SELECTING MARKERS FOR GENETIC DIVERSITY CHARACTERISATION IN SWEET CHERRY

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Introduction

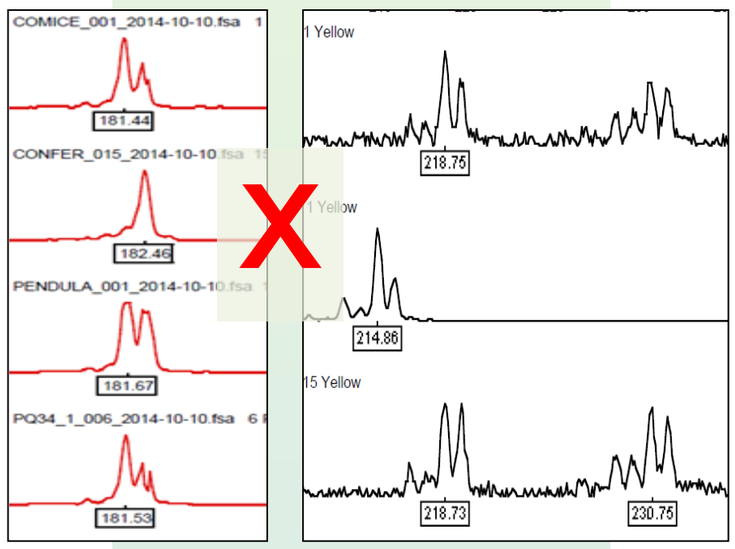
The use of SSR/microsatellite markers for germplasm characterisation has become common practice in the last decade. To compare results across laboratories using different protocols and detection methods depends on using common markers and on the inclusion of known genotypes to allow the standardisation of allele sizes.

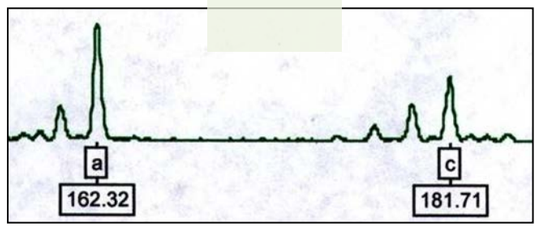
In December 2006, participants of an ECPGR-sponsored workshop met at East Malling Research (now NIAB EMR) to discuss the ideal characteristics of genotyping marker sets and to set out guideless on how to best compare results across experiments (Tobutt & Evans 2007).

Primer	Dye	LG:cM†	Rank	Size range‡	*P. avium* F12/1 (AFT)	*P. avium* 'Goodnestone Black' (AGB)	*P. avium* 'Napoleon' (ANA)	*P. avium* 'Noble' (ANB)	*P. avium* 'Noir de Meched' (ANM)	*P. incisa* E621 (INE)	*P. mahaleb* SL64 (NIF)	*P. nipponica* F1292 (NIF)
EMPA002*	6-FAM	G1:46	4	103-131	105	103/105	103/105	105	103/105	119/121	121	115/131
EMPA003	VIC	G1:115	16	157-175	175	175	171/175	175	157/171	175	166	167/175
EMPA017*	6-FAM	G2:00	3	221-242	238/242	238	238§	232/238	238	223/228	221/223	223/*n*
PceGA34	NED	G2:87	9	100-237	140	138/214	160/203	132/143	140/154	100/237	139	100/237
EMPaS12	6-FAM	G3:38	6	108-144	122/144	136/138	138§	138/144	138/144	110/136	108	122/124
EMPaS02	6-FAM	G3:78	11	129-159	143	129/137	137/139	139/143	137/145	142/187	139/156	159§
EMPaS06	6-FAM	G4:25	10	159-218	201	203/211	203/205	205/221	201	159/209	218	160/207
EMPaS10	VIC	G4:51	5	134-184	166/184	164/166	151§	151/166	151/166	142/152	134/166	145/172
BPPCT037	6-FAM	G5:31	8	120-172	150	131/144	137/144	137	139/144	126	172	120/126
EMPaS14	NED	G5:46	13	164-208	194/208	196	196/208	194/208	194/196	164	174/176	168/188
EMPaS01	6-FAM	G6:28	7	210-250	220/226	226/228	228/230	226/230	226/230	210/250	211	198/212
UDP98-412	6-FAM	G6:77	12	098-157	116/123	100/123	119§	116	119/125	101/116	98	157/*n*
CPPCT022*	VIC	G7:25	2	228-327	253	255/259	253/255	245	245	261	228	247/327
PS05C03	NED	G7:75	15	101-159	144	118/125	142/159	127/146	125/127	109/129	101	125/127
EMPA026	NED	G8:00	14	189-217	200	200/214	200/214	200/214	200/214	189/205	211/217	201/217
CPPCT006*	VIC	G8:31	1	173-203	182	182/184	184/200	184	184/200	173	174	176/203

*"Universal' primers
† Linkage group and map position in *Prunus avium* 'Napoleon' × *P. nipponica*
‡ Alleles in base pairs sized using an ABI 3100 sequencer
§ Homozygous at loci in EMR's interspecific mapping population

As a result, Clarke & Tobutt (2009) proposed a set of SSR markers and control cultivars for sweet cherry that has been used by several groups (all or part) to characterize a range of wild and cultivated germplasm. Often, other markers have also been used. The performance some markers has been unsatisfactory.
Additionally, markers linked to traits of interest for breeders including fruit size (Rosyara *et al* 2013) and flesh colour (Sandefur et al 2016) have been identified.
Validation of those markers in diverse germplasm sets is essential to their deployment for marker-assisted breeding (MAB).

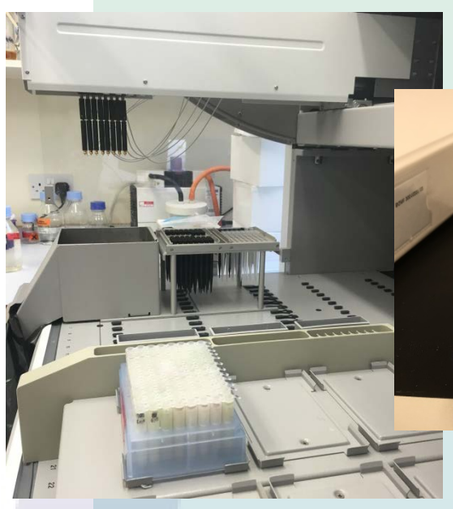


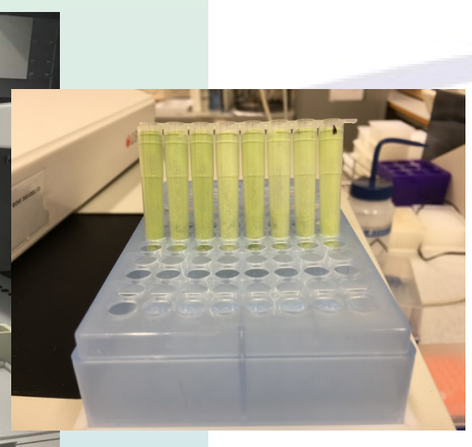


Ideally markers used for germplasm screening would be:

- robust and consistent in A-addition during amplification
- Easy to score:
  - Alleles with clear/no stuttering
  - 2 or more bp intervals

The EU.CHERRY project will select a diversity panel for cultivated cherries and landraces across Europe.
Phenotypic information will be provided by the curators in the originating collection. Genotypic information will be produced for these accessions at NIAB EMR using a set of SSR markers based on the original ECPGR recommendations but modified to take into account feedback from numerous studies. The 'CherryMakerSurvey' file has now been distributed to ECPGR and COST members and we welcome your feedback!





References:

Clarke JB, Tobutt KR (2009) A standard set of accessions, microsatellites and genotypes for harmonising the fingerprinting of cherry collections for the ECPGR. ISHS, 814:615-618

Rosyara UR, Bink M, van de Weg E, Zhang G, Wang D, Sebolt A, Dirlewanger E, Quero-Garcia J, Schuster M, Iezzoni AF (2013) Fruit size QTL identification and the prediction of parental QTL genotypes and breeding values in multiple pedigreed populations of sweet cherry. Molecular Breeding 32 (4): 875-887

Sandefur P, Oraguzie N, Peace C (2016) A DNA test for routine prediction in breeding of sweet cherry fruit color, Pav-Rf-SSR. Molecular Breeding Online first (36:33)

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EMR is part of the NIAB group

Acknowledgements and contacts