AFLP Protocol

27 June 2014

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

<u>Cycler Protocol</u>
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	13.6	1428	714
NEB 10X CutSmart	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 (20mg/mL)	0.1	10.5	5.25
DNA (50ng/μl)	4		
Total	**20**		

<u>Cycler Protocol</u>
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	/4
dH_2O	22.8	2394	598.5
E Primer (10μM)	5	525	131.25
M Primer (10μM)	5	525	131.25
$MgSO_4$	5	525	131.25
10x BioBasic Buffer	5	525	131.25
dNTPs (0.5mM)	5	525	131.25
Taq	0.25	26.25	6.5625
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	/2
H_20	11.4	1197	598.5
10x BioBasic Buffer	2.5	262.5	131.25
dNTPs (0.5mM)	2.5	262.5	131.25
Mse1 selective primer (2µM)	2.5	262.5	131.25
EcoR1 labeled selective primer (2µM)	2.5	262.5	131.25
$MGSO_4$	2.5	262.5	131.25
Taq	0.125	13.125	6.5625
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 Pipeline

Submit Plate to Genomics Center

Download Data from FGC
- Genotyping tab, click on Download Your Fluorescent DNA Fragment Analysis Results
- Unzip files

Process data in PeakScanner

- export data

Further process in RawGeno

- export data
- Use conversion script 'RawGeno_2_AFLPScore'

AFLPScore
- Determine locus and phenotype thresholds
- Calculate error rates
- Export final matrix