Title: **Enhancing Disease Management through Whole Genome Sequencing.**


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**Background**

Plant diseases, along with pests and weeds, present significant impediments to agricultural economic prosperity. The global movement of plant materials has dramatically increased risks of introducing and spreading diseases. The rapidity with which diseases can spread, both naturally and artificially through trade, means plant disease diagnostic laboratories have a critical need to increase their capacity to help stakeholders. This can be done using whole genome sequencing (WGS). This technology reveals all the genetic information contained within an organism, which can be analyzed in powerful ways to transform disease diagnostics. This technology is cost-effective and will accelerate diagnosis, shorten time of response to outbreaks, provide more accurate and sustainable management strategies, and disrupt transmission chains earlier to enable stakeholders to better mitigate disease and reduce economic losses. This proposal is aimed at implementing WGS in the OSU Plant Clinic.

Our stakeholders have expressed interest in services that leverage genomics to define disease management options, such as learning which treatments would be efficacious. Technologies for generating WGS were transformational to biology and we are at a point where they can be used in diagnostics. Currently, depending on the technology used, the equivalent of about 20 bacterial genomes can be sequenced in a few days for ~ $25. Similarly, costs for sequencing instruments have declined. This ability to inexpensively sequence whole genomes has already been implemented at a national scale to prevent the spread of food-borne pathogens (Jackson et al., 2016). The power of WGS has been repeatedly demonstrated in these cases, allowing early action to identify sources, intervene, and prevent further spread of disease.

WGS holds similar promise for plant disease diagnosis and can provide additional layers of information to aid in disease management. For example, crown gall disease, a focus of the OSU Plant Clinic, is a significant problem in the Oregon nursery industry, particularly within herbaceous ornamentals, grapes, ornamental and fruiting apple, and *Prunus* species such as cherries. Losses due to crown gall have been estimated to exceed $100 million annually across the US. There is no control for an infected plant, which must be destroyed. However, some strains of the causal agent of crown gall, *Agrobacterium*, are susceptible to the biocontrol agent K1026, which is effective when used preventively. In the near past, several Oregon growers have reported extensive losses due to crown gall disease. Recently, a wholesale nursery in Oregon recently had an infestation of crown gall in the field, and losses were estimated to exceed one million dollars. The grower had no knowledge of the source of the infection or the means to know whether biocontrol products

would help his situation. With WGS, the Plant Clinic would be able to identify likely sources of the pathogen, e.g. whether it was resident or obtained from incoming plant material. Importantly, we could search for the presence of genes that confer susceptibility to K1026 and make recommendations on whether the product would control the pathogen.

We at OSU have used WGS to trace pathogens among nurseries and inform growers on disease management strategies. In one example, by using WGS we were able to link two nurseries to an offshore producer, suggesting it was a common source for disease (Savory et al., 2017). In the same study we were able, using WGS, to determine that previous conclusions on the cause of a novel disease in tissue cultured plant material were erroneous (Putnam, 2018). In a second example, we capitalized on the great resolution provided by WGS to trace the global movement of pathogens across the world (Weisberg et al., 2020)

**Project goal**: To implement standard computational tools to enable the OSU Plant Clinic to use WGS in disease diagnostics.

**Rationale:** WGS has not been adopted in disease diagnostic clinics because analyses require a computational infrastructure, tools, and appropriate expertise to process and analyze the large-scale datasets, none of which are common in plant diagnostic labs. The Plant Clinic has long-term collaborations with OSU faculty who have worked together to develop databases and computational tools for "genomic epidemiology" (Savory et al., 2017; Weisberg et al., 2020). We currently rely on services provided by a genome sequencing center for WGS. However, we have no quality control over the process and months can elapse before the center processes the samples. Direct access to a sequencing instrument devoted exclusively to plant disease diagnostics is crucial for using WGS to advance interests of Oregon agriculture.

**Objective:** The overall objective is to make WGS integral to the current workflow at the OSU Plant Clinic.

**Timeline:**
1st quarter – Purchase Illumina iSeq100 sequencer and associated supplies and materials.
2nd-4th quarters – Use the Illumina iSeq100 sequencer to develop rigorous protocols and standards for methods associated with sequencing such that we can draw confident conclusions and make recommendations to stakeholders.

We will first use bacterial pathogens such as *Agrobacterium* as targets for this technology because of their importance to the Oregon nursery industry. Funds from other sources will be used to support staff to carry out the objectives of developing protocols and quality control measures. Once established, we will be able to expand services in extremely transformative ways, such as in diagnosing pathogenic fungi, oomycetes, and viral pathogens, and eventually we hope to be able to detect pathogens directly from plant tissues without prior culturing.

In summary, this is a low risk proposal with high rewards in changing how the OSU Plant Disease Clinic serves growers in the state of Oregon. A one-time award will have a long term impact that will stretch into future decades, transforming diagnostic support for growers and allowing growers to manage diseases effectively, without guessing.

## Budget

We are asking for a total of **$30,665** for the instrument and sequencing supplies.

| **Equipment** | **Estimated cost** |
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| Illumina iSeq100 | $19,900 |
| Support plan | $3,640 |
| Reagent 4 pack (3 packs) @ $2,375 ea | $7,125 |
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| **Total** | **$30,665** |

**References cited:**

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Putnam, M.L. 2018. *Rhodococcus* revisited: Does it have any role in PBTS? Pacific Nut Producer 24:40-42.

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Weisberg et al., 2020. Unexpected conservation and global transmission of agrobacterial virulence plasmids. Science 368:256. DOI: 10.1126/science.aba5256