

# Meeting of the ECPGR Activity 'Prunus Alignment'

18 February 2020, Athens, Greece

## HRM analysis on plum samples

Marco Pietrella

# Materials and Methods

Samples analysed → leaves of *P. domestica*

- Italy: 14
- Greece: 6
- Latvia: 9
- UK: 7

## Extraction:

Samples were extracted using DNeasy® Plant Mini Kit (Qiagen)

## Quality and quantification assessment:

[DNA] was measured with Pico100 (Picodrop) and run on a 0.6% gel to assess DNA integrity

## Amplification & analysis

DNA was finally amplified with BrightGreen 2X qPCR MasterMix-Low ROX (ABM) in 14 ul volume in duplicates

HRM was performed on a 7500 Fast (ABI) QPCR system

Analyses performed with GenAIEx v6

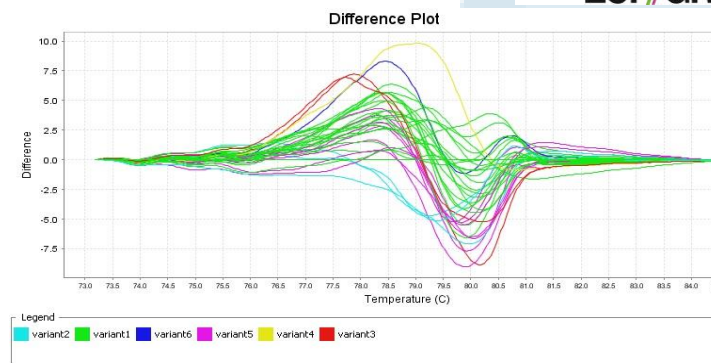
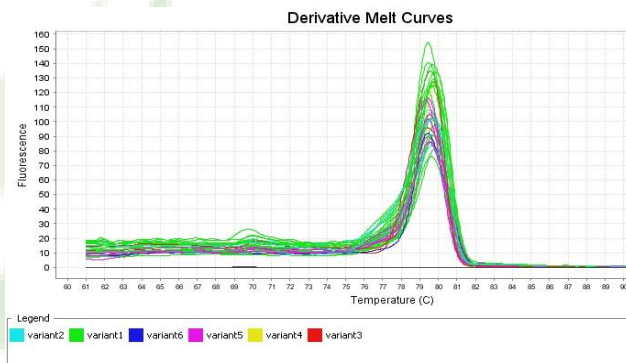


# Molecular Markers used

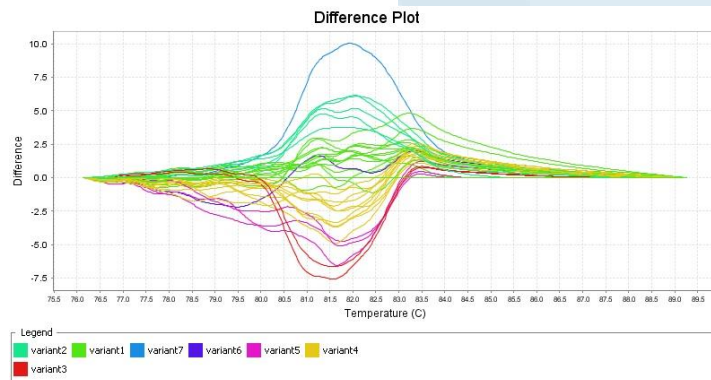
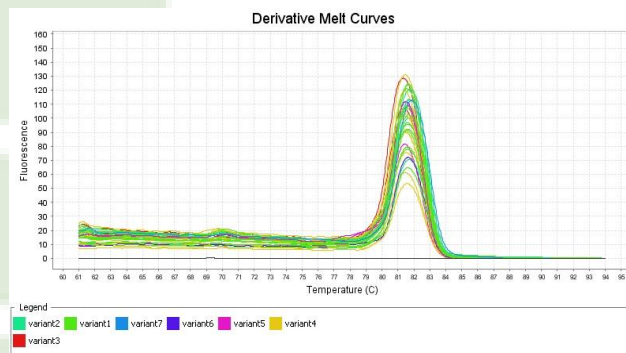
Microsatellite loci	Forward (5'-3')	Reverse (5'-3')	Amplicon size	Motif*	Tm range	Species of origin	Reference
BPPCT034	CTACCTGAAATAAGCAGAGCC AT	CAATGGAGAATGGGGTGC	228	(GA) <sub>19</sub>	77.4- 79.1	<i>P. persica</i>	1
BPPCT037	CATGGAAGAGGATCAAGTGC	CTTGAAGGTAGTGCCAAAGC	155	(GA) <sub>25</sub>	76.7- 78.92	<i>P. persica</i>	1
PceGA34	GAACATGTGGTGTGCTGGTT	TCCACTAGGAGGTGCAAATG	155	NA	81.38- 81.70	<i>P. cerasus</i>	2
PS12A02	GCCACCAATGGTTCTTCC	AGCACCAGATGCACCTGA	200	NA	77.73- 79.62	<i>P. cerasus</i>	2
UDP96-001	AGTTTGATTTTCTGATGCATCC	TGCCATAAGGACCGGTATGT	120	(CA) <sub>17</sub>	76.5- 78.73	<i>P. persica</i>	3
UDAp-404	CATGAACAGGGTCAAAAGCA	TATATCCTTACGCGGCCTCA	181	(GA) <sub>21</sub>	79.75- 82.4	<i>P. armeniaca</i>	4
UCD-CH17	TGGACTTCACTCATTTTCAGAGA	ACTGCAGAGAATTTCCACAAC CA	188	(CT) <sub>11</sub>	75.4- 77.42	<i>P. avium</i>	5

\*The repeat motif of each marker is given based on information provided, or not, by the original reference that published primers for the marker. 1=Dirlewanger et al., 2002, 2=Downey and lezzoni, 2000, 3=Cipriani et al., 1999, 4=Messina et al., 2004, 5=Struss et al., 2003.

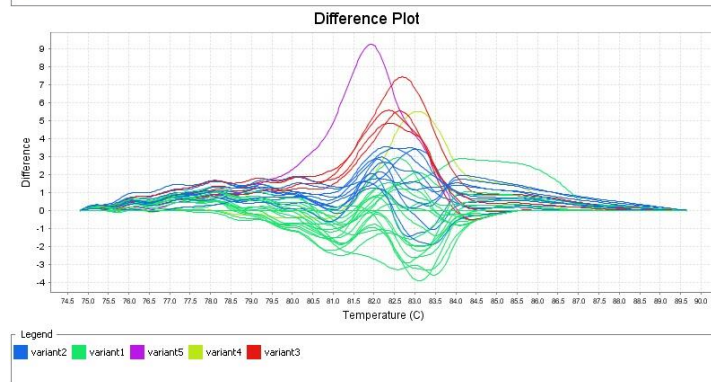
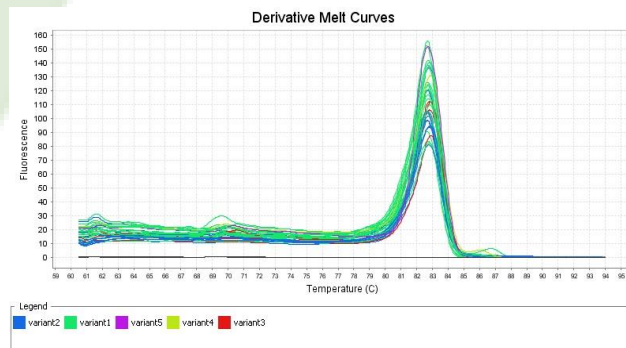
**BPPCT034**



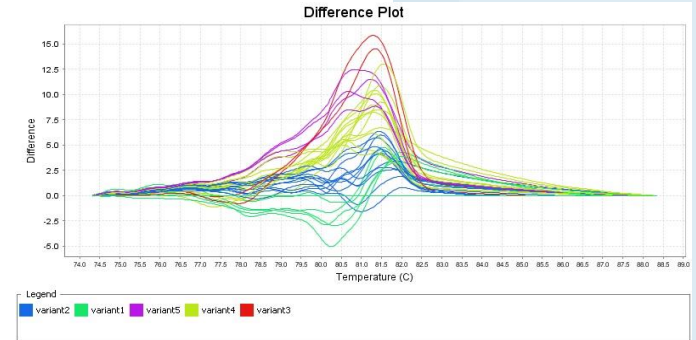
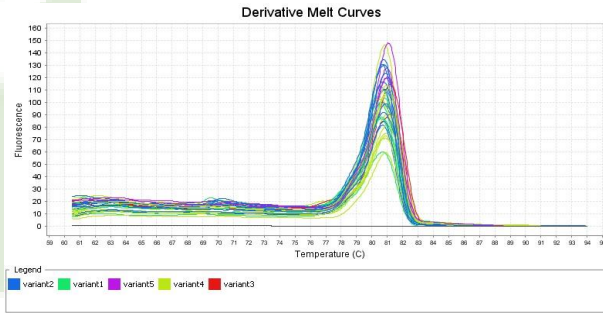
**BPPCT037**



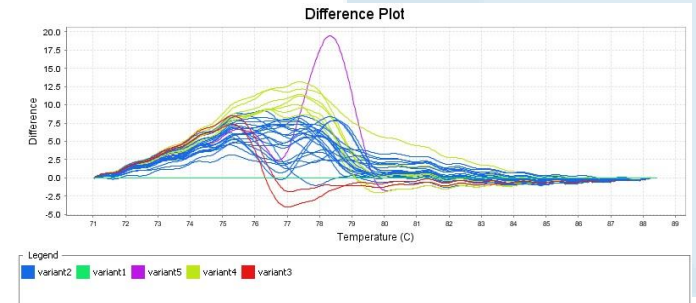
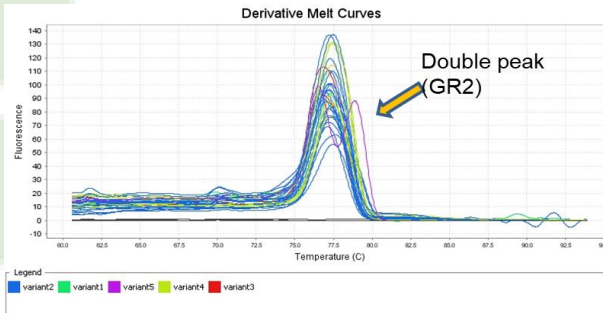
**PceGA34**



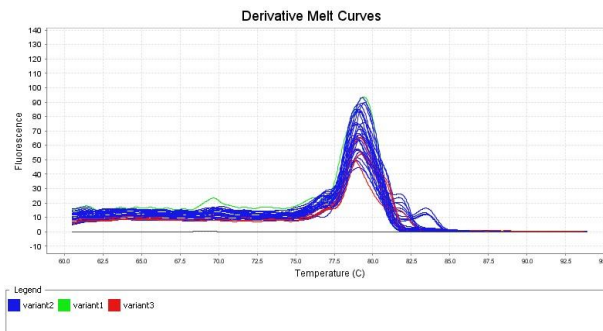
PS12A02



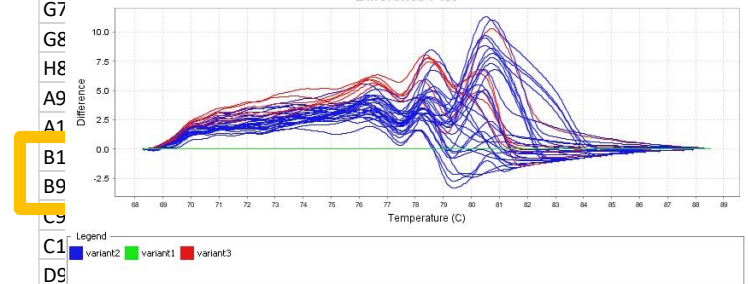
UCD-CH14

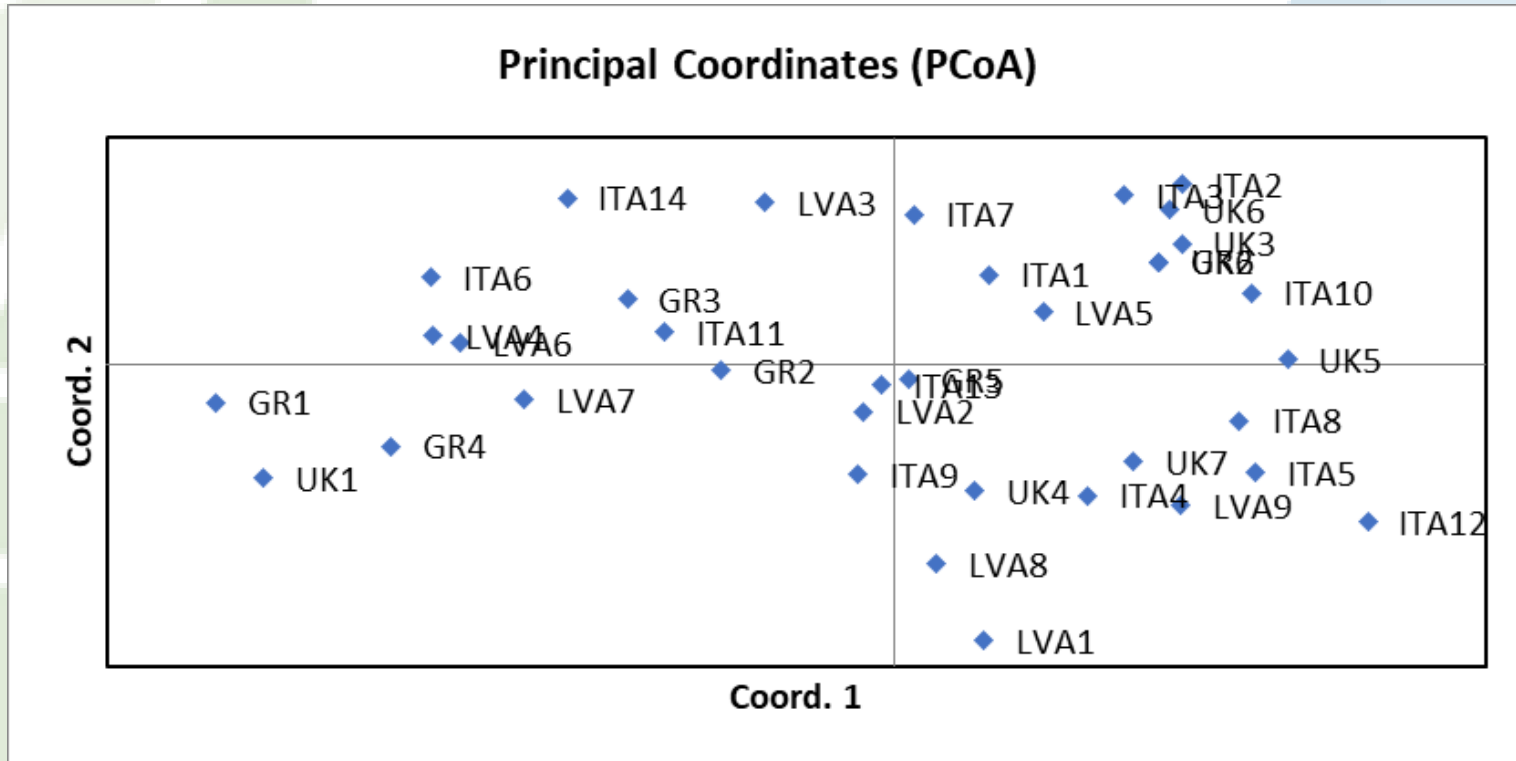


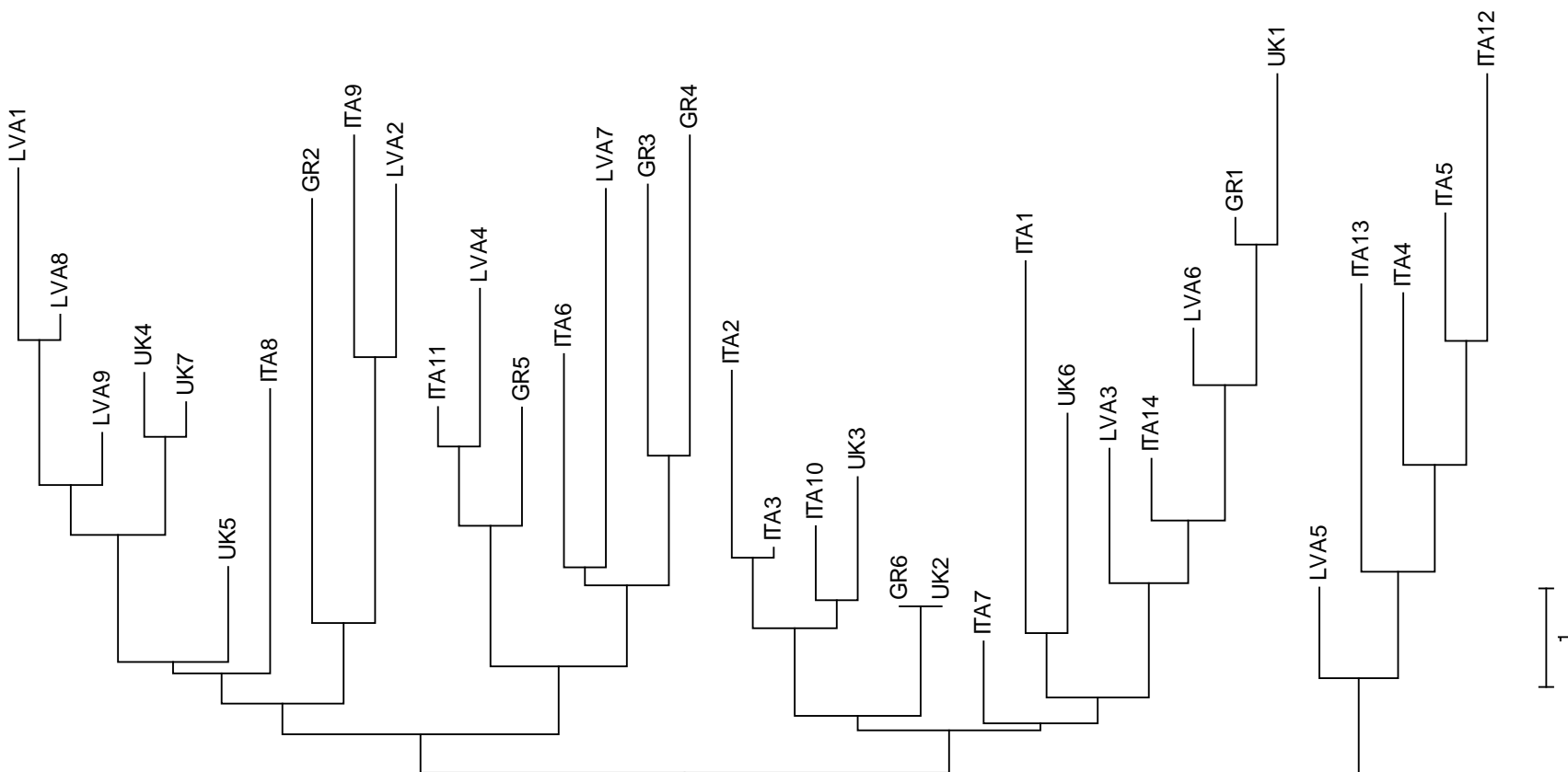
UDP96-001



Well	Sample Name	Variant Call	Confidence (%)	Tm
G7				
G8				
H8				
A9				
A1				
B1				
B9				
C3				
C1				
D9				
D10	GR6	variant1	61.5092	78.7288







# Conclusions

- Data showed relatively few (max 7) variants
  - In some cases hard to unambiguously call a variant
  - In some cases difference in variant call among replicates
- ➔ Replicates and an accurate and careful scoring system are needed to achieve reproducible results → training people essential