

Breeding for fruit quality- Linking phenotype to genotype

Julie Graham



Key quality traits of interest

- **Ripening/season**
- **Size**
- **Colour**
- **pH**
- **Anthocyanins**
- **Sugars/acids**
- **volatiles**
- **Sensory/taste**
- **Not crumbly**



Susan McCallum
Danny Cullen
Kay Smith
Sandie Williamson
Rob Hancock
Craig Simpson
Pete Hedley
Linda Milne
Runxuan Zhang
Christine Hackett
Katharine Preedy

Quality traits on raspberry chromosomes

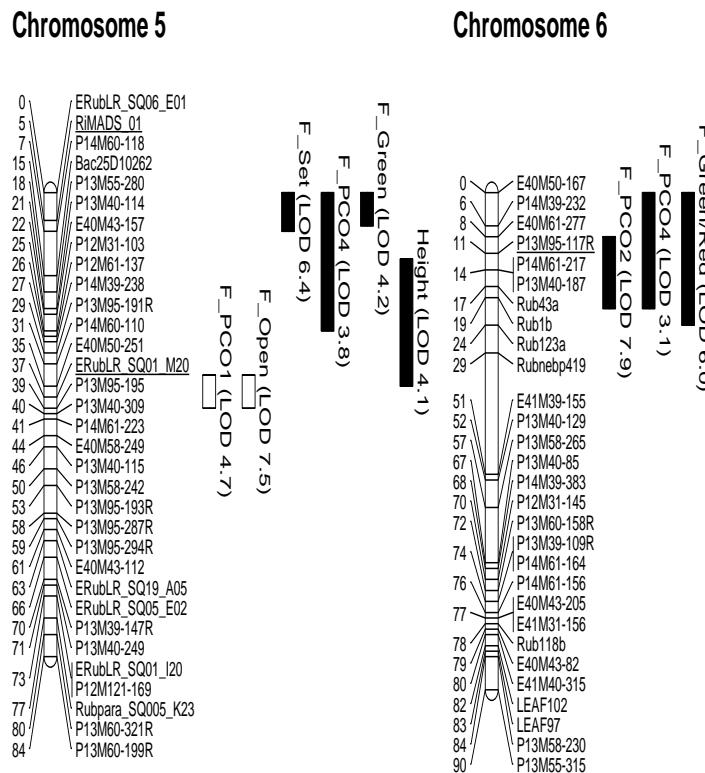
To associate traits with genes on chromosomes need – mapping populations

Mapping population is a cross between two parents varying for the traits of interest – reference population Latham x Glen Moy

Grow populations under different environments across seasons and record data of interest

Make a map by applying molecular markers to DNA of each progeny from the cross and look for variations in DNA sequence representing different ‘versions’ of the sequence of genes

Add trait data to map



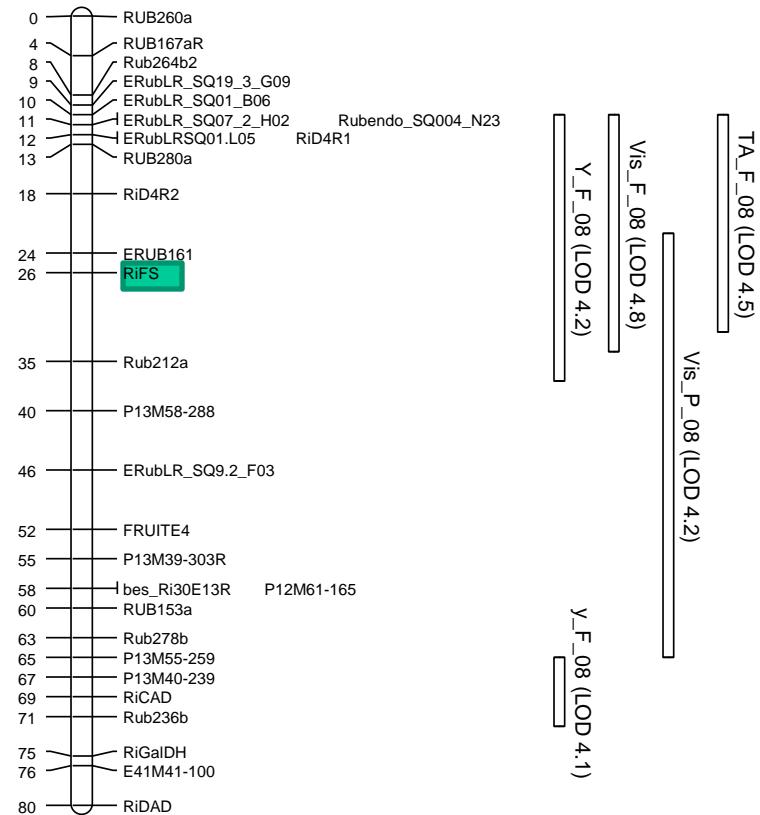
Fruit colour



Marker RiFLS
LG 4

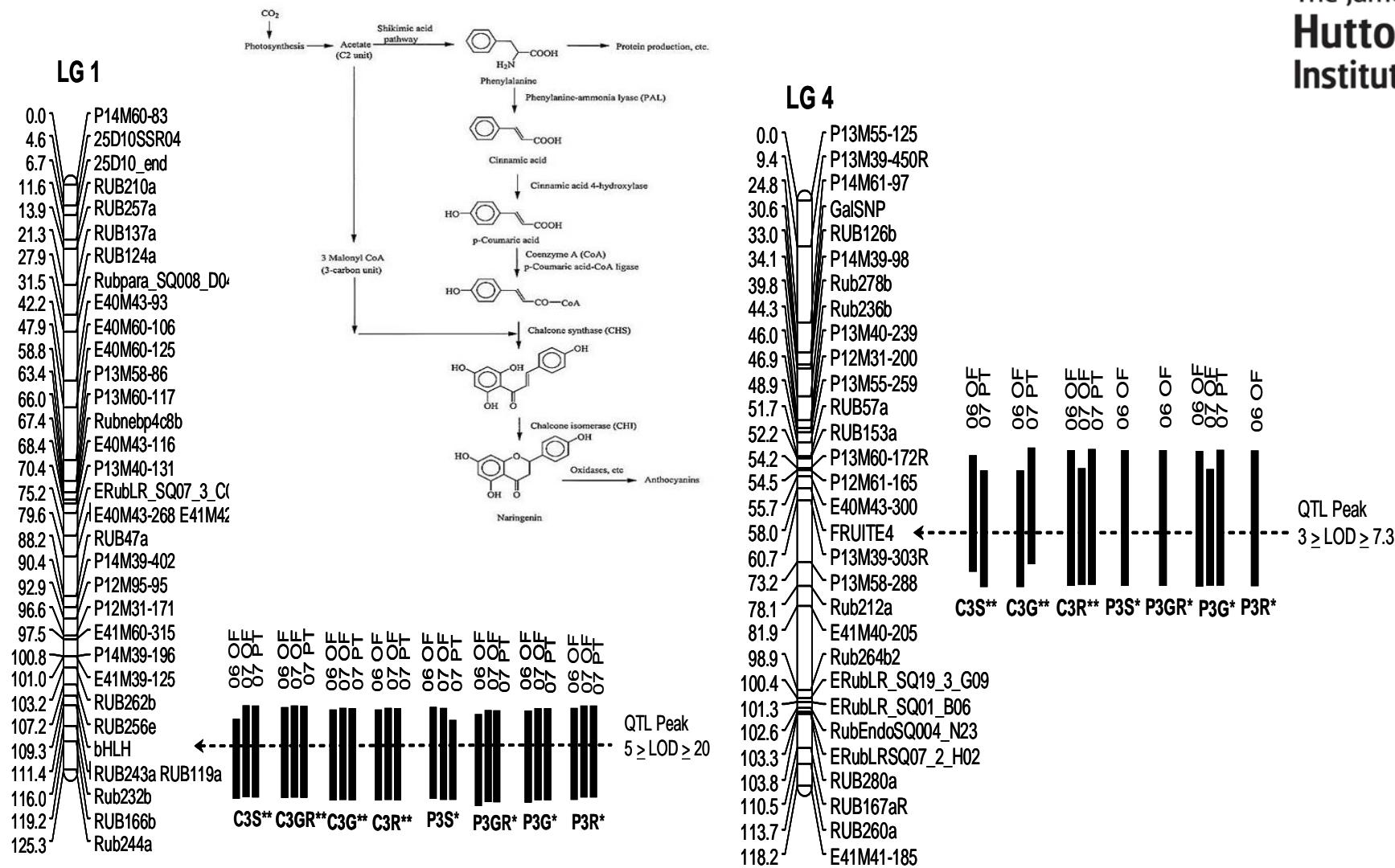
Flavanol synthase impact of alleles

	aa	ab	bb	SED	Sig
Y	15.5	15.0	14.6	0.20	<.001
Visual	2.92	3.25	3.66	0.149	<.001
TA	-0.39	0.06	0.28	0.157	<.001
C3S	200.2	237.5	256.6	22.32	.019
C3GR	62.5	78.1	89.8	10.58	.043





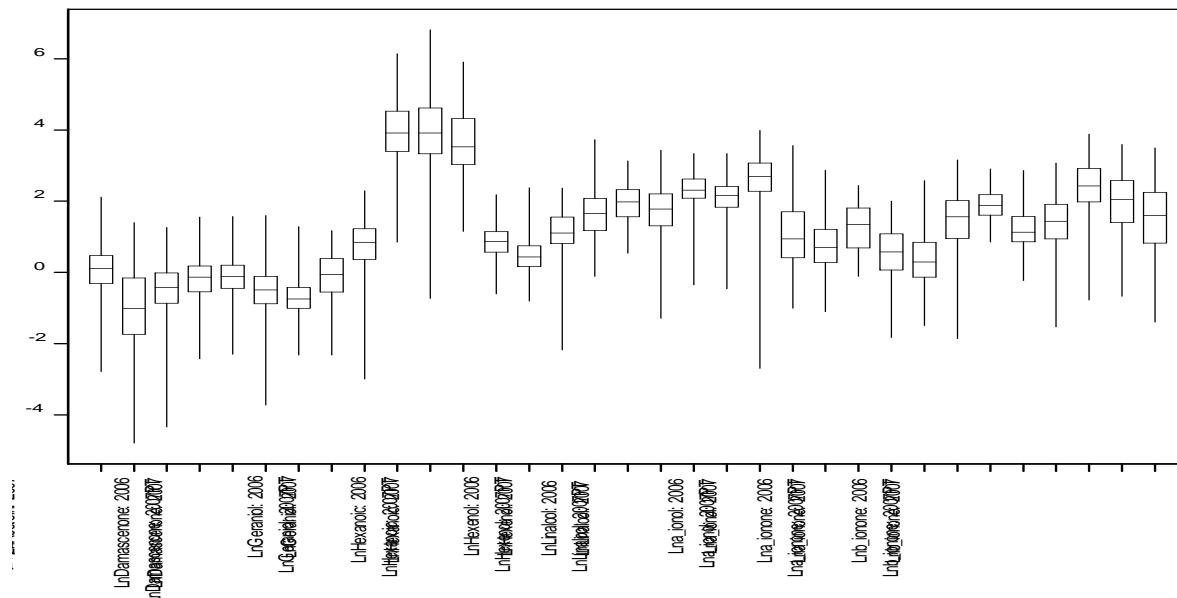
QTL for anthocyanin production



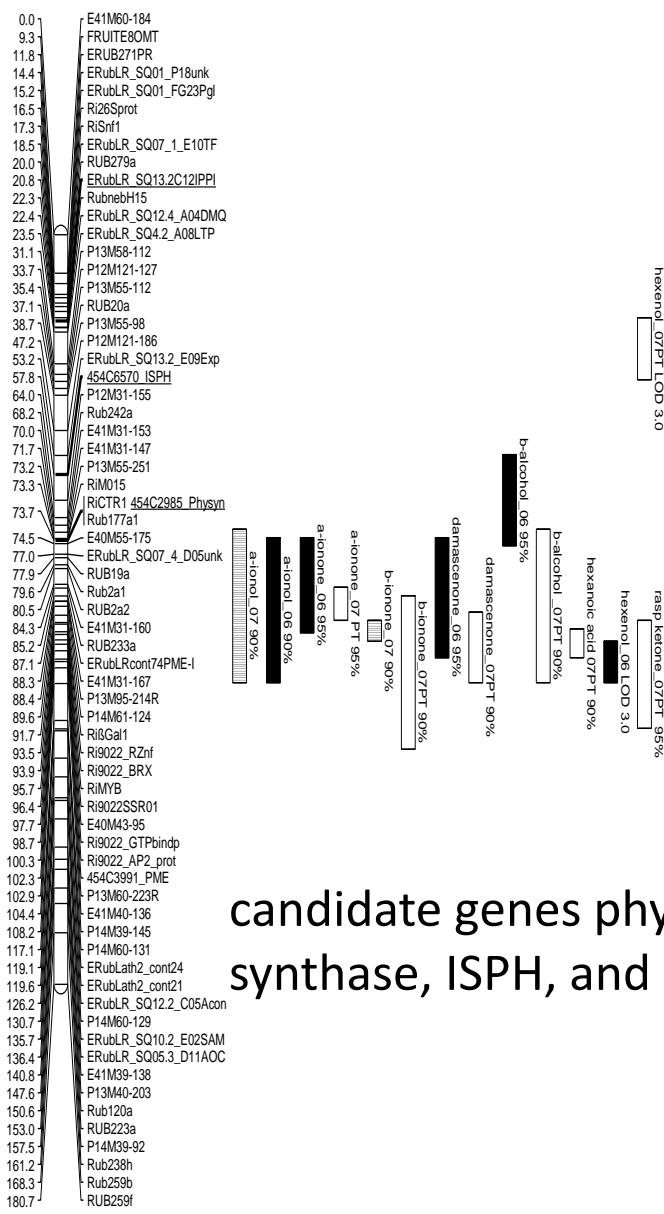
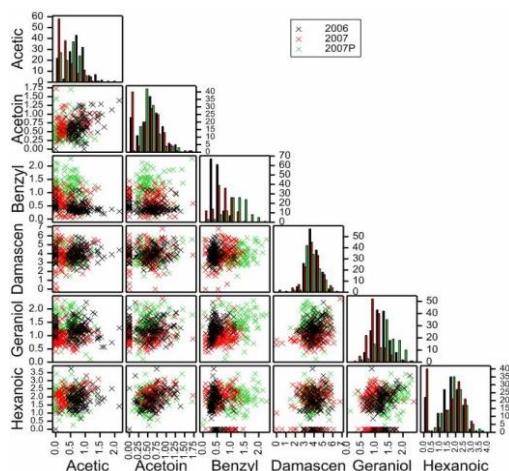
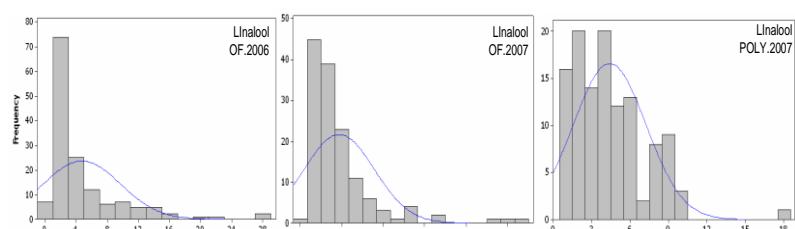
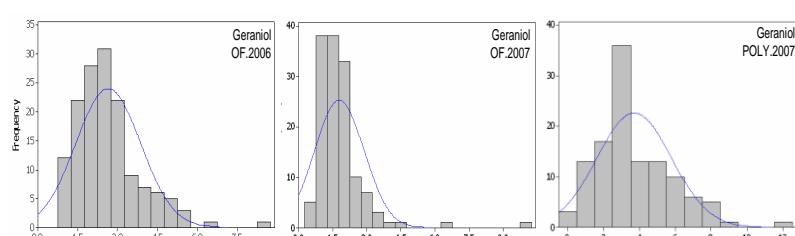
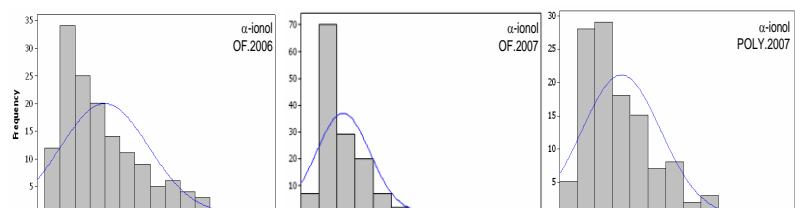
- Understanding what drives flavour



- Sensory analysis both taste panels and individuals sampling straight from cane
- Compositional analysis



Volatiles in red raspberry



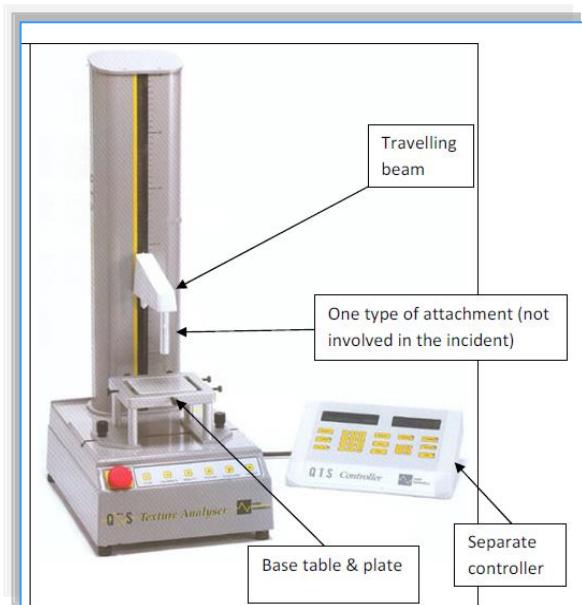
candidate genes phytoene
synthase, ISPH, and CTR1

- Modelling to link sensory and composition
 - Sugar is major driver with acids and Geraniol also impacting
 - No impact from raspberry ketone
 - No impact from anthocyanins
- Final taste/compositional data set collected in 2013 and compositional analysis underway
- Determine breeding objectives

Fruit softening

Mapping population had range of soft/firm phenotypes

Texture analyser



Breeders score – squash test





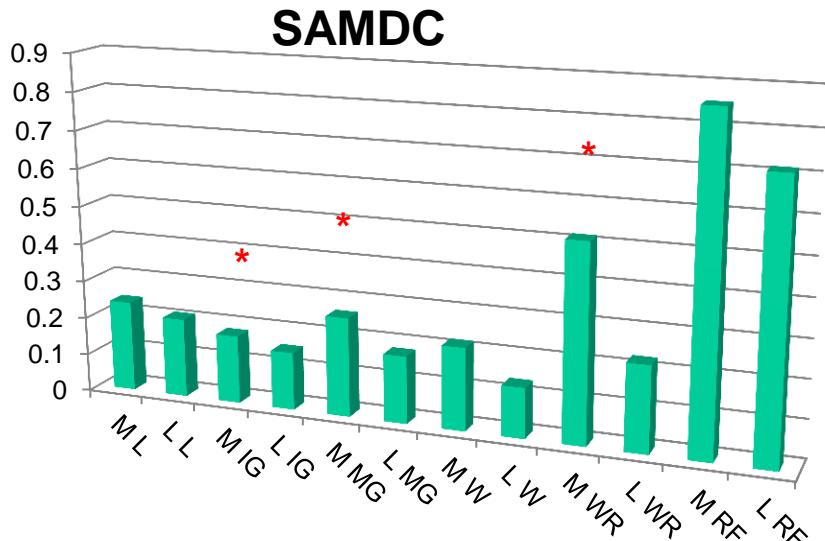
The James
Hutton
Institute

Regulation of fruit softening

0.0 E41M60-184
9.3 ERUITE8OMT
11.3 ERUB271PR
14.1 ERubLR_SQ01_P18unk
15.2 ERubLR_SQ01_FG23Pgl
16.5 Ri26Sprot
17.3 RiSnf1
18.3 ERubLR_SQ07_1_E10TF
20.1 RUB279a
20.1 ERubLR_SQ13.2C12IPPI
22.1 RubnebH15
22.1 ERubLR_SQ12.4_A04DMQ
23.1 ERubLR_SQ4.2_A08LTP
31.1 P13M58-112
33.1 P12M121-127
35.1 P13M55-112
37.1 RUB20a
38.1 P13M55-98
47.1 P12M121-186
53.1 ERubLR_SQ13.2_E09Exp
57.3 454C6570 ISPH
64.1 P12M31-155
68.1 Rub242a
70.1 E41M31-153
71.1 E41M31-147
73.1 P13M55-251
73.1 RIM015
73.1 RiCT454C2985 Physyn
73.1 Rub177a1
74.5 E40M55-175
77.0 ERubLR_SQ07_4_D05unk
77.9 RUB19a
79.6 Rub2a1
80.5 RUB2a2
84.7 E41M31-160
85.4 RUB233a
87.7 ERubLRcont74PME-I
88.7 E41M31-167
88.7 P13M95-214R
89.7 P14M61-124
91.7 Ri&Gal1
93.7 Ri9022_RZnf
93.7 Ri9022_BRX
95.7 RiMYB
96.7 Ri9022SSR01
97.7 E40M43-95
98.7 Ri9022_GTPbindp
100.7 Ri9022_AP2_pro
102.7 454C3991_PME
102.7 P13M60-223R
104.7 E41M40-136
108.7 P14M39-145
117.7 P14M60-131
119.7 ERubLat2_conn24
119.7 ERubLat2_conn21
126.7 ERubLR_SQ12.2_C05Acon
130.7 P14M60-129
135.7 ERubLR_SQ10.2_E02SAM
136.7 ERubLR_SQ05.3_D11AOC
140.8 E41M39-138
147.6 P13M40-203
150.6 Rub120a
153.0 RUB223a
157.5 P14M39-92
161.2 Rub238h
168.3 Rub259b
180.7 RUB259f

SELECTED CALCULATIONS	Current test data								Arithmetic Mean	Standard Deviation	Lowest	Highest	Units
	R 234 R1_1.qdf	R 234 R1_2.qdf	R 234 R1_3.qdf	R 234 R1_4.qdf	R 234 R1_5.qdf	R 234 R1_6.qdf	R 234 R1_7.qdf	R 234 R1_8.qdf					
Hardness	78.00	85.00	88.00	96.00	77.00	58.00	64.00	66.00	76.50	12.23	58.00	96.00	g
Apparent modulus	0.00	0.00	5.97	13.09	112.00	12.00	18.29	0.00	20.17	35.31	0.00	112.00	g/s
Springiness index	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-
Area cycle 1	629.21	548.25	708.75	786.56	639.19	504.86	487.39	555.90	607.51	96.78	487.39	786.56	gs
Area cycle 2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hardness 1 work done	587.40	514.88	669.00	743.55	600.79	482.25	465.15	526.91	573.74	89.89	465.15	743.55	gs
Recoverable deformation 1	-1.96	-1.33	-1.76	-1.58	-1.56	-1.21	-0.98	-1.18	-1.44	0.31	-1.96	-0.98	mm
Recoverable deformation 2	5.05	4.16	4.58	5.43	5.00	5.00	4.20	5.19	4.83	0.43	4.16	5.43	mm
Recoverable work done 1	41.81	33.38	39.75	43.01	38.40	22.61	22.24	28.99	33.77	7.81	22.24	43.01	gs
Recoverable work done 2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	gs
Rigidity 1	29.00	20.00	35.50	31.00	29.50	26.00	26.00	24.00	27.63	4.41	20.00	35.50	g
Rigidity 2	49.00	51.00	61.00	-2.00	53.00	42.00	45.00	-2.00	37.13	23.19	-2.00	61.00	g
Sample length	21.16	19.82	20.47	21.73	21.07	21.09	19.86	21.36	20.82	0.65	19.82	21.73	mm
Final load	66.00	72.00	73.00	82.00	65.00	49.00	52.00	57.00	64.50	10.55	49.00	82.00	g

Gene expression levels of SAMDC between Latham and Glen Moy



KEY: L, leaf

IG, immature green

MG, mature green

W, white fruit

WR, white/red fruit

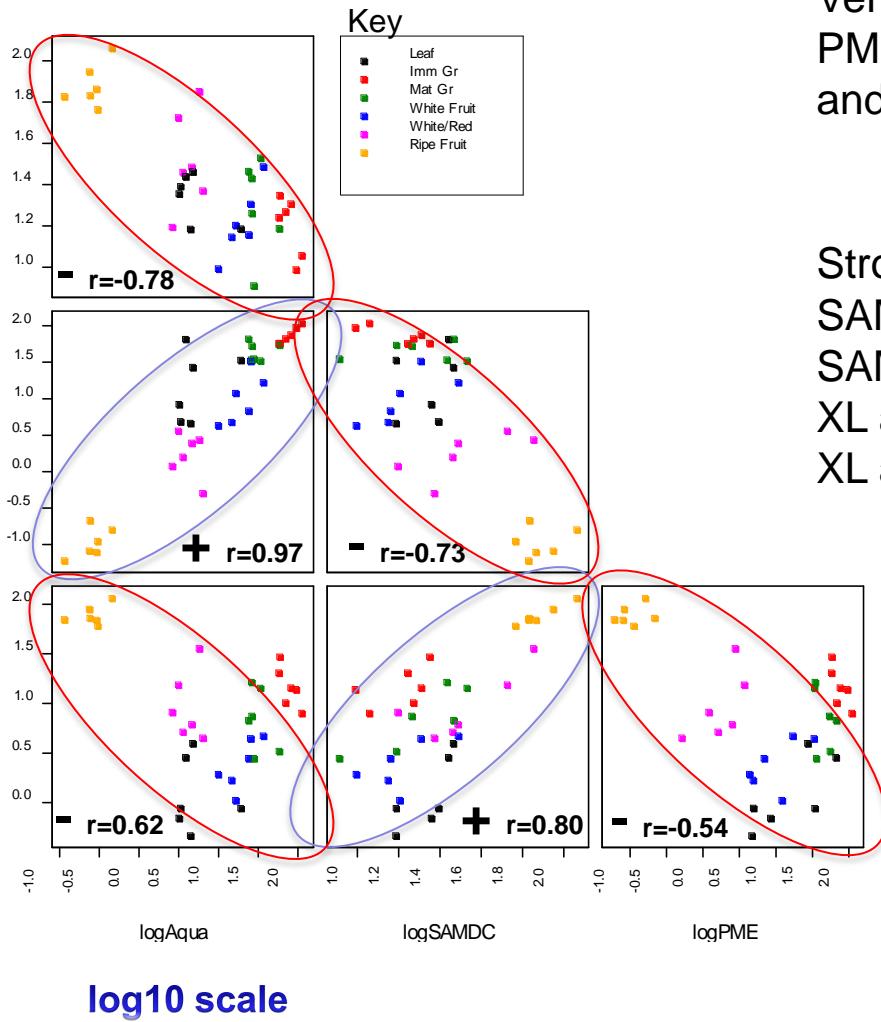
RF, ripe fruit

Material collected for Gene Expression Studies 2010



Breeder score of Firmness (scale):		
Soft (4)	Medium (2-3)	Hard (1)
Latham	R42	Glen Moy
R1	R56	R7
R61	R62	R13
R66	R88	R73
R102	R104	R93
R114	R169	R163
R136	R171	R201
R210	R172	R241
R254	R184	R252
R289	R206	
	R214	
	R234	
	R244	
	R248	

Coordinated expression between candidate genes



Very strong **positive** relationship between:
PME and Aquaporin
and XL and SAMDC.

Strong **negative** relationship between:
SAMDC and Aquaporin,
SAMDC and PME,
XL and Aquaporin,
XL and PME.

The inhibition of PME activity coincided with the increased levels of SAMDC and XL.

Together these enzymes may help coordinate the process of fruit ripening with cell wall degradation process.

Flowering and ripening

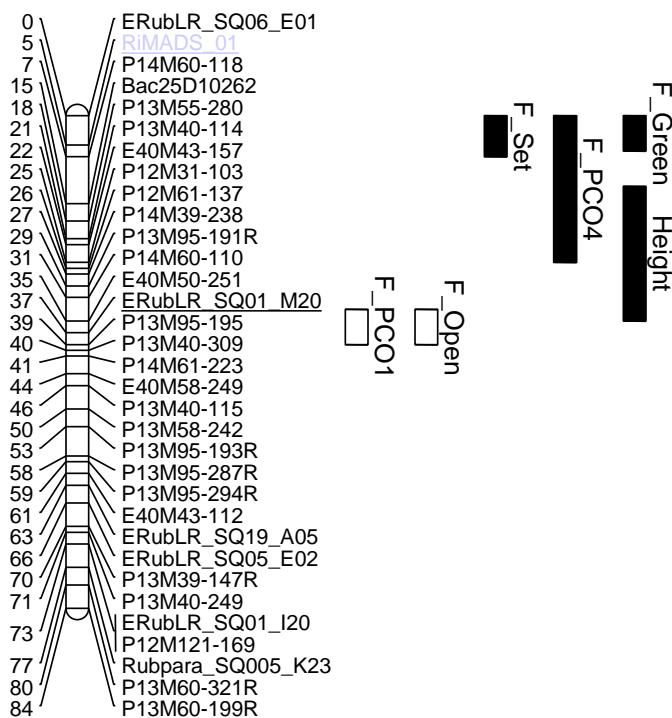


- Flowering time/ripening variation is highly relevant to quality and yield.
- Flowering at the appropriate time ensures best use of the available growing season, promoting sustainability and reducing the need for inputs.
- There is now evidence that flowering time has shifted in response to changes in climate.

Flowering/Ripening QTLs

- LG 2, 3, 5 and 6

Chromosome 5



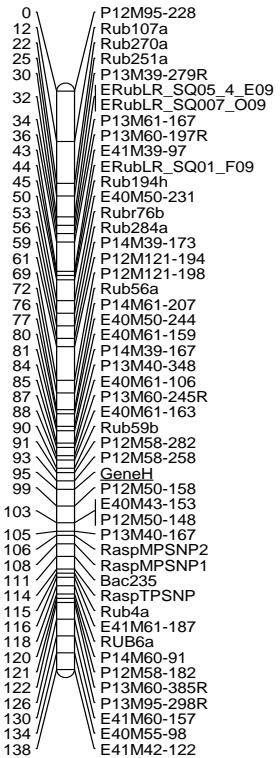
MADS box gene responsible for regulating developmental transition to flowering. MADS box associated with earlier ripening in colder season.

M20 BAC sequenced –
Squamosa promoter, floral transition

Ripening and Gene H?



Chromosome 2



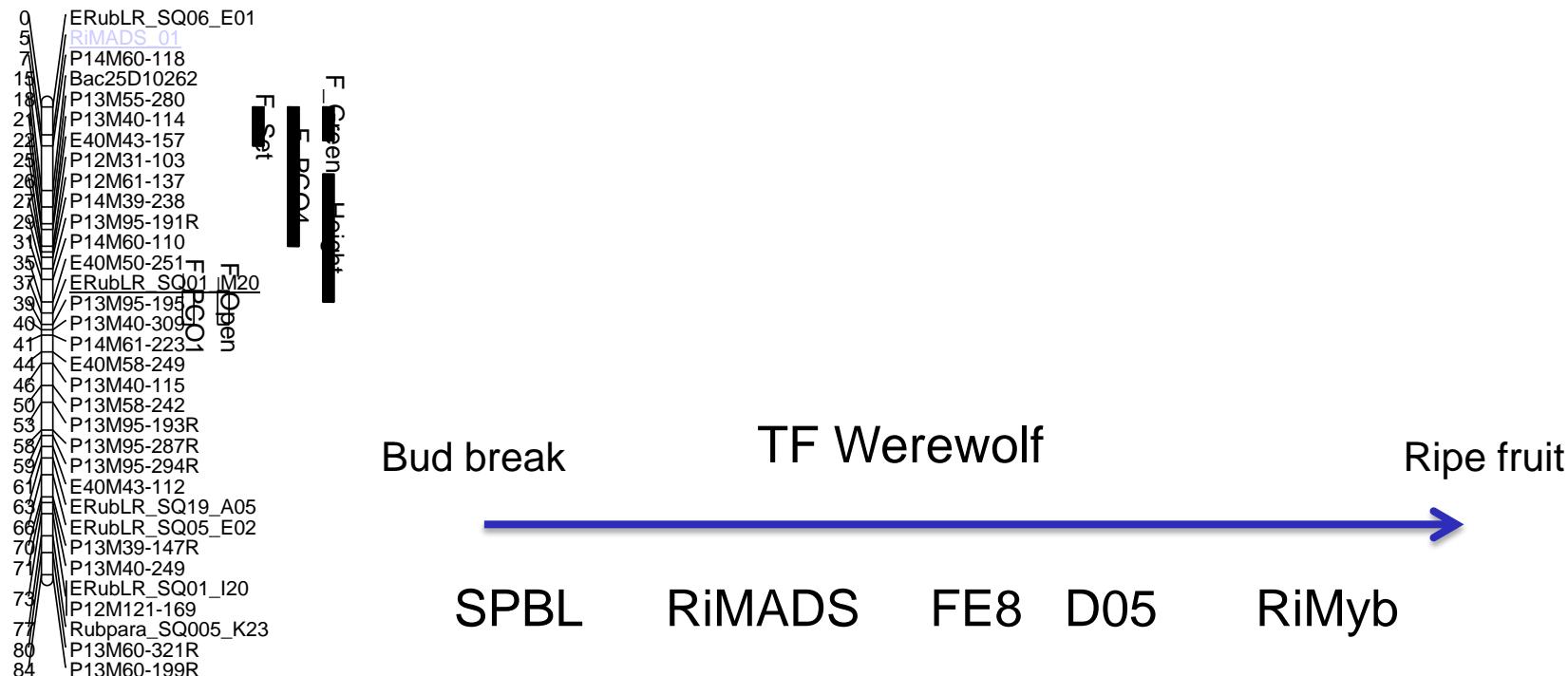
The *Hh* genotype of Gene H is associated with a slowing down of ripening across all stages from open flowers to the green/red stage compared to the *hh* genotype

Myb transcription factor on bac 11G23 could be controlling both trichomes and flowering time. Similarity to WEREWOLF which has been shown to post-transcriptionally regulate FT, a key floral regulator

QTL impacting on specific stages

- LG 5

Chromosome 5



Complex nature of trait regulation



- Traits controlled by a number of genes and under environmental regulation
- Ways to look at ‘bigger picture’

Links between genes and biochemistry

Rubus Fruit Development Array

60, 000 genes

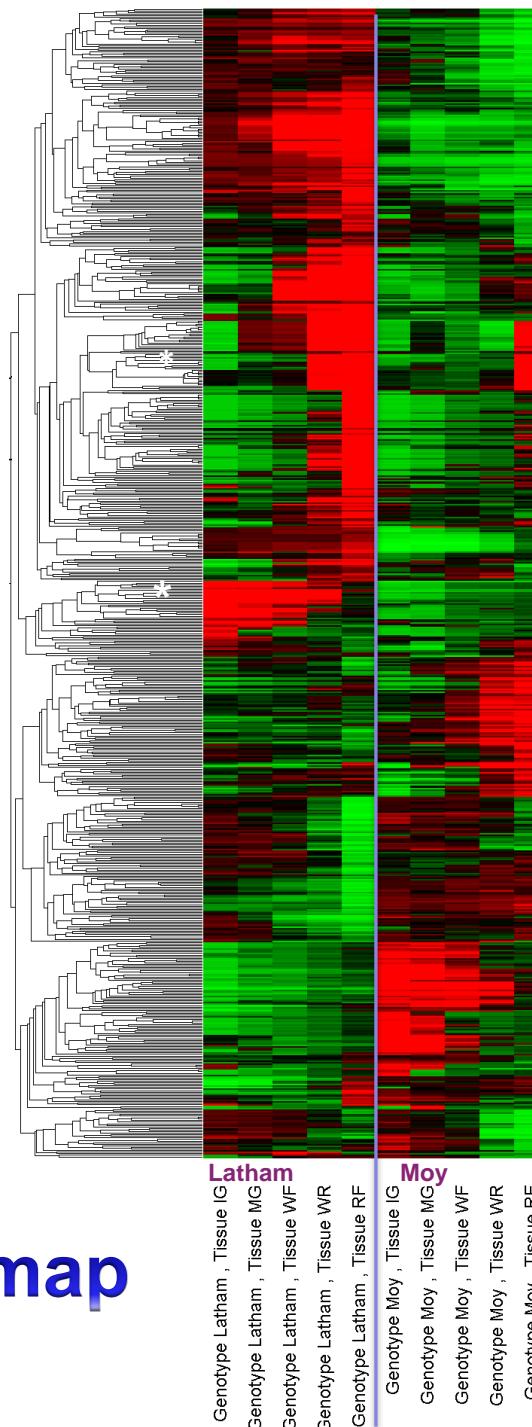
Fruit development time course



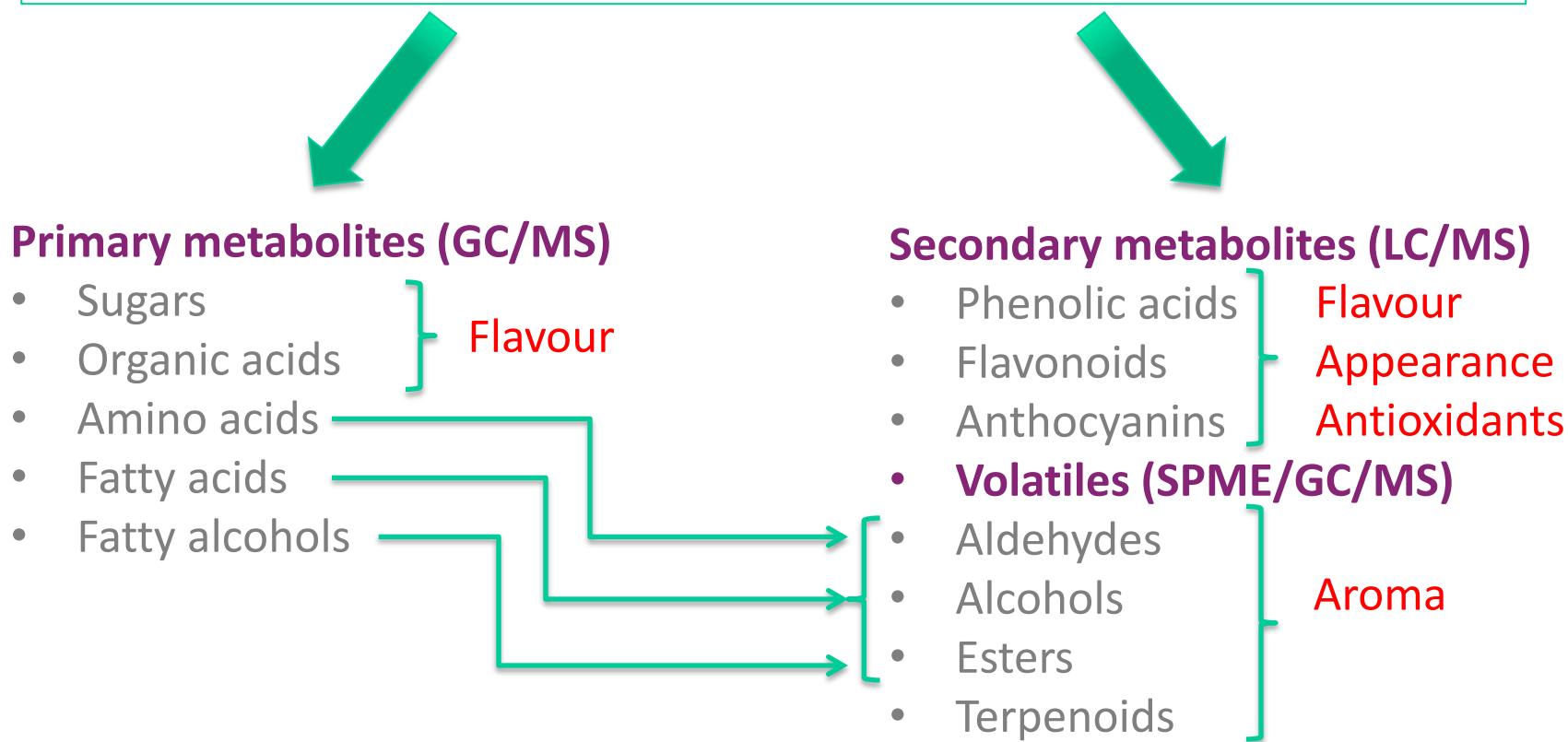
IG MG W WR RF

- Examples of both gene expression increasing or decreasing during fruit development.
- Genotypic differences.
- Relate to metabolomics data

Heatmap



Fruit chemistry is a significant driver of fruit quality

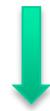


Fruit samples for analysis

- Three replicate samples of the following fruit stages collected:



Snap frozen in liquid N₂ then stored at -80°C until processing



GC/MS
Primary metabolites

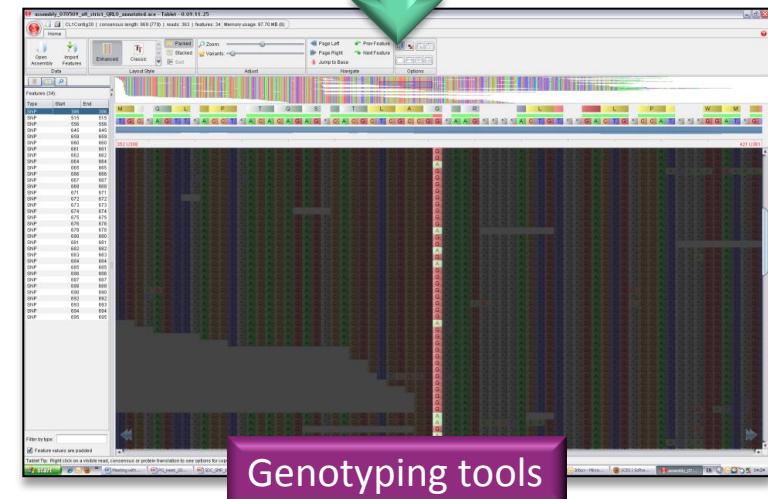
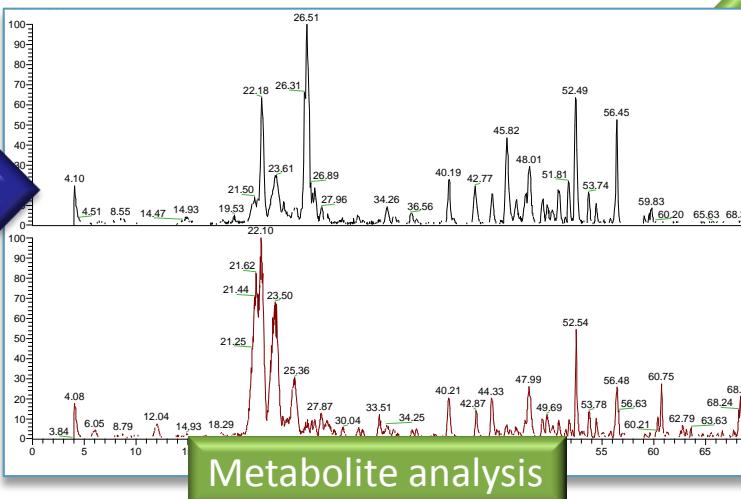
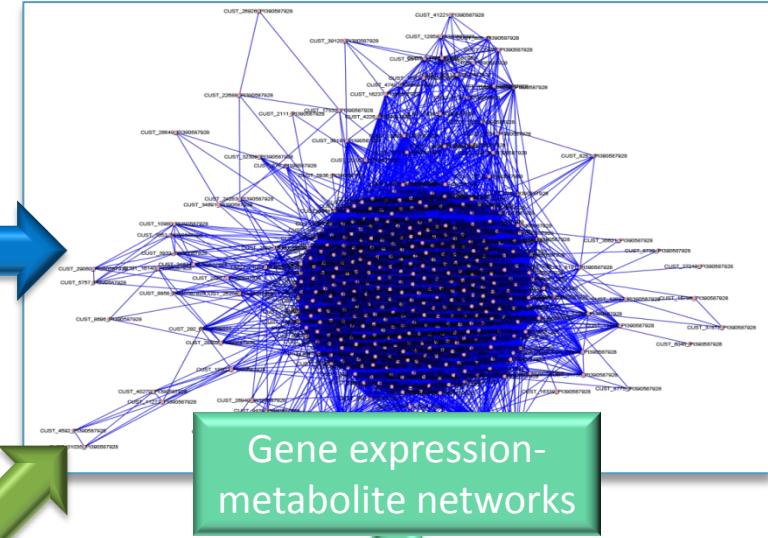
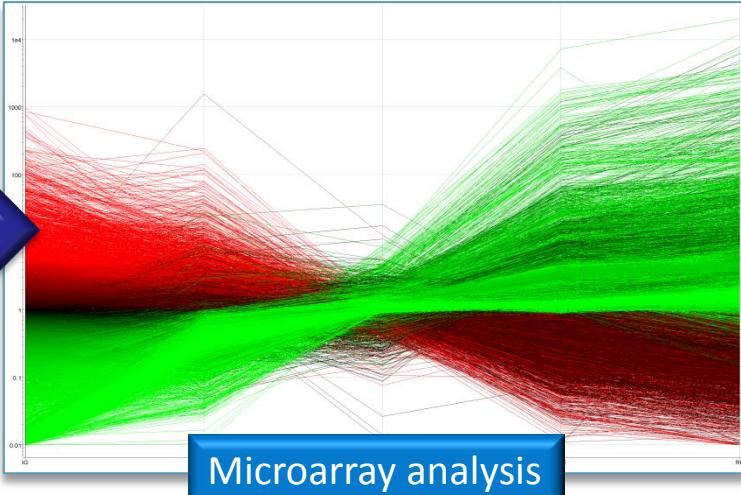
GC/MS
Volatiles

LC/MS
Polyphenols

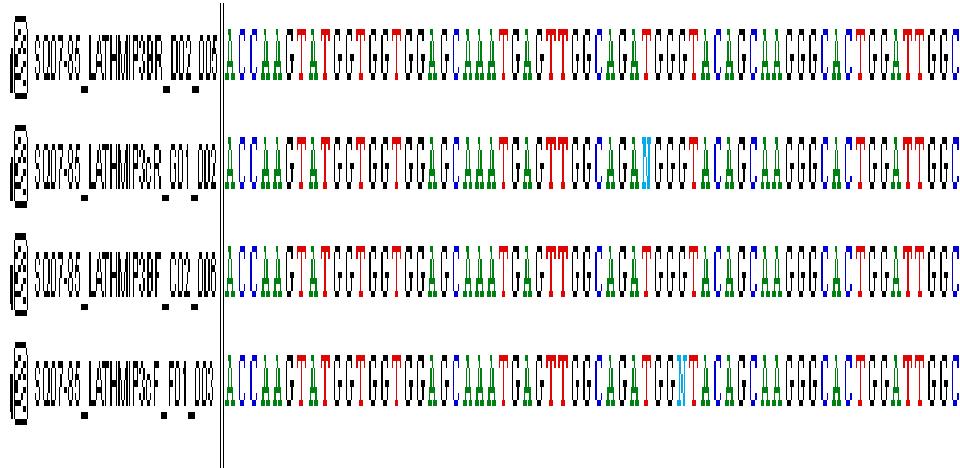
Microarray
Transcriptome

High throughput breeding tool

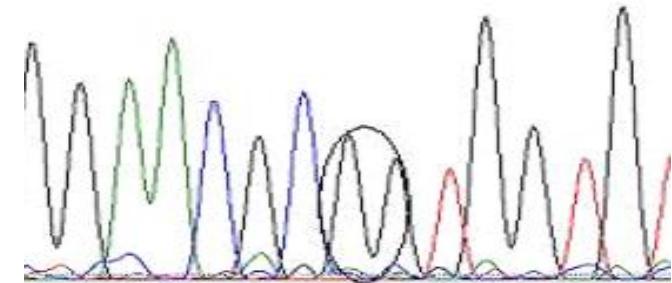
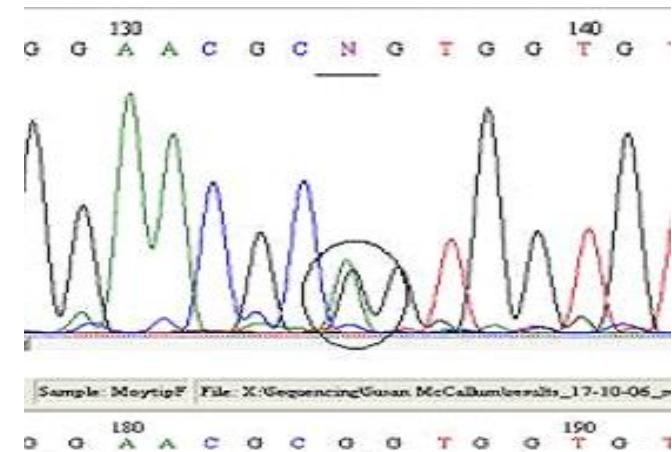
Developing fruit



Sequence Analysis



SNP Identification



DNA markers linked to key alleles forming
MAB toolkit

Identify targets for marker assisted breeding for complex traits

Scottish Government
Horticulture LINK
TSB
HDC
Industry partners

