722	92	60.1	512	65
<i>Lotus japonicus</i>	ccb203	ASGTTCTACGGACCGATGCC 288028 ← 288047 88834 ← 88853	95 95	56.9 56.9	ccb203	CACGGGGAGGGAGCRGGCGA 287536 → 287555 88342 → 88361 162916 → 162935 105732 ← 105751 308693 ← 308712	98 98 82 72 72	64.0 64.0 57.1 20.3 34.8	512	65
<i>Glycine max</i>	ccb203	ASGTTCTACGGACCGATGCC 244563 → 244582 394154 ← 394173	95 95	58.0 58.0	ccb203	CACGGGGAGGGAGCRGGCGA 245055 ← 245074 385278 ← 385297	92 72	60.1 35.8	512	65

Locus 4: ccb206 (reference: 3)

Species	F primer	5'-3' position and orientation	Com (%)	T _m (°C)	R primer	5'-3' position and orientation	Com (%)	T _m (°C)	Fragm length	T _a (°C)
<i>Vicia faba</i>	ccb206	TCAATCTTGTRAACTAATCG 402031 → 402050	68	19.7	ccb206	CYYCTCCACCAATCACGA 155816 ← 155835 88577 ← 88596 26398 → 26417	95 70 70	52.6 34.4 21.2	No product	
<i>Vigna angularis</i>	ccb206	TCAATCTTGTRAACTAATCG —			ccb206	CYYCTCCACCAATCACGA 12011 → 12030	95	52.6	No product	
<i>Vigna radiata</i>	ccb206	TCAATCTTGTRAACTAATCG —			ccb206	CYYCTCCACCAATCACGA 209669 → 209688	95	52.6	No product	
<i>Millettia pinnata</i>	ccb206	TCAATCTTGTRAACTAATCG —			ccb206	CYYCTCCACCAATCACGA 7188 → 7207	95	52.6	No product	
<i>Lotus japonicus</i>	ccb206	TCAATCTTGTRAACTAATCG —			ccb206	CYYCTCCACCAATCACGA 292768 → 292787	95	52.6	No product	
<i>Glycine max</i>	ccb206	TCAATCTTGTRAACTAATCG 301796 → 301815	72	4.0	ccb206	CYYCTCCACCAATCACGA 164805 ← 164824	95	52.6	No product	

Locus 5: *ccb256* (reference: 3)

Species	F primer	5'-3' position and orientation	Com (%)	T _m (°C)	R primer	5'-3' position and orientation	Com (%)	T _m (°C)	Fragm length	T _a (°C)
<i>Vicia faba</i>	ccb256	GGAAGTTAGCAAAGTTAGAC			ccb256	TTGTTCTTAACAGCGATGGC				
		287580 <- 287599	100	49.0		287071 -> 287090	100	54.4	529	55
		556026 -> 556045	100	49.0		556535 <- 556554	100	54.0	529	55
						52782 <- 52801	65	31.8		
<i>Vigna angularis</i>	ccb256	GGAAGTTAGCAAAGTTAGAC			ccb256	TTGTTCTTAACAGCGATGGC				
		396898 <- 396917	100	49.0		396389 -> 396408	100	54.4	529	55
<i>Vigna radiata</i>	ccb256	GGAAGTTAGCAAAGTTAGAC			ccb256	TTGTTCTTAACAGCGATGGC				
		288274 <- 288293	100	49.0		287765 -> 287784	100	54.4	529	55
		353935 -> 353954	75	30.8						
<i>Millettia pinnata</i>	ccb256	GGAAGTTAGCAAAGTTAGAC			ccb256	TTGTTCTTAACAGCGATGGC				
		264194 -> 264213	100	49.0		264703 <- 264722	100	54.4	529	55
<i>Lotus japonicus</i>	ccb256	GGAAGTTAGCAAAGTTAGAC			ccb256	TTGTTCTTAACAGCGATGGC				
		342808 <- 342827	100	49.0		342299 -> 342318	100	54.4	529	55
<i>Glycine max</i>	ccb256	GGAAGTTAGCAAAGTTAGAC			ccb256	TTGTTCTTAACAGCGATGGC				
		2240 <- 2259	100	49.0		1731 -> 1750	100	54.4	529	55

Locus 6: *ccb452* (reference: 3)

Species	F primer	5'-3' position and orientation	Com (%)	T _m (°C)	R primer	5'-3' position and orientation	Com (%)	T _m (°C)	Fragm length	T _a (°C)
<i>Vicia faba</i>	ccb452	TATTACCAGACATAATTGGG			ccb452	TGAACTGTATCTTCTTG TG				
		446364 <- 446383	100	46.4		444877 -> 444896	100	48.8	1507	54
		376579 <- 376598	100	46.4		375092 -> 375111	100	48.8	1507	54
<i>Vigna angularis</i>	ccb452	TATTACCAGACATAATTGGG			ccb452	TGAACTGTATCTTCTTG TG				
		365258 -> 365277	100	46.4		369875 <- 369894	100	48.8	4637	55
						60297 <- 60316	70	25.1		
						382707 <- 382726	70	21.6		
<i>Vigna radiata</i>	ccb452	TATTACCAGACATAATTGGG			ccb452	TGAACTGTATCTTCTTG TG				
		32943 -> 32962	100	46.4		37560 <- 37579	100	48.8	4637	55
						57841 <- 57860	70	23.2		
<i>Millettia pinnata</i>	ccb452	TATTACCAGACATAATTGGG			ccb452	TGAACTGTATCTTCTTG TG				
		326571 -> 326590	100	46.4		331182 <- 331201	100	48.8	4631	55
						53769 -> 53788	75	28.0		
						302057 <- 302076	65	24.6		
						31249 <- 31268	75	28.0		
<i>Lotus japonicus</i>	ccb452	TATTACCAGACATAATTGGG			ccb452	TGAACTGTATCTTCTTG TG				
		227648 <- 227667	100	46.4		226323 -> 226342	100	48.8	1345	54
						205032 <- 205051	65	24.6		
						341645 <- 341664	75	26.9		

<i>Glycine max</i>	ccb452	TATTACCAGACATAATTGGG 57609 → 57628	100	45.4	ccb452	TGAACTGTATCTTCTTTGTG 62241 ← 62260	100	48.8	4652	55
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Locus 7: *cox1* (reference: 3)

Species	F primer	5'-3' position and orientation	Com (%)	T _m (°C)	R primer	5'-3' position and orientation	Com (%)	T _m (°C)	Fragm length	T _a (°C)	
<i>Vicia faba</i>	cox1	TTGTTACGACCACGAAGA 381799 → 381816	100	49.4	cox1	TCGGTGCCATTGCTGGAG 383140 ← 383157	100	58.8	1359	58	F primers give 4804 bp product
<i>Vigna angularis</i>	cox1	TTGTTACGACCACGAAGA 221237 ← 221254 216451 → 216468	100 78	49.4 34.4	cox1	TCGGTGCCATTGCTGGAG 219896 → 219913	100	58.8	1359	58	
<i>Vigna radiata</i>	cox1	TTGTTACGACCACGAAGA 1409 ← 1426 397899 → 397916	100 78	49.4 34.4	cox1	TCGGTGCCATTGCTGGAG 68 → 85	100	58.8	1359	58	
<i>Milletia pinnata</i>	cox1	TTGTTACGACCACGAAGA 1409 ← 1426 83194 → 83211	100 78	49.4 34.4	cox1	TCGGTGCCATTGCTGGAG 68 → 85	100	58.8	1359	58	
<i>Lotus japonicus</i>	cox1	TTGTTACGACCACGAAGA 1409 ← 1426 379322 → 379339	94 78	44.5 34.4	cox1	TCGGTGCCATTGCTGGAG 68 → 85	100	58.8	1359	58	F primers give 4822 bp product
<i>Glycine max</i>	cox1	TTGTTACGACCACGAAGA 52052 ← 52069 47248 → 47265	100 78	49.4 34.4	cox1	TCGGTGCCATTGCTGGAG 50711 → 50728	100	58.8	1359	58	

Locus 8: *cox2/1-2* (reference: 2, 3)

Species	F primer	5'-3' position and orientation	Com (%)	T _m (°C)	R primer	5'-3' position and orientation	Com (%)	T _m (°C)	Fragm length	T _a (°C)	
<i>Vicia faba</i>	cox2/1	TTTCTTCCTCATTCTKATTT 17145 → 17165 67233 → 67253 124264 → 124284 430100 → 430120 88385 → 88405 254374 ← 254394 130249 ← 130269	98 98 64 69 74 74 74	45.8 45.8 4.0 12.0 12.8 4.0 4.0	cox2/2	CCACTCTATTGTCCACTTCTA 17502 ← 17522 67590 ← 67610	100 100	50.6 50.6	378 378	56 56	F primers give 6006 bp product
<i>Vigna angularis</i>	cox2/1	TTTCTTCCTCATTCTKATTT 18175 ← 18195 376794 → 376814	76 64	31.7 4.0	cox2/2	21072 → 21092 CCACTCTATTGTCCACTTCTA –	67	20.9		No product	
<i>Vigna radiata</i>	cox2/1	TTTCTTCCTCATTCTKATTT 215833 ← 215853 368659 ← 368679 201053 → 201073	76 64 74	31.7 4.0 23.6	cox2/2	CCACTCTATTGTCCACTTCTA –				No product	

<i>Millettia pinnata</i>	cox2/1	TTTTCTTCCTCATTCTKATTT			cox2/2	CCACTCTATTGTCCACTTCTA					
		96231 → 96251	97	45.8		96588 ← 96608	100	50.6	378	53	
		106083 ← 106103	64	4.0							
		33934 → 33954	69	12.0							
		51083 ← 51103	69	12.0							
		28130 → 28150	60	15.5							
		189247 ← 189267	64	4.0							
<i>Lotus japonicus</i>	cox2/1	TTTTCTTCCTCATTCTKATTT			cox2/2	297776 → 297796	67	12.0			
		371365 ← 371385	98	45.8		CCACTCTATTGTCCACTTCTA					
		299514 ← 299534	64	4.0		371008 → 371028	95	43.1	378	53	
		307521 → 307541	64	4.0							
		334641 ← 334661	64	4.0							
<i>Glycine max</i>	cox2/1	TTTTCTTCCTCATTCTKATTT			cox2/2	CCACTCTATTGTCCACTTCTA					
		353032 ← 353052	98	45.8		352675 → 352695	100	50.6	378	53	
		144072 ← 144092	83	21.8							
		207872 → 207892	83	21.8							
		11212 → 11232	64	4.0							
		126159 ← 126179	74	23.8							
		292075 ← 292095	69	9.6							
		373153 ← 373173	64	4.0							
				</							

Locus 9: cox2/2–3 (reference: 3)

Species	F primer	5'-3' position and orientation	Com (%)	Tm (°C)	R primer	5'-3' position and orientation	Com (%)	Tm (°C)	Fragm length	Ta (°C)	
<i>Vicia faba</i>	cox2/2	TAGRAACAGCTTCTACGACG			cox2/3	GRGTTTACTATGGTCAGTGC					
		67810 <- 67829	98	49.3		67752 -> 67771	98	48.3	78	56	
		17722 <- 17741	98	49.3		17664 -> 17683	98	48.3	78	56	
		418505 -> 418524	72	28.8							
<i>Vigna angularis</i>	cox2/2	TAGRAACAGCTTCTACGACG			cox2/3	GRGTTTACTATGGTCAGTGC					
		141471 -> 141490	72	28.8							
						38364 <- 38383	88	42.1			
						38424 <- 38443	72	33.1			
<i>Vigna radiata</i>	cox2/2	TAGRAACAGCTTCTACGACG			cox2/3	GRGTTTACTATGGTCAGTGC	65	31.9			
		164512 <- 164531	72	28.8							
<i>Millettia pinnata</i>	cox2/2	TAGRAACAGCTTCTACGACG			cox2/3	GRGTTTACTATGGTCAGTGC					
		96808 <- 96827	98	49.3		96750 -> 96769	98	48.3	78	56	
		234363 -> 234382	72	28.8							
		126335 -> 126354	82	30.8							

<i>Lotus japonicus</i>	cox2/2	TAGRAACAGCTTCTACGACG 370789 → 370808 104259 → 104278 182985 ← 183004	98 72 82	49.3 28.8 30.8	cox2/3	GRGTTTACTATGGTCAGTGC 370847 ← 370866	98	48.3	78	56
<i>Glycine max</i>	cox2/2	TAGRAACAGCTTCTACGACG 352456 → 352475 42080 ← 42099	92 72	43.5 28.8	cox2/3	GRGTTTACTATGGTCAGTGC 352514 ← 352533	98	48.3	78	56

Locus 10: *cox3* (reference: 3)

Species	F primer	5'-3' position and orientation	Com (%)	T _m (°C)	R primer	5'-3' position and orientation	Com (%)	T _m (°C)	Fragm length	T _a (°C)		
<i>Vicia faba</i>	cox3	CCGTAGGAGGTGTGATGT 124044 → 124061	100	53.0	cox3	CTCCCCACCAATAGATAGAG 124729 ← 124748	100	51.9	705	58		
<i>Vigna angularis</i>	cox3	CCGTAGGAGGTGTGATGT 376574 → 376591 379395 ← 379412	100 78	53.0 33.2	cox3	CTCCCCACCAATAGATAGAG 377259 ← 377278	100	51.9	705	58	F primers give 2839 bp product	
<i>Vigna radiata</i>	cox3	CCGTAGGAGGTGTGATGT 368882 ← 368899 366060 → 366077	100 78	53.0 33.2	cox3	9049 ← 9068 73157 → 73176 CTCCCCACCAATAGATAGAG 368195 → 368214	75 70 100	12.5 10.5 51.9		705	58	F primers give 2840 bp product
<i>Millettia pinnata</i>	cox3	CCGTAGGAGGTGTGATGT 106306 ← 106323 99026 → 99043	100 78	53.0 33.2	cox3	206713 ← 206732 70682 → 70701 CTCCCCACCAATAGATAGAG 105619 → 105638	75 70 100	12.5 10.5 51.9		705	58	F primers give 7298 bp product
<i>Lotus japonicus</i>	cox3	CCGTAGGAGGTGTGATGT 299737 ← 299754	100	53.0	cox3	193923 ← 193942 CTCCCCACCAATAGATAGAG 299050 → 299069	70 100	16.6 51.9		705	58	
<i>Glycine max</i>	cox3	CCGTAGGAGGTGTGATGT 10992 → 11009 350213 ← 350230	100 78	53.0 33.2	cox3	CTCCCCACCAATAGATAGAG 11677 ← 11696	100	51.9	705	58	R primers give 7049 bp product	
						4648 → 4667 15187 → 15206	75 75	30.2 12.5				

Locus 11: *nad1* intron 2/3 (reference: 1, 3)

Species	F primer	5'-3' position and orientation	Com (%)	T _m (°C)	R primer	5'-3' position and orientation	Com (%)	T _m (°C)	Fragm length	T _a (°C)
<i>Vicia faba</i>	nad1 exon B	GCATTACGATCTGCAGCTCA 380141 ← 380160 449926 ← 449945	100 100	55.0 55.0	nad1 exon C	GGAGCTCGATTAGTTTCTGC 378525 → 378544 448310 → 448329 178244 ← 178263 493293 ← 493312	100 100 65 65	52.9 52.9 9.2 9.2	1636 1636	60 60

<i>Vigna angularis</i>	nad1 exon B	GCATTACGATCTGCAGCTCA 222894 → 222913 35027 ← 35046	100 65	55.0 40.6	nad1 exon C	GGAGCTCGATTAGTTTCTGC 224527 ← 224546	100	52.9	1653	60
<i>Vigna radiata</i>	nad1 exon B	GCATTACGATCTGCAGCTCA 3065 → 3084 179257 ← 179276	100 65	55.0 40.6	nad1 exon C	GGAGCTCGATTAGTTTCTGC 4698 ← 4717	100	52.9	1653	60
<i>Millettia pinnata</i>	nad1 exon B	GCATTACGATCTGCAGCTCA 3032 → 3051	100	55.0	nad1 exon C	202612 → 202631 GGAGCTCGATTAGTTTCTGC 4647 ← 4666	75	34.1		
<i>Lotus japonicus</i>	nad1 exon B	GCATTACGATCTGCAGCTCA 3086 → 3105 93352 → 93371 292546 → 292565	100 65 65	55.0 30.6 30.6	nad1 exon C	GGAGCTCGATTAGTTTCTGC 4697 ← 4716	100	52.9	1635 1631	60 60
<i>Glycine max</i>	nad1 exon B	GCATTACGATCTGCAGCTCA 53727 → 53746	100	55.0	nad1 exon C	GGAGCTCGATTAGTTTCTGC 55346 ← 55365	100	52.9	1639	60

Locus 12: *nad1* intron 4/5 (reference: 2, 3)

Species	F primer	5'-3' position and orientation	Com (%)	Tm (°C)	R primer	5'-3' position and orientation	Com (%)	Tm (°C)	Fragm length	Ta (°C)	
<i>Vicia faba</i>	nad1/4	GCCAATATGATCTTAATGAG 89487 → 89506	100	46.8	nad1/5r	TCACCTTGATACTAAACCAG 92731 ← 92750 4953 → 4972 457758 → 457777	100 70 70	48.4 22.1 22.1	3264	54	Contains matR gene
<i>Vigna angularis</i>	nad1/4	GCCAATATGATCTTAATGAG 67987 ← 68006	100	46.8	nad1/5r	TCACCTTGATACTAAACCAG 64738 → 64757	100	48.4	3269	54	Contains matR gene
<i>Vigna radiata</i>	nad1/4	GCCAATATGATCTTAATGAG 65531 ← 65550	100	46.8	nad1/5r	TCACCTTGATACTAAACCAG 62282 → 62301	100	48.8	3269	54	Contains matR gene
<i>Millettia pinnata</i>	nad1/4	GCCAATATGATCTTAATGAG 370508 → 370527	100	46.8	nad1/5r	TCACCTTGATACTAAACCAG 373724 ← 373743	100	48.8	3236	54	Contains matR gene
<i>Lotus japonicus</i>	nad1/4	GCCAATATGATCTTAATGAG 43089 → 43108	100	46.8	nad1/5r	TCACCTTGATACTAAACCAG 46333 ← 46352	100	48.8	3264	54	Contains matR gene
<i>Glycine max</i>	nad1/4	GCCAATATGATCTTAATGAG 340687 ← 340706	100	46.8	nad1/5r	TCACCTTGATACTAAACCAG 337437 → 337456 32482 ← 32501	100 70	48.8 22.1	3270	54	Contains matR gene

Locus 13: *nad2* intron 1/2 (reference: 3)

Species	F primer	5'-3' position and orientation	Com (%)	Tm (°C)	R primer	5'-3' position and orientation	Com (%)	Tm (°C)	Fragm length	Ta (°C)
<i>Vicia faba</i>	nad 2/1	AATGTGGGTTGGCTTGGWTT 27785 → 27804 2331 ← 2350 99499 → 99518 455136 ← 455155	98 78 62 78	54.6 34.7 41.6 34.7	nad 2/2	AATATGTAAAATTGTCCCTC 29101 ← 29120	100	47.1	1336	53

<i>Vigna angularis</i>	nad 2/1	AATGTGGGTTGGCTTGGWTT 147624 → 147643 142527 → 142546 402176 → 402195	98 78 68	54.6 37.9 17.5	nad 2/2	AATATGTAAAATTGTCCTC 148978 ← 148997	100	47.1	1374	53
<i>Vigna radiata</i>	nad 2/1	AATGTGGGTTGGCTTGGWTT 193922 ← 193941 163456 ← 163475	98 78	54.6 37.9	nad 2/2	AATATGTAAAATTGTCCTC 192568 → 192587	100	47.1	1374	53
<i>Millettia pinnata</i>	nad 2/1	AATGTGGGTTGGCTTGGWTT 194706 → 194725 351448 → 351467	98 68	54.6 28.0	nad 2/2	AATATGTAAAATTGTCCTC 196058 ← 196077	100	47.1	1372	53
<i>Lotus japonicus</i>	nad 2/1	AATGTGGGTTGGCTTGGWTT 60253 → 60272 186976 ← 186995 125385 → 125404	98 62 62	54.6 35.2 37.2	nad 2/2	AATATGTAAAATTGTCCTC 61603 ← 61622	100	47.1	1370	53
<i>Glycine max</i>	nad 2/1	AATGTGGGTTGGCTTGGWTT 134827 ← 134846	98	54.6	nad 2/2	AATATGTAAAATTGTCCTC 133475 → 133494	100	47.1	1372	53

Locus 14: *nad2* intron 3/4 (reference: 3)

Species	F primer	5'-3' position and orientation	Com (%)	Tm (°C)	R primer	5'-3' position and orientation	Com (%)	Tm (°C)	Fragm length	Ta (°C)
<i>Vicia faba</i>	nad 2/3	AGAAARGAATGCTGTAACCG 415414 → 415433 332368 ← 332387	98 70	50.0 40.7	nad 2/4	ATGGGGATTKTYARTATCGC			No product	
<i>Vigna angularis</i>	nad 2/3	AGAAARGAATGCTGTAACCG 99140 ← 99159	98	50.0	nad 2/4	152757 ← 152776 ATGGGGATTKTYARTATCGC	58	4.0	No product	
<i>Vigna radiata</i>	nad 2/3	AGAAARGAATGCTGTAACCG 96655 ← 96674	98	50.0	nad 2/4	324667 ← 324686 ATGGGGATTKTYARTATCGC	62	23.8	No product	
<i>Millettia pinnata</i>	nad 2/3	AGAAARGAATGCTGTAACCG 134762 ← 134781	98	50.0	nad 2/4	267633 ← 267652 ATGGGGATTKTYARTATCGC	62	23.8	No product	
<i>Lotus japonicus</i>	nad 2/3	AGAAARGAATGCTGTAACCG 101591 → 101610 152048 ← 152067	98 70	50.0 40.7	nad 2/4	21179 ← 21198 ATGGGGATTKTYARTATCGC	58	4.0	No product	
<i>Glycine max</i>	nad 2/3	AGAAARGAATGCTGTAACCG 320446 → 320465 382533 → 382552 233979 ← 233998	98 70 78	50.0 35.4 28.6	nad 2/4	37094 ← 37113 377333 → 377352 ATGGGGATTKTYARTATCGC –	62 62	23.8 4.0	No product	

Locus 15: *nad2* intron 4/5 (reference: 3)

Species	F primer	5'-3' position and orientation	Com (%)	T _m (°C)	R primer	5'-3' position and orientation	Com (%)	T _m (°C)	Fragm length	T _a (°C)
<i>Vicia faba</i>	nad 2/4	TTCATATAGAATCCATGTCC 413337 → 413356 139973 ← 139992	95 70	41.2 22.2	nad 2/5	CTATTGTTCTTCGCCGCTT 414960 ← 414979	100	56.3	1643	54
<i>Vigna angularis</i>	nad 2/4	TTCATATAGAATCCATGTCC 101195 ← 101214	95	41.2	nad 2/5	356348 → 356367 CTATTGTTCTTCGCCGCTT 99594 → 99613 89580 → 89599	70 100 75	28.6 56.3 29.3	1612	54
<i>Vigna radiata</i>	nad 2/4	TTCATATAGAATCCATGTCC 98711 ← 98730	95	41.2	nad 2/5	CTATTGTTCTTCGCCGCTT 97109 → 97128 87090 → 87109	100 75	56.3 29.3	1622	54
<i>Millettia pinnata</i>	nad 2/4	TTCATATAGAATCCATGTCC 136816 ← 136835 168261 ← 168280 25450 → 25469	95 70 70	41.2 26.3 22.2	nad 2/5	CTATTGTTCTTCGCCGCTT 135216 → 135235	100	56.3	1620	54
<i>Lotus japonicus</i>	nad 2/4	TTCATATAGAATCCATGTCC 99499 → 99518 317477 → 317496	95 70	41.2 19.8	nad 2/5	353013 ← 353032 CTATTGTTCTTCGCCGCTT 101137 ← 101156	80 100	40.4 56.3	1658	54
<i>Glycine max</i>	nad 2/4	TTCATATAGAATCCATGTCC 318390 → 318409	95	41.2	nad 2/5	134794 ← 134813 CTATTGTTCTTCGCCGCTT 319992 ← 320011 99708 ← 99727	75 100 75	29.3 56.3 29.3	1622	54

Locus 16: *nad4* intron 1/2 (reference: 1, 3)

Species	F primer	5'-3' position and orientation	Com (%)	T _m (°C)	R primer	5'-3' position and orientation	Com (%)	T _m (°C)	Fragm length	T _a (°C)
<i>Vicia faba</i>	nad4 exon 1	CAGTGGGTTGGTCTGGTATG 353611 → 353630	95	53.1	nad4 exon 2	TCATATGGGCTACTGAGGAG 355634 ← 355653 418785 → 418804	100 70	53.9 33.1	2043	61
<i>Vigna angularis</i>	nad4 exon 1	CAGTGGGTTGGTCTGGTATG 86853 → 86872 13644 → 13663	95 80	53.1 41.8	nad4 exon 2	TCATATGGGCTACTGAGGAG 88877 ← 88896	100	53.9	2044	61
<i>Vigna radiata</i>	nad4 exon 1	CAGTGGGTTGGTCTGGTATG 84363 → 84382 211303 → 211322	95 80	53.1 41.8	nad4 exon 2	141750 → 141769 TCATATGGGCTACTGAGGAG 86387 ← 86406	70 100	33.1 53.9	2044	61
<i>Millettia pinnata</i>	nad4 exon 1	CAGTGGGTTGGTCTGGTATG 355750 ← 355769 8323 → 8342	95 80	53.1 41.8	nad4 exon 2	164233 ← 164252 TCATATGGGCTACTGAGGAG 353734 → 353753	70 100	33.1 53.9	2036	61

<i>Lotus japonicus</i>	nad4 exon 1	CAGTGGGTTGGTCTGGTATG	95	53.1	nad4 exon 2	235175 → 235194	70	33.1	2054	61
		137536 ← 137555				TCATATGGGCTACTGAGGAG				
		232982 ← 233001	80	31.7		135502 → 135521	100	53.9		
<i>Glycine max</i>	nad4 exon 1	CAGTGGGTTGGTCTGGTATG	95	53.1	nad4 exon 2	104535 → 104554	70	33.1	2041	61
		102458 ← 102477				TCATATGGGCTACTGAGGAG				
		157527 ← 157546	80	31.7		100437 → 100456	100	53.9		
						238484 → 238503	85	33.5		
						41801 ← 41820	70	33.1		

Locus 17: *nad4* intron 2/3 (reference: 2, 3)

Species	F primer	5'-3' position and orientation	Com (%)	T _m (°C)	R primer	5'-3' position and orientation	Com (%)	T _m (°C)	Fragm length	T _a (°C)
<i>Vicia faba</i>	nad4/2	CTCCTCAGTAGCCCATATGA	100	52.5	nad4/3r	AACCAGTCCATGACTTAACA	100	53.4	3265	60
		355634 → 355653				358879 ← 358898				
		82203 ← 82222	70	21.7						
<i>Vigna angularis</i>	nad4/2	CTCCTCAGTAGCCCATATGA	100	52.5	nad4/3r	AACCAGTCCATGACTTAACA	100	53.4	3340	60
		88877 → 88896				92197 ← 92216				
		206274 → 206293	75	22.7						
		134816 ← 134835	70	21.7						
<i>Vigna radiata</i>	nad4/2	CTCCTCAGTAGCCCATATGA	100	52.5	nad4/3r	AACCAGTCCATGACTTAACA	100	53.4	3341	60
		86387 → 86406				89708 ← 89727				
		132278 ← 132297	70	21.7						
<i>Milletia pinnata</i>	nad4/2	CTCCTCAGTAGCCCATATGA	100	52.5	nad4/3r	AACCAGTCCATGACTTAACA	100	53.4	3396	60
		353734 ← 353753				350358 → 350377				
		12541 ← 12560	70	21.7						
<i>Lotus japonicus</i>	nad4/2	CTCCTCAGTAGCCCATATGA	100	52.5	nad4/3r	AACCAGTCCATGACTTAACA	100	53.4	3361	60
		135502 ← 135521				132161 → 132180				
<i>Glycine max</i>	nad4/2	CTCCTCAGTAGCCCATATGA	100	52.5	nad4/3r	AACCAGTCCATGACTTAACA	100	53.4	3393	60
		100437 ← 100456				97064 → 97083				
		304256 ← 304275	70	21.7						

Locus 18: *nad4* intron 3/4 (reference: 2, 3)

Species	F primer	5'-3' position and orientation	Com (%)	T _m (°C)	R primer	5'-3' position and orientation	Com (%)	T _m (°C)	Fragm length	T _a (°C)
<i>Vicia faba</i>	nad4 exon 3	GGAGCTTTCCAAAGAAATAG	100	49.7	nad4 exon 4	GCCATGTTGCACTAAGTTAC	100	52.8	3030	57
		359082 → 359101				362092 ← 362111				
		319535 → 319554	65	16.3						
<i>Vigna angularis</i>	nad4 exon 3	GGAGCTTTCCAAAGAAATAG	100	49.7	nad4 exon 4	38312 → 38331	70	34.5	2800	57
		92400 → 92419				GCCATGTTGCACTAAGTTAC				
		125508 → 125527	75	34.9		95180 ← 95199	95	50.6		

<i>Vigna radiata</i>	nad4 exon 3	GGAGCTTTCCAAAGAAATAG 89911 → 89930	100	49.7	nad4 exon 4	GCCATGTTGCACTAAGTTAC 92694 ← 92713 122970 → 122989	95	50.6	2803	57
<i>Milletia pinnata</i>	nad4 exon 3	GGAGCTTTCCAAAGAAATAG 350155 ← 350174	100	49.7	nad4 exon 4	GCCATGTTGCACTAAGTTAC 347872 → 347891	95	50.6	2303	57
<i>Lotus japonicus</i>	nad4 exon 3	GGAGCTTTCCAAAGAAATAG 131958 ← 131977 332014 ← 332033	100	49.7	nad4 exon 4	GCCATGTTGCACTAAGTTAC 129148 → 129167	100	52.8	2830	57
<i>Glycine max</i>	nad4 exon 3	GGAGCTTTCCAAAGAAATAG 96861 ← 96880 148855 → 148874	100	49.7	nad4 exon 4	235247 → 235266 GCCATGTTGCACTAAGTTAC 94051 → 94070	65	32.5		
			75	24.9			95	50.6	2830	57

Locus 19: *nad4* (reference: 1)

Species	F primer	5'-3' position and orientation	Com (%)	Tm (°C)	R primer	5'-3' position and orientation	Com (%)	Tm (°C)	Fragm length	Ta (°C)
<i>Vicia faba</i>	nad4 exon 2*	TGTTTCCCGAAGCGACACTT 355516 → 355535	95	52.0	nad4 exon 4	GGAACACTTTGGGGTGAACA 362048 ← 362067	100	53.2	6552	64
<i>Vigna angularis</i>	nad4 exon 2*	TGTTTCCCGAAGCGACACTT 88759 → 88778	95	52.0	nad4 exon 4	GGAACACTTTGGGGTGAACA 95136 ← 95155	100	53.2	6397	64
<i>Vigna radiata</i>	nad4 exon 2*	TGTTTCCCGAAGCGACACTT 86269 → 86288	95	52.0	nad4 exon 4	GGAACACTTTGGGGTGAACA 92650 ← 92669	100	53.2	6401	64
<i>Milletia pinnata</i>	nad4 exon 2*	TGTTTCCCGAAGCGACACTT 353852 ← 353871	95	52.0	nad4 exon 4	GGAACACTTTGGGGTGAACA 347916 → 347935	100	53.2	5956	64
<i>Lotus japonicus</i>	nad4 exon 2*	TGTTTCCCGAAGCGACACTT 135620 ← 135639	95	52.0	nad4 exon 4	GGAACACTTTGGGGTGAACA 129192 → 129211	100	53.2	6448	64
<i>Glycine max</i>	nad4 exon 2*	TGTTTCCCGAAGCGACACTT 100555 ← 100574	95	52.0	nad4 exon 4	GGAACACTTTGGGGTGAACA 94095 → 94114	100	53.2	6480	64

Locus 20: *nad4* intron 1/2 (reference: 4)

Species	F primer	5'-3' position and orientation	Com (%)	Tm (°C)	R primer	5'-3' position and orientation	Com (%)	Tm (°C)	Fragm length	Ta (°C)
<i>Vicia faba</i>	nad4 innerF	TGTTTGTTTCGCGAGAATGGATTCC 354078 → 354101	100	58.5	nad4 innerR	TGTAATCGGCGGTCCCTGTTTGG 355282 ← 355305	100	61.7	1228	65
<i>Vigna angularis</i>	nad4 innerF	TGTTTGTTTCGCGAGAATGGATTCC 87316 → 87339 46659 → 46682 222526 → 222549	100	58.5	nad4 innerR	TGTAATCGGCGGTCCCTGTTTGG 88526 ← 88549	100	61.7	1234	65
<i>Vigna radiata</i>	nad4 innerF	TGTTTGTTTCGCGAGAATGGATTCC 84826 → 84849 225920 ← 225943 2697 → 2720	100	58.5	nad4 innerR	TGTAATCGGCGGTCCCTGTTTGG 86036 ← 86059	100	61.7	1234	65
			67	27.9						
			62	23.3						
			67	27.9						
			62	23.3						

<i>Milletia pinnata</i>	nad4 innerF	TGTTTGTTTCGCGAGAATGGATTCC 355283 <-- 355306	100	58.5	nad4 innerR	TGTAAATCGGCGGTCCCTGTTTGG 354081 -> 354104	100	61.7	1226	65
<i>Lotus japonicus</i>	nad4 innerF	TGTTTGTTTCGCGAGAATGGATTCC 137065 <-- 137088 319796 <-- 319819	100 67	58.5 28.5	nad4 innerR	TGTAAATCGGCGGTCCCTGTTTGG 135849 -> 135872	100	61.7	1240	65
<i>Glycine max</i>	nad4 innerF	TGTTTGTTTCGCGAGAATGGATTCC 101991 <-- 102014 291305 <-- 291328 53355 -> 53378	100 71 62	58.5 25.0 23.3	nad4 innerR	TGTAAATCGGCGGTCCCTGTTTGG 100784 -> 100807	100	61.7	1231	65

Locus 21: *nad4L/orf25* (reference: 3)

Species	F primer	5'-3' position and orientation	Com (%)	Tm (°C)	R primer	5'-3' position and orientation	Com (%)	Tm (°C)	Fragm length	Ta (°C)
<i>Vicia faba</i>	orf25	CTGTYTTTTCGCACTTAGGC 69443 <-- 69462	98	51.1	nad4L	GTCCGRGGTACTATTGCTGT 68857 -> 68876	92	46.2	606	59
<i>Vigna angularis</i>	orf25	CTGTYTTTTCGCACTTAGGC 215024 <-- 215043	98	51.1	nad4L	GTCCGRGGTACTATTGCTGT 214446 -> 214465	92	46.2	598	59
<i>Vigna radiata</i>	orf25	CTGTYTTTTCGCACTTAGGC 396472 <-- 396491	98	51.1	nad4L	GTCCGRGGTACTATTGCTGT 395894 -> 395913	92	46.2	598	59
<i>Milletia pinnata</i>	orf25	CTGTYTTTTCGCACTTAGGC 81807 <-- 81826 385021 -> 385040	98 70	51.1 28.5	nad4L	GTCCGRGGTACTATTGCTGT 81218 -> 81237	92	46.2	609	59
<i>Lotus japonicus</i>	orf25	CTGTYTTTTCGCACTTAGGC 97776 -> 97795	98	51.1	nad4L	131590 <-- 131609 GTCCGRGGTACTATTGCTGT 98353 <-- 98372	65 92	31.3 46.2	597	59
<i>Glycine max</i>	orf25	CTGTYTTTTCGCACTTAGGC 45825 <-- 45844	98	51.1	nad4L	312930 -> 312949 GTCCGRGGTACTATTGCTGT 45260 -> 45279 146031 -> 146050 205914 <-- 205933 323611 -> 323630	62 92 92 92 70	19.3 46.2 46.2 46.2 28.8	585	59

Locus 22: *nad5* intron a/b (reference: 2, 3)

Species	F primer	5'-3' position and orientation	Com (%)	Tm (°C)	R primer	5'-3' position and orientation	Com (%)	Tm (°C)	Fragm length	Ta (°C)
<i>Vicia faba</i>	nad5/1	TTTTTTCGGACGTTTTCTAG 71584 -> 71603	100	49.1	nad5/2r	TTTGCCCAAGTATCCTACAA 73834 <-- 73853	100	50.3	2270	57
<i>Vigna angularis</i>	nad5/1	TTTTTTCGGACGTTTTCTAG 120992 -> 121011	100	49.1	nad5/2r	TTTGCCCAAGTATCCTACAA 118744 <-- 118763	100	50.3	2268	57
<i>Vigna radiata</i>	nad5/1	TTTTTTCGGACGTTTTCTAG 118473 <-- 118492	100	49.1	nad5/2r	TTTGCCCAAGTATCCTACAA 116224 -> 116243	100	50.3	2269	57
<i>Milletia pinnata</i>	nad5/1	TTTTTTCGGACGTTTTCTAG 116985 -> 117004	100	49.1	nad5/2	TTTGCCCAAGTATCCTACAA 119220 <-- 119239	100	50.3	2255	57

<i>Lotus japonicus</i>	nad5/1	TTTTTTCGGACGTTTCTAG 73532 <- 73551 92539 -> 92558 291733 -> 291752	100 85 85	49.1 29.0 29.0	nad5/2r	TTTGGCCAAGTATCCTACAA 71281 -> 71300	100	50.3	2271	57
<i>Glycine max</i>	nad5/1	TTTTTTCGGACGTTTCTAG 252085 -> 252104	100	49.1	nad5/2r	TTTGGCCAAGTATCCTACAA 254320 <- 254339	100	50.3	2255	57

Locus 23: nad5 intron d/e (reference: 2, 3)

Species	F primer	5'-3' position and orientation	Com (%)	Tm (°C)	R primer	5'-3' position and orientation	Com (%)	Tm (°C)	Fragm length	Ta (°C)
<i>Vicia faba</i>	nad5/4	CCAATTTTGGGCCAATTCC 279736 -> 279755 563870 <- 563889 309974 <- 309993 367879 <- 367898 437664 <- 437683	100 100 70 70 70	55.4 55.4 29.4 29.4 29.4	nad5/5r	CATTGCAAAGGCATAATGAT 281047 <- 281066 562559 -> 562578	100 100	49.2 49.2	1331 1331	57 57
<i>Vigna angularis</i>	nad5/4	CCAATTTTGGGCCAATTCC 183939 -> 183958	100	55.4	nad5/5r	CATTGCAAAGGCATAATGAT 185255 <- 185274 367370 -> 367389 401251 -> 401270	100 70 70	49.2 26.4 20.2	1336	57
<i>Vigna radiata</i>	nad5/4	CCAATTTTGGGCCAATTCC 324793 <- 324812	100	55.4	nad5/5r	CATTGCAAAGGCATAATGAT 323477 -> 323496 35055 -> 35074 292633 -> 292652	100 70 70	49.2 26.1 20.2	1336	57
<i>Millettia pinnata</i>	nad5/4	CCAATTTTGGGCCAATTCC 241833 -> 241852 29337 -> 29356 55681 <- 55700	100 70 70	55.4 29.4 29.4	nad5/5r	CATTGCAAAGGCATAATGAT 243149 <- 243168 31269 -> 31288 115933 <- 115952 53749 <- 53768 328691 -> 328710	100 75 75 75 70	49.2 19.0 19.0 19.0 26.1	1336	57
<i>Lotus japonicus</i>	nad5/4	CCAATTTTGGGCCAATTCC 222134 -> 222153 196964 <- 196983	100 65	55.4 33.0	nad5/5r	CATTGCAAAGGCATAATGAT 223462 <- 223481 143573 -> 143592	100 75	49.2 19.0	1348	57
<i>Glycine max</i>	nad5/4	CCAATTTTGGGCCAATTCC 267594 -> 267613	100	55.4	nad5/5r	CATTGCAAAGGCATAATGAT 268910 <- 268929 353319 <- 353338 59715 -> 59734	100 75 70	49.2 19.0 26.1	1336	57

Locus 24: nad6 (reference: 3)

Species	F primer	5'-3' position and orientation	Com (%)	T _m (°C)	R primer	5'-3' position and orientation	Com (%)	T _m (°C)	Fragm length	T _a (°C)
<i>Vicia faba</i>	nad6	TGAGTGGGTCWGTGCCTC 100860 → 100879	98	56.7	nad6	TGATACTTTCTGTTTGTCTG 101446 ← 101465 38170 ← 38189	100 100	47.5 47.5	606	54
<i>Vigna angularis</i>	nad6	TGAGTGGGTCWGTGCCTC 17581 ← 17600	98	56.7	nad6	TGATACTTTCTGTTTGTCTG 16995 → 17014 134218 → 134237	100 65	47.5 32.9	606	54
<i>Vigna radiata</i>	nad6	TGAGTGGGTCWGTGCCTC 215244 ← 215263	98	56.7	nad6	TGATACTTTCTGTTTGTCTG 214658 → 214677	100	47.5	606	54
<i>Milletia pinnata</i>	nad6	TGAGTGGGTCWGTGCCTC 127469 ← 127488	92	51.5	nad6	131680 → 131699 TGATACTTTCTGTTTGTCTG 126883 → 126902 155287 → 155306 316209 → 316228 11951 → 11970 260274 → 260293	65 100 90 90 70 70	32.9 47.5 39.8 39.8 39.6 17.8	606	54
<i>Lotus japonicus</i>	nad6	TGAGTGGGTCWGTGCCTC 234514 → 234533 323310 → 323329 126706 ← 126725	92 72 88	51.5 37.9 49.2	nad6	TGATACTTTCTGTTTGTCTG 235100 ← 235119	100	47.5	606	54
<i>Glycine max</i>	nad6	TGAGTGGGTCWGTGCCTC 159212 → 159231 223637 ← 223656	68 72	43.5 27.2	nad6	305332 → 305351 TGATACTTTCTGTTTGTCTG 159798 ← 159817 68845 ← 68865 303653 → 303672 88148 ← 88167 74534 ← 74553	90 100 90 70 75 80	39.8 47.5 39.8 39.6 24.3 23.8	606	54

Locus 25: nad7 intron 1/2 (reference: 2, 3)

Species	F primer	5'-3' position and orientation	Com (%)	T _m (°C)	R primer	5'-3' position and orientation	Com (%)	T _m (°C)	Fragm length	T _a (°C)
<i>Vicia faba</i>	nad7/1	ACCTCAACATCCTGCTGCTC 226324 ← 226343 302233 → 302252 541373 ← 541392	100 100 100	59.2 59.2 59.2	nad7/2r	CGATCAGAATAAGGTAAAGC 225275 → 225294 303282 ← 303301 540324 → 540343 34233 → 34252 94184 → 94203	100 100 100 75 70	48.8 48.8 48.8 11.3 27.9	1069 1069 1069	55 55 55
<i>Vigna angularis</i>	nad7/1	ACCTCAACATCCTGCTGCTC 356308 → 356327	100	59.2	nad7/2r	CGATCAGAATAAGGTAAAGC 357341 ← 357360	100	48.8	1053	55

R primers give 3244 bp product

<i>Vigna radiata</i>	nad7/1	ACCTCAACATCCTGCTGCTC 343682 → 343701	100	59.2	nad7/2r	354117 → 354136	75	27.9	1035	48.8
						199933 ← 199952	70	13.5		
						115191 → 115210	65	22.8		
						266002 ← 266021	75	11.3		
						CGATCAGAATAAGGTAAAGC				
<i>Milletia pinnata</i>	nad7/1	ACCTCAACATCCTGCTGCTC 410243 → 410262	100	59.2	nad7/2r	344715 ← 344734	100	48.8	1063	55
						308812 → 308831	70	13.5		
						284540 → 284559	75	24.9		
						112666 → 112685	65	22.8		
						141365 → 141384	75	11.3		
<i>Lotus japonicus</i>	nad7/1	ACCTCAACATCCTGCTGCTC 25022 → 25041	100	59.2	nad7/2r	126095 ← 126114	65	16.5	1072	55
						CGATCAGAATAAGGTAAAGC				
						411286 ← 411305	100	48.8		
						345772 → 345791	80	34.3		
						380632 ← 380651	75	11.3		
<i>Glycine max</i>	nad7/1	ACCTCAACATCCTGCTGCTC 170054 → 170073	100	59.2	nad7/2r	402305 ← 402324	70	12.0	1059	55
						CGATCAGAATAAGGTAAAGC				
						26074 ← 26093	100	48.8		
						6790 → 6809	75	21.1		
						236621 ← 236640	70	21.1		
						74538 → 74557	75	11.3		
						273732 → 273751	75	11.3		
						CGATCAGAATAAGGTAAAGC				
						171093 ← 171112	100	48.8		
						180479 → 180498	75	26.8		
						260412 → 260431	75	26.8		
						359133 → 359152	75	26.8		
						237791 ← 237810	75	26.8		
						66485 ← 66504	75	26.8		
						21825 ← 21844	75	11.3		
						289054 ← 298073	70	12.0		

Locus 26: nad7 intron 2/3 (reference: 2, 3)

Species	F primer	5'-3' position and orientation	Com (%)	Tm (°C)	R primer	5'-3' position and orientation	Com (%)	Tm (°C)	Fragm length	Ta (°C)
<i>Vicia faba</i>	nad7/2	GCTTTACCTTATTCTGATCG			nad7/3r	TGTTCTTGGGCCATCATAGA				
		225275 ← 225294	100	49.3		223919 → 223938	95	49.8	1376	55
		303282 → 303301	100	49.3		304638 ← 304657	95	49.8	1376	55
		540324 ← 540343	100	49.3		538968 → 538987	95	49.8	1376	55
		221305 → 221324	70	17.9						
		307252 ← 307271	70	17.9						
<i>Vigna angularis</i>	nad7/2	536354 → 536373	70	17.9	nad7/3r					
		GCTTTACCTTATTCTGATCG				TGTTCTTGGGCCATCATAGA				
		357341 → 357360	100	49.3		358711 ← 358730	95	49.8	1390	55

F primers give 3990 bp product

Vigna radiata	nad7/2	344425 → 344444	75	12.3	nad7/3r	TGTTCTTGGGCCATCATAGA	95	49.8	1391	55	F primers give 7972 bp product
		324826 → 324845	70	23.3							
		GCTTTACCTTATTCTGATCG									
		344715 → 344734	100	49.3							
Millettia pinnata	nad7/2	231076 → 231095	75	12.3	nad7/3r	TGTTCTTGGGCCATCATAGA	95	49.8	1387	55	
		267792 → 267811	70	23.3							
		GCTTTACCTTATTCTGATCG									
		411286 → 411305	100	49.3							
Lotus japonicus	nad7/2	GCTTTACCTTATTCTGATCG			nad7/3r	TGTTCTTGGGCCATCATAGA	95	49.8	1385	55	
		26074 → 26093	100	49.3							
		34026 ← 34045	70	11.8							
		GCTTTACCTTATTCTGATCG									
Glycine max	nad7/2	171093 → 171112	100	49.3	nad7/3r	TGTTCTTGGGCCATCATAGA	95	49.8	1384	55	

Locus 27: nad7 intron 3/4 (reference: 2, 3)

Species	F primer	5'-3' position and orientation	Com (%)	Tm (°C)	R primer	5'-3' position and orientation	Com (%)	Tm (°C)	Fragm length	Ta (°C)
<i>Vicia faba</i>	nad7/3	TCTATGATGGCCCAAGAACA			nad7/4r	ACACCAAATTCTCCTTTAGG				
		223919 ← 223938	95	48.7		220057 → 220076	100	52.5	3882	58
		304638 → 304657	95	48.7		308500 ← 308519	100	52.5	3882	58
		538968 ← 538987	95	48.7		535106 → 535125	100	52.5	3882	58
						436190 ← 436209	100	52.5		
<i>Vigna angularis</i>	nad7/3	TCTATGATGGCCCAAGAACA			nad7/4r	ACACCAAATTCTCCTTTAGG				
		358711 → 358730	95	48.7		362576 ← 362595	100	52.5	3885	58
		266477 ← 266496	65	35.9						
						214018 ← 214037	80	16.4		
						ACACCAAATTCTCCTTTAGG				
<i>Vigna radiata</i>	nad7/3	TCTATGATGGCCCAAGAACA			nad7/4r	349940 ← 349959	100	52.5	3874	58
		346086 → 346105	95	48.7						
		140890 → 140909	65	35.9						
						395462 ← 395481	75	7.4		
						ACACCAAATTCTCCTTTAGG				
<i>Milletia pinnata</i>	nad7/3	TCTATGATGGCCCAAGAACA			nad7/4r	416523 ← 416542	100	52.5	3890	58
		412653 → 412672	95	48.7						
		381115 ← 381134	65	35.9						
		94661 → 94680	70	13.0						
						200486 ← 200505	70	16.4		
<i>Lotus japonicus</i>	nad7/3	TCTATGATGGCCCAAGAACA			nad7/4r	ACACCAAATTCTCCTTTAGG				
		27439 → 27458	95	48.7		31336 ← 31355	100	52.5	3917	58
		203103 → 203122	65	28.0						
<i>Glycine max</i>	nad7/3	TCTATGATGGCCCAAGAACA			nad7/4r	ACACCAAATTCTCCTTTAGG				
		172457 → 172476	95	48.7		176325 ← 176344	100	52.5	3888	58
						354979 ← 354998	100	52.5		
						256258 ← 256277	100	52.5		
						280076 ← 280095	65	17.7		

Locus 28: nad7 intron 4/5 (reference: 3)

Species	F primer	5'-3' position and orientation	Com (%)	T _m (°C)	R primer	5'-3' position and orientation	Com (%)	T _m (°C)	Fragm length	T _a (°C)
<i>Vicia faba</i>	nad5/4	TGTCCTCCATCAGATVTCG			nad5/5	CCAAATTCTCCTTTAGGTGC				
		222223 <- 222242	97	52.8		220060 -> 220079	100	51.8	2183	60
		306334 -> 306353	97	52.8		308497 <- 308516	100	51.8	2183	60
		537272 <- 537291	97	52.8		535109 -> 535128	100	51.8	2183	60
		17125 -> 17144	72	13.3						
		67213 -> 67232	72	13.3						
<i>Vigna angularis</i>	nad5/4	TGTCCTCCATCAGATVTCG			nad5/5	366402 <- 366421	100	51.8		
		360433 -> 360452	97	52.8		436187 <- 436206	100	51.8		
						CCAAATTCTCCTTTAGGTGC				
<i>Vigna radiata</i>	nad5/4	TGTCCTCCATCAGATVTCG			nad5/5	362573 <- 362592	100	51.8	2160	60
		347810 -> 347829	97	52.8		59241 <- 59260	70	25.3		
						CCAAATTCTCCTTTAGGTGC				
<i>Millettia pinnata</i>	nad5/4	TGTCCTCCATCAGATVTCG			nad5/5	349937 <- 349956	100	51.8	2147	60
		414364 -> 414383	97	52.8		56773 <- 56792	70	25.3		
		96211 -> 96230	72	13.3		CCAAATTCTCCTTTAGGTGC				
<i>Lotus japonicus</i>	nad5/4	TGTCCTCCATCAGATVTCG			nad5/5	416520 <- 416539	100	51.8	2176	60
		29157 -> 29176	97	52.8		332142 -> 332161	65	28.5		
		371386 <- 371405	72	13.3		CCAAATTCTCCTTTAGGTGC				
<i>Glycine max</i>	nad5/4	TGTCCTCCATCAGATVTCG			nad5/5	31333 <- 31352	100	51.8	2196	60
		174175 -> 174194	97	52.8		CCAAATTCTCCTTTAGGTGC				
		232196 -> 232215	72	12.3		176322 <- 176341	100	51.8	2167	60
		353053 <- 353072	72	13.3						
						354976 <- 354995	100	51.8		
						256255 <- 256274	100	51.8		

Locus 29: nad7 intron 4/5 (reference: 4)

Species	F primer	5'-3' position and orientation	Com (%)	T _m (°C)	R primer	5'-3' position and orientation	Com (%)	T _m (°C)	Fragm length	T _a (°C)
<i>Vicia faba</i>	nad7 innerF1	GAAACGCGGCGCATAGGAACC			nad7 innerR1	TCCCGCTTCCTCGCATCTGCGC				
		306934 -> 306955	100	63.1		307973 <- 307994	100	64.9	1061	70
		536670 <- 536691	100	63.1		535631 -> 535652	100	64.9	1061	70
		221621 <- 221642	100	63.1		220582 -> 220603	100	64.9	1061	70
<i>Vigna angularis</i>	nad7 innerF1	GAAACGCGGCGCATAGGAACC			nad7 innerR1	TCCCGCTTCCTCGCATCTGCGC				
		361033 -> 361054	100	63.1		362060 <- 362081	100	64.9	1049	70

<i>Vigna radiata</i>	nad7 innerF1	GAAACGCGGCGGCATAGGAACC 348410 → 348431	100	63.1	nad7 innerR1	TCCCGCTTCCTCGCATCTGCGC 349424 ← 349445	100	64.9	1036	70
<i>Milletia pinnata</i>	nad7 innerF1	GAAACGCGGCGGCATAGGAACC 414964 → 414985	100	63.1	nad7 innerR1	TCCCGCTTCCTCGCATCTGCGC 415994 ← 416015	100	64.9	1052	70
<i>Lotus japonicus</i>	nad7 innerF1	GAAACGCGGCGGCATAGGAACC 29757 → 29778	100	63.1	nad7 innerR1	TCCCGCTTCCTCGCATCTGCGC 30820 ← 30841	95	58.5	1085	70
<i>Glycine max</i>	nad7 innerF1	GAAACGCGGCGGCATAGGAACC 174775 → 174796	100	63.1	nad7 innerR1	TCCCGCTTCCTCGCATCTGCGC 175805 ← 175826	100	64.9	1052	70

Locus 30: *nad7* intron 4/5 (reference: 4)

Species	F primer	5'-3' position and orientation	Com (%)	T _m (°C)	R primer	5'-3' position and orientation	Com (%)	T _m (°C)	Fragm length	T _a (°C)
<i>Vicia faba</i>	nad7 innerF2	CCCAATGATAAACCACTAACACCC 307486 → 307509	100	55.9	nad7 innerR2	GCGAAGCGTTCTATTGGTTTCCC 307400 ← 307422	100	57.8	No product	
		536116 ← 536139	100	55.9		536203 → 536225	100	57.8	No product	
		221067 ← 221090	100	55.9		221154 → 221176	100	57.8	No product	
						438673 ← 438695	61	34.3		
<i>Vigna angularis</i>	nad7 innerF2	CCCAATGATAAACCACTAACACCC 361582 → 361605	96	52.0	nad7 innerR2	368888 ← 368910	61	34.3		
						GCGAAGCGTTCTATTGGTTTCCC 361494 ← 361516	100	57.8	No product	
						168560 → 168582	57	30.7		
						42177 ← 42199	65	26.9		
<i>Vigna radiata</i>	nad7 innerF2	CCCAATGATAAACCACTAACACCC 348946 → 348969	96	52.0	nad7 innerR2	178496 → 178581	65	31.4		
						GCGAAGCGTTCTATTGGTTTCCC 348858 ← 348880	100	57.8	No product	
						186387 ← 186409	65	26.9		
						330238 ← 330260	65	31.4		
<i>Milletia pinnata</i>	nad7 innerF2	CCCAATGATAAACCACTAACACCC 415518 → 415541	96	52.0	nad7 innerR2	GCGAAGCGTTCTATTGGTTTCCC 415430 ← 415452	100	57.8	No product	
		61674 ← 61697	62	26.5						
<i>Lotus japonicus</i>	nad7 innerF2	CCCAATGATAAACCACTAACACCC 30338 → 30361	96	52.0	nad7 innerR2	GCGAAGCGTTCTATTGGTTTCCC 30250 ← 30272	100	57.8	No product	
						361811 → 361833	61	19.0		
<i>Glycine max</i>	nad7 innerF2	CCCAATGATAAACCACTAACACCC 175323 → 175346	96	52.0	nad7 innerR2	GCGAAGCGTTCTATTGGTTTCCC 175235 ← 175257	100	57.8	No product	
						88309 ← 88331	57	30.7		
						382385 → 382407	57	25.1		

Locus 31: *nad9* (reference: 3)

Species	F primer	5'-3' position and orientation	Com (%)	T _m (°C)	R primer	5'-3' position and orientation	Com (%)	T _m (°C)	Fragm length	T _a (°C)
<i>Vicia faba</i>	nad9	GGTCATCTCAATGGGYTCAG 262573 → 262592	98	51.9	nad9	TATAGTTGGGAGACTTTACC			No product	

		581033 <-- 581052	98	51.9									
		110991 -> 111010	78	9.6									
						559153 -> 559172	70	35.5					
						284453 <-- 284472	70	35.5					
<i>Vigna angularis</i>	nad9	GGTCATCTCAATGGGYTCAG			nad9	TATAGTTGGGAGACTTTACC							
		291217 -> 291236	98	51.9		291689 <-- 291708	100	47.3	492		54		
						49189 -> 49208	75	24.4					
<i>Vigna radiata</i>	nad9	GGTCATCTCAATGGGYTCAG			nad9	TATAGTTGGGAGACTTTACC							
		234230 -> 234249	98	51.9		234702 <-- 234721	100	47.3	492		54		
<i>Milletia pinnata</i>	nad9	GGTCATCTCAATGGGYTCAG			nad9	TATAGTTGGGAGACTTTACC							
		147747 -> 147766	98	51.9		148219 <-- 148238	95	41.3	492		54		
		308669 -> 308688	98	51.9		309141 <-- 309160	95	41.3	492		54		
<i>Lotus japonicus</i>	nad9	GGTCATCTCAATGGGYTCAG			nad9	TATAGTTGGGAGACTTTACC							
		256869 -> 256888	98	51.9		257341 <-- 257360	100	47.3	492		54		
		201634 -> 201653	78	9.6									
<i>Glycine max</i>	nad9	GGTCATCTCAATGGGYTCAG			nad9	TATAGTTGGGAGACTTTACC							
		289644 <-- 289663	98	51.9		289172 -> 289191	100	47.3	492		54		

Locus 32: orf25 (reference: 3)

Species	F primer	5'-3' position and orientation	Com (%)	Tm (°C)	R primer	5'-3' position and orientation	Com (%)	Tm (°C)	Fragm length	Ta (°C)
<i>Vicia faba</i>	orf25	AAGACCRCCAAGCYTCTCG			orf25	TTGCTGCTATTCTATCTATT				
		69633 <-- 69652	92	51.5		69148 -> 69167	95	44.9	505	52
		152094 -> 152113	65	36.8						
		166697 -> 1667716	62	6.2						
		146862 <-- 146881	72	16.9						
		87214 -> 87233	68	21.9						
						74728 -> 74747	80	15.3		
						525810 <-- 525829	75	16.5		
						210761 <-- 210780	75	16.5		
<i>Vigna angularis</i>	orf25	AAGACCRCCAAGCYTCTCG			orf25	TTGCTGCTATTCTATCTATT				
		215214 <-- 215233	92	51.5		214729 -> 214748	95	44.9	505	52
		296378 <-- 296397	60	23.5						
		192210 -> 192229	68	28.6						
		254100 -> 154119	62	6.2						
		106592 -> 106611	72	23.4						
		16874 -> 16893	62	4.0						
						129843 <-- 129862	70	20.9		
						250756 <-- 250775	75	16.5		
<i>Vigna radiata</i>	orf25	AAGACCRCCAAGCYTCTCG			orf25	TTGCTGCTATTCTATCTATT				
		396662 <-- 396681	92	51.5		396177 -> 396196	95	44.9	505	52
		239395 <-- 239414	60	23.5						
		316527 <-- 316546	68	28.6						
		153509 <-- 153528	62	6.2						

		104081 → 104100	72	23.4									
		214537 → 214556	62	4.0									
						228290 → 228309	65	19.2					
						127301 ← 127320	70	20.9					
						387948 ← 387967	70	7.7					
						156851 → 156870	75	16.5					
<i>Milletia pinnata</i>	orf25	AAGACCRCCAAGCYTCTCG			orf25	TTGCTGCTATTCTATCTATT							
		81997 ← 82016	92	51.5		81512 → 81531	95	44.9	505		52		
		313048 ← 313067	60	23.5									
		152126 ← 152145	60	23.5									
		38926 → 38945	68	28.6									
		216496 ← 216515	62	6.2									
		383898 ← 383917	72	36.0									
						256570 ← 256589	80	23.9					
						43137 → 43156	75	21.7					
<i>Lotus japonicus</i>	orf25	AAGACCRCCAAGCYTCTCG			orf25	TTGCTGCTATTCTATCTATT							
		97586 → 97605	92	51.5		98071 ← 98090	95	44.9	505		52		
		21014 → 21033	68	28.6									
		207945 → 207964	62	6.2									
		196432 → 196451	72	23.4									
						70389 ← 70408	85	22.9					
						21576 → 21595	80	19.9					
						21641 → 21660	70	25.9					
						147833 ← 147852	65	22.5					
<i>Glycine max</i>	orf25	AAGACCRCCAAGCYTCTCG			orf25	TTGCTGCTATTCTATCTATT							
		46015 ← 46034	92	51.5		45530 → 45549	95	44.9	505		52		
		25596 ← 25615	65	36.0									
		190673 ← 190692	62	6.2									
		391061 → 391080	72	23.4									
		159922 ← 159941	62	4.0									
						146289 → 146308	95	44.9					
						205656 ← 205675	95	44.9					
						194033 → 194052	75	16.5					

Locus 33: *rpI5* (reference: 3)

Species	F primer	5'-3' position and orientation	Com (%)	T _m (°C)	R primer	5'-3' position and orientation	Com (%)	T _m (°C)	Fragm length	T _a (°C)
<i>Vicia faba</i>	rpI5	AGTGGTAAAGTCTCATCT			rpI5	ATYGTGTGAAATAAGAGTAG				
		36761 → 36778	94	44.5		37167 ← 37186	92	40.0	426	52
		435202 ← 435219	94	44.5						
		365417 ← 365434	94	44.5						
		476674 ← 476691	78	19.5						
		240172 ← 240189	78	19.5						

<i>Vigna angularis</i>	rpl5	AGTGGTAAAGTCTCATCT 263811 <- 263828 29136 <- 29153 399441 -> 399458	94 78 72	44.5 19.5 33.6	rpl5	ATYGTGTGAAATAAGAGTAG 263403 -> 263422	98	44.8	426	52
<i>Vigna radiata</i>	rpl5	AGTGGTAAAGTCTCATCT 143555 -> 143572 43886 -> 43903 290823 -> 290840	94 78 72	44.5 19.5 33.6	rpl5	ATYGTGTGAAATAAGAGTAG 143961 <- 143980	98	44.8	426	52
<i>Millettia pinnata</i>	rpl5	AGTGGTAAAGTCTCATCT 378475 <- 378492 346441 -> 346458	94 78	44.5 22.8	rpl5	ATYGTGTGAAATAAGAGTAG 378064 -> 378083	98	44.8	429	52
<i>Lotus japonicus</i>	rpl5	AGTGGTAAAGTCTCATCT 77154 -> 77171 276348 -> 276365 217745 -> 217762	94 94 78	44.5 44.5 25.1	rpl5	363101 -> 363120 ATYGTGTGAAATAAGAGTAG 77560 <- 77579 276754 <- 276773	72 98 98	19.8 44.8 44.8		426 426
<i>Glycine max</i>	rpl5	AGTGGTAAAGTCTCATCT 19617 <- 19634 92546 -> 92563	94 78	44.5 22.8	rpl5	228814 <- 228833 ATYGTGTGAAATAAGAGTAG 19209 -> 19228	80 98	11.8		426 52

Locus 34: *rps3* (reference: 3)

Species	F primer	5'-3' position and orientation	Com (%)	Tm (°C)	R primer	5'-3' position and orientation	Com (%)	Tm (°C)	Fragm length	Ta (°C)
<i>Vicia faba</i>	rps3	GGCGTATTTCCGGATGCTT 502869 <- 502886 187820 <- 187837 552735 -> 552752 290873 <- 290890	100 100 78 78	53.3 53.3 41.4 41.4	rps3	TCAAGTYGGTTCAGTGAG 500601 -> 500618 185552 -> 185569	97 97	47.3 47.3	2286 2286	59 59
<i>Vigna angularis</i>	rps3	GGCGTATTTCCGGATGCTT 228061 <- 228078 103274 <- 103291 117745 -> 117762	100 78 78	53.3 28.4 39.6	rps3	TCAAGTYGGTTCAGTGAG 226195 -> 226212	97	47.3	1884	59
<i>Vigna radiata</i>	rps3	GGCGTATTTCCGGATGCTT 8228 <- 8245 100763 <- 100780 115225 -> 115242	100 78 78	53.3 28.4 39.6	rps3	TCAAGTYGGTTCAGTGAG 6366 -> 6383	97	47.3	1880	59
<i>Millettia pinnata</i>	rps3	GGCGTATTTCCGGATGCTT 293770 <- 293787 91804 <- 91821	100 72	53.3 26.1	rps3	9769 -> 9786 TCAAGTYGGTTCAGTGAG 291787 -> 291804 91321 -> 91338 295317 -> 295334 245396 <- 245413	75 97 69 75 69	33.6 47.3 14.5 33.6 26.1		501 59 57

<i>Lotus japonicus</i>	rps3	GGCGTATTTCCGATGCTT	100	53.3	rps3	TCAAGTYGGTTCAGTGAG	97	47.3	2163	59
		54419 → 54436				56564 ← 56581				
		118637 → 118654								
<i>Glycine max</i>	rps3	252570 ← 252587	100	53.3	rps3	52878 ← 52895	97	47.3	1871	59
						TCAAGTYGGTTCAGTGAG				
						311506 ← 311523				
						272131 → 272148				
						308115 ← 308132	75	33.6		

Locus 35: *rps4* (reference: 3)

Species	F primer	5'-3' position and orientation	Com (%)	Tm (°C)	R primer	5'-3' position and orientation	Com (%)	Tm (°C)	Fragm length	Ta (°C)
<i>Vicia faba</i>	rps4	CSTTTCYGCTCCGAAGAG	94	44.9	rps4	TCTCCGAAGATTGAGG	100	48.8	947	55
		400362 → 400379				401293 ← 401308				
<i>Vigna angularis</i>	rps4	CSTTTCYGCTCCGAAGAG	94	44.9	rps4	TCTCCGAAGATTGAGG	100	48.4	947	55
		314583 → 314600				315514 ← 315529				
		282148 ← 282165								
<i>Vigna radiata</i>	rps4	CSTTTCYGCTCCGAAGAG	94	44.9	rps4	109815 ← 109830	88	32.5	947	55
		257574 → 257591				TCTCCGAAGATTGAGG				
						258505 ← 258520				
<i>Milletia pinnata</i>	rps4	CSTTTCYGCTCCGAAGAG	94	44.9	rps4	107304 ← 107319	100	48.4	947	55
		146071 → 146088				TCTCCGAAGATTGAGG				
		306993 → 307010				147002 ← 147017				
		161021 → 161038				307924 ← 307939				
<i>Lotus japonicus</i>	rps4	CSTTTCYGCTCCGAAGAG	94	44.9	rps4	251095 → 251110	88	32.5	947	55
		180836 → 180853				49296 ← 49311				
						TCTCCGAAGATTGAGG				
						181767 ← 181782				
						85732 → 85747				
<i>Glycine max</i>	rps4	CSTTTCYGCTCCGAAGAG	94	44.9	rps4	284926 → 284941	81	32.1	947	55
		250451 ← 250468				49607 ← 49622				
		224377 ← 224394				TCTCCGAAGATTGAGG				
						249522 → 249537				
			75	25.4		247664 ← 247679	81	32.1		

Locus 36: *rps12-1/nad3-2* (reference: 3)

Species	F primer	5'-3' position and orientation	Com (%)	Tm (°C)	R primer	5'-3' position and orientation	Com (%)	Tm (°C)	Fragm length	Ta (°C)
<i>Vicia faba</i>	rps12	TTTCTTCTCTACCATGACGA	100	50.4	nad3	TGATCCYACTCGGTSTTCCT	90	46.9	393	56
		364685 ← 364704				364312 → 364331				

<i>Vigna angularis</i>	rps12	TTTCTTCTCTACCATGACGA 171122 <- 171141	100	50.4	nad3	25644 -> 25663	90	46.8	389	56
						521806 <- 521825	90	48.1		
						206757 <- 206776	90	48.1		
						TGATCCYACTCGGTSTTCCT				
						170753 -> 170772	90	46.9		
<i>Vigna radiata</i>	rps12	TTTCTTCTCTACCATGACGA 335961 -> 335980	100	50.4	nad3	54556 -> 54575	60	34.3	389	56
						135943 <- 135962	65	20.7		
						TGATCCYACTCGGTSTTCCT				
						336330 <- 336349	90	46.9		
						52084 -> 52103	60	34.3		
<i>Milletia pinnata</i>	rps12	TTTCTTCTCTACCATGACGA 342441 <- 342460	100	50.4	nad3	133405 <- 133414	65	20.7	393	56
						TGATCCYACTCGGTSTTCCT				
						342068 -> 342087	95	52.5		
						185511 -> 185530	68	13.2		
						13650 <- 13669	65	20.7		
<i>Lotus japonicus</i>	rps12	TTTCTTCTCTACCATGACGA 333314 <- 333333	100	50.4	nad3	TGATCCYACTCGGTSTTCCT	95	52.5	393	56
<i>Glycine max</i>	rps12	TTTCTTCTCTACCATGACGA 43355 <- 43374	100	50.4	nad3	332941 -> 332960			393	56
						TGATCCYACTCGGTSTTCCT				
						42982 -> 43001	90	46.9		
						73302 -> 73321	68	13.2		
						305360 <- 305379	65	20.7		
						29677 <- 29696	65	24.6		

Locus 37: rps12-2/nad3-1 (reference: 3)

Species	F primer	5'-3' position and orientation	Com (%)	Tm (°C)	R primer	5'-3' position and orientation	Com (%)	Tm (°C)	Fragm length	Ta (°C)
<i>Vicia faba</i>	rps12	ACCATATTTDGATCTGCCDC			nad3	YACGATHGGATTCTMTATG				
		364996 <- 365015	93	46.2		364562 -> 364581	84	34.0	454	52
		227862 -> 227881	68	27.3		230717 <- 230736	64	10.5	2875	54
		300695 <- 300714	68	27.3		297840 -> 297859	64	10.5	2875	54
		542911 -> 542930	68	27.3		545766 <- 545785	64	10.5	2875	54
		71337 <- 71356	68	21.7						
		237146 -> 237168	63	4.0						
		473651 -> 473670	63	4.0						
		180810 -> 180829	58	4.0						
		495859 -> 495878	58	4.0						
		109030 <- 109049	68	4.0						
		108927 <- 108946	63	26.4						
						194772 -> 194791	59	14.2		
						509821 -> 509840	59	14.2		
						138565 -> 138584	67	10.6		
						19583 -> 19602	69	9.7		
						325544 -> 325563	62	4.0		

Vigna angularis	rps12	ACCATATTTDGATCTGCCDC	nad3	429479 → 429498	64	4.0						
		252416 ← 252435		63	4.0	249094 → 249113	64	6.8	3342	54		
		171433 ← 171452		93	46.2	171003 → 171022	84	34.0	450	52		
		121239 → 121258		63	22.1							
		28222 ← 28241		63	10.9							
		349344 ← 349363		68	27.3							
Vigna radiata	rps12	ACCATATTTDGATCTGCCDC	nad3	240794 → 240813	59	23.3						
		335650 → 335669		93	46.2	271471 ← 271490	74	13.8				
		155187 → 155206		63	4.0	YACGATHGGATTTCTMTATG						
		118720 → 118739		68	21.7	336080 ← 336099	84	34.0	450	52		
		44798 → 44817		63	10.9							
		279776 ← 279795		68	27.3							
Millettia pinnata	rps12	ACCATATTTDGATCTGCCDC	nad3	29230 → 29249	64	6.8						
		342752 ← 342771		93	46.2	YACGATHGGATTTCTMTATG						
		116754 ← 116773		63	22.1	342318 → 342337	84	34.0	454	52		
		22755 ← 22774		73	4.0							
		408687 ← 408706		68	27.3							
		166630 → 166649		58	4.0							
		360939 → 360958		68	4.0							
		6749 → 6768		58	9.4							
Lotus japonicus	rps12	ACCATATTTDGATCTGCCDC	nad3	394995 ← 395014	64	11.1						
		333625 ← 333644		93	46.2	26833 ← 26852	67	10.6				
		341509 ← 341528		58	4.4	YACGATHGGATTTCTMTATG						
		23477 ← 23496		68	27.3	333191 → 333210	84	34.0	454	52		
		347382 ← 347401		68	4.0							
		347279 ← 347298		63	26.4							
Glycine max	rps12	ACCATATTTDGATCTGCCDC	nad3	230822 ← 230841	69	9.5						
		43666 ← 43685		93	46.2	YACGATHGGATTTCTMTATG						
		251840 ← 251859		68	21.7	43232 → 43251	84	34.0	454	52		
		400239 → 400258		73	4.0							
		168511 ← 168530		68	27.3							
		1048 → 1067		63	4.3							
						199839 ← 199858	59	12.7				
						399231 ← 399250	69	11.6				
						347396 → 347415	67	14.6				

Locus 38: *rps14-cob* (reference: 1)

Species	F primer	5'-3' position and orientation	Com (%)	T _m (°C)	R primer	5'-3' position and orientation	Com (%)	T _m (°C)	Fragm length	T _a (°C)
<i>Vicia faba</i>	rps14	CACGGGTCGCCCTCGTTCCG 36501 <- 36520	100	52.0	cob	GTGTGGAGGATATAGGTTGT 35125 -> 35144	100	52.0	1396	58
<i>Vigna angularis</i>	rps14	CACGGGTCGCCCTCGTTCCG 264069 -> 264088	100	65.0	cob	GTGTGGAGGATATAGGTTGT 265110 <- 265129	95	48.0	1061	58
<i>Vigna radiata</i>	rps14	CACGGGTCGCCCTCGTTCCG 143295 <- 143314	100	65.0	cob	GTGTGGAGGATATAGGTTGT 142257 -> 142276 188424 -> 188443	95 70	48.0 36.2	1058	58
<i>Milletia pinnata</i>	rps14	CACGGGTCGCCCTCGTTCCG 378733 -> 378752	100	65.0	cob	GTGTGGAGGATATAGGTTGT 379740 <- 379759	100	52.4	1027	58
<i>Lotus japonicus</i>	rps14	CACGGGTCGCCCTCGTTCCG 276088 <- 276107 76894 <- 76913	100 100	65.0 65.0	cob	GTGTGGAGGATATAGGTTGT 274624 -> 274643 75430 -> 75449	100 100	52.4 52.4	1484 1484	58 58
<i>Glycine max</i>	rps14	CACGGGTCGCCCTCGTTCCG 19879 -> 19898	100	65.0	cob	GTGTGGAGGATATAGGTTGT 20933 <- 20952	95	48.0	1074	58

Locus 39: *rrn5/rrn18-1* (reference: 3)

Species	F primer	5'-3' position and orientation	Com (%)	T _m (°C)	R primer	5'-3' position and orientation	Com (%)	T _m (°C)	Fragm length	T _a (°C)
<i>Vicia faba</i>	rrn5	GAGGTCGGAATGGGATCGGG 320283 -> 320302 53713 -> 53732	100 80	59.2 43.5	rrn18-1	GGGTGAAGTCGTAACAAGGT 320541 <- 320560	100	54.4	278	61
<i>Vigna angularis</i>	rrn5	GAGGTCGGAATGGGATCGGG 125741 -> 125760 203511 <- 203530	100 75	59.2 38.2	rrn18-1	GGGTGAAGTCGTAACAAGGT 125991 <- 126010	100	54.4	270	61
<i>Vigna radiata</i>	rrn5	GAGGTCGGAATGGGATCGGG 123199 -> 123218 305238 -> 305257	100 75	59.2 38.2	rrn18-1	GGGTGAAGTCGTAACAAGGT 123449 <- 123468	100	54.4	270	61
<i>Milletia pinnata</i>	rrn5	GAGGTCGGAATGGGATCGGG 399440 -> 399459	100	59.2	rrn18-1	GGGTGAAGTCGTAACAAGGT 399690 <- 399709	100	54.4	270	61
<i>Lotus japonicus</i>	rrn5	GAGGTCGGAATGGGATCGGG 176770 <- 176789 10416 -> 10435	100 75	59.2 46.2	rrn18-1	GGGTGAAGTCGTAACAAGGT 176512 -> 176531	100	54.4	278	61
<i>Glycine max</i>	rrn5	GAGGTCGGAATGGGATCGGG 295167 -> 295186 87770 -> 87789 153537 -> 153556	100 75 75	59.2 38.2 46.2	rrn18-1	GGGTGAAGTCGTAACAAGGT 295411 <- 295430 243391 -> 243410 395326 <- 395345 5573 <- 5592 202913 -> 202932	100 80 80 95 85	54.4 34.2 34.2 50.9 45.6	264	61

Position and orientation of primers in mtDNA genomes; Com - complementarity of a primer and template DNA expressed through the percentage of binding nucleotides; *T_m* – melting temperature of a primer in °C; Fragm length – the length of the PCR products obtained via *in silico* PCR method; *T_a* – annealing temperature of a primer pair in °C; * - primer *nad4* exon2 is not located within exon2 but within exon1.

References:

- 1 – DEMESURE, B., N. SODZI and R.J. PETIT (1995): A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. *Mol. Ecol.* 4: 129-134.
- 3 - DUMINIL, J., M.H. PEMONGE and R.J. PETIT (2002): A set of 35 consensus primer pairs amplifying genes and introns of plant mitochondrial DNA. *Mol. Ecol. Notes* 2: 428-430.
- 2 – DUMOLIN-LAPEGUE, S., M.H. PEMONGE and R.J. PETIT (1997): An enlarged set of consensus primers for the study of organelle DNA in plants. *Mol. Ecol.* 6: 393-397.
- 4 – HAVANANDA, T., E.C. BRUMMER, I.J. MAUREIRA-BUTLER and J.J. DOYLE (2010): Relationships among diploid members of the *Medicago sativa* (Fabaceae) species complex based on chloroplast and mitochondrial DNA sequences. *Syst. Bot.* 35: 140-150.