14-002

FY16 Application for Nursery Research Funding
Washington State Department of Agriculture - Nursery License Surcharge
(Please use one application packet, including the Progress Report page for each proposal. You must use our form - *failure to do so may result in not funding your project.*)

Project Title: Use of advanced sequencing to facilitate the introduction of virus-tested fruit trees

Project Leader: Ken Eastwell

Institution (if any): Washington State University

Mailing Address: 24106 North Bunn Road, Prosser, WA 99350

Email: keastwell@wsu.edu **Project Phone Number:** (509)786-9385 **Cell Number:** (509)781-1445

Note: Project leader or his/her designee must be available at above project phone number on **February 27, 2015** between the hours of 10:00-12:00 and 1:00-3:00.

(Check One) New Project _____ ***Continuing*** ✓

Start Date: July 1, 2013 **Completion Date:** June 30, 2016

Amount Requested for (FY16) July 1, 2015 to June 30, 2016: $31,892

If this is a multiple year project, please estimate and list the following information for each future July 1 - June 30 period listed below through project completion:

Fiscal Years (FY)	July 1, 2016 to June 30, 2017	July 1, 2017 to June 30, 2018	July 1, 2018 to June 30, 2019	July 1, 2019 to June 30, 2020	July 1, 2020 to June 30, 2021
$ Amount Needed					

If you are increasing the above amounts since your last application, please explain why:

*Please list **all** other sources and amounts of funding for this project for the current year only: **(Please notify us by February 15 if other funding has been approved and from where.)**

Source	$ Amount Applied For	Approved	Pending Date of Notification
USDA-APHIS (NCPN)	$33,575	$33,575	

Total Amount Needed to Fund Project (include all sources*) $ 65,467

If total amount from all sources is not granted, will you be able to complete the project? No

Explain: This project compares current standard virus testing procedures with the accuracy of deep sequencing for the detection of viruses. USDA-APHIS funding to the Clean Plant Center Northwest (CPCNW) at WSU partially offsets the cost of testing fruit tree selections by current protocols. Nursery Research Funding is requested for deep sequencing of a representative subset of these selections. Both testing protocols are necessary for the comparison to be valid.

Please indicate which sector(s) of the nursery industry stand to benefit from the results of your research: (Letters of support from the industry are encouraged.)
All nurseries that propagate and sell temperate climate fruit trees will benefit. If the technology explored in this study is adopted, implementation will lead to more rapid access to new fruit tree clones and more reliable diagnosis of viruses.

Submit 16 copies of this proposal to: Tom Wessels, Plant Services Program Manager, P.O. Box 42560, Olympia, WA 98504-2560, twessels@agr.wa.gov, or fax (360) 902-2094
All applications must be postmarked by December 31, 2014.

Please summarize the purpose of this research: (you may attach additional sheets if necessary or submit this summary in your own format)

Continued success of the U.S. tree fruit industry depends on its ability to access new varieties quickly, and to minimize the economic and environmental impacts of managing diseases in the orchard. Adapting new, rapid technology for virus detection will greatly enhance the achievement of both of those objectives. In the current study, results of deep sequencing (also known as next generation sequencing) will be compared to results obtained from current accepted testing procedures. The latter evolved over many years and relies heavily on biological reactions of select indicator plant species that were chosen because they respond to known diseases. Deep sequencing offers the potential to detect viruses that do not produce symptoms on this limited number of plant species and cultivars. Moreover, deep sequencing does not require any prior knowledge of the virus detected, but only that some sequence information of a related virus has been deposited in the public database.

For those nurseries engaged in the international movement of fruit trees to reap maximum benefit from the introduction of new technology, it must also be recognized and accepted by U.S. trading partners. In cooperation with the Government of Canada's Genomics Research and Development Initiative (GRDI), samples are being exchanged in a blind test to validate procedures used in both countries. This will facilitate the future exchange of propagation material.

Methods of research:

Relative to methods used at the CPCNW, the Plant Health Laboratory of the Canadian Food Inspection Agency (CFIA) uses a different strategy to prepare samples for deep sequencing, and for subsequent data analysis. The two laboratories are participating in a reciprocal blind test in which ten samples are exchanged from each facility and the results of the twenty deep sequencing analyses compared. This will provide a measure of the robustness of the methods and the general applicability of deep sequencing for virus detection. Work performed by CFIA is funded through the GRDI. In anticipation of this project, 10 selections recently introduced into CPCNW were subjected to current testing protocols approved by USDA-APHIS for virus detection in trees from foreign sources. Samples from these selections were shipped to CFIA for analysis using their deep sequencing protocols and the same samples will be submitted for deep sequencing and the results analyzed at the CPCNW. In both cases, the virus status of the samples is not disclosed until all analyses are complete and the composite data compiled. Five additional selections from the CPCNW program will be subjected to deep sequencing.

The experimental design of this study will contribute to two objectives: it will reveal whether different strategies employed for sample preparation and analysis will affect the results, and it will provide additional information regarding the relative reliability of deep sequencing versus currently accepted protocols for virus detection.

Expenditure Breakdown:
(Please include salaries, supplies, travel, etc.)

Salaries (0.30 FTE Post Doctoral research associate)	$12,330
Benefits (State standard rate = 37%)	4,562
Supplies for analyzing 15 samples:	15,000
• materials for isolating RNA from plants; enzymes for cDNA labeling	
• Illumina deep sequencing	
• computer access for sequence analysis	
Travel	0
Overhead	0
TOTAL REQUEST	**$31,892**

The information requested on this page will have a direct bearing on whether your research request is approved or denied. Letters of support from the industry are also encouraged.

Note: Funding is not available for general overhead cost.

Progress Report on Funded Nursery Projects
Washington State Department of Agriculture

Date: 11/28/2014

Project Title: Use of advanced sequencing to facilitate the introduction of virus-tested fruit trees

Project Leader: Ken Eastwell

Progress:

At the conclusion of the previous year of this three year project, we reported that deep sequencing revealed the presence of luteovirus-like sequences in the nucleic acid samples prepared from a domestically sourced nectarine. There are reports in the literature of virus-like sequences occurring within the genomic sequences of plants, so the viral nature of these sequences needed to be confirmed. Based on the sequence data obtained from the deep sequencing process, primers for detection by reverse transcription polymerase chain reaction (RT-PCR) were designed, and reaction conditions optimized. This provided a rapid assay method specific for this virus sequence; the RT-PCR assay was employed in the subsequent studies. The original nectarine budwood was grafted onto several woody species including *P. avium, P. domestica* and *P. tomentosa*. In each case, the recipient trees were tested prior to graft inoculation, and at intervals after inoculation. The trees yielded negative RT-PCR results prior to graft inoculation but positive results after inoculation, confirming that the sequence was associated with a graft-transmissible agent.

The complete genome of the virus-like sequence was determined and its genome organization is consistent with that of luteoviruses. This constitutes only the second luteovirus known to infect a member of the family Rosaceae; most luteoviruses infect cereals where they cause significant damage to agriculture world-wide.

In many cases, luteoviruses cause disease only when in association with other viruses, particularly those of the genus *Marafivirus*. The sequences obtained from the original deep sequencing reaction were reexamined using a modified protocol and revealed a marafivirus-like sequence in the same sample. Using the same strategy described above, it was demonstrated that the marafivirus-like sequence is also graft transmissible to several *Prunus* species.

The original budwood source was subjected to standard virus testing procedures as approved by USDA-APHIS; all test results were negative indicating that the two new viruses do not produce detectable reactions in any of the diagnostic methods (biological, serological or molecular) currently relied upon for interception of viruses. This single case demonstrates the superior ability of deep sequencing to reveal the presence of viruses that are not detected by current testing methods, and are not associated with diseases previously described in the literature. It is not known at this time whether either of these viruses, alone or in combination, causes significant economic impact. This latter point raises the question of whether deep sequencing is TOO sensitive in that it reveals the presence of viruses of no immediate significance. However, this new technology is being employed in an ever-increasing number of programs around the world, and new viruses are being revealed and reported every few months. If a trading partner detects virus or viruses in material originating from the U.S. that was/were not detected by our programs, it would have repercussions for future trade. Therefore, in response to new virus reports emerging from international research programs, the CPCNW is bound to repeatedly test at substantial cost the entire collection as each new virus is reported. The adoption of deep sequencing would be a significant step forward in being proactive, and detecting novel viruses before they become embodied in trade and certification regulations (international and/or domestic). To a certain extent, it would also relieve the need to subject trees to repeated virus elimination therapy should they be found infected with a novel virus after initial treatment and screening. All novel viruses would likely be detected in the initial virus testing procedure and eliminated as a matter of standard procedure. This project will contribute significantly to the development of the most appropriate methods for applying this advanced technology.