Multiplex PCR for amplifying mitochondrial and nuclear products in plants

CURRENT STATUS: POSTED

Noemí Colombo
Instituto de Genética, INTA Castelar, Argentina
ncolombo@cnia.inta.gov.ar*Corresponding Author*

Magdalena Lütz
IIB-Intech-UNSAM, Argentina

DOI:
10.1038/protex.2013.098
SUBJECT AREAS
Genetics
KEYWORDS
Multiplex PCR, plant genomes

Abstract

PCR amplification of a single copy nuclear region and a multicopy mitochondrial region together results in the competition between targets unequally represented in a single tube PCR reaction. This protocol allows a correct amplification of both products by adopting a sequential lay out of the PCR reaction which enhances the amplification of the nuclear gene in the first step and the mitochondrial gene in the final step.

Introduction

Multiplex PCR was first described by Chamberlain et al., 1988. It allows simultaneous amplification of many targets in a single reaction by using more than one pair of primers. Our objective was to amplify a nuclear region and a mitochondrial region which are present in different numbers in the cell due to the polyploid nature of mitochondrial DNA. Multiplex PCR of a single copy nuclear region and a multicopy mitochondrial region together results in the competition between targets unequally represented. We designed a two-steps PCR reaction: in the first one, a 25µl reaction was set adding only the primers amplifying the nuclear target. In this case, the nuclear target was a 170 bp SCARE3M12 marker associated to the restorer of fertility allele Rf3 in maize (Zhang et al., 2006). After 10 cycles the reaction was stopped and the primers to amplify the mitochondrial region were added, maintaining constant concentrations for the buffer, Mg2 Cl and dNTPs in a 27 µl reaction. The mitochondrial primers amplify a 799 bp region of the cytoplasmic male sterility-associated orf 355 in CMS-S maize (Liu et al., 2002). Both sets of primers were chosen so that they had the same annealing temperature. The sequential lay out of the reaction allowed for efficient amplification of both products.

Reagents

Plant material: Inbred line Va58-CMS-S/Rf3Rf3 from Maize Genetics and Genomics Database -Maize GDB- (Lawrence et al., 2007). Inbred line LE 08-309-Mito N/rf3rf3 from IGEAF INTA.

Genomic maize DNA was extracted from leaves of seedlings according to Saghai-Maroof et al., (1984).

PCR

-Taq DNA Polymerase (recombinant) (5 U/µL) Thermo Scientific Fermentas; includes 10 X PCR Buffer (Tris-HCl pH 8.8 75 mM, (NH4)2SO4 20 mM) and 25 mM Mg2CL

-dNTPs

-Nuclear primers:

SCARE12M7-F: 5´-ATGGAGATTGAAGGGACG-3´

SCARE12M7-R: 5´-ACACGGAAGACCATGACC-3´

Mitochondrial primers:

CMSSF: 5´-CAACTTATTACGAGGCTGATGC-3´

CMSSR: 5´-AGTTCGTCCCATATACCCGTAC-3´

Agarose gel electrophoresis

-Agarose D1 Max Biodynamics.

-Buffer TBE 0,5X (Tris Base 45 mM, Boric acid 45 mM; EDTA 1 mM)

-6X Xylene cyanol dye

-Ethidium bromide (10 mg/mL)

-100 bp ladder DNA marker. Axygen Biosciences.

Equipment

Thermocycler Biometra T3

Mini-Sub-cell GT electrophoresis system. Bio-Rad

Power Pack 300 Power supply. Bio-Rad

Procedure

1. First step: 1.1. Prepare the PCR master mix according to Table 1.

1.2. Add 24 µl of master mix to each PCR eppendorf tube.

1.3. Add 1 µl of genomic DNA to each tube. Mix well.

1.4. Set the thermal profile in the thermocycler according to Table 2 and run the PCR reaction for 10 cycles.

2. Second step

    2.1. Prepare the master mix according to Table 3.

2.2. Add 2 µl of the master mix to each PCR tube.

2.3. Set the thermal profile in the thermocycler according to Table 2 and run the PCR reaction for 35

cycles.

3. Agarose Gel Electrophoresis 3.1. Make a 2% agarose gel (w/v) in 0.5 X TBE with ethidium bromide.

3.2. Load the gel adding 1X xylene cyanol dye to each sample. Include a 100bp ladder DNA marker.

3.3. Run the gel at 80 volts for 60 minutes and visualize under UV light.

Timing

The time requiered for preparing the PCR master mix is variable depending on the number of samples. The complete PCR multiplex run takes about 3 hours. The agarose gel is run for 1 hour.

Anticipated Results

Two PCR products are expected in the inbred line Va58-CMS-S/Rf3Rf3: a 799 bp product corresponding to the mitochondrial orf 355 and a 170 bp corresponding to the nuclear SCARE12M7 associated to the Rf3 allele. No PCR product is expected in the inbred line LE 08-309-Mito N/rf3rf3, which carries neither the orf355 nor the Rf3 allele (Figure 1).

References

Chamberlain, J.S., Gibbs, R.A., Ranier, J.E., Nguyen, P.N., Caskey C.T. 1988. Deletion screening of the Duchenne muscular dystrophy locus via multiplex DNA amplification. Nucleic Acids Res., 16:11141-11156.

Lawrence, C.J., Schaeffer, M.L., Seigfried, T.E., Campbell, D.A. Harper, L.C. 2007. MaizeGDB's new data types, resources and activities. Nucleic Acids Res., 35: D895 – 900.

Liu, Z., Peter, S.O., Long, M., Weingartner, U., Stamp, P. Kaeser, O. 2002. A PCR Assay for Rapid Discrimination of Sterile Cytoplasm Types in Maize. Crop Sci., 42: 566 – 569.

Saghai-Maroof, M., Soliman, K., Jorgensen, R. Allard, R. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. P. Natl. Acad. Sci. USA, 81: 8014 - 8018.

Zhang ZF, Wang Y, Zheng YL (2006) AFLP and PCR-based markers linked to Rf3, a fertility restorer gene for S cytoplasmic male sterility in maize. Mol. Gen. Genomics, 276:162-169.

Acknowledgements

Funds from PNCER-021321 INTA.

Figures

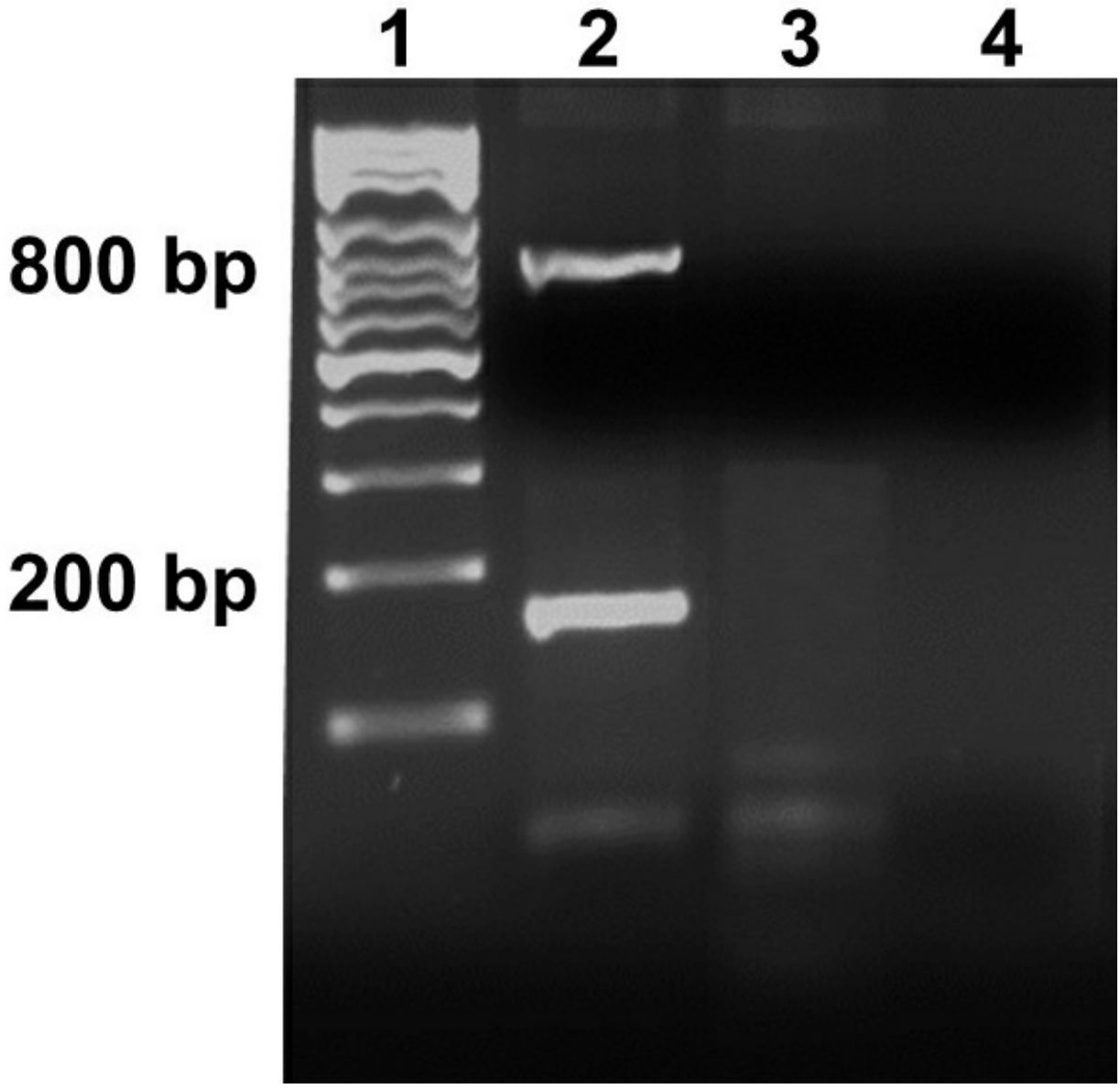

Figure 1

Expected multiplex PCR products Lane1: 100 bp DNA ladder Axygen Lane 2: Va58-CMS-S/Rf3Rf3 Lane 3: LE 08-309-Mito N/rf3rf3 Lane 4. Negative cotrol: no DNA added

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

table_1.doc
table_2.doc
Table_3.doc