Laboratório de Citogenética e Evolução Vegetal

Protocolo de PCR para sequenciamento Sanger usando *primers* universais

Versão 1.0 (12/11/2020)

Para uma reação de volume final (V_f) de 50 µl:

Reagentes	**C_i**	**C_f**	**V_i**	**V_i (Promega)**
DNA	10 - 100 ng/µl	20 - 200 ng	2	
Tampão da Taq	10×	1	5	10
TBT	5×	1	10	
dNTP	2,5 mM	0,2	4	
MgCl	50 mM	3	3	1,5
Primer Forward	10 µM	0,1	0,5	
Primer Reverse	10 µM	0,1	0,5	
Taq caseira	-	-	0,2	
H_2O			24,8	21,3

C_i= concentração inicial; C_f = concentração final; V_i= volume inicial

Obs. 1: A amplificação de cada região de interesse deve ser inicialmente testada num volume total de 25 µl. Para algumas regiões/espécies problemáticas, a Taq Promega (kit) pode mostrar melhor resultado.

Obs. 2: Para amplificação de várias amostras simultaneamente, uma Master Mix deve ser preparada de acordo com o número de amostras com um excesso de 10% para todos os reagentes.

Obs. 3: A quantidade ideal de DNA na reação pode ser testada em série de diluição.

Obs. 4: Para preparar a solução de 5× TBT pH 8,0, segundoSAMARAKOON et al, 2013.

Reagente	**C_f**
BSA (Soro de albumina bovina)	1 g/L
Tris- HCL ph 8,0	8.5 mM
Tween-20	1% (v/v)
Trehalose	750 mM

Programa do termociclador

5 min – 95 °C Desnaturação do DNA dupla fita

1 min – 95°C
1 min – 48 - 65°C } 35 Ciclos Desnaturação, Anelamento e Extensão
1 min – 72°C

10 min – 72°C Extensão final

∞ 10°C Temperatura de conservação do produto

Obs. 4: Tempos mais curtos podem ser testados nas diferentes etapas para diferentes regiões. O tempo de extensão varia de acordo com o tamanho do produto final.

Obs. 5: A temperatura de anelamento depende dos *primers* usados (ver tabela abaixo) e pode ser ajustada para cada espécie por gradiente, se necessário.

Tabela 1. *Primers* universais disponíveis para análises filogenéticas e filogeográficas

Nome da região	Sequência do primer *forward*	Nº estoque	Sequência do primer *reverse*	N° estoque	Temperatura de anelamento	Tamanho de fragmento	Referência
atpβ-rbcL	atpβF (GTGGAAACCCCGGGACGAGAAGTAGT)	192	atpβR (ACTTGCTTTAGTTTCTGTTTGTGGTGA)	193	53ºC (original) 56ºC (otimizada)	~580-850 pb	Hodges e Arnold 1994
ITS1-5.8S-ITS2	ITS5 (GGAAGTAAAAGTCGTAACAAGG)	475, 870	ITS4 (TCCTCCGCTTATTGATATGC)	576	50-60ºC	~600-850 pb	White *et al*, 1990
	17SE (ACGAATTCATGGTCCGGTGAAGTGTTCG)	U9	26SE (TAGAATTCCCCGGTTCGCTCGCCGTTAC)	U10	50-60ºC	~800 pb	Sun et al,
ITS1	ITS5 (GGAAGTAAAAGTCGTAACAAGG	475	ITS2 (GCTGCGTTCTTCATCGATGC)	76	50°C	1500 pb	White *et al*, 1990
ITS2	ITS3 (GCATCGATGAAGAACGCAGC)	69	ITS4 (TCCTCCGCTTATTGATATGC)	576	55°C	300 pb	White *et al*, 1990
*mat*K	matK3F (AAGATGCCTCTTCTTTGCAT)	638	matK1R (GAACTAGTCGGATGGAGTAG)	637	52ºC	~1500 pb	Sang *et al*, 1997
*ndh*F	1318F (GGATTAACYGCATTTTATATGTTTCG)	868	2110R (CCCCCTAYATATTTGATACCTTCTCC)	869	50ºC	~800-2000 pb	Olmstead e Sweere, 1994
*ndh*F–*rpl*32	ndhF-rpl32 nested F (TTTTTCTGATTCACCTGC)	464	ndhF-rpl32 nested R (CATCTATTGTTCAAAACG)	465	50-55°C	~700 pb	Steele *et* al, 2010

*psb*A-*trn*H	psbAF (GTTATGCATGAACG TAATGCTC)	470	trnHR (CGCGCATGGTG GATTCACAAATC) trnH (GUG)	471	50ºC		Sang *et* al, 1997
	psbA (CGAAGCTCCATCTA CAAATGG)	641	(ACTGCCTTGATC CACTTGGC)	642	53ºC	~495 pb	Hamilton *et* al, 1999
*psb*J-*pet*A	psbJ (ATAGGTACTGTARC YGGTATT)	162	petA (AACARTTYGARA AGGTTCAATT)	163	50ºC	~700-1300 pb	Shaw *et al*, 2007
*psb*E-*pet*L	psbE-petL F (TGCTATGAATGACC CAGTATCG)	436	psbE-petL R (CAGACCGATAA ATAGAGCTGAG G)	437	50-55°C	~1200 pb	Steele *et* al, 2010
*rbc*L	rbcL N (a F NY1151) (ATGTCACCACAAAC AGAAACTAAAGC)	536	a R (NY1152) (TCACAAGCAGC AGCTAGTTCAGG ACT)	537	53°C	~500-1400 pb	Käss and Wink, 1996/ Knopf et al, 2012
*rps*16	*rps*F (GTGGTAGAAAGCA ACGTGCGACTT)		*rps*R2 (TGCGGATCGAA CATCCAATTGCA AC)		56ºC		Schaferhoff et al, 2010
*rps*16-*trn*Q	*rps*16-*trn*Q F (GTCATTGGTTTAGT TGGTCC)	438	*rps*16-*trn*Q R (GCCAAGTGGTA AGGCAACG)	439	50-59°C	~1200 pb	Steele *et* al, 2010
*trn*L-*trn*F	c (CGAAATCGGTAGA CGCTACG)	636	f (ATTTGAACTGGT GACACGAG)	635	50-56ºC	~900 pb	Taberlet *et al*, 1991
*trn*L-*trn*T	a (CATTACAAATGCGA TGCTCT)	81	b (TCTACCGATTTC GCCATATC)	82	50-57ºC	~250-830 pb	Taberlet *et al*, 1991
*trns*S-*trns*G	trnG^{UUC} (GTAGCGGGAATCG	191	trnS^{GCU}(AGATAG GGATTCGAACCC TCGGT)	190	50-66ºC	~600-1000 pb	Shaw *et al*, 2005

	AACCCGCATC)						
*ycf*1	ycf1bF (TCTCGACGAAAATC AGATTGTTGTGAAT)	645	ycf1bR (ATACATGTCAA AGTGATGGAAA A)	646	52-53°C	900 pb	Dong *et al,* 2015

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