

# HRM-based locus-specific DNA methylation analyses & Post-TILLING HRM-based screening

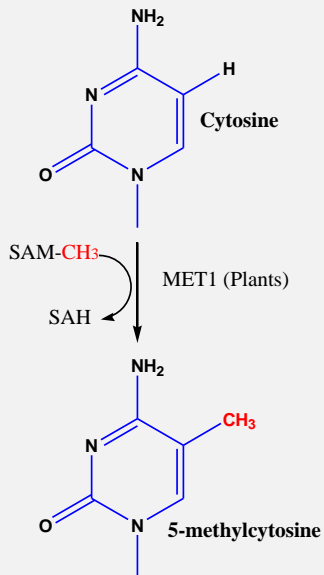
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Graham King.



- **Epigenetics:**

- Heritable changes in phenotype or gene expression caused by mechanisms other than changes in the underlying DNA sequence

### DNA methylation



### Experimental material

Rapid cycling *Brassica rapa* ssp. *trilocularis* (Roxb.) Hanelt (Yellow Sarson) line (R-o-18)

$2n=20$

Possesses the 'A' genome



# Technical challenges

- Bisulfite sequencing “the golden standard” is **expensive** and **lengthy**.
- Enzymatic-based methods – limited to few **restriction sites; incomplete digestion; low resolution**.
- HPLC-based methods – not **sequence-specific**.

# HRM-Assisted Bisulfite Sequencing

## Advantages

- **Locus-specific**
- **Quick**
- **Cheap**
- **PCR-based - small amount of DNA required**



**Rotor-Gene**



**LightScanner**

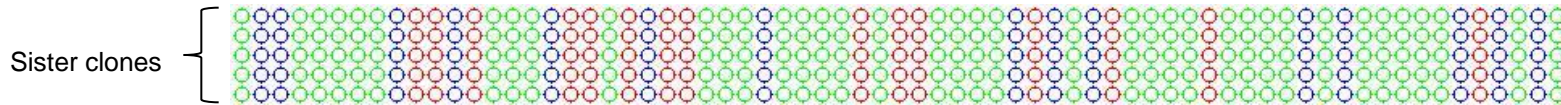
# Bisulfite sequencing revealed hypermethylation of Brassica *BANYULS* gene

Circles in the diagram represent cytosines in DNA. There may be other bases (A, T, or G) between the circles but they are not shown here. DNA was treated by two rounds of Bisulfite conversion (Qiagen). A section of *BANYULS* gene was amplified, cloned and sequenced and analysed using Kismeth software.

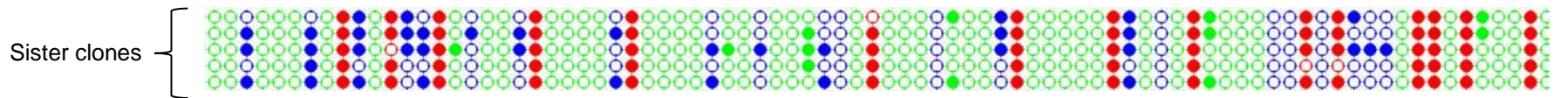
CG   
CHG   
CHH 

Filled circle = methylated (C not converted)  
Empty circle = unmethylated (C converted to T)

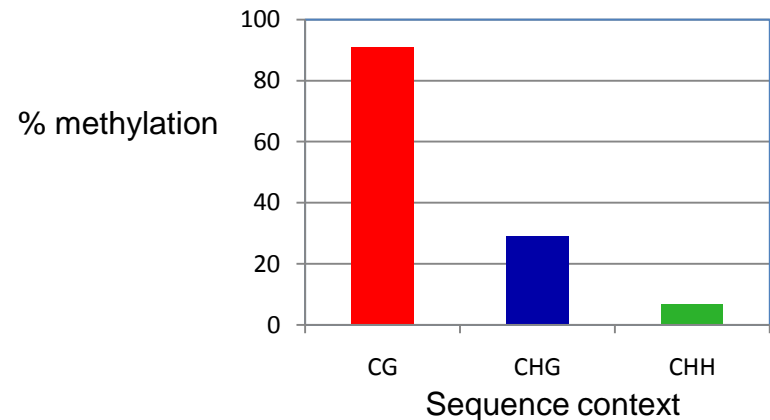
**Internal control:** Unmethylated piasmid DNA @ 1:10,000.



***BraA.BAN* gene**

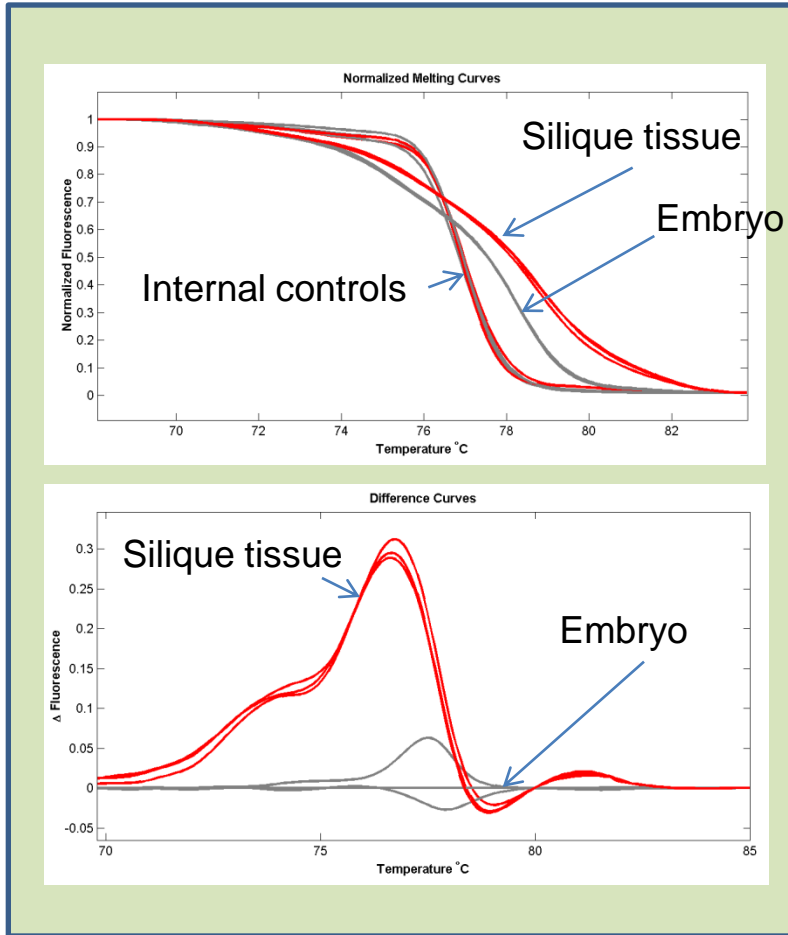


***BAN (BANYULS) Atg61720***

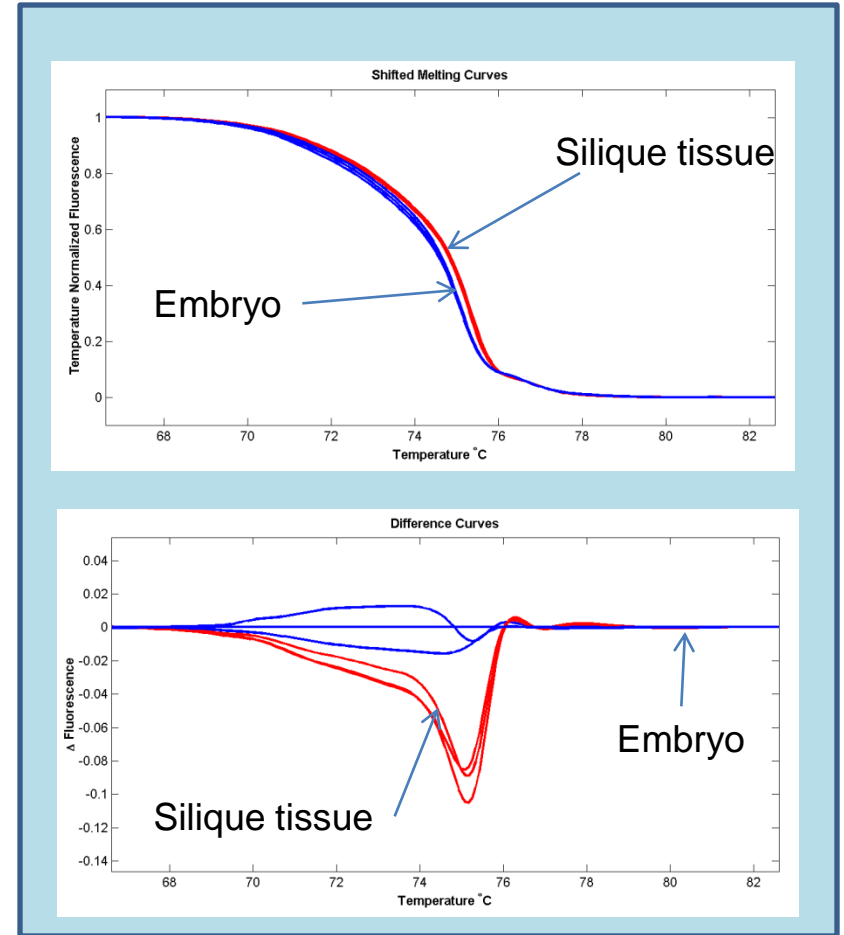


# Differential methylation of *Brassica rapa* *ABI3a* and *ATS1a* in silique and developing embryos 30DAP

*B. rapa* orthologue of *ATS1a* is differentially methylated between developing embryo and silique pericarp tissue

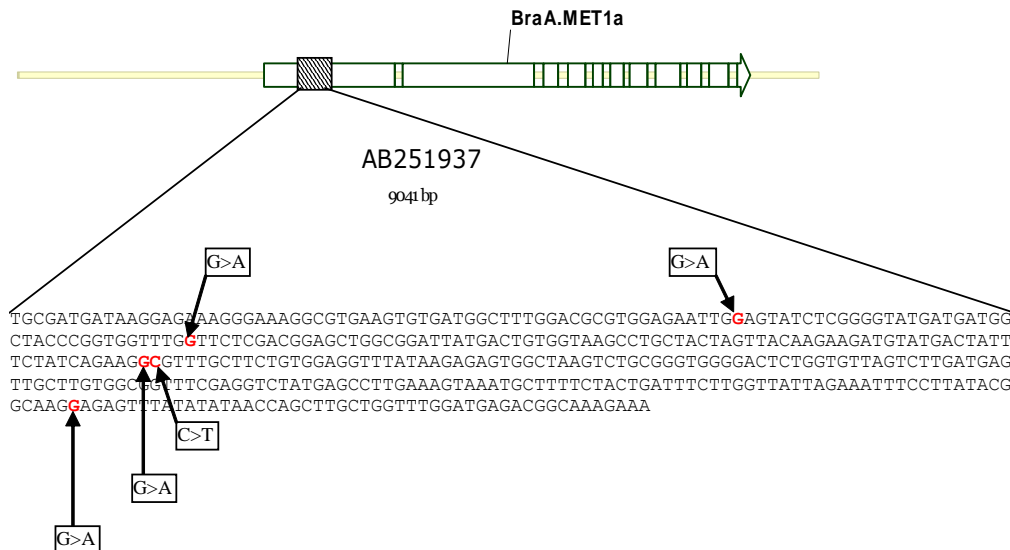


*B. rapa* orthologue of *ABI3a* is differentially methylated between developing embryo and silique pericarp tissue



The **HRM** is a highly sensitive technique for detecting differences in DNA molecule population. The internal control which is unmethylated plasmid DNA did not show any differences between the two tissues.

# Post-TILLING HRM assay for *BraA.MET1* genes



*BraA.MET1a*

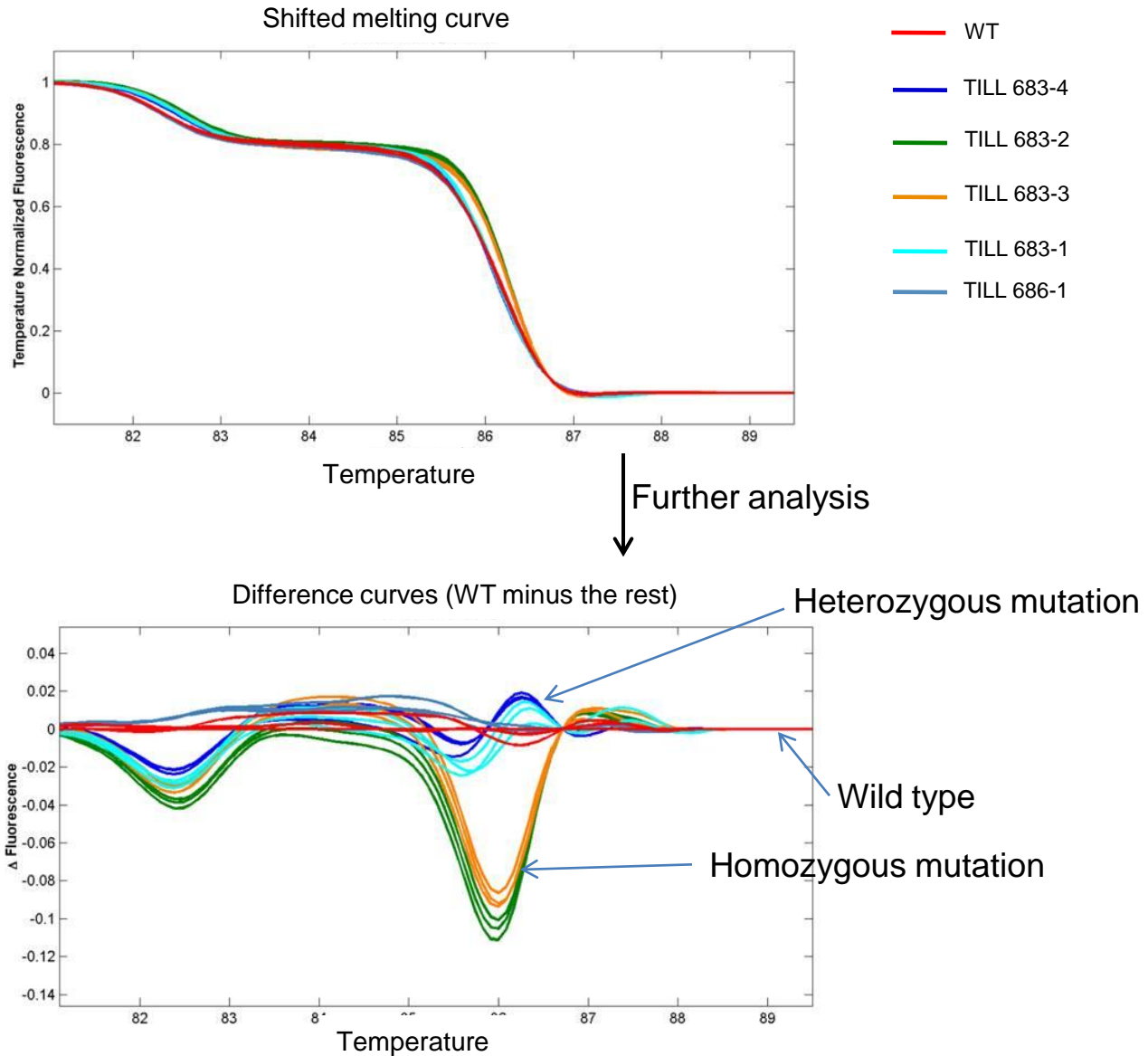
Sequence	440	460	480	Position
R-o-18 MET1a WT	ATSYKKMYDYFYOKIFASVEVYKRVAKSAGGDSGVSLDELLVAVSRMSLESKCFSTDFLV	440	460	234
met1a G/E mutation	ATSYKKMYDYFYOKIFASVEVYKRVAKSAGGDSGVSLDELLVAVSRMSLESKCFSTDFLV	440	460	234
met1a A/T mutation	ATSYKKMYDYFYOKIFASVEVYKRVAKSAGGDSGVSLDELLVAVSRMSLESKCFSTDFLV	440	460	234
met1a A/V mutation	ATSYKKMYDYFYOKIFASVEVYKRVAKSAGGDSGVSLDELLVAVSRMSLESKCFSTDFLV	440	460	234
Brassica rapa MET1a	ATSYKKMYDYFYOKIFASVEVYKRVAKSAGGDSGVSLDELLVAVSRMSLESKCFSTDFLV	440	460	234
Brassica rapa MET1b	ATTYKKVYDYFQKAYASVEVYKRVANS---DLMSLDELLVAVSRMSLETKCFSSDYLA	440	460	232
Arabidopsis thaliana MET1	AASYRKVYDYFYEKIRASVAVYKRLSKSSGGDPDGLDELLAAVVRSMSSGSKYFSSG-AA	440	460	227
Arabidopsis thaliana MET2	SKKYKLYDYFYEKACACQVEVFKLSK---NPDTSLDELLAAVVRSMSSGSKYFSSG-GA	440	460	217
Nicotiana tabacum MET1	SGGYKFFYDHLAKATAQIEVYKRLSKSSGGNPDLSLDELLAGVVRAMS-GLKCFSGG-VS	440	460	252
Daucus carota MET1	SASYKHHYEHLLAKATAQIEVYKRLSKSSGGNPDLSLDELLAGVVRAMS-GMKCFSSG-VS	440	460	251
Daucus carota MET2	SAAYKHHMEHFHAKATAQIEVYKRLSKSSGGNPDLSLDELLAGVVRAMN-GMKCFSSG-VS	440	460	467
Solanum lycopersicum CAA	SGSYKFFYDHLAKATAQIEVYKRLSKSSGGNPDLSLDELLAGVVRAMT-GIKCFSSG-VS	440	460	257
Pisum sativum AAC49931	AGTYKYYLDFEKAQACIEVYKRLAKSSGGDPDLSLDELLAGVVRAMS-GSKYFSSG-AS	440	460	254
Oryza sativa MET1A	ASSYRSYFHFSEKARVQVEVYKRLARSVGGNPDVDELLIAGVVRISIN-SNBSFNGT--V	440	460	252
Elaeis guineensis MET1	ASSYRRFYDHFYDKACIQVEVYKRLARSAGGNPDLSLDELLAAVVRSMN-GIKNASGG-FV	440	460	256
Oryza sativa MET1B	AGNYKSYDYDFYEKAQVQVEVYKRLARSVGGNPNIGDELLIAGVVRISIN-AIKCYSGT--L	440	460	252
Zea mays AAC16389	STNYKSYDFHFYEKAQVQVEVYKRLAKSSVGGNPNQGLDELLIAGVVRISIN-AMKCYSGT--M	440	460	238

Conserved Alanine (hydrophobic) changes to Threonine (polar)

Conserved Alanine (small) changes to Valine (medium)

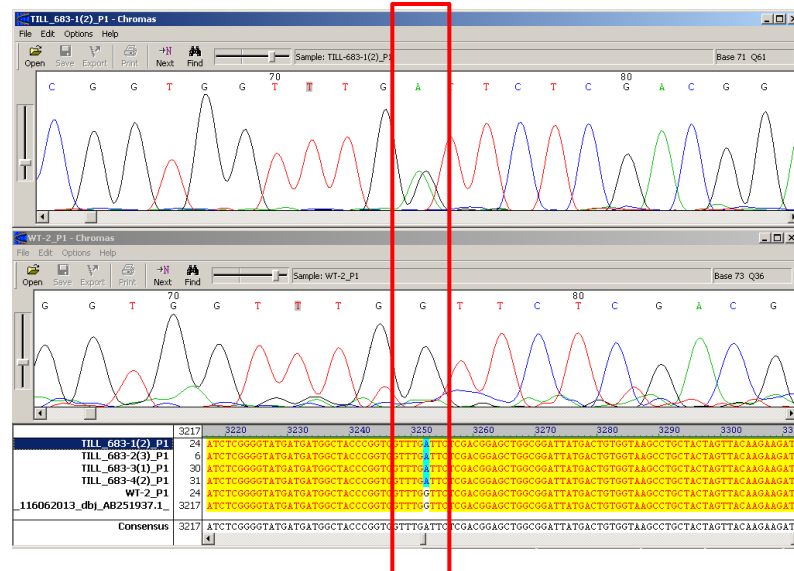
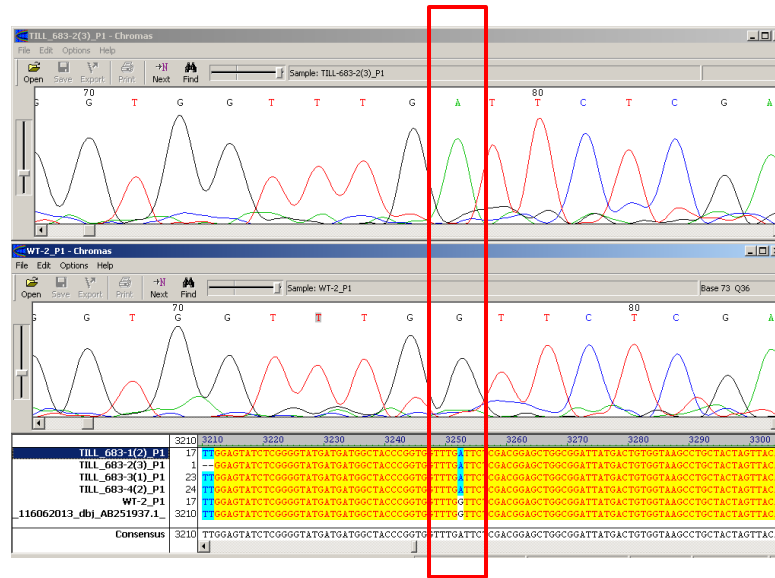
## HRM analysis of *BraA.MET1a* "TILLED".

High resolution melt (HRM) was used to screen plants for mutations.





# Sequences of *BraA.MET1a* TILLING lines



# Backcross\_1

Heterozygous were phenotypically indistinguishable from wild type

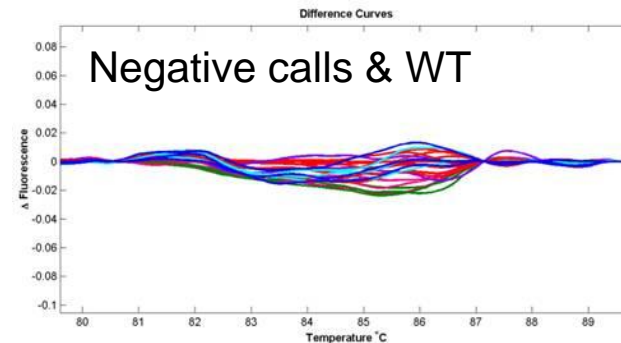
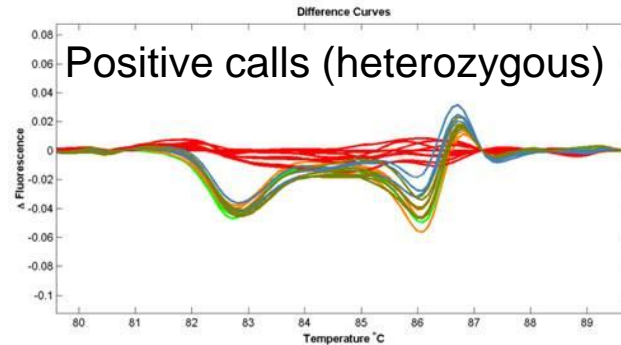


WT

*BraA.met1a* heterozygous

Mutations were introgressed into wild type background.

HRM was used to screen for mutations. Second Backcross has been made.



# Acknowledgements

Lars Østergaard's group (JIC)

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**THANK YOU**

