Protocol - PCR amplification

David M. Francis
Horticulture and Crop Science
OARDC
1680 Madison Ave
Wooster, OH 44691

Reagents:

dNTP stock:
988 ul water
13 ul each of four 100 mM dNTPs (Pharmacia Ultrapure set #27-2035-01)
= 5 mM dNTP (1.25 mM ea)

PCR buffer (10X stock):
100 mM Tris pH 8.3
500 mM KCl
15 mM $MgCl_2$
0.01% w/v gelatin

Primers

Marker		Primer(5'----3')	Temp	Cycles
CosOH44	f:	TGCTTCTTGCACCACAAACT	56	36
	r:	TGTTGTCATGGTCCCTTTGA		
CosOH45	f:	TGAGACTGAAAATGTGCGATATG	56	36
	r:	CTCCATTTCACAGAACTGCTTG		
COSOH49	f:	TTCGCGTGGTGACACAGTTA	56	36
	r:	GCTCTAGCTTCTCTTTCTCGGACT		
SSR383	f:	CACGACGTTGTAAAACGACATTGTACAAAGACCCGTGGC	45	36
	r:	GTTGCACACTGGATCAATGC		
SSR96	f:	CACGACGTTGTAAAACGACGGGTTATCAATGATGCAATGG	45	36
	r:	CCTTTATGTCAGCCGGTGTT		
SSR32	f:	CACGACGTTGTAAAACGACTGGAAAGAAGCAGTAGCATTG	45	36
	r:	CAACGAACATCCTCCGTTCT		
SSR134	f:	CACGACGTTGTAAAACGACCCCTCTTGCCTAAACATCCA	45	36
		CGTTGCGAATTCAGATTAGTTG		

Annealing Temp. based on *L. esculentum*.
Stocks should be 10 uM.

T1/10E Stock
10 mM Tris (7.5-8.0)
0.1 mM EDTA

This stock contains 1/10 the amount of EDTA found in “TE” and is used for diluting DNA templates for use in PCR. Water can be used to dilute templates that will not be stored for any length of time.

PCR Amplification Procedures

Mix for 1 50 ul reaction:
Can easily scale down to 20 or 25 ul

water	33.6
DNA	5.0 (1 ng/ul)*
buffer	5.0
dNTP	2.0
Forward Primer	2.0
Reverse Primer	2.0
Taq polymerase	0.4

Make up PCR mix leaving out the DNA. Add 45 ul of the mix to tubes; then, using fresh pipette tips, add DNA. Cover with a drop of mineral oil and amplify.

amplification protocol for primers:

step 1 94 C for 2 min
step 2 94 C for 1 min
step 3 __C for 1 min (55 C-62 C)
step 4 72 C for 2 min
step 5 go to "step 2" 34 times (30-34)
step 6 72 C for 5 min
step 7 4 C for 00 00 00
step 8 end

Protocol: PCR AMPLIFICATION OF SSRs

David M. Francis
Horticulture and Crop Science
OARDC
1680 Madison Ave
Wooster, OH 44691

Reagents:

dNTP stock:
988 ul water
13 ul each of four 100 mM dNTPs (Pharmacia Ultrapure set #27-2035-01)
= 5 mM dNTP (1.25 mM ea)

PCR buffer A (10X stock):
100 mM Tris pH 8.3
500 mM KCl
15 mM $MgCl_2$
0.01% w/v gelatin

PCR buffer B (10X stock):
100 mM Tris pH 8.3
500 mM KCl
0.01% w/v gelatin

DNA template
Approximate concentration 1-5 ng/ul

Primers
Stocks should be 25X (25 uM)

Marker		Primer(5'----3')	Temp	Cycles
SSR383	f:	CACGACGTTGTAAAACGACATTGTACAAAGACCCGTGGC	45	36
	r:	GTTGCACACTGGATCAATGC		
SSR96	f:	CACGACGTTGTAAAACGACGGGTTATCAATGATGCAATGG	45	36
	r:	CCTTTATGTCAGCCGGTGTT		
SSR32	f:	CACGACGTTGTAAAACGACTGGAAAGAAGCAGTAGCATTG	45	36
	r:	CAACGAACATCCTCCGTTCT		
SSR134	f:	CACGACGTTGTAAAACGACCCCTCTTGCCTAAACATCCA	45	36
		CGTTGCGAATTCAGATTAGTTG		

T1/10E Stock
10 mM Tris (7.5-8.0)
0.1 mM EDTA

This stock contains 1/10 the amount of EDTA found in “TE” and is used for diluting DNA templates for use in PCR. Water can be used to dilute templates that will not be stored for any length of time.

Proceedure:

PCR reaction mix:

Component	1 X (μl)	
ddH_2O	12.6	
10 X buffer B	2.0	
25 mM $MgCl_2$	1.2	
dNTPs	1.6	
Forward primer	0.2	
Reverse primer	0.2	
Taq polymerase	0.2	
DNA	2.0	
Total	20	

Component	1 X (μl)	
ddH_2O	12.6	
10 X buffer A	2.0	
dNTPs	1.6	
Forward primer	0.2	
Reverse primer	0.2	
Taq polymerase	0.2	
DNA	2.0	
Total	20	

PCR conditions:

Step	Temperature (C)	Time	
1	94	3 min.	
2	94	45 sec.	
3	50	45 sec.	
4	72	1 min.	
5	35 cycles to step 2		
6	72	5 min.	
7	4	continuous	
8		end	

Electrophoresis:
Use 2%-4% agarose gel