

CryoConnect
**A Regional Initiative for a Cross-Border Cryobank in the Western
Balkans and the Caucasus**

Sanitary evaluation of the material for cryopreservation purposes

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CRYOPRESERVATION

- ✓ **Preserve pathogen-free material**
- ✓ **Careful preparation the mother plants**
- ✓ **Virus infections can reduce *in vitro* multiplication**
- ✓ **Virus infections can affect plant survival and regeneration**
- ✓ **Cryopreservation can influences viruses
(elimination or preservation)**

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CryoConnect genetic material

- ✓ **Plum (*Prunus domestica*)**
6 viruses + 1 phytoplasma
- ✓ **Grapevine (*Vitis vinifera*)**
11 viruses + phytoplasmas
- ✓ **Apple (*Malus x domestica*)**
4 viruses + 1 viroid + 1 phytoplasma



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Case study - plum

- plum pox virus (PPV)
- prune dwarf virus (PDV)
- *Prunus* necrotic ringspot virus (PNRSV)
- apple chlorotic leaf spot virus (ACLSV)
- apple mosaic virus (ApMV)
- plum bark necrosis stem pitting-associated virus (PBNPaV)
- myrobalan latent ringspot virus (MLRSV)
- '*Candidatus* Phytoplasma prunorum'



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- Biological tests
- **Enzyme-linked immunosorbent assay (ELISA)**
- **Reverse transcription–polymerase chain reaction (RT-PCR) and PCR**
- **Real-time reverse transcription–polymerase chain reaction (Real-time RT-PCR)**

Virus	Primer name	Primer sequence	Amplified fragment (bp)	Reference
PPV	P1	ACCGAGACCACTACACTCCC	243	Wetzel et al., 1991
	P2	CAGACTACAGCCTCGCCAGA		
PDV	PDV2	TAGTGCAGGTTAACCAAAAGGAT	172	Parakh et al., 1995
	PDV1	ATGGATGGGATGGATAAAATAGT		
PNRSV	PNRSV-R	TCACTCTAGATCTCAAGCAG	616	Spiegel et al., 1999
	PNRSV-F	GAGCTCTGGTCCCACTCAGG		
ACLSV	A53	GGCAACCCTGGAACAGA	358	Candresse et al., 1995
	A52	CAGACCCATTATTGAAGTCGAA		
ApMV	ApMV1R	CCTCAAATTCIGCTTAAAGCGGCG	312	Massart et al., 2009
	ApMV1F	AGAAGTGACTGCCACGGTTGAAG		
PBNSPaV	PBNSPaV det-R	AGTCGCACCACCAGTCTTCT	400	Al Rwahnih et al., 2007
	PBNSPaV det-F	TACCGAAGAGGGTTTGGATG		
'Candidatus Phytoplasma prunorum'	P1	AAGAGTTTGATCCTGGCTCAGGATT	1800	Deng and Hiruki, 1991; Schneider et al., 1995
	P7	CGTCCTTCATCGGCTCTT		
	R16F2	ACGACTGCTAAGACTGG	1245	Lee et al., 1993
	R16R2	TGACGGGCGGTGTGTACAAACCCG		

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- Explants for the establishment of aseptic culture of stated genotype for the conservation purpose

