Genetic and Phenotypic Effects of Chromosome Segments Introgressed From Gossypium barbadense into Gossypium hirsutum

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Abstract

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Protocol

Gently crumble about 2 leaf tissue by the freezing grinding machine (MM400) in a microfuge tube.

Step 1.

Add 700 µl of CTAB buffer which had been preheated in 65 ℃ water bath.

Step 2.

Incubate the CTAB/plant extract mixture for about 45 min 65 ℃ in a recirculating water bath.

Step 3.

Add equal volume of chloroform/iso-amyl alcohol (24:1), Mix for about 3min. Spin the mixture at 12000 g for 15 min to spin down cell debris.

Step 4.

Transfer the upper aqueous phase only (contains the DNA) to a clean microfuge tube.

Step 5.

Precipitate DNA with 500 µl of cold isopropanol.

Step 6.

Following precipitation, the DNA can be pipetted off by slowly rotating/spinning a tip in the cold solution. To wash the DNA, transfer the precipitate into a microfuge tube containing 500 µl of ice cold 70 % ethanol and slowly invert the tube. Repeat.

Step 7.

Resuspend the DNA in sterile DNase free water.

Step 8.

Mix the following in 96-well plates for PCR applications:Water□6.2µl□□Template□1.2µl□□F-Primer□0.5µl□□R-Primer□0.5µl□□dNTP□0.5µl□□10xPCRbuffer□1µl□□TaqDNApolymerase□0.1µl□

Step 9.

Put the 96-well plates in PCR machine with heated lid.

Step 10.

Prepare polyacrylamide gel

Step 11.

Pour the gel mix in plate carefully to avoid bubbles. Insert comb into plate. Leave to polymerise for about 1 hour. Pour 1X TBE into electrophoresis apparatus, remove the comb.

Step 12.

Load 1.2 ul of each sample into individual wells of the gel .

Step 13.

Run gel until the dark blue just runs off the bottom of the gel or as appropriate.

Step 14.

Remove the gel and separate the plates carefully with a single-edged razor blade.

Step 15.

Place the gel in tray with the fixer and leave shaking in a fume hood for 10 minutes. Pour off fixer(400ml H_2O+50ml 90%alcohol+30ml 10% acetic acid).

Step 16.

Wash with water and pour off the water.

Step 17.

Add silver-stain(0.9g $AgNO_3$ +300ml H_2O) and leave shaking for 12 minutes

Step 18.

Wash with water, rinse again and pour off the water.

Step 19.

Immediately agitate the gel in the developer(6g NaOH +7ml methanol +5ml o.2% $Na_2S_2O_3$ +400ml H_2O) until band development progress sufficiently.

Step 20.

Rinse gel in water.

Step 21.

Scan the gel with a computer flatbed scanner, or photograph.

Step 22.