INTRODUCTION

This protocol describes the use of allele-specific amplification followed by fluorescence detection (KASPar) for genotyping. The method is compatible with any real-time PCR setup and has the advantage that no specifically labeled oligonucleotides are needed.

RELATED INFORMATION

For more information, see http://www.kbioscience.co.uk.

MATERIALS

Reagents

Genomic template DNA

MgCl₂ (50 mM)

Reaction mix (KASPar, KBioscience)

Taq polymerase (KBioscience)

Equipment

Fluorescence plate reader or real-time PCR machine (compatible with FAM, VIC, and ROX)

KASPar primer design utility (http://www.kbioscience.co.uk/primer-picker/)

Klustercaller software

PCR machine

Plate in which to perform reaction

METHOD

1. Design oligonucleotides (two allele-specific 40-mers and one common 20-mer) using the Web-based KASPar primer design utility.

2. Prepare the following reaction mixture (4-µL total volume):

<table>
<thead>
<tr>
<th>µL</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Genomic template DNA (10 to 100 ng)</td>
</tr>
</tbody>
</table>
1 µL Reaction mix (KASPar)
0.055 µL Oligonucleotide mix (12 µM of each allele-specific oligonucleotide and 30 µM of the common oligonucleotide designed in Step 1)
0.013 µL Taq polymerase
0.032 µL MgCl₂ (50 mM)
0.9 µL H₂O

3. Perform PCR using the following protocol:

<table>
<thead>
<tr>
<th>Number of cycles</th>
<th>Denaturation</th>
<th>Annealing</th>
<th>Extension</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15 min at 94°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>10 sec at 94°C</td>
<td>5 sec at 57°C</td>
<td>10 sec at 72°C</td>
</tr>
<tr>
<td>18</td>
<td>10 sec at 94°C</td>
<td>20 sec at 57°C</td>
<td>40 sec at 72°C</td>
</tr>
</tbody>
</table>

4. Scan the plate in a fluorescence plate reader or real-time PCR machine (compatible with FAM, VIC, and ROX).

5. Analyze the data using Klustercaller software.