

# Use of Genetic Markers to Assess Pedigrees of Grape Cultivars and Breeding Program Selections

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In a grape breeding program, it is necessary to keep accurate records of pedigrees. Occasionally, breeder's record can contain mistakes. Using genetic markers, "DNA Fingerprinting", it is possible to identify and confirm parent progeny relationships. In this project, Simple Sequence Repeat (SSR) markers are used to confirm or correct pedigrees for grape varieties from the Cornell breeding program. As expected, most (20 of 24) reported pedigrees were confirmed.

Keeping accurate records can allow plant breeders to continue breeding grapes with desired traits. Since 1906 Cornell University has named and released 56 grape cultivars. Many of these cultivars have been selected for cold-tolerance, often times fungal disease-resistance, and high fruit quality. Like all plant breeding programs, many previously selected cultivars are used again as parents to create a new variety. An example is Emerald Seedless, a selection made by Dr. Harold Olmo from a cross of Emperor and Sultana Moscato. Emerald Seedless was later used as a parent, crossed with Athens, to obtain the new variety Marquis (Figure 1).

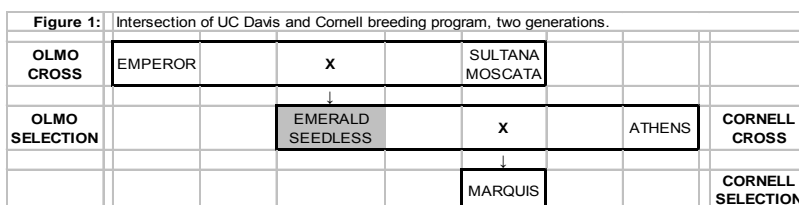
Tests were performed on DNA extracted from dried young leaves using a DNeasy Plant Mini Kit (Quigen, Valencia, California, USA) following the manufacturer's protocol. PCR amplifications were performed following typical protocols (Dangl et al. 2005). Each sample was analyzed with a set of eight markers which included the international six SSR marker set (This et al., 2004). An additional seven loci were used for specific samples to confirm unexpected results (Table 1). Forward primers were labeled with one of three fluorescent dyes. Fragment amplifications were verified on 2% agarose gel. Samples are run in multiplexes on a capillary electrophoresis on an ABI Prism 3100 Genetic Analyzer.

As expected, most (20 of 24) reported pedigrees were confirmed. Analysis of three pedigrees, Groups A, B, and C showed a heritable null allele in Ontario at VVMD25 (Table 1). Russian Seedless is a synonym for Black Kishmish (Dangl et al. 2001) and has been used, inappropriately, as a synonym for Black Monukka. Russian Seedless was reported to be a parent of both Glenora and Suffolk Red (Groups A and D, Table 1). In both of the original publications (Einset, 1973; Pool et al., 1977), the Russian Seedless parent was described as "probably" being Black Monukka. This study shows only Black Kishmish, not Black Monukka, produced SSR fingerprints consistent with parentage of Glenora and Suffolk Red.

Alden (Group C) is reported to be from a cross of Ontario and Gros Guillaume. As Gros Guillaume is not available, Ribier, a suspected synonym, was tested. The results confirm the pedigree and the synonymy. The tetraploid nature of the seedless table grape selection NY 88.0515.01 and its two reported parents is clearly illustrated by this analysis (Group E). Group F is an example pedigree for which one of the reported parents is no longer available; the associate grandparents were assessed to support the reported pedigrees.

We tested several accessions of Vignoles (Ravat 51), an important parent in the Cornell grape breeding program. They were found to be identical and the data were consistent with Vignoles being a parent of a particular selection (data not shown). However, this study shows neither of the reported parents of Vignoles (Seibel 6905 x Pinot de Corton, a clone of Pinot noir) could be a parent of Vignoles (Group G). To the best of our knowledge, the two parents are correctly identified; therefore, it may be possible the tested Vignoles may not be Ravat's actual selection 51.

This study shows SSR markers are a powerful tool for confirming and correcting pedigrees of grape breeding programs. This study also provides some specific, valuable information to future grape breeding efforts. In some cases scoring heritable null alleles was necessary to confirm pedigrees. We also demonstrated the use of “grand parents” to confirm a pedigree when one reported parent is no longer available for analysis.



**Table 1:** SSR marker analysis at loci designated in first row of table. Allele sizes (in base pairs) for grape releases and selections addressed in this study are arranged in parent/progeny groups, with the putative offspring in the middle. SSR alleles display typically Mendelian segregation, with alleles at each locus contributed by parents, permitting assessment of parent/progeny relationship..

Accession	VVMD5 <sup>a</sup>	VVM D 7 <sup>a</sup>	VVM D 27 <sup>a</sup>	VVM D 31	VVM D 32	VVS2 <sup>a</sup>	VrZA G 6 <sup>2a</sup>	VrZA G 7 <sup>9</sup>	VVM D 21	VVM D 28	VVM D 34	VVM D 6	VVM D 25	VVM D 36	VrZA G 9 <sup>3</sup>
<b>A Ontario</b>	238:238	235:247	185:185	204:216	241:251	123:125	203:205	247:265	241:249	229:233	240:242	205:205	244:null	264:295	189:189
Glenora	234:238	235:253	185:194	212:216	251:251	125:151	189:203	251:265	249:256	221:229	240:240	205:214	253:null	250:296	189:215
Black Kishmish	234:234	249:253	181:194	196:212	251:251	151:155	189:203	247:251	249:256	221:249	240:240	214:214	249:253	244:250	189:215
Black Monukka	234:240	253:253	181:194	212:212	251:257	141:151	189:189	247:257	249:249	221:247	240:248	212:214	253:259	250:268	189:199
<b>Conclusion: Markers consistent with Ontario x Black Kishmish as parents for Glenora, scoring null allele from Ontario at VVMD25</b>															
<b>B Ontario</b>	238:238	235:247	185:185	204:216	241:251	123:125	203:205	247:265	241:249	229:233	240:242	205:205	244:null	264:295	189:189
Himrod	234:238	239:247	185:194	212:216	251:251	125:151	189:205	259:265	241:249	221:229	240:248	205:214	253:null	268:295	189:199
Thompson Seed	234:234	239:253	181:194	212:212	251:251	145:151	189:189	247:259	249:256	221:247	240:248	212:214	243:253	250:268	189:199
<b>Conclusion: Markers consistent with Ontario x Thompson Seedless as parents for Himrod, scoring null allele from Ontario at VVMD25</b>															
<b>C Ontario</b>	238:238	235:247	185:185	204:216	241:251	123:125	203:205	247:265	241:249	229:233	240:242	205:205	244:null	264:295	189:189
Alden	238:238	235:249	185:185	204:216	241:273	123:135	187:203	239:265	249:249	233:247	240:248	205:214	259:null	254:264	189:215
Ribier	226:238	249:255	185:185	210:216	253:273	133:135	187:205	239:251	249:249	247:247	240:248	194:214	243:259	254:264	189:215
<b>Conclusion: Markers consistent with Ontario x Ribier as parents for Alden, scoring null allele from Ontario at VVMD25, is Ribier synonym for Grosse Guillaume</b>															
<b>D Fredonia</b>	236:290	235:235	183:185	204:204	251:273	125:125	203:203	247:259	249:249	229:247	240:248	212:null	247:247	264:270	187:189
Suffolk Red	234:236	235:253	181:185	204:212	251:273	125:155	189:203	247:259	249:256	229:249	240:240	214:null	247:249	244:270	187:189
Black Kishmish	234:234	249:253	181:194	196:212	251:251	151:155	189:203	247:247	249:256	221:249	240:240	214:214	249:253	244:250	189:215
Black Monukka	234:240	253:253	181:194	212:212	251:257	141:151	189:189	247:257	249:249	221:247	240:248	212:214	253:259	250:268	189:199
<b>Conclusion: Black Monukka not Suffolk Red parent. Markers consistent with Fredonia x Black Kishmish as parents for Suffolk Red with null allele at VVMD6</b>															
<b>E Niabell</b>	236:236	241:247	179:185	204:212	241:249:273	123:133	203:205:207	237:247							
NY 88.0515.01	234:236:238	239:241	179:185:194	204:212	249:251:273	123:125:151	189:203	237:247:259:265							
Himrod 4X	234:238	239:247	185:194	212:216	251:251	125:151	189:205	259:265							
<b>Conclusion: Markers consistent with Niabell x Himrod 4X as parents for NY 88.0515.01, note that all members of this group are tetraploid</b>															
<b>F Seyval</b>	226:252	237:243	189:189	212:220	251:273	133:133	181:189	259:261							
Melody	238:252	235:243	185:189	204:220	241:273	133:137	189:203	247:261							
Ontario <sup>b</sup>	238:238	235:247	185:185	204:216	241:251	123:125	203:205	247:265							
Pinot noir <sup>b</sup>	228:238	239:243	185:189	216:216	241:273	137:151	189:195	239:245							
<b>Conclusion: Markers consistent with Seyval x GW5 as parents for Melody, using Ontario and Pinot Noir as parents of GW5</b>															
<b>G Pinot noir<sup>c</sup></b>	228:238	239:243	185:189	216:216	241:273	137:151	189:195	239:245	249:249	221:239	240:240	205:205	243:253	254:254	189:189
Vignoles	226:244	239:251	185:194	212:214	251:251	125:149	189:195	251:259	243:249	241:263	240:242	212:219	253:259	240:270	189:199
Seibel 6905 <sup>c</sup>	226:236	237:243	181:189	212:212	241:273	143:145	181:189	243:261	229:249	239:261	240:243	211:219	245:259	264:270	209:209
<b>Conclusion: Markers exclude both Pinot noir and Seibel 6905 as parents for Vignoles, though these are the reported parents</b>															

<sup>a</sup>Six internatal reference markers used in this study. <sup>b</sup>Parent used in the study. <sup>c</sup>Parent used in the study. <sup>d</sup>Parent used in the study.

## References

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