Automated and Rapid Genotyping of Mouse Colonies with the WAVE® Nucleic Acid Fragment Analysis System

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Introduction
The ability to rapidly and reliably genotype mice is an important concern for researchers. Traditional methods employ labor-intensive and time-consuming techniques such as test-crossing, gel-electrophoresis, or nucleic acid hybridization. Here we show that the WAVE® System can easily resolve PCR* products with subtle differences in base pair lengths in under 7 minutes. By exploiting such DNA polymorphisms around a mutation of interest, genotyping of research animals becomes quick and efficient.

Example
A recessive, embryonic lethal mutation that was chemically-induced on chromosome 7 of the BALB/c mouse strain can be followed when crossed to FRCH mice since the closely linked D7Mit352 marker shows a DNA-length polymorphism between the two strains. The BALB/c allele (linked to the mutation of interest) yields a D7Mit352 PCR* product of 120 base pairs (bp) while the FRCH wild-type allele is only 116 bp.
The BALB/c allele yielded a PCR* product of 120 bp with retention time of 6.14 min as shown in Figure 1. The small indent (double peak) observed on the peak corresponds to A-tailed PCR* fragments. Tag de-carboxylases tend to add a deoxyribo-nucleotide, preferentially dATP, to the 3'-hydroxyl terminus of a blunt-ended substrate in a non-template fashion. This results in a PCR* product one base longer than its predicted size. The high-sensitivity of the WAVE® System makes it capable of registering these A-tailed products. The FRCH allele gave a 116 bp PCR* fragment with retention time of 5.85 min. For presentation purposes the FRCH chromatogram was aligned with the BALB/c and two embryo samples (numbered 6 and 7), as shown in Figure 2. The samples yielded two peaks corresponding to the 120 bp and 116 bp signals indicating heterozygosity. A third peak was observed with retention time of 5.15 min. This peak corresponds to the heteroduplex form produced by the annealing of the different-size PCR* products of the two alleles. Heteroduplexes are retained differentially and can be separated from the homoduplex fragments.²

![Figure 2. Heterozygous mice (1-5) yield consistent chromatographic patterns which differ from the corresponding wild-type (6) and mutant (7) mice. pUC18 HaeIII digest (pUC18) was used as a ladder standard.](image)

**Table 1. Separation gradient for 80 - 200 bp fragments**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Buffer A: 0.1 M TEAA (%)</th>
<th>Buffer B: 0.1 M TEAA, 25% ACN (%)</th>
<th>Flow Rate (mL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>55</td>
<td></td>
<td>45</td>
</tr>
<tr>
<td>4.4</td>
<td>40</td>
<td></td>
<td>60</td>
</tr>
<tr>
<td>7.5</td>
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<td>0</td>
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<td>0</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>11</td>
<td>55</td>
<td></td>
<td>45</td>
</tr>
<tr>
<td>13</td>
<td>55</td>
<td></td>
<td>45</td>
</tr>
</tbody>
</table>

**Discussion**

The typical three peak patterns are consistent in heterozygous mice and differ from the corresponding homozygous mice. They are easy to score visually, and the precise retention times are documented by the instrument, which permits accurate data processing. The results can be monitored on-line or stored electronically. The time for each sample is less than 8 minutes, which allows for the processing of 192 samples (2 x 96-well plate) in 24 hours. The only hands-on time with the WAVE® System is when placing the sample tubes in the 96-well plate and choosing the detection method on the computer, which is facilitated by the WAVE® System software. Total hands-on time is less than 5 min. In contrast to current gel methods, the technique omits sample preparation with gel-loading dyes, gel preparation, pipetting during sample loading, running the gel, and documenting by image equipment. In most cases, scoring of transgenic animals is performed by Southern blotting, which delays the availability of data by several days and involves radioactive chemicals.

Genotyping of mice will usually involve a marker for a mutation or for a transgene insertion. The fragments in most cases are 150-600 bp. Three different gradient programs were developed at Transgenic that are optimized for the separation of fragments in the range 80-200 bp, 180-350 bp, and 300-600 bp, respectively. These gradients are described in Tables 1, 2, and 3, and can be used successfully in genotyping of mouse colonies.

**Experimental Methods**

Genomic DNA was isolated from individual 8.5-day mouse embryos. Primers specific to the marker locus D7Mit352 (Gibco BRL) were used to amplify a PCR* product of either 120 bp (the BALB/c allele linked to the mutation of interest) or 116 bp (the FRCH wild-type allele). The forward primer has the sequence 5'-AGCCTATTGCAACCAATTT-3' and...
the reverse primer's sequence is 5’-AGCATGGAAATTGACAATTC-3’. PCR was performed in 25-μL reactions amplified for 30 cycles at 94°C (30 s), 55°C (2 min), and 72°C (2 min).

For automated analysis on the WAVE® System, PCR reactions were aliquoted in 7 μL, loaded on the 96-well plate of the WAVE® System, and 5 μL were automatically injected. Analysis was carried out at non-denaturing conditions. The column oven temperature was set at 50°C, and triethyl-ammonium acetate (TEAA) was used to make the mobile phase with pH 7.0. The mobile phase consisted of buffer A (0.1 M TEAA) and buffer B (0.1 M TEAA and 25% acetonitrile). Buffer B was prepared by adding 50 mL of 2M TEAA stock solution to 250 mL of acetonitrile in a 1000-mL volumetric flask, and the final volume was brought to 1000 mL. The wavelength detector was set at 254 nm. HaeIII digested plasmid pUC18 DNA was used as a standard.

Conclusions

The procedure reported here is convenient for high-throughput genomic facilities and research laboratories. Moreover, the different analytical methods available on the WAVE® System allow for polymorphism detection, mutation screening, and PCR control.

References


* PCR is a process covered by patents issued and applicable in certain countries. Transgenomic does not encourage or support the unauthorized or unlicensed use of the PCR process.

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