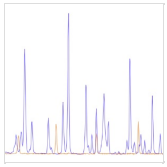

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AFLP RedTaq protocol

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1 Works for me dx.doi.org/10.17504/protocols.io.rpvd5n6

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MATERIALS

NAME	CATALOG #	VENDOR
EcoRI - 10,000 units	R0101S	New England Biolabs
MseI - 500 units	R0525S	New England Biolabs
BSA-Molecular Biology Grade - 12 mg	B9000S	New England Biolabs
Water, nuclease free		
NaCl 0.5 M		
T4 DNA Ligase (5U/uL)	10 799 009 001	Roche
REDTaq DNA Polymerase (1U/uL)	D4309	Sigma Aldrich
dNTP mix (2.5 mM each)		

BEFORE STARTING

Dilute BSA to 1 mg/ml

Oligonucleotides used:

EcoR1-Adapters 5-CTCGTAGACTGCGTACC 5-AATTGGTACGCAGTCTAC final: 5 µM of each	MseI-Adapters 5-GACGATGAGTCCTGAG 5-TACTCAGGACTCAT final: 50 µM of each
Presel Primer Eco: gactgcgtaccaattca final: 5 µM of each	Presel Primer Mse: gatgagtcctgagtaac final: 5 µM of each
Eco-Primer gac tgc gta cca att cxx x final: 1 µM	Mse Primer gat gag tcc tga gta axx x (FAM-6 labeled) final: 5 µM

Prepare Eco adapters at 5 µM and Mse adapters at 50 µM (working solutions):

Adapter E1 stock (100 µM)	10 µl
Adapter E2 stock (100 µM)	10 µl
ddH_2O	180 µl

Adapter M1 stock (100 µM)	100 µl
Adapter M2 stock (100 µM)	100 µl
ddH_2O	0 µl

Incubate for 5 min at 95°C on the thermoblock/thermocycler and let it cool down slowly; eg. at 37°C on thermoblock/thermocycler.

Restriction-ligation reaction

1 **0.53 µl water**

1.2 µl T4 DNA Ligase buffer (10×)

0.6 µl BSA (1 mg/ml)

1.2 µl NaCl (0.5 M)

1 µl Adapter MseI (50 µM)

1 µl Adapter EcoRI (5 µM)

0.1 µl MseI (10 U/µl)

0.25 µl EcoRI (20 U/µl)

0.12 µl T4 DNA Ligase (5U/µl)

2 Add 6 µl of DNA to 6 µl of the restriction-ligation mix.

3 Incubate at 37ºC for 3 h and at 17 °C overnight.

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4 Dilute ligated DNA fragments 10-fold.

Preselective PCR

5 Prepare the reaction mix:

- **6.09 µl water**
- **1.14 µl RedTaq Buffer (10x)**
- **0.22 µl dNTP (2.5 mM each)**
- **0.15 µl Presel. primer Eco-A (10 µM)**
- **0.15 µl Presel. primer Mse-C (10 µM)**
- **0.25 µl Sigma RedTaq (1U/ µl)**

6 Add 2 µl of the 10x diluted restriction-ligation product to 8 µl of the reaction mix (total volume 10 µl).

7 Run the PCR program:

- 72°C for 2 min
- 25 cycles of:
- 94°C for 1 s
- 56°C for 30 s
- 72°C for 120 s
- 60°C for 30 min
- hold at 4°C

8 Dilute the PCR product 10-fold.

Selective PCR

9 Prepare reaction mix:

- **5.45 µl water**
- **1 µl RedTaq Buffer (10x)**
- **0.22 µl dNTP (2.5 mM each)**
- **0.25 µl Sigma RedTaq (1U/ µl)**
- **0.54 µl Sel. primer Mse-Cxx (5 µM)**
- **0.54 µl Sel. primer Eco-Axx (1 µM)**

10 Add 2 µl of the 10x diluted preselective PCR product to 8 µl of the reaction mix (total volume 10 µl).

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11 Run the PCR program:
- 94°C for 2 min
- 10 cycles of:
- 94°C for 1 s
-
- 65°C for 30 s, with a ramp of -1°C per step (up to 56°C)
-
- 72°C for 120 s
-
- 22 cycles of:
- 94°C for 1 s
-
- 56°C for 30 s
-
- 72°C for 120 s
-
- 60°C for 30 min
- hold at 4°C

Purification

12 Proceed with Sephadex purification protocol and loading on ABI 3130.

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