AFLP Protocol

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Anthony J Geneva

Modified from:

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. Lee, M. Hornes, et al. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research 23:4407-4414.

and

https://www.msu.edu/user/hazensam/aflp/AFLPprotocolMSU.html

Oligos

Adaptors	
EcoR1adapterA	CTCGTAGACTGCGTACC
EcoR1adapterB	AATTGGTACGCAGTCTAC
Mse1adapterA	GACGATGAGTCCTGAG
Mse1adapterB	TACTCAGGACTCAT

Pre Selective Primers	
PreselE	GACTGCGTACCAATTCA
PreselM	GATGAGTCCTGAGTAAC

Mse Selective Unlabeled Primer	
SelampM47	GATGAGTCCTGAGTAACAA
SelampM51	GATGAGTCCTGAGTAACCA
SelampM55	GATGAGTCCTGAGTAACGA
SelampM59	GATGAGTCCTGAGTAACTA
SelampM49	GATGAGTCCTGAGTAACAG
SelampM53	GATGAGTCCTGAGTAACCG
SelampM61	GATGAGTCCTGAGTAACTG

EcoR1 Selective 5′-Labeled Primer	
SelAmpE1	GACTGCGTACCAATTCAGG
SelAmpE2	GACTGCGTACCAATTCATC
SelAmpE3	GACTGCGTACCAATTCACA

Dilute all stocks to 100μM and store -20°C.

Adaptor Construction

Component	Amount (ml)
Mse1adapterA (100μM)	7.5
Mse1adapterB (100μM)	7.5
dH_2O	135
Total (5μM)	150

Component	Amount (ml)
EcoR1adapterA (100μM)	75
EcoR1adapterB (100μM)	75
Total (50μM)	150

Cycler Protocol
95°C 5 mins
70X 94°C 30 secs/ -1°C per cycle
4°C hold
Store at -20C

Digestion

Component	Amount (μl)	Per plate	/2
dH_2O	13.5	1417.5	708.75
10X NEB buffer #4	2	210	105
NEB EcoR1 (20 units/μl)	0.25	26.25	13.125
NEB Mse1 (10 units/μl)	0.05	5.25	2.625
NEB 100X BSA	0.2	21	10.5
DNA (50ng/μl)	4		
Total	**20**		

Cycler Protocol
37°C 180 mins
60°C 15 mins

Ligation

Component	Amount (μl)	Per plate
dH_2O	5.8	609
10X T4 ligase buffer (with ATP)	2	210
EcoR1 adapter (5μM)	1	105
Mse1 adapter (50μM)	1	105
T4 DNA ligase (400 units/μl)	0.2	21
Entire Digestion Product	20	
Total	**30**	

Incubate overnight at 37°C.

Run 4μL of Ligation product on a 1.5% agarose gel. A diffuse smear (or sometimes distinct bands) should be visible between ~200-1000 bp. If these appear, perform a full of plate preselective amplifications. If not, test a small number of samples via preselective amplification and continue if these are successful.

Preselective Amplification

Component	Amount (μl)	Per plate
dH_2O	22.8	2394
E Primer (10μM)	5	525
M Primer (10μM)	5	525
$MgSO_4$	5	525
10x BioBasic Buffer	5	525
dNTPs	5	525
Taq	0.25	26.25
Ligation Product	2	
Total	**50.05**	

Cycler Protocol

94°C 120 secs
 94°C 60 secs
26x 56°C 60 secs
 72°C 60 secs
72°C 60 secs
4°C hold

Run 4μL of Preselective Product on a 1.5% agarose gel. A DNA smear or distinct bands should appear between 50-500 bp. Store product at -20°C.

Selective Amplification

Component	Amount (µl)	Per Plate
H_20	11.4	1197
Buffer	2.5	262.5
dNTPs	2.5	262.5
Mse1 selective primer (2µM)	2.5	262.5
EcoR1 labeled selective primer (2µM)	2.5	262.5
$MGSO_4$	2.5	262.5
Taq	0.125	13.125
Template	1	
Total	**25.025**	

Cycler Protocol

94°C 120 secs

	94°C	30 secs
12x	65°C	30 secs/ -1°C per cycle
	72°C	60 secs
	94°C	30 secs
23x	56°C	30 secs
	72°C	60 secs

72°C 60 secs

4°C hold

Run 4µL of Selective Product. Bands should appear between 50-500 bp. Store product at 4°C in the dark by wrapping in foil.

Analysis

TBA