Development of New Primers and TaqMan® Probes for the Detection of Grapevine Viruses Associated with Red Blotch and Leafroll by qPCR and Digital PCR

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TaqMan® assays are highly sensitive and specific in the detection of infectious agents. In this research TaqMan® probes and primers were developed for the detection of several new genetic variants of leafroll 1 & 3 associated grapevine viruses and, a new DNA virus, Grapevine red blotch-associated virus (GRBaV), that is found to be widespread in wine grape production areas in the US and Canada for which a TaqMan® assay had yet to be developed. Method – Primers were developed by DNA sequencing, using the National Center for Biotechnology Information Nucleotide Database and tested using field samples in a qPCR system. Optimized TaqMan® assays was also used for the quantification of these three viruses in nucleic acid extracts prepared from virus-infected grapevines by using a digital PCR. Results – Successfully developed primers to detect three separate viruses, validated using field samples in qPCR system. A single extraction kit was used for all viruses. Digital PCR system was used to precisely quantify the amount of virus in the sample. Conclusions – This research has demonstrated the ability to develop single primer probes for 3 separate viruses vs. separate primers for each virus, resulting in significantly reducing time and cost in detection of viruses. Digital PCR, an emerging process used to quantify the virus is very beneficial in early detection, monitoring virus elimination, and to identify linkages between multiple viruses. Upon request, primer has been licensed to California plant and seed lab to begin commercial use of the primer and with interest from New Zealand.

Awards Won: