

LG Clark Lab: Sequencing Primers and PCR Protocols

Region	Size (bp)	Primer Sets	PCR Parameters (All end with 4°C hold)
<b>atpI-atpH</b>	~930	atpI: CCG GTC ATG TTT CTT GGA TT (53.0°C) atpH: CAA TAA CRG AAG CAG CAG CA (54.8°C)	94°C, 2m; 25x (96°C, 45s; 56°C, 1m; 72°C, 1m); 72°C, 3m.
<b>ndhF (5' end)</b>	~1300	5F: ATG GAA CAK ACA TAT SAA TAT GC (49.7°C) 1318R: CGA AAC ATA TAA AAC GCA GTT AAT CC (53.0°C) <u>SEQ</u> : 536F: TTG TAA CTA ATC GTG TAG GGG A (~800bp) 803R: GAA AAA TCC CCG CAG CAA CCA TAG (~800bp) [274R: ATT AGT ATT AAC ATA ATA GAA GTA AG (~300bp)]	94°C, 1m; 30x (94°C, 1m; touchdown* 58-48°C, 1m; 72°C, 1m 30s); 72°C 10m.
<b>ndhF (3' end)</b>	~1140	972F: GTC TCA ATT GGG TTA TAT GAT G (48.9°C) 2110R: CCC CCT AYA TAT TTG ATA CCT TCT CC (55.2°C) <u>SEQ</u> : 1318F: GGA TTA ACT GCG TTT TAT ATG TTT CG (~800bp) 1603R: GCA TAG TAT TTC CCG TTT CAT GAG G (~650bp) [1821F: TTK GGT YTA TYC ATA GCA TA (~300bp)]	94°C, 1m; 30x (94°C, 1m 30s; touchdown* 53-43°C, 2m; 72°C, 3m); 72°C 10m.
<b>psaA-ORF170</b>	~970	psaA: TCG AAA TCG TGA GCA TCA GC (55.4°C) ORF170: TCT CAA GTA CGG TTC TAG G (50.4°C)	95°C, 2m; 35x (95°C, 1m; 50°C, 10s; +15°C, 0.3°C/s; 65°C, 5m); 65°C, 5m.
<b>rpL16 intron</b>	~1100	F71: GCT ATG CTT AGT GTG TGA CTC GTT G R1661: CGT ACC CAT ATT TTT CCA CCA CGA C <u>SEQ</u> : SAK8: CCA TCC CAC CCA ATG AAG (~700bp) R1516: CCC TTC ATT CTT CCT CTA TGT TG (~700bp) (Do not use published primers F220 or R270; poor for bamboo)	95°C, 2m; 35x (95°C, 1m; 50°C, 10s; +15°C, 0.3°C/s; 65°C, 4m); 65°C, 5m  SK LAB: 80°C, 5m; 35x (95°C, 1m; 50°C, 10s; +15°C, 0.3°C/s; 65°C, 5m); 65°C, 4m
<b>rpL16 intron</b> Untested internal primers (JT 2007)		jt320F: GGA TGT GGA TAA AAG GAG G (~550bp) jt580R: GCA GTC ATA GGT TCT GTC G (~600bp) jt680F: GGA TGG CGG AAC AAA CCA A (~550bp)	
<b>rpS16 intron</b>	~860	rps16F: AAA CGA TGT GGT ARA AAG CAA C (53.0°C) rps16R: AAC ATC WAT TGC AAS GAT TCG ATA (52.9°C)	94°C, 2m; 35x (94°C, 45s; touchdown* 58-48°C, 30s; 72°C, 1m); 72°C, 5m.
<b>rps16-trnQ v1</b>	~1120	1F: GCA CGT TGC TTT CTA CCA CA (55.9°C) 1574R: ATC CTT CCG TCC CAG ATT TT (53.8°C) [rps16F: GTT GCT TTC TAC CAC ATC G (51.2°C)] [trnQ: GTT CGA ATC CTT YCG TCC C (54.4°C)] <u>SEQ</u> : 334F: CGA GAT GGT CAA TCC TGA AAT G (~700bp) 628R: CTT TTG GTA TTC KAG TCG AAG (~550bp)	95°C, 2m; 35x (95°C, 1m; 50°C, 10s; +15°C, 0.3°C/s; 65°C, 5m); 65°C, 5m.
<b>rps16-trnQ v2</b> For NT taxa and others with the 3' insert.	~1550 (929; 1050)	5' end: 1F: GCA CGT TGC TTT CTA CCA CA (55.9°C) 929R: TTC TGT CTA CTC GGC TTT CG (54.3°C) 3' end: 538F: CGA CTC GAA TAC CAA AAG AGG (53.1°C) 1574R: ATC CTT CCG TCC CAG ATT TT (53.8°C) <u>SEQ</u> : (5') 16Q 650R: GTT CGT TGG ATA GAA TGG ATT C (~650bp) (3') jt16Qin-for: GCC GAG TAG ACA GAA TAT ATG (~650bp) (3') 16Q 1100R: GGC CAG ATT AAA GAA TAG GAA G (~650bp)	95°C, 2m; 35x (95°C, 1m; 48°C, 10s; +17°C, 0.3°C/s; 65°C, 5m); 65°C, 5m.  <u>SEQ note</u> : Shibataeinae/ Thamnocalaminae, also use 628R with the 5' amplicon (see rps16-trnQ v1 for primer sequence); <i>i.e.</i> , 4 seq. primers total.
<b>[NT insert in rps16-trnQ]</b>	~425	jt16Qin-for: GCC GAG TAG ACA GAA TAT ATG (50°C) jt16Qin-rev: CCT ACT ACT ACT CAT TAC TC (48°C)	94°C, 2m; 35x (94°C, 1m; 44°C, 1m; 72°C, 1m); 72°C, 5m

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<b>trnC-rpoB</b>	~1185	trnC: TGG GGA TAA AGG ATT TGC AG (55.4°C) rpoB: ATT GTG GAC ATT CCC TCR TT (53.0°C) <u>SEQ</u> : jt400-for: CAG GTC CGA ACA GCA TTA (~750bp) jt700-rev*: CGT AGT AGT AGA ATT GCT AG (~750bp)	94°C, 2m; 35x (96°C, 1m; touchdown* 56-46°C, 2m; 72°C, 3m); 72°C, 5m. (total = 5 hrs) *jt700-rev is bad for Olyreae & non-bamboo; use the "trnC" primer for 5' end.
<b>trnD-trnT (rev. 10/2/06)</b>	~1100	trnD-for: ACC AAT TGA ACT ACA ATC CC (50.2°C) trnT-rev: CCC TTT TAA CTC AGT GGT A (48.8°C) <u>SEQ</u> : trnY-rev: CTC TTT GCT TTG GAT CTA G (~700bp) trnE-for: GCC TCC TTG AAA GAG AGA TG (~700bp) SAK1F: GGA TTT GAA CCA GCG TAT ACA (~850bp)	94°C, 2m; 35x (94°C, 45s; touchdown* 58-48.5°C, 1m; 72°C, 1m15s); 72°C, 5m.  <u>SEQ note</u> : trnE-for & SAK1: choose 1. SAK1 provides better overlap but poorer 3' end.
<b>[trnD-trnT] Kelchner</b>	~1100	SAK9F: CTT GAC AGG GCG GTG CTC T SAK10R: GCA TAA GTC ATC GGT TCA AAT C <u>SEQ</u> : SAK1F: GGA TTT GAA CCA GCG TAT ACA SAK2R: TGC CCC TAT CGT CTA GTG GT	80°C, 5m; 35x (95°C, 1m; 50°C, 10s; +15°C, 0.3°C/s; 65°C, 5m); 65°C, 4m
<b>trnG intron</b>	~745	3' trnG: GTA GCG GGA ATC GAA CCC GCA TC (62.1°C) 5' trnG: GCG GGT ATA GTT TAG TGG TAA AA (52.5°C)	95°C, 2m; 35x (95°C, 1m; 50°C, 10s; +15°C, 0.3°C/s; 65°C, 5m); 65°C, 5m.
<b>trnH-psbA</b>	~670	trnH: CGC GCA TGG TGG ATT CAC AAT CC (61.1°C) psbA: GTW ATG CAY GAA CGT AAT GCT C (53.3°C)	80°C, 5m; 35x (94°C, 30s; touchdown* 58-48°C, 30s; 72°C, 1m); 72°C, 10m.
<b>trnK-rps16</b>	~664	trnK: TAC TCT ACC RTT GAG TTA GCA AC (53.1°C) rps16: AAA GGK GCT CAA CCT ACA RGA AC (57.2°C)	80°C, 5m; 35x (94°C, 30s; touchdown* 58-48°C, 30s; 72°C, 1m); 72°C, 10m.
<b>trnS-trnfM</b>	~1400	trnS: GAG AGA GAG GGA TTC GAA CC (54.2°C) trnfM: CAT AAC CTT GAG GTC ACG GG (55.3°C)	94°C, 5m; 35x (94°C, 1m; 62°C, 1m; 72°C, 2m); 72°C, 10m.
<b>trnT-trnL</b>	~830	trnT-L F: CAT TAC AAA TGC GAT GCT CT (51.0°C) trnT-L R: TCT ACC GAT TTC GCC ATA TC (51.8°C)	95°C, 2m; 35x (95°C, 1m; 48°C, 10s; +17°C, 0.3°C/s; 65°C, 5m); 65°C, 5m. (total 5.5 hrs)

### Notes

1. First pair is for PCR amplification of target region. "SEQ" are internal primers for sequencing reactions; if none are listed, use the PCR primers.
2. Brackets [] indicate alternate primers used only in special cases (*e.g.*, to complete the ends of otherwise poor reads).
3. Touchdown PCR: Initial annealing temperature in cycle 1 (*e.g.*, 58°C), with a reduction (typically -1°C) each cycle until a target annealing temperature is reached (*e.g.*, 48°C), followed by additional cycles at this new temperature (Don, R.H., Cox, P.T., Wainwright, B.J., Baker, K., and Mattick, J.S. 1991. 'Touchdown' PCR to circumvent spurious priming during gene amplification. *Nucleic Acids Res.* 19: 4008).
4. For additional information: contact Jimmy Triplett: triplett@iastate.edu

Region	Primer Name	Published name, if diff.	Purpose	Sequence (5' to 3')	Source	Notes
atpI-atpH	atpI	atpI/643F	AMP/SEQ	CCG GTC ATG TTT CTT GGA TT	Yamane & Kawahara (2005)	
	atpH	atpH/18R	AMP/SEQ	CAA TAA CRG AAG CAG CAG CA	Yamane & Kawahara (2005); modified by Triplett	
ndhF (5' end)	5F		AMP, 5' end	ATG GAA CAK ACA TAT SAA TAT GC	Olmstead & Sweere (1994)	
	1318R		AMP, 5' end	CGA AAC ATA TAA AAC GCA GTT AAT CC	Olmstead & Sweere (1994)	
	274R		SEQ, 5' end	ATT AGT ATT AAC ATA ATA GAA GTA AG	Olmstead & Sweere (1994); modified by Triplett	
	536F		SEQ, 5' end	TTG TAA CTA ATC GTG TAG GGG A	Olmstead & Sweere (1994)	
	803R		SEQ, 5' end	GAA AAA TCC CCG CAG CAA CCA TAG	Olmstead & Sweere (1994); modified by Triplett	
ndhF (3' end)	972F		AMP, 3' end	GTC TCA ATT GGG TTA TAT GAT G	Olmstead & Sweere (1994)	
	2110R	3'R	AMP, 3' end	CCC CCT AYA TAT TTG ATA CCT TCT CC	Olmstead & Sweere (1994)	
	1318F		SEQ, 3' end	GGA TTA ACT GCG TTT TAT ATG TTT CG	Olmstead & Sweere (1994); modified by Triplett	
	1603R		SEQ, 3' end	GCA TAG TAT TTC CCG TTT CAT GAG G	Olmstead & Sweere (1994)	
	1821F		SEQ, 3' end	TTK GGT YTA TYC ATA GCA TA	Clark et al. (1995); modified by Triplett	
psaA-ORF170	psaA		AMP/SEQ	TGC AAA TCG TGA GCA TCA GC	Saltonstall, K. (2001)	
	ORF170		AMP/SEQ	TCT CAA GTA CGG TTC TAG G	Saltonstall, K. (2001)	
rpL16 intron	F71		AMP	GCT ATG CTT AGT GTG TGA CTC GTT G	Jordan et al. (1996)	
	R1661		AMP	CGT ACC CAT ATT TTT CCA CCA CGA C	Jordan et al. (1996)	
	SAK8		SEQ	CCA TCC CAC CCA ATG AAG	Kelchner	
	R1516		SEQ	CCC TTC ATT CTT CCT CTA TGT TG	Kelchner & Clark (1996)	
	jt320F		[seq]	GGA TGT GGA TAA AAG GAG G	Triplett (2007), unpublished	Untested
	jt580R		[seq]	GCA GTC ATA GGT TCT GTC G	Triplett (2007), unpublished	Untested
	jt680F		[seq]	GGA TGG CCG AAC AAA CCA A	Triplett (2007), unpublished	Untested
	F220		[drop]	CTG ATT ATG AGT TGT GAA GC	Zhang (2000)	Poor for bamboo
	R270		[drop]	TCA CCC TTT CAT TTA TCC	Zhang (2000)	Poor for bamboo
	rpS16 intron	rpS16F		AMP/SEQ	AAA CGA TGT GGT ARA AAG CAA C	Oxelmann et al. (1997), modified by Shaw et al. (2005)
rpS16R			AMP/SEQ	AAC ATC WAT TGC AAS GAT TCG ATA	Oxelmann et al. (1997), modified by Shaw et al. (2005)	
rps16-trnQ v1 (Non-NT clade)	1F		AMP, 5' end	GCA CGT TGC TTT CTA CCA CA	Triplett (2006), unpublished	
	1574R		AMP, 3' end	ATC CTT CCG TCC CAG ATT TT	Triplett (2006), unpublished	
	rps16F		[amp, seq]	GTT GCT TTC TAC CAC ATC G	Saltonstall, K. (2001)	
	trnQ		[amp, seq]	GTT CGA ATC CTT YCG TCC C	Saltonstall, K. (2001)	
	334F		SEQ	CGA GAT GGT CAA TCC TGA AAT G	Triplett & Ho (2006), unpublished	
	628R		SEQ	CTT TTG GTA TTC KAG TCG AAG	Triplett & Ho (2006), unpublished	
rps16-trnQ v2 (NT Clade)	1F		AMP, 5' end	GCA CGT TGC TTT CTA CCA CA	Triplett (2006), unpublished	
	929R		AMP, 5' end	TTC TGT CTA CTC GGC TTT CG	Triplett (2006), unpublished	
	538F		AMP, 3' end	CGA CTC GAA TAC CAA AAG AGG	Triplett (2006), unpublished	
	1574R		AMP, 3' end	ATC CTT CCG TCC CAG ATT TT	Triplett (2006), unpublished	
	16Q 650R		SEQ, 5' end	GTT CGT TGG ATA GAA TGG ATT C	Triplett (2006), unpublished	
	16Q 900F	(jt16Q in-for)	SEQ, 3' end	GCC GAG TAG ACA GAA TAT ATG	Triplett (2006), unpublished	
16Q 1100R		SEQ, 3' end	GGC CAG ATT AAA GAA TAG GAA G	Triplett (2006), unpublished		
Insert in rps16-trnQ (NT Clade)	jt16Q in-for		AMP, SEQ	GCC GAG TAG ACA GAA TAT ATG	Triplett (2007), unpublished	
	jt16Q in-rev		AMP, SEQ	CCT ACT ACT ACT CAT TAC TC	Triplett (2007), unpublished	
trnC-rpoB	trnC	trnC/22F	AMP	TGG GGA TAA AGG ATT TGC AG	Yamane and Kawahara (2005)	
	rpoB	rpoB/16R	AMP	ATT GTG GAC ATT CCC TCR TT	Yamane and Kawahara (2005); modified by Triplett (2006)	
	jt400-for		SEQ	CAG GTC CGA ACA GCA TTA	Triplett (2007); unpublished	
	jt700-rev		SEQ	CGT AGT AGT AGA ATT GCT AG	Triplett (2007); unpublished	Bambuseae only.
trnD-trnT	trnD-for		AMP	ACC AAT TGA ACT ACA ATC CC	Demesure et al. (1995)	
	trnT-rev		AMP	CCC TTT TAA CTC AGT GGT A	Triplett (2005), unpublished	
	trnE-for		SEQ	GCC TCC TTG AAA GAG AGA TG	Doyle et al. (1992)	
	trnY-rev		SEQ	CTC TTT GCT TTG GAT CTA G	Triplett (2006), unpublished	
	SAK1F		[seq]	GGA TTT GAA CCA GCG TAT ACA	Kelchner	
Alt: trnD-trnT (Kelchner)	SAK9F		[amp]	CTT GAC AGG GCG GTG CTC T	Kelchner	
	SAK10R		[amp]	GCA TAA GTC ATC GGT TCA AAT C	Kelchner	
	SAK2R		[seq]	TGC CCC TAT CGT CTA GTG GT	Kelchner	
trnG intron	5' trnG	5' trnG2G	AMP/SEQ	GCG GGT ATA GTT TAG TGG TAA AA	Shaw et al. (2005)	
	3' trnG		AMP/SEQ	GTA GCG GGA ATC GAA CCC GCA TC	Shaw et al. (2005)	
trnH-psbA	trnH		AMP/SEQ	CGC GCA TGG TGG ATT CAC AAT CC	Tate & Simpson (2003)	
	psbA		AMP/SEQ	GTW ATG CAY GAA CGT AAT GCT C	Sang et al. (1997); modified by Triplett	
trnK-rps16	trnK	trnK5'r	AMP/SEQ	TAC TCT ACC RTT GAG TTA GCA AC	Johnson & Soltis (1995)	
	rps16		AMP/SEQ	AAA GKG GCT CAA CCT ACA RGA AC	Johnson & Soltis (1995), modified by Kress et al. (2005)	
trnS-trnM	trnS		AMP/SEQ	GAG AGA GAG GGA TTC GAA CC	Demesure et al. (1995)	
	trnM		AMP/SEQ	CAT AAC CTT GAG GTC ACG GG	Demesure et al. (1995)	
trnT-trnL	trnTL for	trnT F; TabA	AMP/SEQ	CAT TAC AAA TGC GAT GCT CT	Taberlet et al. (1991)	
	trnTL rev	5' trnL R; TabB	AMP/SEQ	TCT ACC GAT TTC GCC ATA TC	Taberlet et al. (1991)	

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