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PCR protocol for MgSTS marker genotyping

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1 Works for me dx.doi.org/10.17504/protocols.io.baf3ibqn

Mimulus

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ABSTRACT

This is a standard PCR protocol used to amplify MgSTS markers from genomic DNA for genotyping. MgSTS markers were developed for use in Mimulus species and typically span length polymorphisms between conserved sequence regions.

GUIDELINES

Taq polymerase and any mix containing it should be kept on ice at all times. Keep GoTaq enzyme stock in the -20C freezer except when loading.

MATERIALS

NAME	CATALOG #	VENDOR
MgCl2		
GoTaq(R) Flexi DNA Polymerase, 500u	M8295	Promega
5X Colorless GoTaq(R) Flexi Reaction Buffer, 20ml	M8901	Promega
dNTP Mix, 10mM, 1000ul	U1515	Promega
BSA		
MgSTS Primers		
Molecular Biology Grade Water		
HiDi Buffer		
ROX size standard (500 or 1000)		

MATERIALS TEXT

dNTPs are aliquoted into 2.5mM working stocks and stored at 4C prior to use.
BSA working stock is at 10mg/mL.

MgSTS primers have a matching Forward and Reverse pair. These are combined into a single working stock of 5uM (5uM Forward + 5uM Reverse) and stored at 4C until use.

BEFORE STARTING

dNTPs should be at a working concentration of 2.5 mM.
Forward and Reverse primers should be pre-mixed at 5uM.
Thaw 5X buffer, dNTPs, MgCl2, Primers, and DNA sample.
Keep enzyme in freezer until needed.

Prepare PCR reaction

1 Determine the amount of reagents needed. The following amounts are sufficient for a **single** reaction:

2.95 µl Molecular Biology Grade Water

2 µl 5X GoTaq Flexi Buffer, colorless

0.8 µl MgCl2, 25mM stock

0.8 µl dNTPs, 2.5mM stock

0.5 µl BSA, 10 mg/mL stock

0.2 µl Premixed F+R Primers, 5uM stock

0.15 µl GoTaq G2 polymerase, 5 units/uL stock

2 µl DNA sample

Note: Markers that are known to amplify in different, non-overlapping length ranges can be combined into a single PCR reaction. Add **0.2 µl** of EACH premixed primer pair, and reduce your water volume accordingly so that the final reaction volume is **10 µl** .

2 Thaw mix components except for GoTaq polymerase. Leave the polymerase in the freezer until it is needed.

3 Create a master mix with all ingredients except DNA. Add the polymerase last, mix well, and keep the mix on ice.

4 Transfer **8 µl** of master mix to each well of a PCR plate. Then add **2 µl** sample DNA to each well. Add a plastic seal to the plate and press all edges to ensure the seal is tight.
Mix well, spin the plate to collect droplets, and place in PCR machine.

Run PCR

5 Run the following PCR cycler program:

94 °C 2 mins 00 sec

10 cycles of stepping down annealing temp:

> **94 °C 0 mins 30 sec**

> **62 °C 0 mins 45 sec** : Reduced by 1C every cycle to **53 °C**

> **72 °C 1 min 00 sec**

35 cycles of constant annealing temp:

> **94 °C 0 mins 30 sec**

> **52 °C 0 mins 45 sec**

> **72 °C 1 min 00 sec**

72 °C 10 mins

10 °C Hold

Prepare genotyping reaction

6 Once the PCR is complete, prepare the following reaction in a new plate:

HiDi and ROX size standard can be premixed and aliquoted beforehand. For marker lengths > 500, use ROX1000. For smaller lengths, ROX500 can be used.

8.25 µl HiDi buffer

0.25 µl ROX size standard

1.5 µl PCR sample

7 Briefly spin down the plate to remove air bubbles and sample stuck to the sides.

Then cover with foil PCR covers and submit to your sequencing provider.