

## PROJECT REPORT

**Project Title:** ABI 3730 DNA analyzer to augment tree fruit breeding and research

**PI:** Cameron Peace  
**Organization:** Washington State University  
**Telephone:** (509) 335 6899  
**Email:** [cpeace@wsu.edu](mailto:cpeace@wsu.edu)  
**Address:** Department of Horticulture & Landscape Architecture  
**Address 2:** 39 Johnson Hall  
**City:** Pullman  
**State/Zip:** Washington 99164

**Cooperators:** Deven See (USDA-ARS Western Regional Small Grains Genotyping Laboratory, Pullman), Kate Evans (WSU-TFREC, Wenatchee), Nnadozie Oraguzie (WSU-IAREC, Prosser).

**Total Project Request:** Year 1: \$50,000 Year 2: Year 3:

### Other Funding Sources

**Agency Name:** Washington Wheat Commission (WWC)

**Amount awarded:** \$50,000

**Notes:** WTFRC funds were matched with \$50,000 from the Washington Wheat Commission (separate award, PI: Dr. Deven See).

**Agency Name:** Washington State University - Agricultural Research Center

**Amount awarded:** \$100,000

**Notes:** Funds provided to Dr. Peace for equipment purchase to support tree fruit genetic screening.

**Agency Name:** Various

**Amount awarded:** approximately \$80,000

**Notes:** \$50K from WWC, \$3K from ARC, \$8K from USDA-ARS, and \$8K from ARC (part of above \$100K) for a Biomek "robot".

**Total Project Funding:** \$50,000

### Budget History:

Item	Year 1: 2008-2009	Year 2:	Year 3:
Salaries			
Benefits			
Wages			
Benefits			
Equipment	\$50,000		
Supplies			
Travel			
Miscellaneous			
Total	\$50,000		

## **RECAP ORIGINAL OBJECTIVES**

1. Obtain an ABI 3730 for high-throughput genotyping capability to support marker-assisted breeding needs of the WSU apple and sweet cherry breeding programs.
2. Establish the Pacific Northwest Tree Fruit Genotyping Laboratory (PNWTFGL), based in Pullman.

## **SIGNIFICANT FINDINGS**

- A refurbished 48-capillary ABI 3730 DNA Analyzer was installed in the laboratory of collaborator Dr. Deven See in April 2009.
- A Cooperative Utilization Agreement between the PNWTFGL and the WRSGL (Western Regional Small Grains Genotyping Laboratory; Program Leader Dr. Deven See, USDA-ARS) governing ownership, access, and use of the ABI 3730 was created.
- WSU-ARC provided Dr. Peace with \$100,000 for various smaller equipment items for the genetic screening process, particularly for the steps prior to genotyping on the ABI 3730, to remove technical bottlenecks and enable routine marker-assisted seedling selection on many thousands of tree fruit seedlings every year. Much of this equipment has been purchased and is being installed in the PNWTFGL.
- A Beckman Biomek NX<sup>P</sup> laboratory automation station (total cost approximately \$80,000) is currently being purchased, with approximately \$50,000 from the WWC, \$8000 from Dr. See's funds, and \$8000 from the ARC's provision to Dr. Peace.
- Use of the ABI 3730 is well underway, and the machine is utilized on daily basis. Its high-throughput capacity has previous scheduling difficulties and allows many labs to conduct genotyping. A per-datapoint fee is paid by external users for a repairs & maintenance fund.
- Use of the ABI 3730 is contributing to several current tree fruit projects.

## **RESULTS & DISCUSSION**

The ability to screen thousands of plants in a single season allows genetic marker technologies to truly interface with modern breeding programs for enhanced cultivar development. We have partnered with the Western Regional Small Grains Genotyping Laboratory, run by USDA-ARS scientist Dr. Deven See, to develop a WSU Genotyping Center for crop improvement. WSU's apple and sweet cherry breeding programs are now supported by the Pacific Northwest Tree Fruit Genotyping Laboratory, physically located on the Pullman campus.

In 2009, existing resources were strategically improved with the addition of an Applied Biosystems (ABI) 3730 DNA Analyzer (Figure 1), obtained through grants from WTFRC and the Washington Wheat Commission (WWC). Dr. See's USDA-ARS lab previously used an ABI 3130xl (33% capacity of the 3730), capable of more than 100,000 cereal samples in a season, and we had successfully trialed that machine for apple. The new ABI 3730 can be loaded at a time with up to 16 plates of 384 samples (6144), each "sample" having four different tests, taking 5 hours to process each 384-well plate. So for example, we could prepare tests for 6000 different apple seedlings with each seedling undergoing four genetic tests, and come back to see all the results about three days

later. The ABI 3730 therefore provides sufficient capacity for genotyping needs of WSU's tree fruit breeding programs (apple, cherry, pear) and tree fruit research colleagues, the USDA-ARS's cereal genotyping needs, and surplus capacity for breeding and research programs of other crops.



Figure 1. The ABI 3730 DNA Analyzer of the Pacific Northwest Tree Fruit Genotyping Laboratory.

In addition to genotyping, an advantage of the ABI 3730 is that it can also be efficiently used for DNA sequencing. Such sequencing is at a different scale (fewer sequences) and different screening capacity (more individual samples) than Dr. Amit Dhingra's newly acquired Roche 454 machine. Together, this sequencing capacity will significantly enhance WSU genomics activities.

This key piece of equipment is combined with further equipment support from the Agricultural Research Center (ARC), for DNA extraction (plate centrifuge, waterbath, UV gel imager, pipettes) and Genotyping (384-well thermocyclers, freezer). Our next purchase in 2009 will be a Beckman Biomek NX<sup>P</sup> laboratory automation station, which eliminates much repetitive labor in sample preparation, and reduces consumables. The Biomek "robot" is being purchased primarily with WWC funds, as well as operating funds of Dr. See and ARC funds for Dr. Peace.

Availability of such equipment has positioned WSU's tree fruit genomics, genetics, and breeding team in a highly competitive position for obtaining federal funding. The equipment (and available expertise) is also enabling current projects to be completed more efficiently.

We believe the Genotyping Center concept, partnering with the USDA-ARS cereals lab for full access to combined resources for both groups and managed by See and Peace, is an efficient approach to meet tree fruit breeding needs and enhance the scope of research capability. The momentum of obtaining the ABI 3730 has enabled the addition of \$100K in WSU-ARC support, and soon a Biomek laboratory automation station. These various equipment items were chosen to strategically remove technical bottlenecks and reduce labor costs, allowing high-throughput genetic marker assistance for the region's tree fruit breeding programs and industry.

## **EXECUTIVE SUMMARY**

We seek to establish the Pacific Northwest Tree Fruit Genotyping Laboratory (PNWTFGL), served with an ABI 3730 DNA Analyzer for high-throughput genotyping capability to support marker-assisted breeding needs of the WSU apple and sweet cherry breeding programs.

- A refurbished 48-capillary ABI 3730 DNA Analyzer was installed in April 2009.
- A Cooperative Utilization Agreement between the PNWTFGL and the WRSGL (Western Regional Small Grains Genotyping Laboratory; Program Leader Dr. Deven See, USDA-ARS) governing ownership, access, and use of the ABI 3730 was created.
- WSU-ARC provided Dr. Peace with \$100,000 for various smaller equipment items for an efficient genetic screening process.
- A Beckman Biomek NX<sup>P</sup> laboratory automation station (total cost approximately \$80,000) is currently being purchased as the last major technical component of our efficient genetic screening process.
- Use of the ABI 3730 is well underway, and the machine is utilized on daily basis. The machine is contributing to several current tree fruit projects.