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Butternut Hybridization Report

Report Contents

Report Issued:

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Background and Methodology

Four (N = 4) samples of putative butternut tree (*Juglans cinerea* L.) were submitted by Dillon Consulting Ltd – and received by Precision Biomonitoring Inc. (PBI) - to determine if there has been potential hybridization with Japanese Walnut (*Juglans ailantifolia Carr*.).

Overview

The method employed by PBI for detecting hybrids between Butternut (*Juglans cinerea L*.) and Japanese Walnut (*Juglans ailantifolia Carr*.) species is a re-creation of the testing that was being conducted at the Ontario Forest Research Institute (OFRI). The assays employed by the OFRI are derived from the publication by Zhao and Woeste (2011). The publication developed a suite of molecular markers to identify potential hybrids between Butternut and Japanese Walnut species. In total, nine different molecular assays were developed in the study. For the hybrid testing used by OFRI, three specific assays were selected to be used in testing of samples to determine hybridity.

Methodology

All three assays are classified as cleaved amplified polymorphic sequences (CAPS) genetic markers. The methodology consists of performing a PCR reaction to amplify a specific gene region and then employ a restriction enzyme digestion on the amplified fragment. The specific restriction enzyme used cleaves (cuts) the PCR fragment differently depending on whether the PCR fragment is from a Butternut or Japanese Walnut tree. That is, the specific DNA sequence that is recognized by the restriction enzyme is located in a different part of the amplified PCR fragment depending on whether the PCR fragment is from a Butternut or Japanese walnut tree. A hybrid of these two species would be cut in such a way as to show each of the unique fragments that are associated with either pure Butternut or Japanese walnut trees. The specifics of the three assays used are as follows:

Assay #1) PCR amplification of chloroplast gene ***trnT-F,*** followed by restriction digest with enzyme ***MboII***.

Assay #2) PCR amplification of ***ITS*** region of ribosomal nuclear DNA, followed by restriction digest with enzyme ***Bsi*EI.**

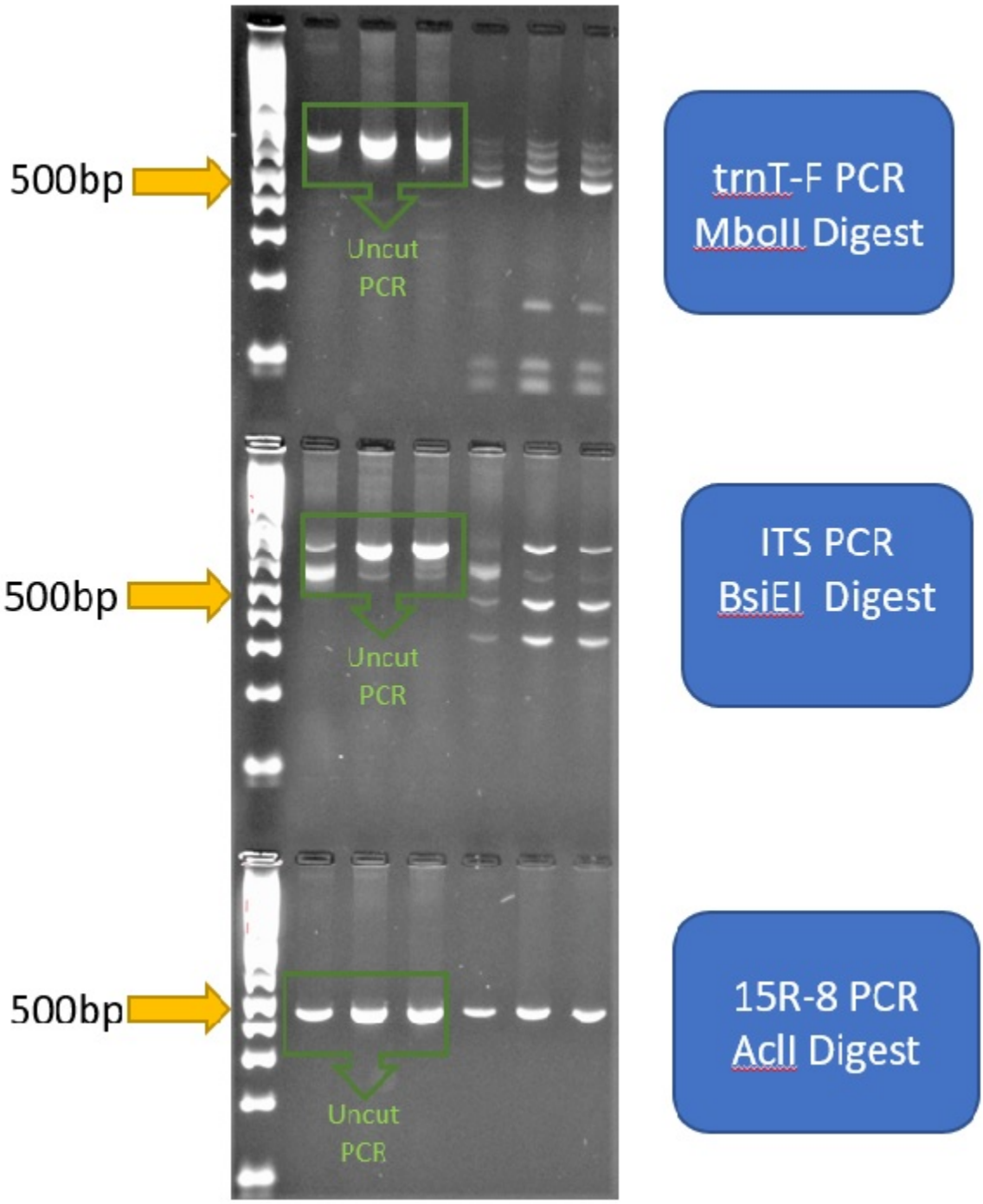
Assay #3) PCR amplification of random nuclear fragment called "***15R-8***", followed by restriction digest with enzyme ***Acl*I.**

Additional Marker for Hybrid Detection

In a comprehensive review of the publication, it was determined that another molecular marker would be of high utility in testing the purity of butternut samples. A PCR assay referred to as "***22-5***" has key benefits to be incorporated as an additional screening tool. It is a simple PCR assay, without need of subsequent restriction digestion and is also a codominant genetic marker. The value of a codominant marker is that in one assay all three phenotypic classes can be detected. That is, pure Butternut, pure Japanese Walnut and the hybrid can be detected in one PCR test. This assay is included in testing protocols as noted in the results section of the report.

The following image is a reference overview of the assays employed for detection of pure butternut samples. The three standard OFRI assays are shown with the DNA sizing ladder (500 base pair band marked) as well the original PCR amplification of the desired gene region and subsequent restriction digestion with respective restriction enzyme.

Reference Overview of Assay Results for Pure Butternut



Results

Figure 1: Gel electrophoresis image of hybridity detection assays

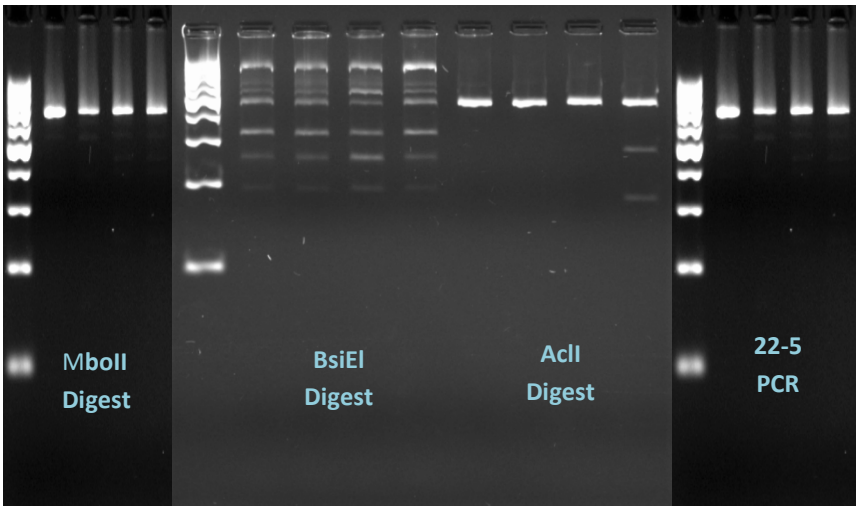


Table 1: Details of Results of Testing Assays

	Assay			
Sample	**MboII digest**	**BsiEI digest**	**AclI digest**	**22-5 PCR**
20-2878-001	J.cinerea	Hybrid	J.cinerea	J.cinerea
20-2878-002	J.cinerea	Hybrid	J.cinerea	J.cinerea
20-2878-003	J.cinerea	Hybrid	J.cinerea	J.cinerea
20-2878-006	J.cinerea	Hybrid	Hybrid	J.cinerea

Conclusion

The DNA tests used to determine hybridity with Japanese walnut for the four samples submitted (20-2878-001, 002, 003 and 006) were observed to be hybrids between *J. cinerea L.* and *J. ailantifolia*. It is important to note that these tests only detect the occurrence of a hybrid event between *J. cinerea L.* and *J. ailantifolia*, similar to the previous OFRI test that was derived from the publication by Zhao and Woeste (2011).

References Cited

Ross-Davis, A. & Woeste, K. E. (2008). Microsatellite markers for *Juglans cinerea* L. and their utility in other Juglandaceae species. *Conservation Genetics*, **9**, 465-469.

Zhao, P. & Woeste, K. E. (2011). DNA markers identify hybrids between butternut (*Juglans cinerea* L.) and Japanese walnut (*Juglans ailantifolia* Carr.). *Tree Genetics & Genomes,* **7**, 511-533.