


---

**CELL BIOLOGY & MOLECULAR GENETICS**

### AFLP Analysis of DNA from Dried Hop Cones

M. Shaun Townsend, John A. Henning,* and Daniel L. Moore

#### ABSTRACT

Hop (Humulus lupulus L.) cones are used extensively in beer brewing to enhance flavor and impart bittering. Verifying hops cultivar identity has traditionally been accomplished by morphological characteristics or a chemical analysis of lupulin glands but these traits may vary according to environmental influences. The objective of this research was to develop an AFLP (amplified fragment length polymorphism) protocol for analyzing DNA extracted from dried hop cones. The DNA was extracted from dried cones of six hop genotypes by a technique published for grapes (Vitis spp). The reagents, MseI primers, and protocol were part of a commercially available kit, while the 6-carboxyfluorescein-labeled EcoRI primers were purchased separately. Eleven primer combinations amplified an average of 546.5 scorable fragments with an average of 49.7 fragments per primer combination. All genotypes were differentiated with the primer combinations studied. Average genetic similarity estimates ranged between 0.956 and 0.995 among the six hop genotypes studied. This research provides the hops industry with a powerful technique to verify accurately hops cultivar identity and purity through an analysis of dried cone DNA.

---

**HOPS** are used in beer brewing to impart bittering, enhance flavor, and as a preservative. The plant organs of interest to brewers are the female flowers, or cones, which contain lupulin glands with a chemical profile desirable for brewing. The wide variation in chemical profiles found among hop cultivars gives the brewer flexibility to impart specific flavor and aroma characteristics to beer (Neve, 1991).

One of the challenges faced by both the brewing industry and hop breeders has been obtaining a reliable technique to identify accurately hop cultivars. Traditionally, hops cultivar identification has been verified by either a chemical analysis of lupulin glands, or by morphological characteristics. Both traits are influenced by environmental conditions during cone development which, in turn, can lead to difficulty in cultivar identification (Hartl and Seefelder, 1998). Since hop cultivars are clonally propagated and all individuals within a cultivar are genetically identical, DNA fingerprinting using a molecular marker approach would be an ideal way to distinguish among hop cultivars.

The AFLP (amplified fragment length polymorphism) technique (Vos et al., 1995) is a recent innovation that has proven both powerful and reliable. This technique has several advantages over earlier marker technologies: very little genomic DNA is required, many polymorphisms can be generated per reaction, prior knowledge of specific DNA sequences is not needed, and the technique has high reproducibility (Vos et al., 1995). In a comparison among AFLP, RFLP (restriction fragment length polymorphism), and RAPD (random amplified polymorphic DNA) analysis of 14 soybean [Glycine max (L.) Merr.] cultivars, Lin et al. (1996) reported AFLP to average six polymorphic bands in 60 of the 64 primer pairs tested, RAPD produced a polymorphic band with only 35% of the primers tested, and more than 50% of the RFLP probes failed to distinguish a polymorphic band. In other work, Lu et al. (1996) found that AFLP generated more polymorphic bands per primer or probe than other molecular marker technologies.

The AFLP technique has been successfully used to differentiate genetically populations or individuals within numerous species including tea (Camellia sinensis L.) (Paul et al., 1997), cotton (Gossypium barbadense L.) (Feng et al., 1997), tef (Eragrostis tef (Zucc) Trotter)
A Core Primer

Technique for analyzing hop cone DNA would allow a AFLP analysis of DNA from dried hop cones. A when an additional 50 primer pairs were used. Con- (v/v) were added. The tubes were inverted several times and to distinguish between three of the hop cultivars, even of 5

5-GACTGCGTACC
CATCCTGACGATGGTTAA-5
5-GATGAGTCCTGAGTAA

5-CTCGTAGACTGCGTACC
5-GACGATGAGTCCTGAG

Eco

Table 1. Primer sequences used for AFLP analysis of DNA extracted from dried hop cones.

<table>
<thead>
<tr>
<th>EcoRI</th>
<th>MseI</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-CTCGTAGACTGCGTACC CATCCTGACGATGGTTAA-5</td>
<td>5-GACGATGAGTCCTGAG</td>
</tr>
<tr>
<td>5-GACTGCGTACC</td>
<td>TACTCGAGACTCAT-5</td>
</tr>
</tbody>
</table>

Adaptors

Core Primes

Preselective Amplification Primers

Selective Amplification Primers

Core Primer + A

Core Primer + AAC + FAM label

Core Primer + ACC + FAM label

Core Primer + AGC + FAM label

Core Primer + +

Core Primer + CAA

Core Primer + CAC

Core Primer + CAG

Core Primer + CAT

Core Primer + CTA

Core Primer + CTC

Core Primer + CTG

Core Primer + CTT

(Bai et al., 1999), soybean (Lin et al., 1996), and wheat (Triticum aestivum L.) (Barrett and Kidwell, 1998). Hartl and Seefelder (1998) used AFLP to evaluate DNA from leaf tissue of eight hop cultivars for genetic diversity. They obtained 523 AFLP fragments from eight primer combinations, and the average polymorphism count per primer combination was 18. They were unable to distinguish between three of the hop cultivars, even when an additional 50 primer pairs were used. Con- versely, Schut et al. (1997) were able to identify 31 barley lines with only eight AFLP primer combinations. They obtained 681 markers with over 43% showing polymorphism, and each primer combination was able to discriminate all of the 31 barley lines studied.

Hop cones are dried and processed on farm before delivery to marketing agents and/or a brewer. We are not aware of any research reports describing the use of AFLP analysis of DNA from dried hop cones. A technique for analyzing hop cone DNA would allow a brewer to verify accurately a hop sample’s purity and identity prior to the brewing process. The objective of this research was to develop an AFLP protocol for analyzing DNA from dried hop cones.

MATERIALS AND METHODS

Plant Material and DNA Extraction

Hop genotypes analyzed were the cultivars Sterling (USDA-21689), Nugget (USDA-21193), Wye Target (USDA-21112), Hallertauer Gold (USDA-21671), and the genotypes USDA-21688 and USDA-21737. The genotype USDA-21688 is a half-sib sister to Sterling, and USDA-21737 is a virus-free Sterling hops that was propagated from meristem culture. Hop cones were dried in a forced-air drier at 65°C prior to analysis. The experiment was repeated twice beginning with DNA extraction.

The DNA extraction protocol used was a slight modification of a protocol reported for grapes (Lodhi et al., 1994). A 0.5-g sample of dried hops cone tissue was ground in liquid nitrogen with a mortar and pestle. After grinding, the sample was transferred to a sterile 20-mL polypropylene tube and 6 mL of pre-heated (65°C) CTAB extraction buffer [100 mM Tris-HCl, pH 8.0, 1.4 M NaCl, 20 mM EDTA, 2% CTAB (w/v)], and 50 mg polyvinylpolypyrrolidone was added and the capped tube was gently inverted several times to mix the contents. Samples were incubated at 65°C for 30 min and allowed to cool at room temperature for 10 min. During incubation, the tubes were gently inverted several times every 10 min. After incubation, 7 mL of chloroform:isoamyl alcohol (24:1) was added, samples were inverted approximately 20 times, and then centrifuged at 2600 x g for 15 minutes. The upper aque- phase was transferred to a new sterile 20-mL polypropylene tube by means of a wide-bore pipette tip. An equal volume of 5 M NaCl and 2 volumes of cold (−20°C) 95% ethanol (v/v) were added. The tubes were inverted several times and refrigerated (4°C) for 60 min. Nucleic acids were pelleted at 3600 x g (4°C) for 15 min. The supernatant was removed and the pellet was washed with cold (−20°C) 70% ethanol (v/v). The ethanol was evaporated by heating the samples to 37°C, the pellet dissolved in 600 μL of sterile TE buffer (pH 8.0), and the samples were treated with 10 units of RNase and stored in a refrigerator (4°C). The DNA was quantified with a Dynanant 200 fluorometer (Hoefer Pharmacia Biotech, Inc., San Francisco, CA).

AFLP Analysis

The protocol, enzymes, most reagents, and the MseI primers used were supplied in a kit (AFLP Analysis System I) manufactured by Life Technologies, Inc. (Rockville, MD). The 6-carboxyfluorescein-labeled (FAM) EcoRI primers were purchased from Integrated DNA Technologies, Inc. (Cor- alville, IA). Primers and DNA sequences used in this study are listed in Table 1. Approximately 250 ng of DNA was digested with 2.5 units each of EcoRI and MseI endonuclease for 2 h at 37°C. EcoRI and MseI adapters were ligated to the DNA fragments using 1 unit of T4 DNA ligase for 2 h at 20°C. The ligated DNA fragments were diluted 1:10 with TE buffer. Preselective and selective PCR amplification reactions were performed in a Genius thermocycler (Techne, Inc., Princeton, NJ).

For preselective amplification, a 51-μL reaction mixture was used containing 5 μL of the diluted DNA with ligated adapters, 40 μL pre-amp primer mix, 5 μL 10× PCR buffer (100 mM Tris-HCl at pH 8.3, 15 mM MgCl2, 500 mM KCl), and 5 units of AmpliTaq DNA polymerase (PE Applied Bio- systems, Foster City, CA). The preselective amplification PCR program was 20 cycles with the following program: 94°C for 30 s, 56°C for 60 s, and 72°C for 60 s. Following preselective amplification, samples were diluted 1:10 with TE buffer.

The total volume for the selective amplification reaction was 20.5 μL and contained 13.9 ng of FAM-labeled EcoRI primer, 30.15 ng of MseI primer and dNTPs, 10 μL of enzyme.
was omitted from the study. The 11 remaining primer 
ments and averaged 18 polymorphisms in hop leaf tissue.

One of the primer combinations (eACC-mCTT) 
failed to amplify fragments in three of the genotypes and 
was omitted from the study. The 11 remaining primer 
combinations amplified 546.5 scorable DNA fragments 
with an average of 49.7 fragments per primer combination 
(Table 2). The primer combination eACC-mCAC 
produced the fewest number of scorable fragments 
while eAAC-mCAG produced the most. Overall, an 
average of 62.5 total polymorphisms were detected with 
an average of 5.7 polymorphisms per primer combina-

Table 2. Average number of AFLP fragments scored and poly-

<table>
<thead>
<tr>
<th>Primer combination</th>
<th>Fragments scored</th>
<th>Polymorphic fragments</th>
<th>Percent polymorphic</th>
</tr>
</thead>
<tbody>
<tr>
<td>eAAC-mCAG</td>
<td>50.5 ± 8.5</td>
<td>4.0 ± 0.0</td>
<td>18.7 ± 11.7</td>
</tr>
<tr>
<td>eAAC-mCAG</td>
<td>76.5 ± 6.5</td>
<td>10.0 ± 2.0</td>
<td>17.0 ± 6.2</td>
</tr>
<tr>
<td>eAAc-mCTA</td>
<td>49.0 ± 0.0</td>
<td>3.0 ± 0.0</td>
<td>7.1 ± 1.0</td>
</tr>
<tr>
<td>eAAC-mCTC</td>
<td>64.0 ± 3.0</td>
<td>8.5 ± 2.5</td>
<td>16.6 ± 7.6</td>
</tr>
<tr>
<td>eACC-mCAC</td>
<td>37.0 ± 7.0</td>
<td>6.5 ± 0.5</td>
<td>23.5 ± 7.6</td>
</tr>
<tr>
<td>eACC-mCAG</td>
<td>40.5 ± 1.5</td>
<td>4.0 ± 0.0</td>
<td>9.9 ± 0.4</td>
</tr>
<tr>
<td>eACC-mCTA</td>
<td>52.5 ± 0.5</td>
<td>4.0 ± 1.0</td>
<td>8.5 ± 2.8</td>
</tr>
<tr>
<td>eAGC-mCAG</td>
<td>49.5 ± 5.5</td>
<td>13.5 ± 0.5</td>
<td>31.8 ± 8.2</td>
</tr>
<tr>
<td>eAGC-mCAT</td>
<td>41.5 ± 0.5</td>
<td>3.5 ± 1.5</td>
<td>7.4 ± 2.6</td>
</tr>
<tr>
<td>eAGC-mCTC</td>
<td>43.0 ± 3.0</td>
<td>4.0 ± 1.0</td>
<td>10.3 ± 2.8</td>
</tr>
<tr>
<td>eAGC-mCTG</td>
<td>42.5 ± 3.5</td>
<td>1.5 ± 1.5</td>
<td>6.3 ± 6.3</td>
</tr>
<tr>
<td>Total</td>
<td>546.5 ± 13.5</td>
<td>62.5 ± 9.5</td>
<td>11.4 ± 2.2</td>
</tr>
</tbody>
</table>

Average genetic similarity estimates and standard deviations across 11 primer combinations based upon AFLP analysis of hop cone DNA.†

Table 3. Average genetic similarity estimates and standard deviations across 11 primer combinations based upon AFLP analysis of hop cone DNA.†

<table>
<thead>
<tr>
<th></th>
<th>Sterling</th>
<th>USDA 21688</th>
<th>Nugget</th>
<th>Hallertauer Gold</th>
<th>Wye Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>USDA-21688</td>
<td>0.991 ± 0.008</td>
<td>0.972 ± 0.023</td>
<td>0.987 ± 0.012</td>
<td>0.981 ± 0.012</td>
<td>0.986 ± 0.012</td>
</tr>
<tr>
<td>Nugget</td>
<td>0.976 ± 0.024</td>
<td>0.972 ± 0.023</td>
<td>0.981 ± 0.012</td>
<td>0.986 ± 0.012</td>
<td>0.987 ± 0.011</td>
</tr>
<tr>
<td>Hallertauer Gold</td>
<td>0.986 ± 0.012</td>
<td>0.972 ± 0.028</td>
<td>0.987 ± 0.011</td>
<td>0.960 ± 0.024</td>
<td></td>
</tr>
<tr>
<td>Wye Target</td>
<td>0.963 ± 0.020</td>
<td>0.963 ± 0.031</td>
<td>0.958 ± 0.027</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† Computed according to Dice (1945).

‡ N = 11 for each estimate.
phic fragments between them. Since USDA-21737 is Sterling hop that has undergone meristem culture, the genetic difference between the two genotypes may have arisen during the meristem culture process, or from the presence of viral DNA in Sterling. Most of the primer combinations studied were able to differentiate between the half-sib sisters Sterling and USDA-21688 with an average of 8.5 polymorphic fragments detected. These data show that closely related individuals in hops can be readily differentiated with an AFLP analysis of dried cone DNA as long as suitable primer combinations are employed.

The lowest genetic similarity estimates observed were between Wye Target and the other genotypes studied (Table 3), which corroborates the work of Hartl and Seefelder (1998). Although Hartl and Seefelder (1998) studied different hop cultivars and used different primer pairs, some data similarities were evident between our work and theirs. Average genetic similarities in our work for comparisons between Sterling vs. Nugget (0.976), Sterling vs. Hallertauer Gold (0.986), Nugget vs. Hallertauer Gold (0.973), and Nugget vs. Wye Target (0.963) were comparable to Saazer vs. Northern Brewer (0.95), Saazer vs. Hallertauer Mittelfruh (0.98), Northern Brewer vs. Hallertauer Mittelfruh (0.95), and Northern Brewer vs. Wye Target (0.93) in Hartl and Seefelder’s (1998) work. Although the genotypes used in the two studies are not identical, some are closely related. Sterling was derived from Saazer 38, both Nugget and Northern Brewer are predominantly Brewer’s Gold, and Hallertauer Gold was derived from Hallertauer Mittelfruh (Al Haunold, 1999, personal communication; Neve, 1991). Our data suggest that AFLP will provide consistent results in hop cone DNA fingerprinting and genetic relationship research.

In conclusion, we report a method for employing AFLP analysis of DNA from dried hop cones. The AFLP technique generates many scorable DNA fragments which increases the chances of detecting genetic differences between closely related hop genotypes. All of the primer combinations used in this research were informative, and the combinations involving eAAC provided a greater number of scorable and polymorphic fragments across the MseI primers used here. Some variation between runs was observed for number of fragments scored and polymorphic fragments. This technique should prove useful for those needing to verify hops identity and purity by providing a method of analyzing DNA extracted from dried hop cones.

ACKNOWLEDGMENTS

The authors thank Caprice Rosato for her assistance with the AFLP analysis.

REFERENCES


