Grapevine Disease Testing Protocol 2010

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Freedom from viruses and other pathogens in grapevine stocks is important because all plants for plantings are produced by vegetative propagation. If present, disease agents will be readily perpetuated in the progeny. Once diseased plants are established in commercial vineyards, they are not amenable to any curative or therapeutic control measures. The most effective disease control option in most instances is removal of infected plant or plants. Further, several disease agents are spread secondarily by natural vector species, i.e. mealybugs and nematodes.

The principal method proven most efficient in controlling virus and virus-like diseases in grapevines involves applying pathogen exclusion protocols in advance of wholesale plant propagations. These protocols are often performed in the framework of clean stock/certification programs. Certification schemes worldwide share a common objective: to identify healthy sources for propagation through the application of time-tested indexing procedures as well as more recently developed molecular assays. Even so, the actual procedures and protocols can vary widely depending on the specific pathogens being targeted, the endemic disease agents in a production region, the availability of techniques and financial resources, and the expectations of industries served. The first step is the establishment of foundation or nuclear source plants; these plants test free from all known harmful viruses and are professionally identified for true-to-type phenotypes.

At Foundation Plant Services (FPS), we produce and maintain grapevine certified nuclear stock materials that become available to nurseries and growers in California, the United States, and foreign countries. The California Department of Food and Agriculture (CDFA) administers the statewide California Registration and Certification (R&C) Program for Grapevines. By the establishment of National Clean Plant Network (NCPN) in 2008, further support was provided through the Federal Government 2008 Farm Bill for specialty crops including grapevines nationwide. "The purpose of NCPN is to ensure the availability of high quality asexually propagated plant material that is free of targeted plant pathogens and pests that cause disease and resulting economic loss, to protect the environment, and ensure for the global competitiveness of specialty crop producers. The NCPN promotes disease and pest free specialty crops, rapid and safe introduction of new varieties from foreign sources, hygienic products for export, and a wholesome and abundant food supply. It attains these objectives by supplying pathogen and pest tested plant material for production of plants for planting. NCPN conducts research to improve its diagnostic and therapeutic service."

However, an important decision reached by the Grape Clean Plant Network (CPN), in conjunction with members of the Core Working Group, at the Grape CPN meeting in February 2009 was to set the future national standard for grapevine foundation material in the United States at a rigorous new level. Compliance with the new NCPN standard will ultimately be required as a prerequisite to NCPN certification for a foundation vineyard on the 100-acre Russell Ranch parcel on the UC Davis campus. All grapevines in the new vineyard will be propagated by microshoot tip tissue culture techniques (used for the elimination of viruses and crown gall). To qualify the grapevine cultivars and selections for planting at Russell Ranch, they should pass a panel of qPCR and/or PCR tests listed in Table 1 (Columns D and E) **in addition to** the biological indexes that qualify the materials for the CDFA Certification program (Table 1, columns F and G). This testing scheme is designated as "**PROTOCOL 2010**." Many nepoviruses exclusively reported in Europe and other parts of the world have been added to the list to ensure the freedom of our foundation material from these exotic and harmful viruses too. Work is underway to develop more sensitive qPCR for all the pathogens listed in Table 1.

To guarantee the success of PCR and qPCR assays for the detection of pathogens listed in Table 1, the FPS laboratory received support from NCPN in 2010 to upgrade the sample processing and testing equipment. This equipment was needed in order to increase the efficiency and accuracy of the tests and included: 1) Genogrinder 2010, that could process and homogenize 96 samples at a time in a matter of 3 minutes; 2) MagMax Epress that could process the samples prepared by Genogrinder and extract total nucleic acid for amplification and disease detection. This machine has the capacity to process 96 samples at a time in approximately 20 minutes and produce high quality of total RNA; 3) 7900HT Fast Real-Time PCR system that is used for the amplification of target RNA or DNA in the sample and has the capacity of running 96 samples at a time in approximately 1:15-2:15 hours depending on the test plate block used. The machine also has the capacity for low density PCR array (LDA) which could be used to test 384 samples at a time.

Table 1: LIST OF AVAILABLE TESTS FOR PROTOCOL 2010

A	B	C	D	E	F	G
Group	**Pathogen**	**Symbols**	**qPCR**	**PCR**	**Herb. Index**	**Woody Index**
Nepoviruses	Grapevine fanleaf virus	GFLV	√	√	√	St. George
	Tomato ringspot virus	ToRSV	√	√	√	
	Tobacco ringspot virus	TRSV		√	√	
	Arabis mosaic virus	ArMV		√	√	
	Strawberry latent ringspot virus	SLRSV		√	√	
	Peach rosette mosaic virus	PRMV		√	√	
	Blueberry leaf mottle virus	BLMV		√	√	
	Grapevine Bulgarian latent virus	GBLV		√	√	
	Grapevine chrome mosaic virus	GCMV		√	√	
	Grapevine Tunisian ringspot virus	GTRV			√	
	Raspberry ringspot virus	RpRSV		√	√	
	Tomato black ring virus	TBRV		√	√	
	Grapevine Anatolian ringspot virus	GARSV		√	√	
	Grapevine deformation virus	GDefV		√	√	
	Artichoke Italian latent virus	AILV		√	√	
Closteroviruses	Grapevine leafroll associated virus 1	GLRaV-1	√	√		Cab. Franc
	Grapevine leafroll associated virus 2	GLRaV-2	√	√		Cab. Franc
	Grapevine leafroll associated virus 2RG	GLRaV-2RG	√	√		
	Grapevine leafroll associated virus 3	GLRaV-3	√	√		Cab. Franc
	Grapevine leafroll associated virus 4	GLRaV-4	√	√		Cab. Franc
	Grapevine leafroll associated virus 5	GLRaV-5	√	√		Cab. Franc
	Grapevine leafroll associated virus 6	GLRaV-6	√	√		Cab. Franc
	Grapevine leafroll associated virus 7	GLRaV-7	√	√		Cab. Franc
	Grapevine leafroll associated virus 9	GLRaV-9	√	√		Cab. Franc
	Grapevine leafroll associated virus 10	GLRaV-10		√		Cab. Franc
	Grapevine leafroll associated virus 11	GLRaV-11		√		Cab. Franc
	Grapevine leafroll associated virus Car.	GLRaV-Car	√	√		Cab. Franc
Vitiviruses	Grapevine virus A	GVA	√	√		Kober 5BB
	Grapevine virus B	GVB	√	√		
	Grapevine virus D	GVD	√	√		
	Grapevine virus E	GVE		√		
Foveavirus	Grapevine rupestris stempitting associated virus (all strains)	GRSPaV	√	√		St. George
Maculavirus	Grapevine fleck virus	GFkV	√	√		St. George
	Grapevine redglobe virus	GRGV		√		
Marafiviruses	Grapevine syrah virus-1	GSyV-1	√	√		
	Grapevine vein feathering virus	GVFV		√		
	Grapevine asteroid mosaic virus	GAMV		√		St. George ?
Trichovirus	Grapevine berry inner necrosis virus	GINV		√		
Phytoplasma	Phytoplasma	Phyto		√		
Pierce's Disease	*Xylella fastidiosa*	PD	√	√		

Note: √= test is available; qPCR= quantitative PCR= real time RT-PCR with TaqMan probe; PCR= will include RT-PCR for RNA viruses; Cab. Franc= Cabernet Franc; St. George= St. George rootstock. Herb. Index.= herbaceous host indicators which will include a panel of: *Chenopodium quinoa*, *C. amaranticolor*, cucumber and tobacco plants.