

Common Bacterial Blight (CBB)

Bacterial blight:
Xanthomonas



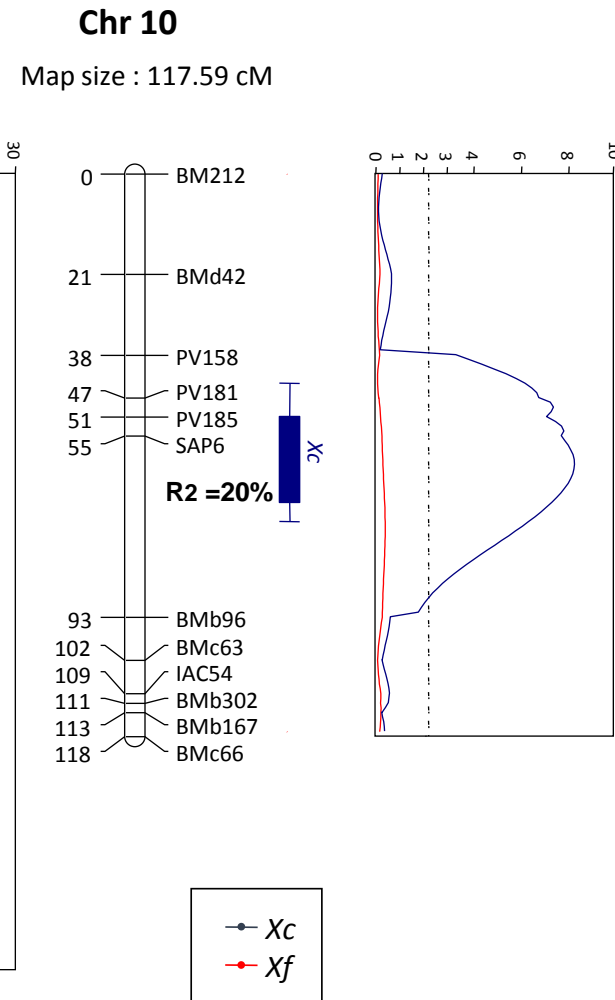
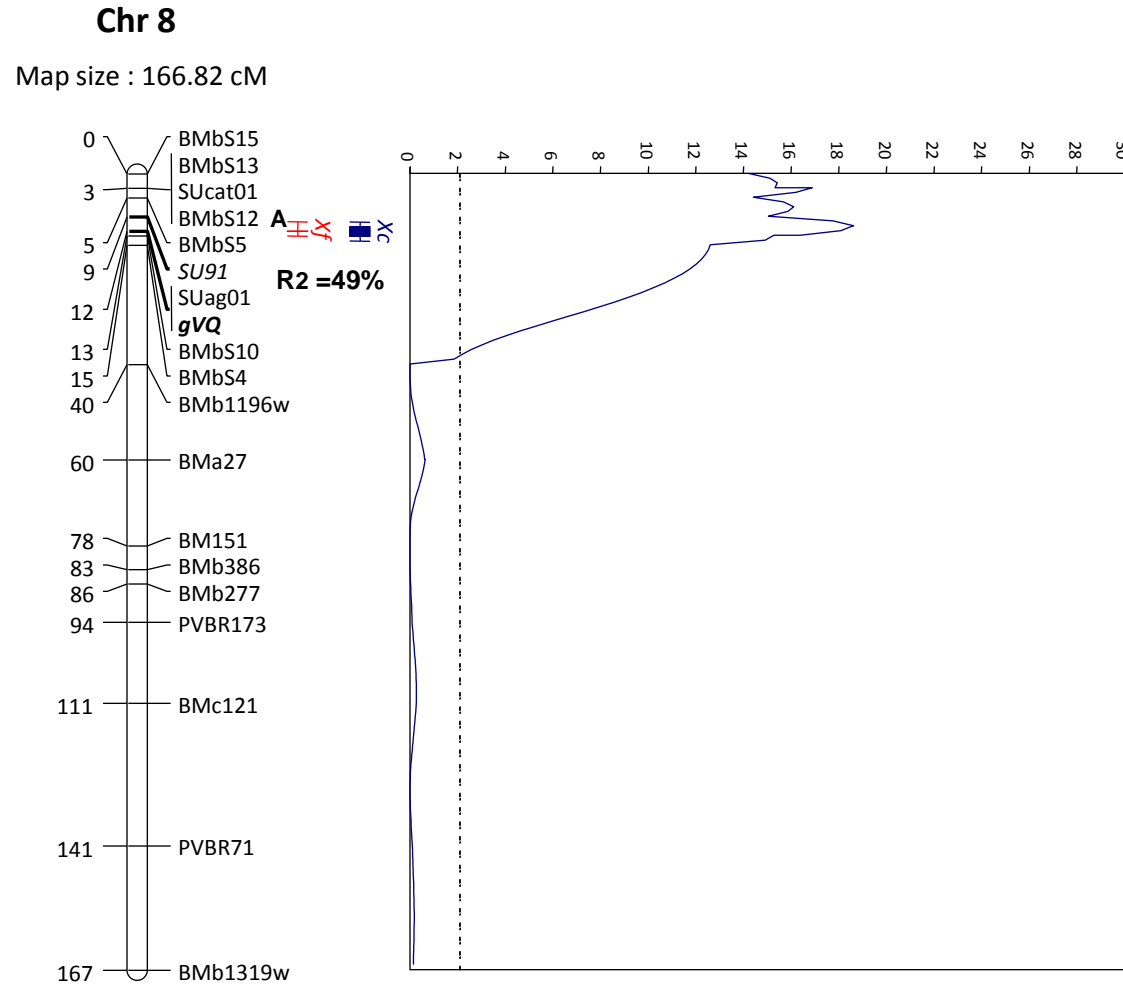
MAS with

- SU91 on Chr8 (Pedraza et al. 1997)
- SCAR's : BC420 on Chr6 (Yu et al. 1998)
- SAP6 on Chr10 (Miklas et al. 2006) .



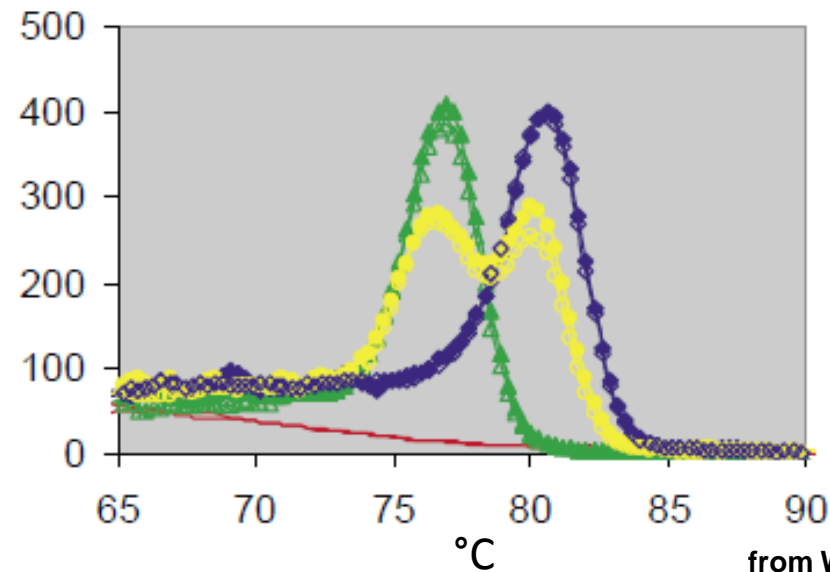
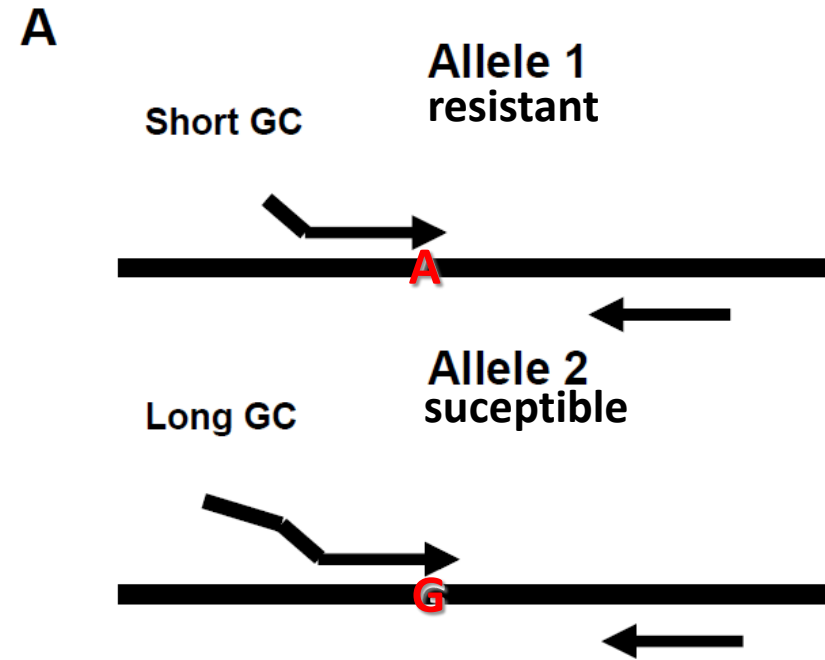
CBB resistance QTLs

BMbS12



SNP genotyping: T_m shift assay

- Based on SNP-specific primers
- Genotyping by melting point analysis
- No fluorescent primers required



from Wang et al. 2005

CONDICIONES PCR

Primers:

eIF4E-2F 5'-ACC GAT GAG CAA AAC CCT A-
3'

eIF4E-R 5'-CTG ACA TTC ACA ACA GCT
CCA-3'

Reactivos	[] STOCK	[] FINAL
Watter	-	-
Buffer	10X	1X
MgCl ₂	25 mM	2 mM
dNTPs Mix	20 mM	0.3mM
Primer L	10 μM	0.2 μM
Primer R	10 μM	0.2 μM
Taq Pol		1U
DNA	5 ng /μL	25 ng /μL

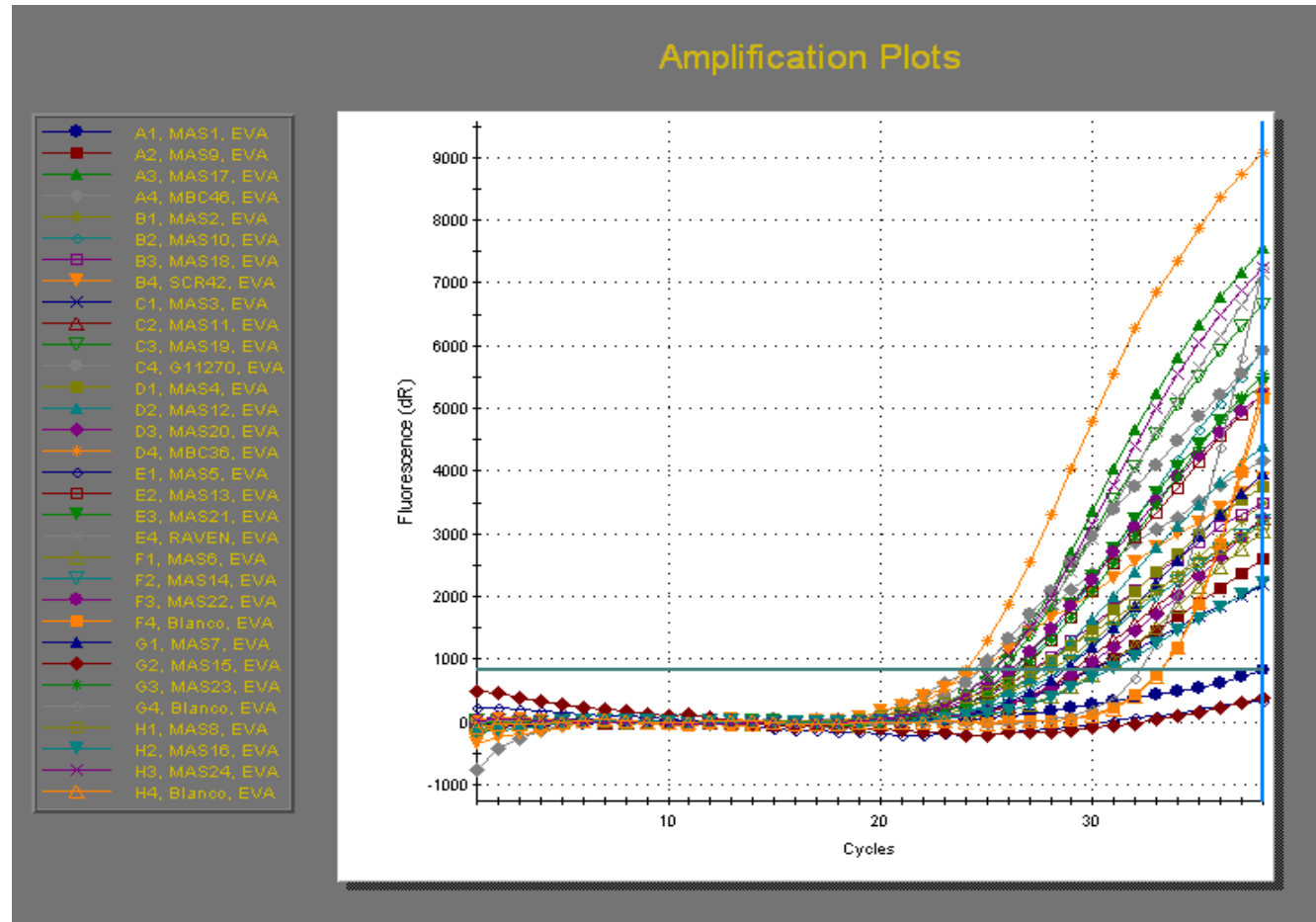
Programa de PCR

- 1.) 94°C x 3 Minutos
- 2.) 92°C x 1 Minutos
- 3.) 60°C x 1 Minutos
- 4.) 72°C x 2 Minutos
- 5.) Ir a paso 2 (x35)
- 6.) 72°C x 8 Minutos
- 6.) 10°C x 10 Minutos
- 7.) FIN

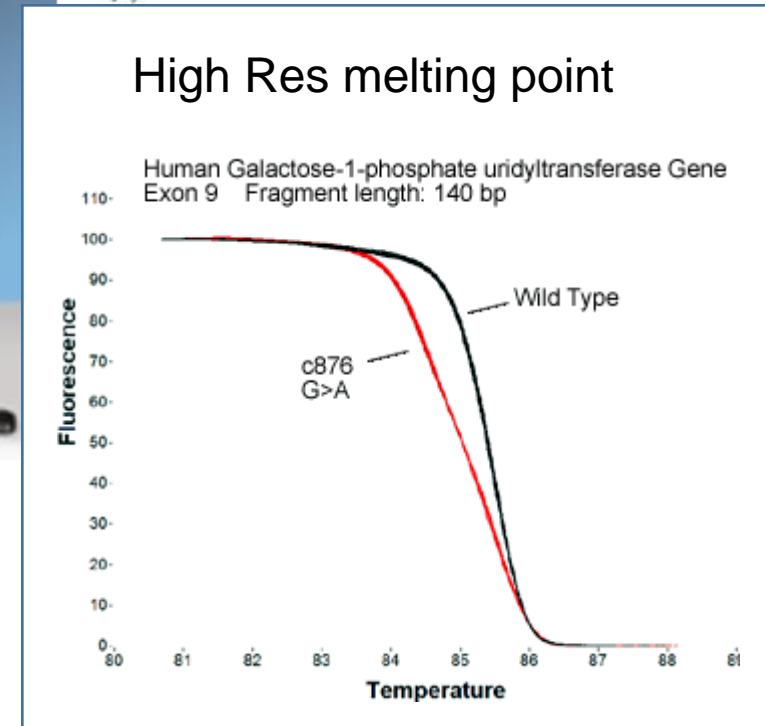
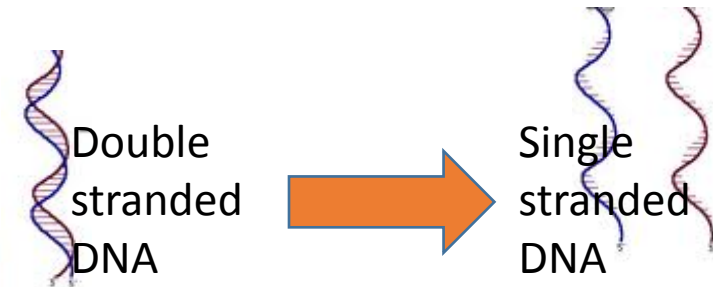
Real-time PCR: Amplification Plots

EGreen, 08-13-2012_Heteros MAS.mxp

Real-time PCR measures double stranded DNA during PCR reaction



Melting point analysis



Dissociation Curve

EGreen, 08-13-2012_Heteros MAS.mxp

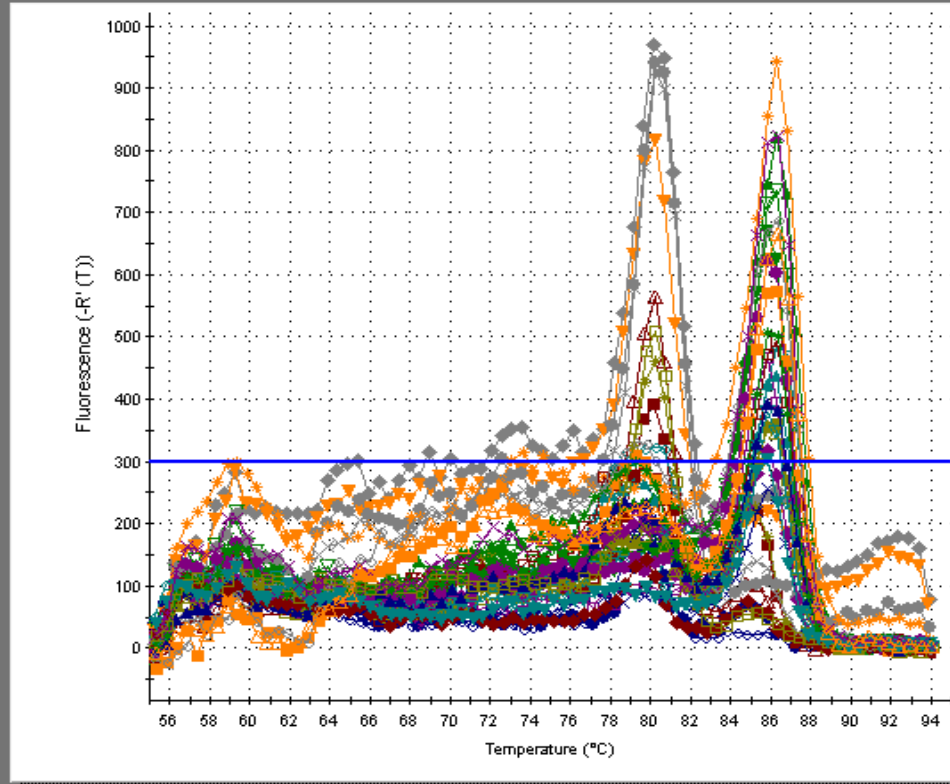
Double
stranded
DNA



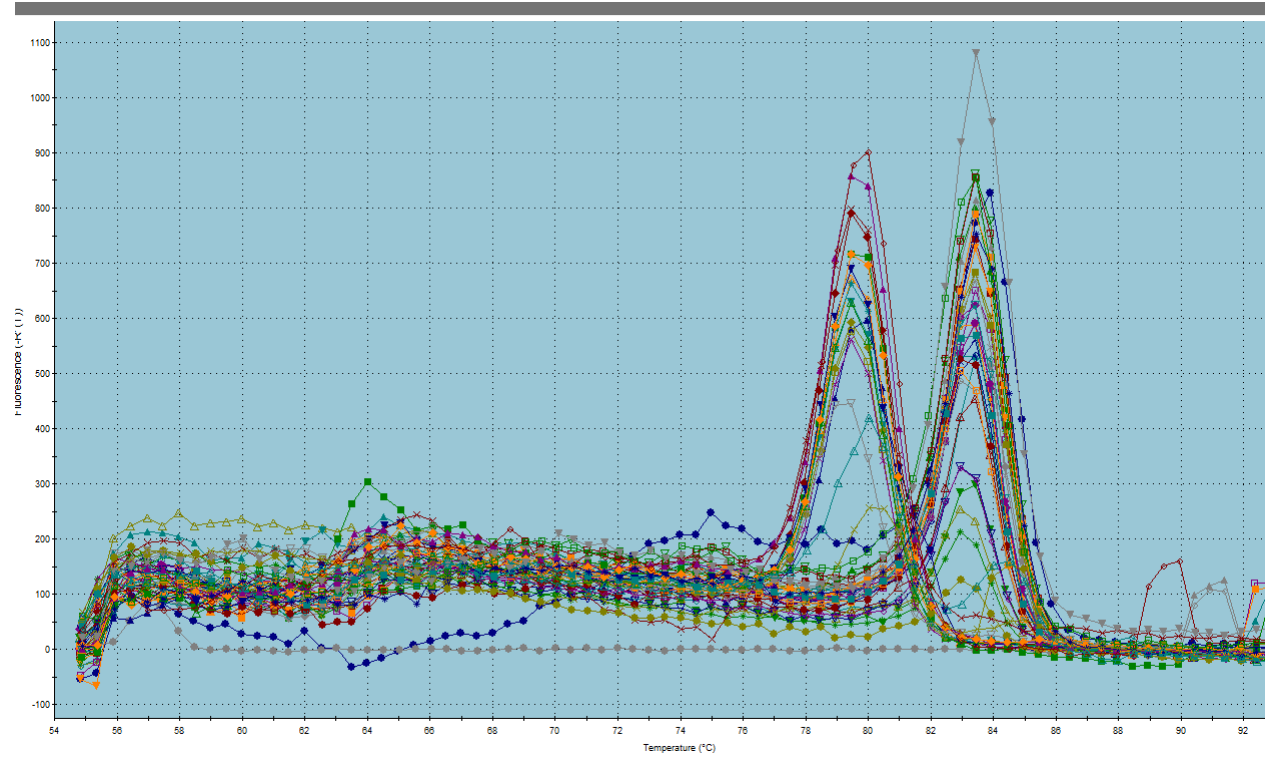
Single
stranded
DNA

Dissociation Curve

- A1, MAS1, EVA
- A2, MAS9, EVA
- ▲ A3, MAS17, EVA
- ◆ A4, MBC46, EVA
- ✱ B1, MAS2, EVA
- ◇ B2, MAS10, EVA
- B3, MAS18, EVA
- ▽ B4, SCR42, EVA
- ✕ C1, MAS3, EVA
- △ C2, MAS11, EVA
- ▽ C3, MAS19, EVA
- ◇ C4, G-11270, EVA
- D1, MAS4, EVA
- ◇ D2, MAS12, EVA
- ◆ D3, MAS20, EVA
- ✱ D4, MBC36, EVA
- ◇ E1, MAS5, EVA
- E2, MAS13, EVA
- ▽ E3, MAS21, EVA
- ◇ E4, RAVEN, EVA
- △ F1, MAS6, EVA
- ▽ F2, MAS14, EVA
- ◆ F3, MAS22, EVA
- F4, Blanco, EVA
- ▲ G1, MAS7, EVA
- ◆ G2, MAS15, EVA
- ✱ G3, MAS23, EVA
- ◇ G4, Blanco, EVA
- H1, MAS8, EVA
- ▽ H2, MAS16, EVA
- ✕ H3, MAS24, EVA
- △ H4, Blanco, EVA

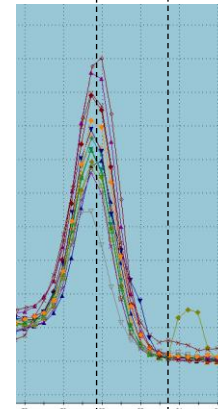
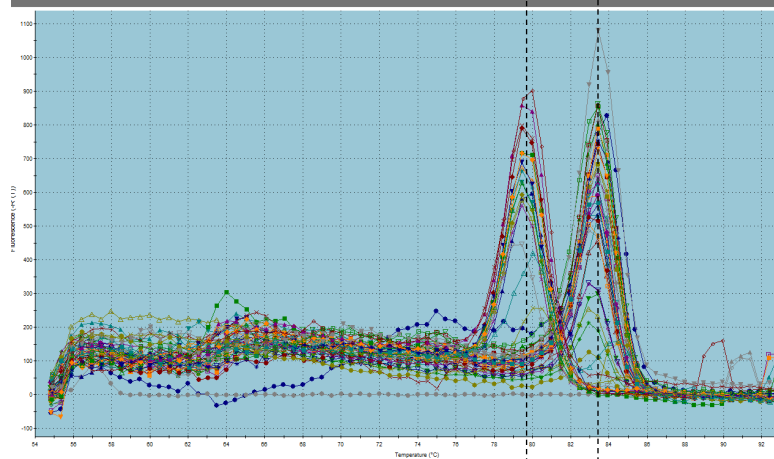


CBB SNP primer: SU91

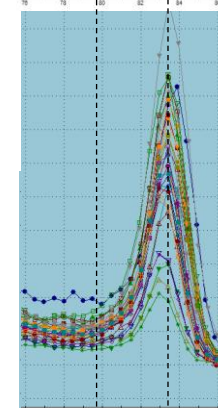


Checked on
~50 parental lines

CBB SNP
primer:
SU91

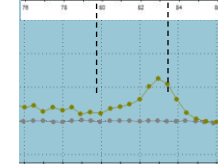


resistentes



susceptibles

no call



By Dr. Mukankusi Clare