

Today's Talk

Tips for Giving Talks

Background of DNA methylation and Plant phenotype

Hypothesis and Results

Summary/Conclusion

Future Experiments

Questions

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MCDB191 Epigenetics
8th January 2024

What constitutes a good presentation

What constitutes a good presentation?

- **Introduction** (10 min):
 - Explain the **relevant background/techniques** needed to understand the paper.
 - **Use material from other papers and reviews** on the reading list or other papers.
- **Paper** (20-30 min):
 - Convey the **paper's main message** concisely while **critically** analyzing the validity of the data presented.
 - **Don't have to explain every figure** of the paper in great detail.
 - Try to **understand the materials and methods**, but you don't need to introduce every detail of the techniques used.
 - Try to end with a **statement of the paper's main findings and the next logical questions** to ask in this field.

What constitutes a good presentation

General Tips:

- **You are responsible for the information you present on your slides:** If you put a figure on your slide, your audience will expect you to be able to explain it and answer questions on reasoning, method, and implications, etc.
- **Its better to say more with less**
 - People will not read slides with paragraphs of text, and you will lose their attention.
- **High contrast is best for people to read your slides**
 - Black on White, for example
 - Avoid highlighter colors like this: **NO**
- **Be conscientious of time limits for presentations and stick to those limits**

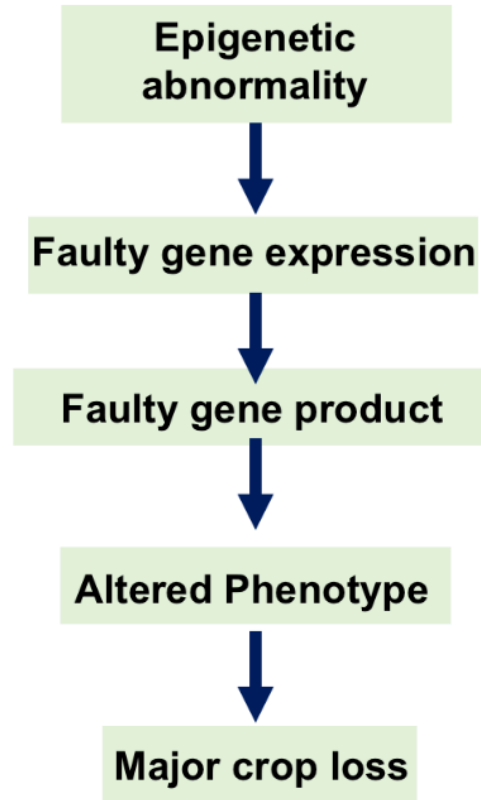
LETTER

doi:10.1038/nature15365

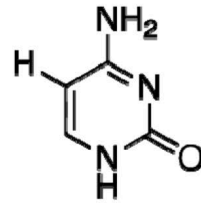
Loss of *Karma* transposon methylation underlies the mantled somaclonal variant of oil palm

Meilina Ong-Abdullah¹, Jared M. Ordway², Nan Jiang², Siew-Eng Ooi¹, Sau-Yee Kok¹, Norashikin Sarpan¹, Nuraziyan Azimi¹, Ahmad Tarmizi Hashim¹, Zamzuri Ishak¹, Samsul Kamal Rosli¹, Fadila Ahmad Malike¹, Nor Azwani Abu Bakar¹, Marhalil Marjuni¹, Norziha Abdullah¹, Zulkifli Yaakub¹, Mohd Din Amiruddin¹, Rajanaidu Nookiah¹, Rajinder Singh¹, Eng-Ti Leslie Low¹, Kuang-Lim Chan¹, Norazah Azizi¹, Steven W. Smith², Blaire Bacher², Muhammad A. Budiman², Andrew Van Brunt², Corey Wischmeyer², Melissa Beil², Michael Hogan^{2†}, Nathan Lakey², Chin-Ching Lim³, Xaviar Arulandoo³, Choo-Kien Wong⁴, Chin-Nee Choo⁴, Wei-Chee Wong⁴, Yen-Yen Kwan⁵, Sharifah Shahrul Rabiah Syed Alwee⁵, Ravigadevi Sambanthamurthi¹ & Robert A. Martienssen⁶

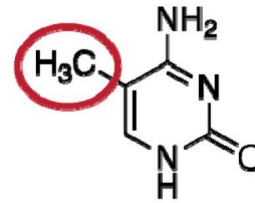
Simplified overview of the paper



DNA cytosine methylation is an epigenetic modification



Cytosine

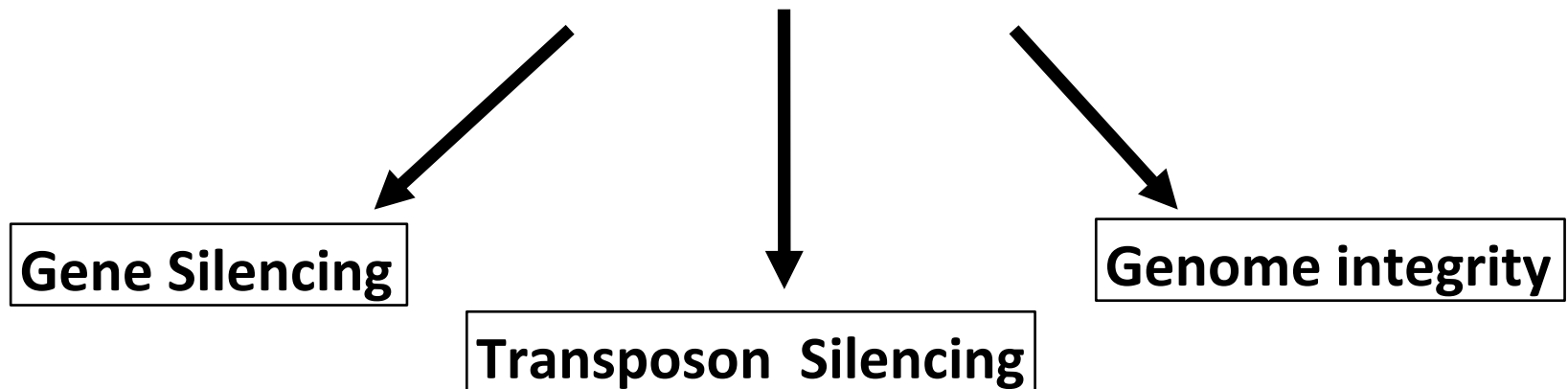


methylated Cytosine

5' ATGTC^mGTAGTTC^mTGATTGTATTC^mATTTTC 3'

- CG, CHG, CHH (H= A, T, C)

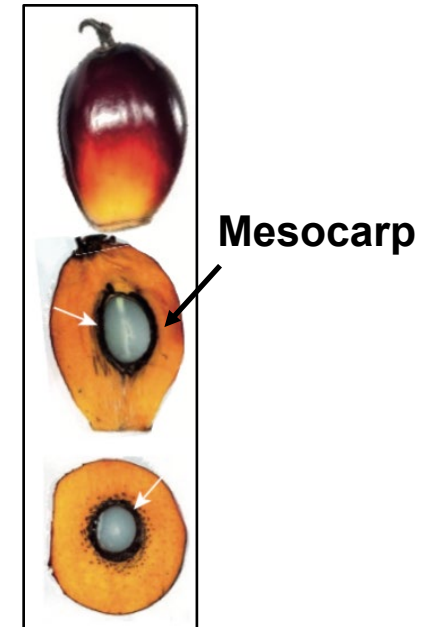
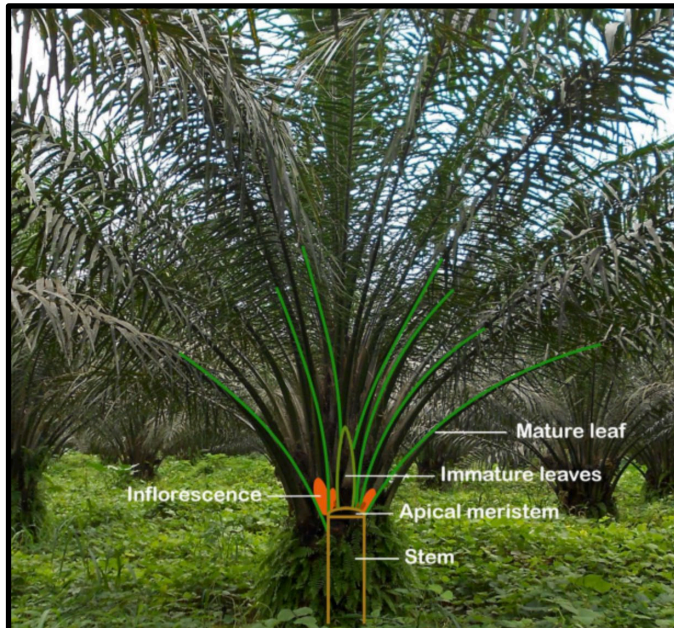
DNA cytosine methylation



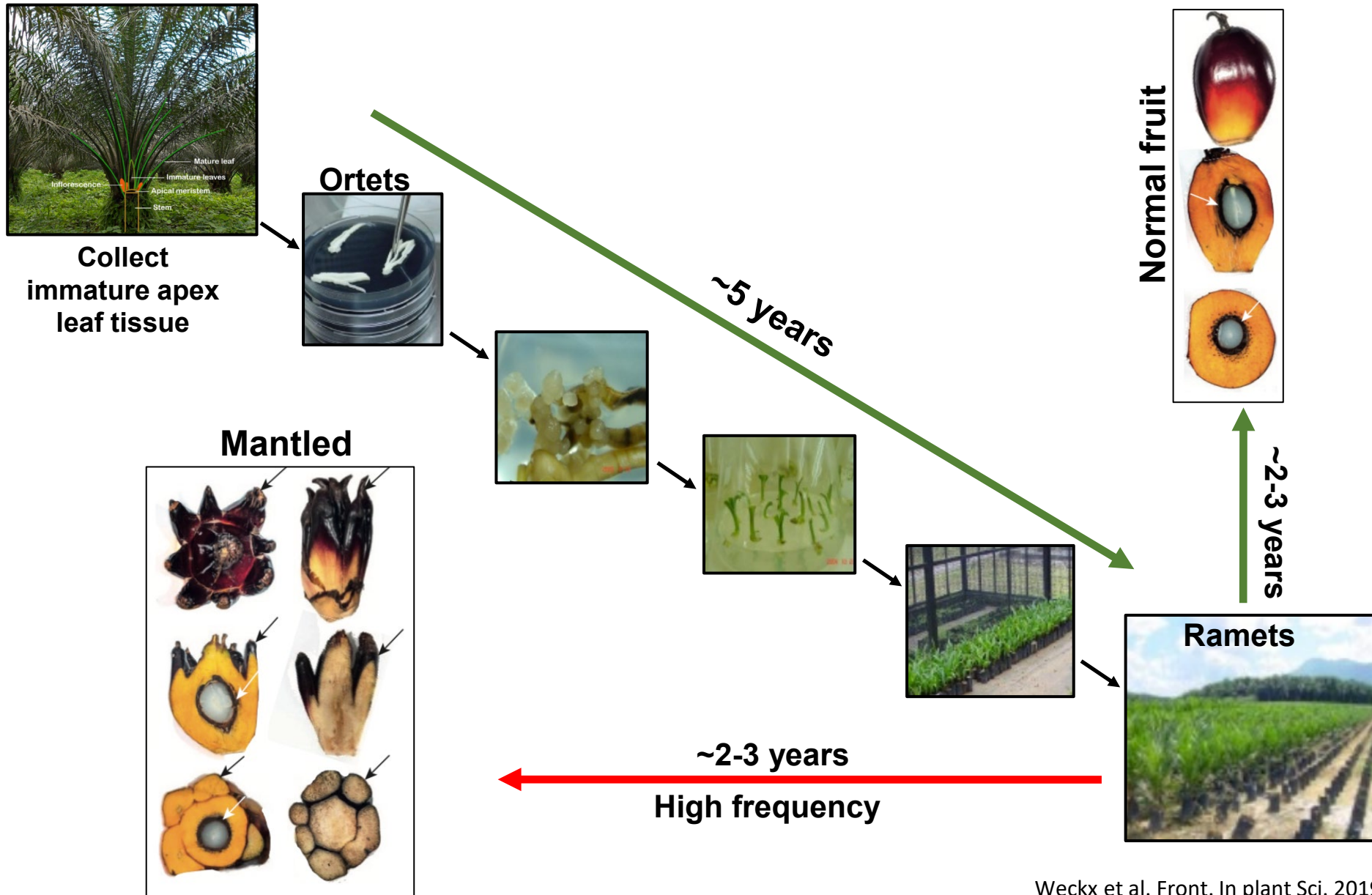
Crop in question: African oil Palm tree

- **Palm oil is the source of**
 - Edible vegetable oil
 - Biofuel
 - Used in other products – cosmetics, soap etc.

- **The mesocarp of the fruit is the source of Palm oil**

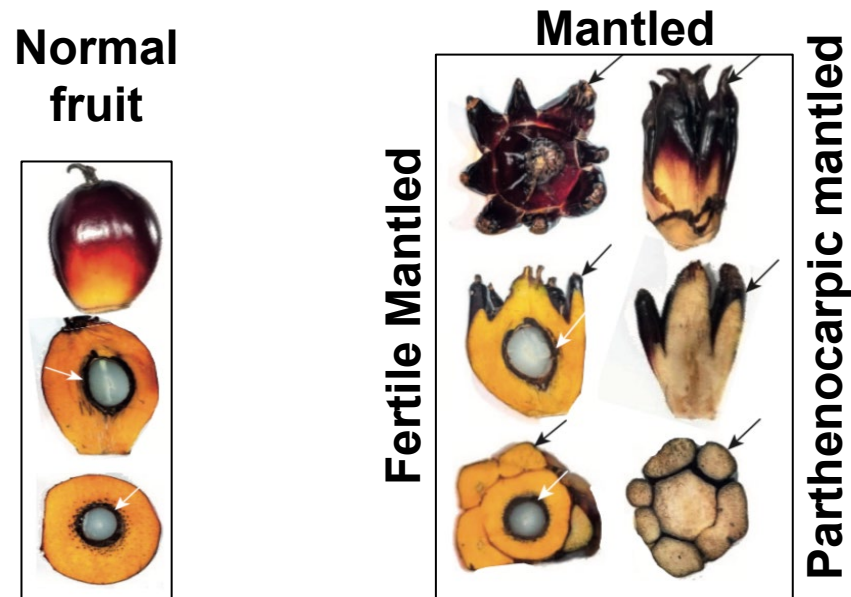


Micropropagation of high yielding varieties of oil Palm



Abnormality in epigenetic modifications might lead to mantled phenotype

- In plant tissue culture genetically identical clones but having variation in phenotype are called Somaclonal Variants



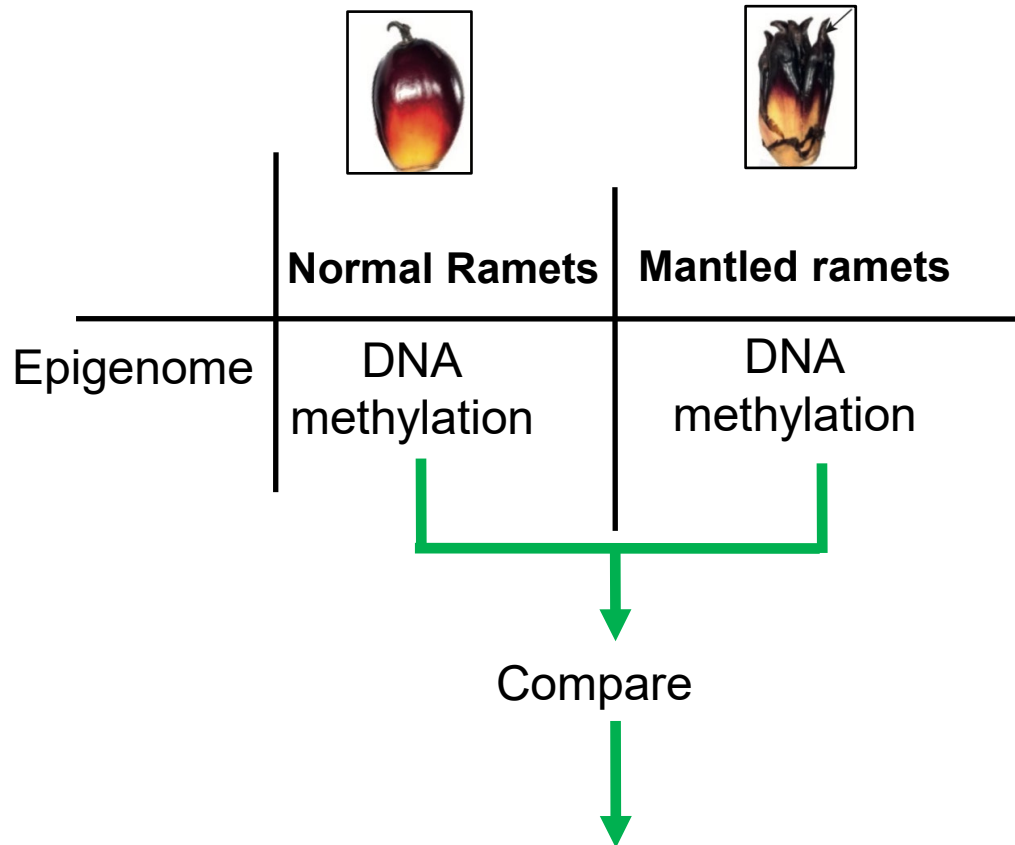
1. Non-Mendelian inheritance of the mantled phenotype
2. Mantled trees exhibit global loss of DNA methylation

What is the cause of the mantled phenotype?

Hypothesis :

Abnormality in DNA methylation at a specific locus leads to mantle phenotype

Epigenome wide Association Study (EWAS)

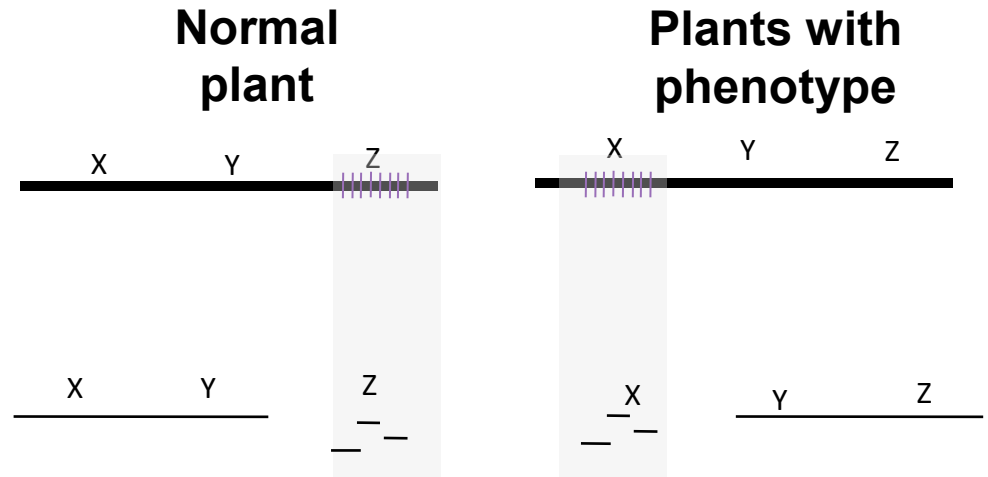


Identification of locus with epigenetic variation among normal and mantled lines

- **Genome wide DNA methylation maps : Methylation sensitive restriction enzymes followed by hybridizing it to genomic sequences**

Methylation sensitive restriction enzyme-based assay for DNA methylation analysis

1. Methylation sensitive restriction enzyme (McrBC) : Digests DNA with methylated cytosines

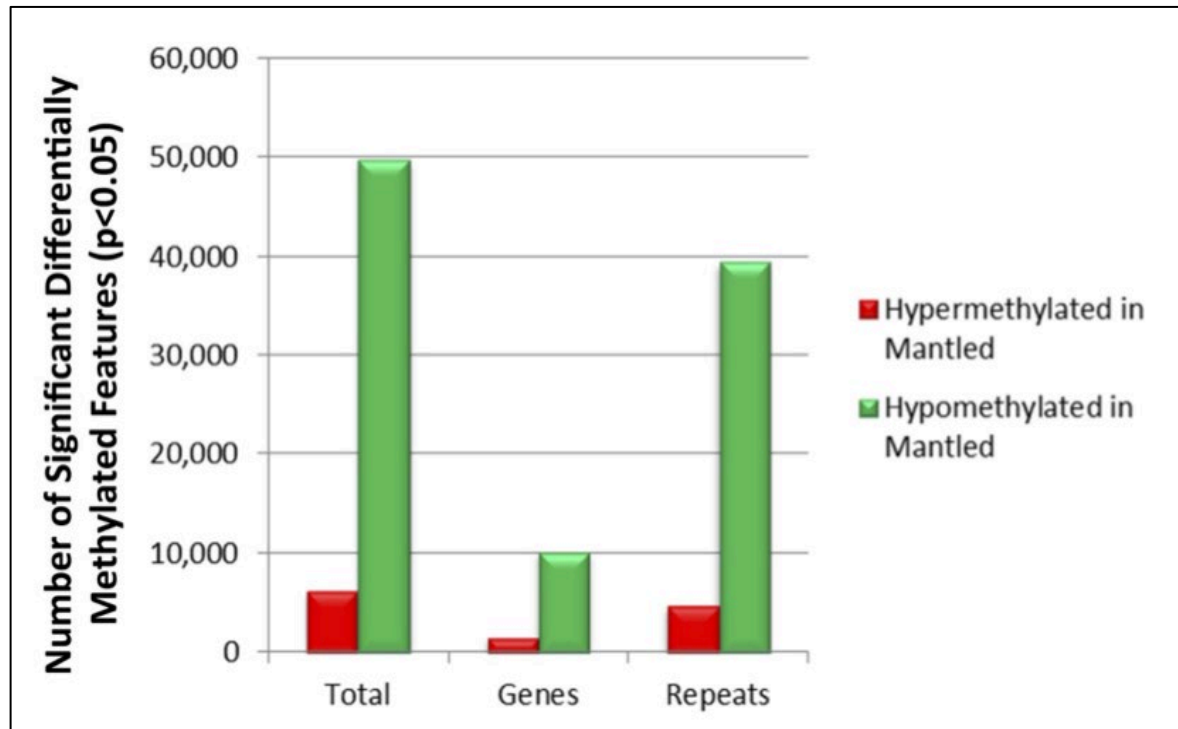


2. Determine the regions of genomic DNA that got digested

DNA Microarray

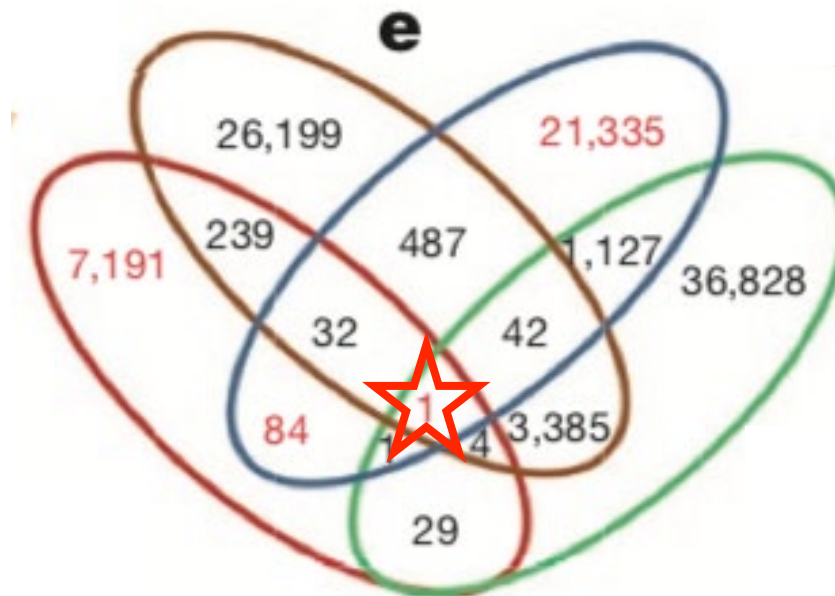
Several thousand hypomethylated regions found from EWAS studies

	Normal	Mantled
Total biological replicates	43 ramets	54 ramets

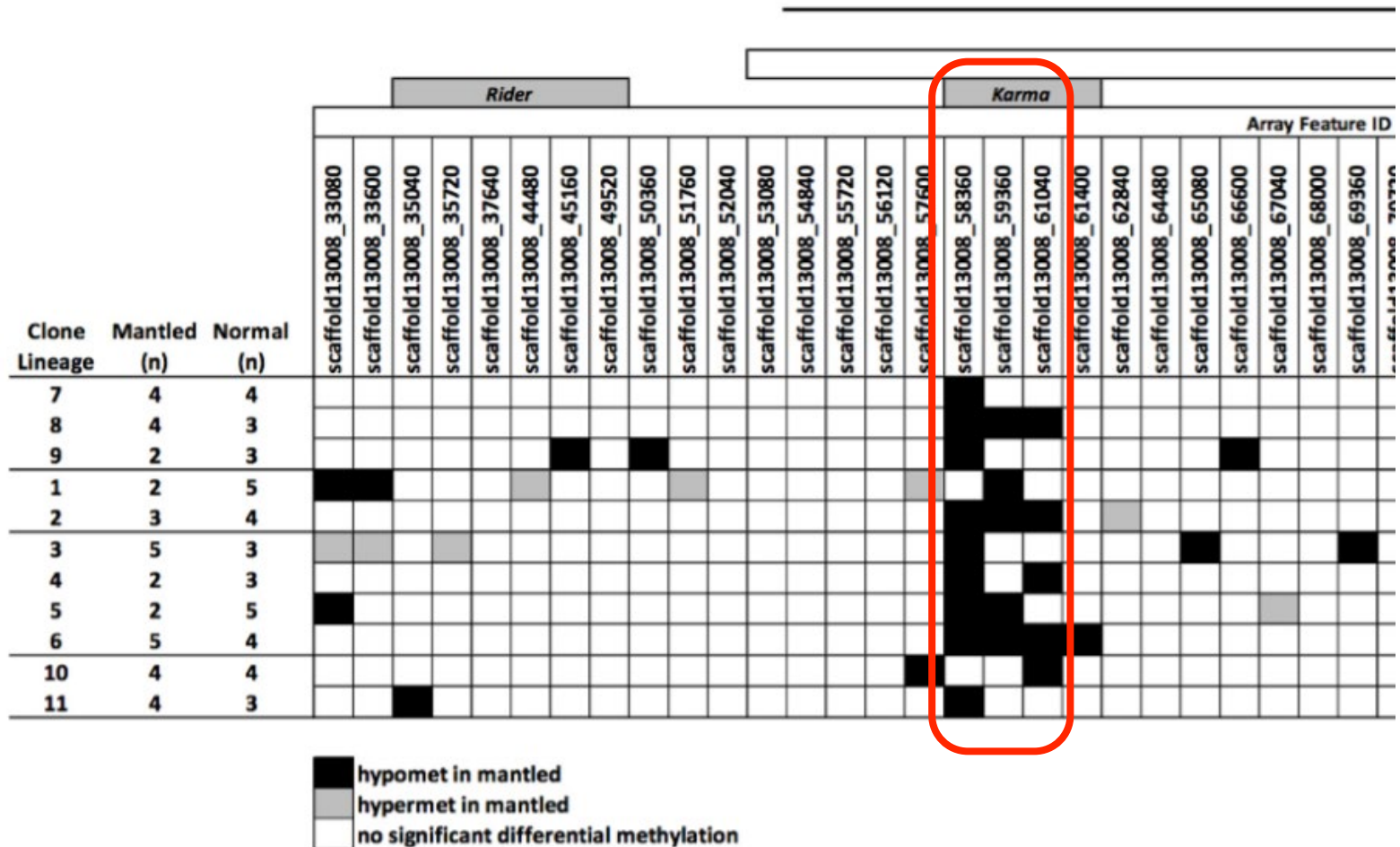


One differential methylated region (DMR) in all four mantled populations

Source Population	Normal (n=43)	Mantled (n=54)
Red	15	15
Brown	6	14
Blue	14	15
Green	8	10

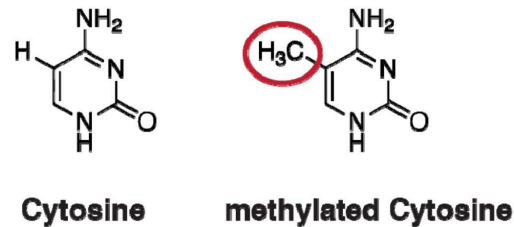


The DMR lies within the 35 kb intron 5 of *EgDEF1* gene



DNA methylation profile of the whole genome at the single nucleotide level

Whole genome bisulfite sequencing



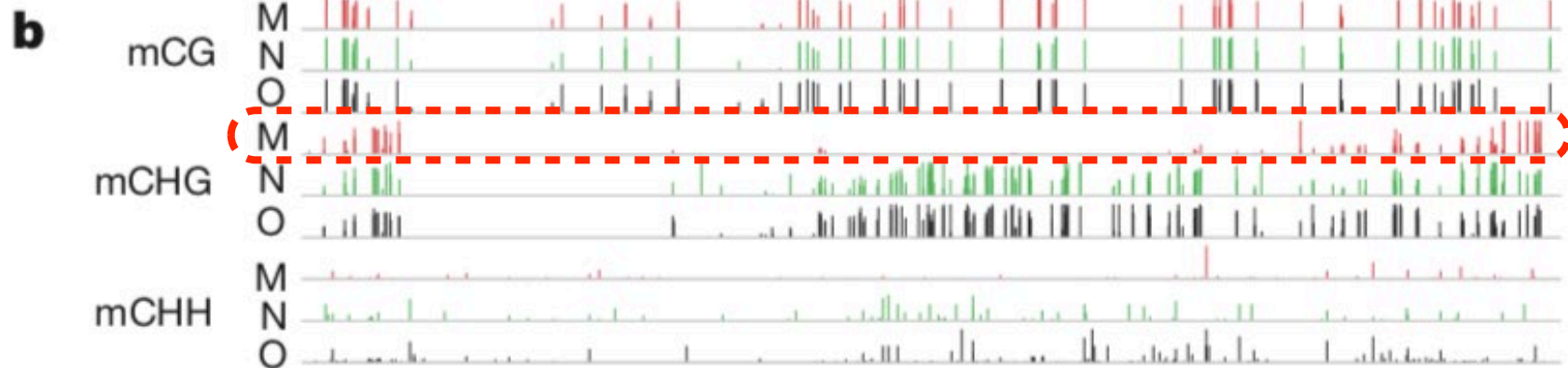
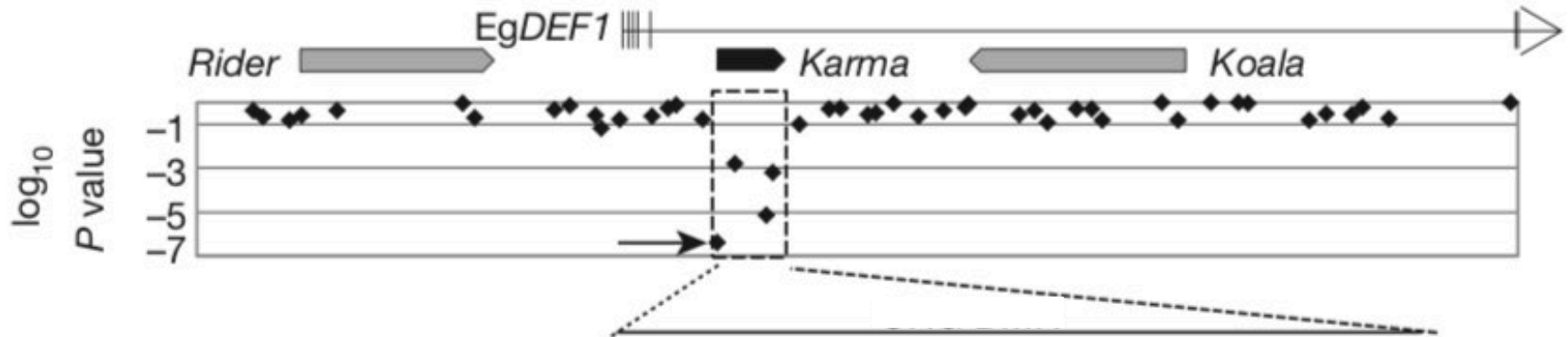
Sequencing the converted DNA

Aligning to a reference genome

Quantify the percentage of methylation for each cytosine of the genome

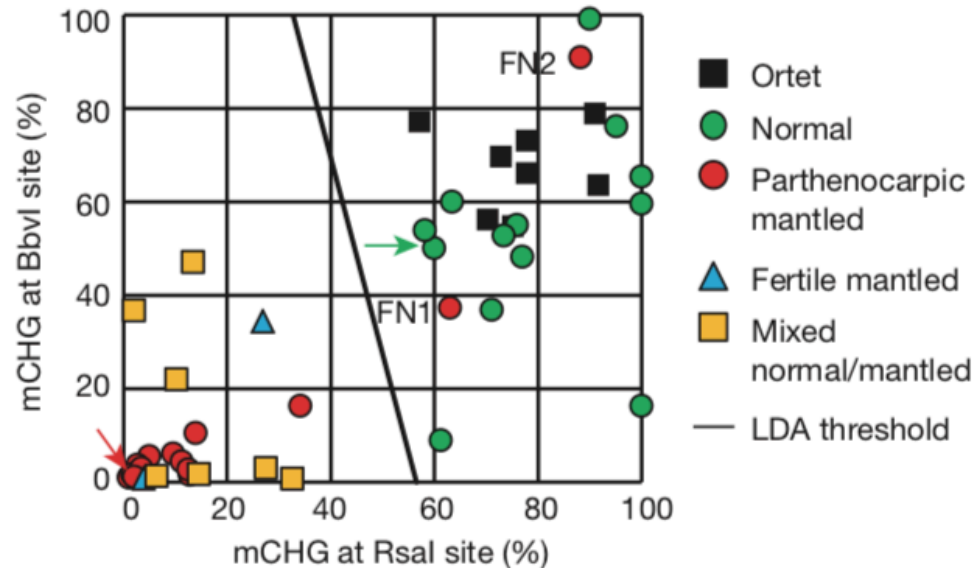
CHG methylation was dramatically reduced in the mantled clones

5' ATGTC^mG TAGTTC^mTG ATTGTATTC^mATTTTC 3'



DNA hypomethylation at Karma: Strong predictor of mantled fruits

Methylation sensitive restriction enzyme assays : BbvI and RsaI



Linear discriminant analysis: **93% sensitive and 100% specific for mantle**

Mantled **hypermethylated** epiallele: **Good karma**

Mantled **hypomethylated** epiallele : **Bad Karma**

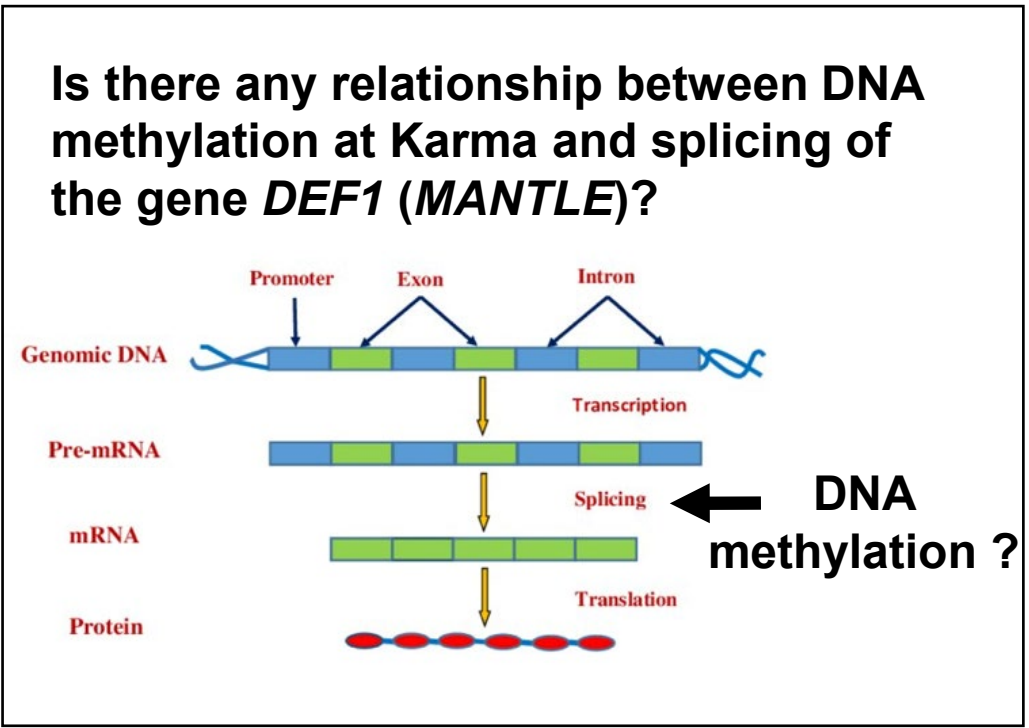
Splice acceptor site
(methylated in all 4 four
populations

Karma sequence

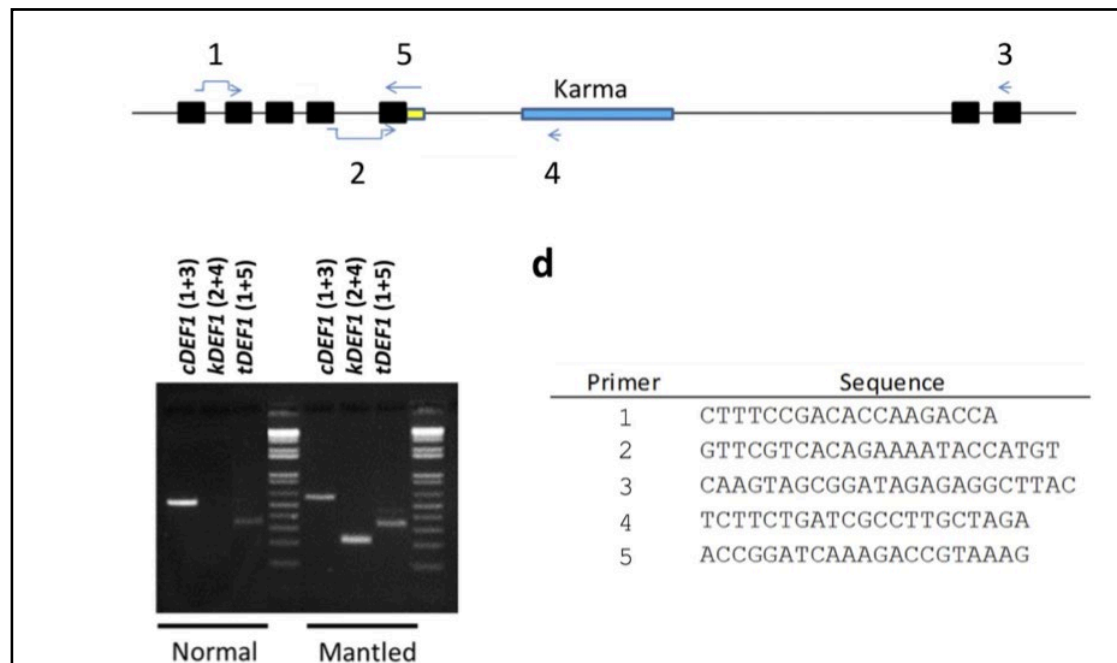
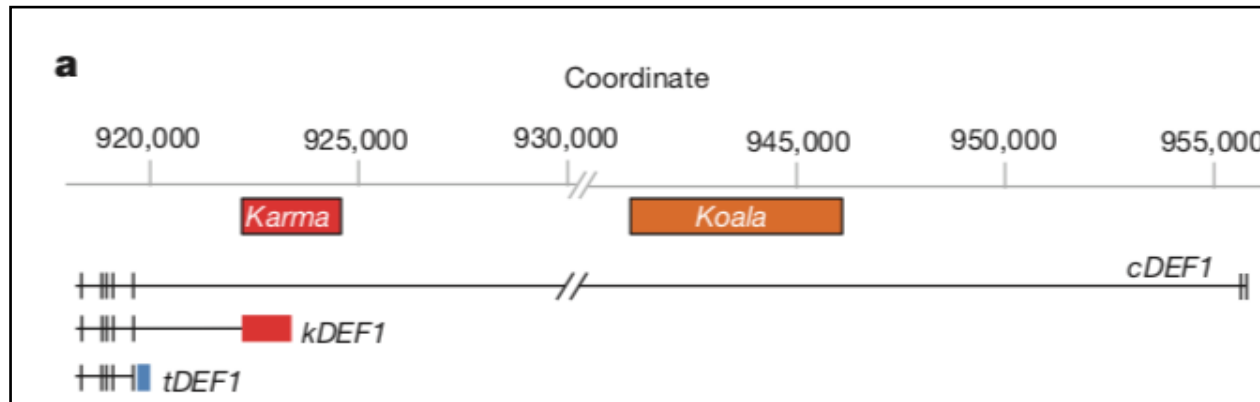
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63 : CTATGGAAACACAGTCCCAATGAGTGGCTTCAAAAAGGGGATTGCAATAGCAAGTTAT : 124
125 : CCATGTTGGGCAAGTAAACAGAAAAGAAATACATCATCAGTCACTGACAGGCACTC : 186
187 : GAAGATTATCGAAATGACAGCAAAATCCATCCACATTCTACACCTTTTCTACCCCTACTAG : 248
249 : GCTCGACTGAGGAATGACTCATCCAGCTGATTAGAAGATTCTTATCCAGAGGACCTCTG : 310
311 : GATCTTCTGACATTGATGCCATTTATGGAGAAAGAAATCCATGATACAGTGTATGACTT : 372
373 : GCGTTGGAAAAGTCAAGCAATGATTTTCCCAATTCCTCTATATGCACTCTAGTGT : 434
435 : ATCACTAAAACATGACTGATGAACTCTTAAAATGAGTAAATGAGCACTCTGAGACTAG : 496
497 : TTCTTCATCACCTTATCCGAAAAGAAATTTGGTGTGATTTGAGTACAGACTCTAGGCA : 558
559 : TAGCCCTGATTAATGGAGTAATAAAAATATTTCAAAAACCTCTATCGAAAAGGCTCTCAG : 620
621 : AAAATGAATTTGTTAATTTATCCACAGAGCTTGCCTTCAACAAGGAAAGAAATATCTCGA : 682
683 : ATATTTTGTATGACTATGGAAACTATACACTTCTGCAAGCTGAAGTACACAAGGATCTCA : 744
745 : ATTATAAAGTCGACTTCGAGAAGCTTTTGCAGATGGATTGGAGCTTCTATTGAAATGG : 806
807 : CBTCAACACAGGGGCTTGAATTCAGGGTGGTGTCAATGGATAGATACTGATATTATACAG : 868
869 : CTAATTTCTCACTCCTTATTAATGGTGATTAAGGTAACCTTTTAAATTTGAGGAAAGATCT : 930
931 : AGGCAAGGAGTCCCTCTTCCGCTAGCTCTTCTTCTAGTGTGATATAGAATGATCAAG : 992
993 : GGAGCAAGTAGGTTCAATCTTTTGGAAATGGATCATAATATCATGGGTAACCTCA : 1054
1055 : AAGCTTTTGTCTCACTGATGACACACTTATTTTGGAGATGATGACTAAAATACATCAAAA : 1116
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1241 : AAGCAAGGTGGCTACATTTCCATTAATTTTGGGCTCCCACTCCATCTCTAAACCTGA : 1302
1303 : GGAACCTTATGGAATCCACTCCTTGAAGGTTGAGAGAAATGATCGGGTGAAGAGGT : 1364
1365 : AAACCTTCAACTTAGGTTAGGCTTATACTAATGAGTGTCTACAGGATCCCACT : 1426
1427 : ACTCTGGAGGATACATCTCTCCCTCAATTCATTATCAAAATAATGATAAAATCCATC : 1488
1489 : GATCATTCAATTTGGAGGAAACGAGGAGTAACTAAGGGCACTTGAATATGTTGGTCC : 1550
1551 : AATATTTGTGATCAAAAATTTGGAGGCTGGGGTCTCCCACTCTAAAATTTTCAATA : 1612
1613 : CAATTTCTTGTAAATGGTGGGAGGCTACTCTAATGCTGGTGCACCCCTGGTGTAGT : 1674
1675 : TTTATTTGCCACTTCCACCACCTTCACTACACTAGAGATCTAAGGTATACACAAATCACTC : 1736
1737 : TTCATTTGGAATGGTTACAGCACACATGAATATTTCTACTCCTAATCCACTTCAAGTT : 1798
1799 : AGCAACTAGTATTTTGGAAAGATAGTGGTTACATAATCCCACTGAAGGATCGATT : 1860
1861 : CCTCACCTTACACAATGACTTGAAGTCAACAACCTAGTGGCAAGGTATTAAGCAATCT : 1922
1923 : ACTTGATAAATCCTTTTGTACTCTCTCTCCAGATACCAAGAGATTTTCAAGATC : 1984
1985 : TATAGGAAGCAATTGAAACAAATTTACATTAACGGAACCTGATACATACAATGGAATGG : 2046
2047 : TTAGTAGCAATTTTTTGGGATGAAGGATCTACTATTTTCTGCAAGATGGAGGATTTG : 2108
2109 : GCCTCTAGTGAATAATATATAAAAACCTCTAATACCAAGAAAGCAAGTATTTGCTT : 2170
2171 : GCCTAAGTCTCACACAATAATCCCAATGAAGCTAATCTTCTAATAGAGGAATTAATGGA : 2232
2233 : ACTGATACCTGACACTTTGGCTGACTATGAAACTAATGATCARTATGCTCATCTA : 2294
2295 : TACTTTTCAAAAGCAATTTGAAATCAAGTACTTTCAGACCTGCAATTTGCAAACTTTAT : 2356
2357 : GCATGCTTAACCCCTATGGGACTTTGGAGACTCATCAATATGCAACAGATAGAGACCT : 2418
2419 : AAACTAGCTCCTATTGCTAATTTGGTCAATGGTGTCTTGAAGAAAGAAATAAAGATT : 2480
2481 : ATTCGACTCTATACTTTTATCCAGATCGAATGCTGAACCTGTGCACTTTTCTTTCTT : 2542
2543 : GGGCATCACACTACACAGGAGCACTAAGAGTGTAGCTCCTGTCGAGAAGTCTCTTA : 2604
2605 : TCTAAGATGAAACACACATCTTACTGAGATATCAAGTGTACAGGCTCAGAGTAT : 2666
2667 : GTTTATAGCACTTTTATAGCTCCAGCTTATATGTGATCTATGGTCAAGGATTAATAT : 2728
2729 : AACCATGGATATTAGTATGTTGACTATCAGAAATCATCTCAATACATCTATGTAACAC : 2790
2791 : TGATCAATCCATGTTCACTAGATAGGAACCTGCCTATATACAGGATGTCCTGATGTA : 2852
2853 : CTATAGTACTATTATTCAATAAATAACGAAGGTTTACCTCTCTCATAAAAGAA : 2914
2915 : AGTACTCTATGCTATCTTATGATGATGATCTCTTTGCTACTCTTTTATTACTTCTT : 2976
2977 : AACCTTGGCTACACTATATTTGAGGCTTTTAAATTTCTTTTGGATATGCAATG : 3038
3039 : CACTCTCACTCACCTCARGCCAGCAACAACTTATTCACACTTGAAAACCAATAAAGAA : 3100
3101 : TACCAAGAAATTTATCATGAAATTTAGAAACTTTGGTTTACTCCTTCTCCATATCA : 3162
3163 : AAAAGTTCAAAAATGATGA : 3181

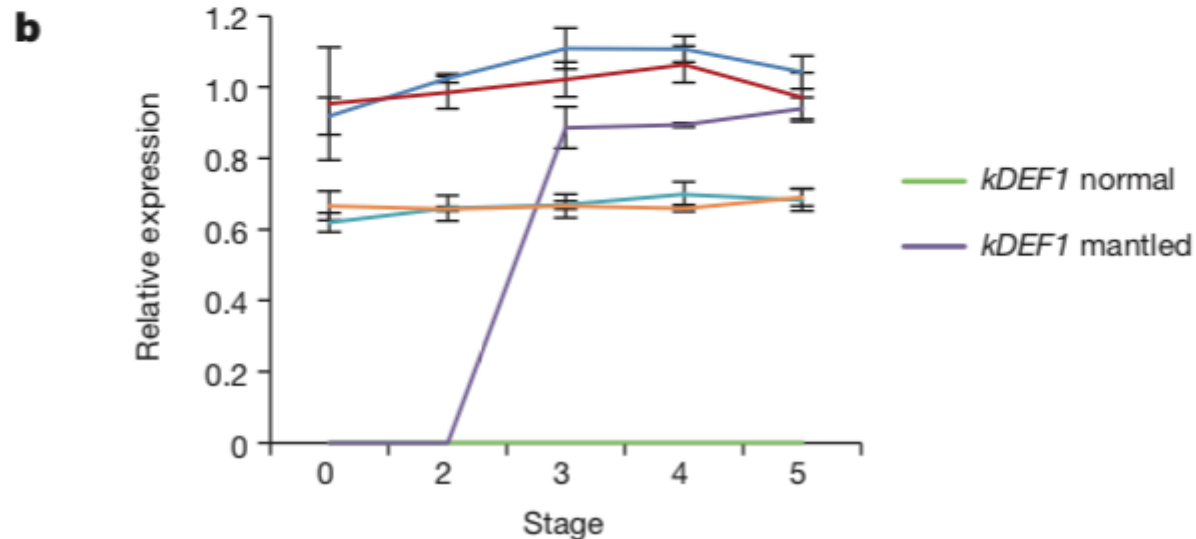
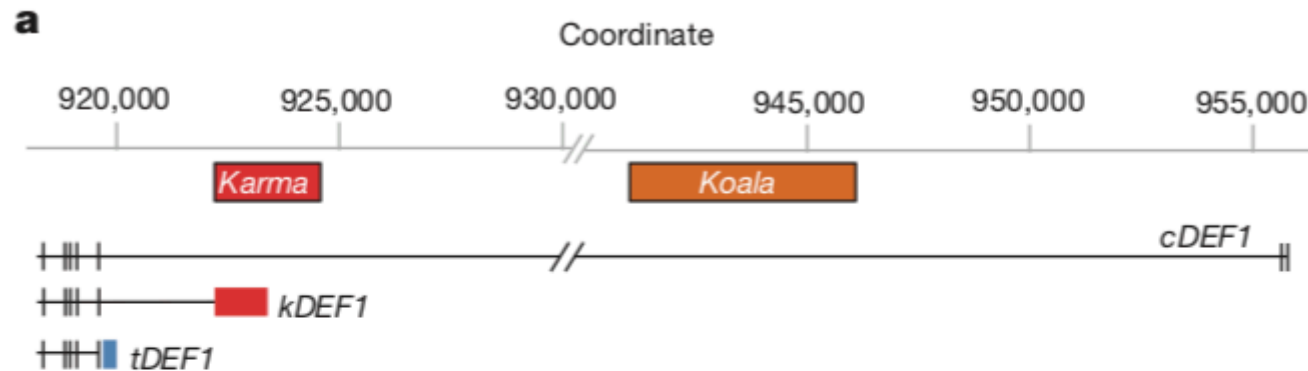
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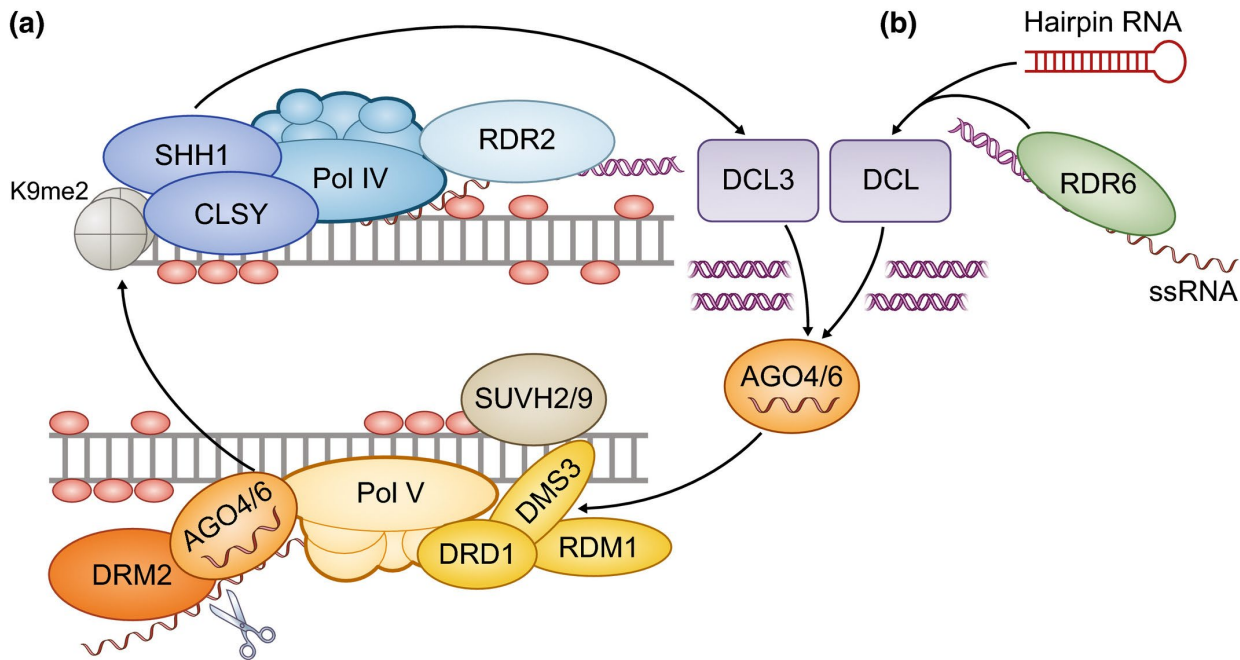
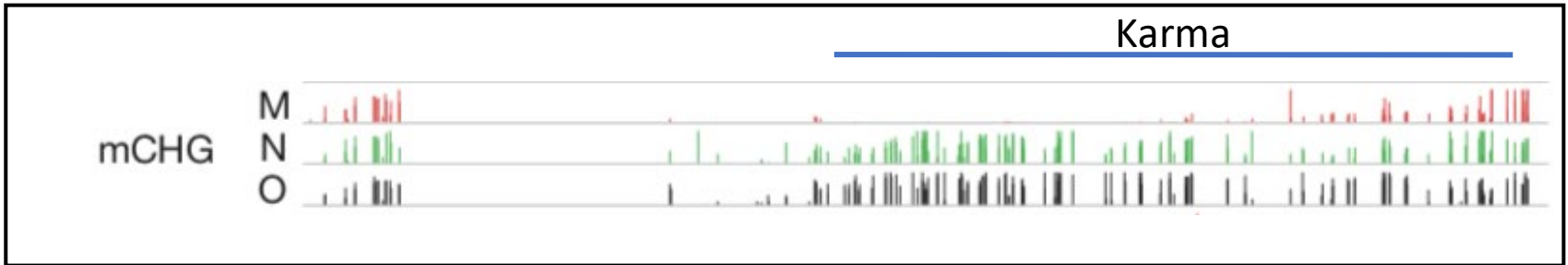
A novel transcript (kDEF1) is identified that gets spliced at the proximal Karma acceptor site in the mantled ramets



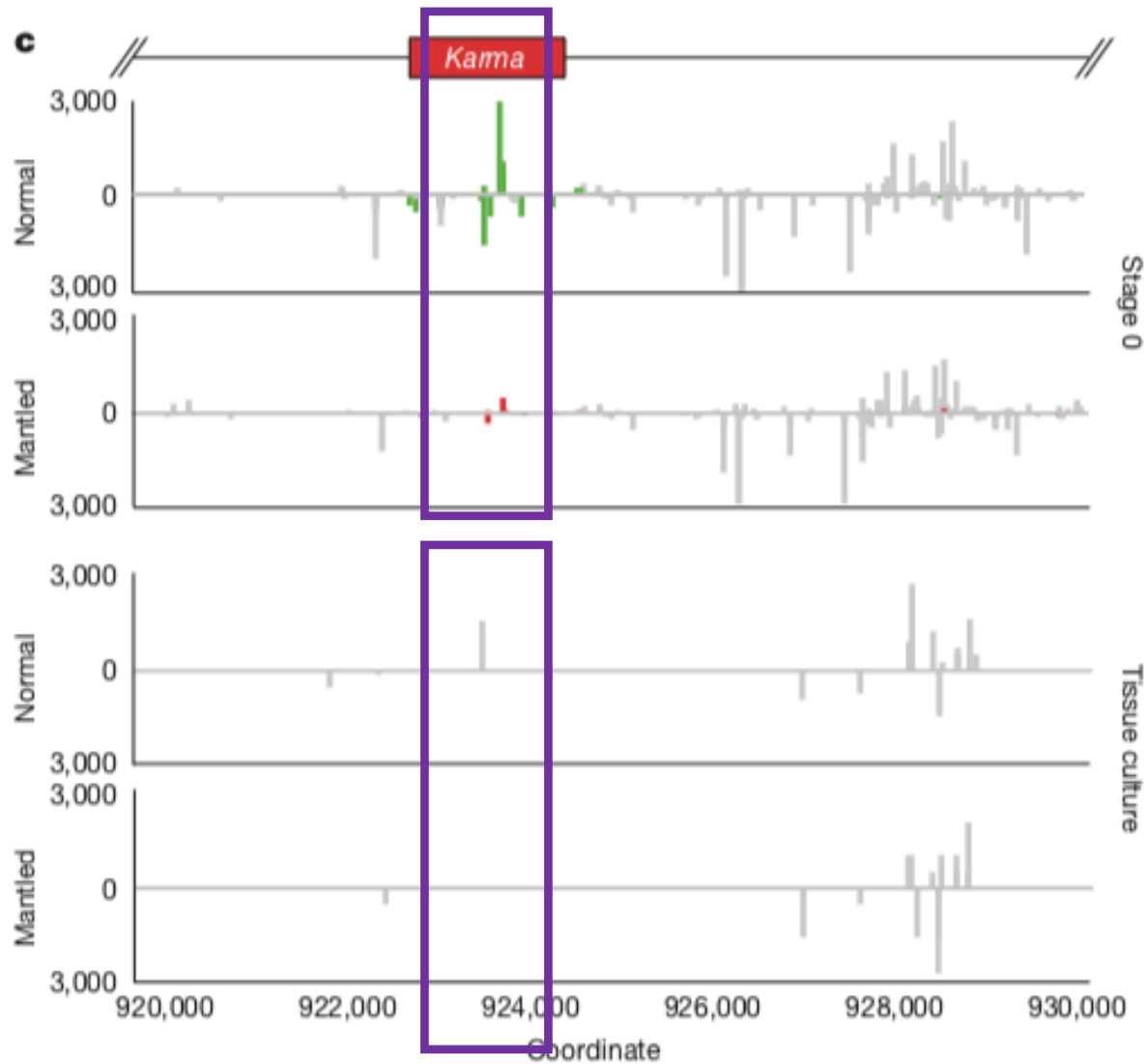
kDEF1 is only restricted to developmental stage 3 to 5 of mantled immature female inflorescence



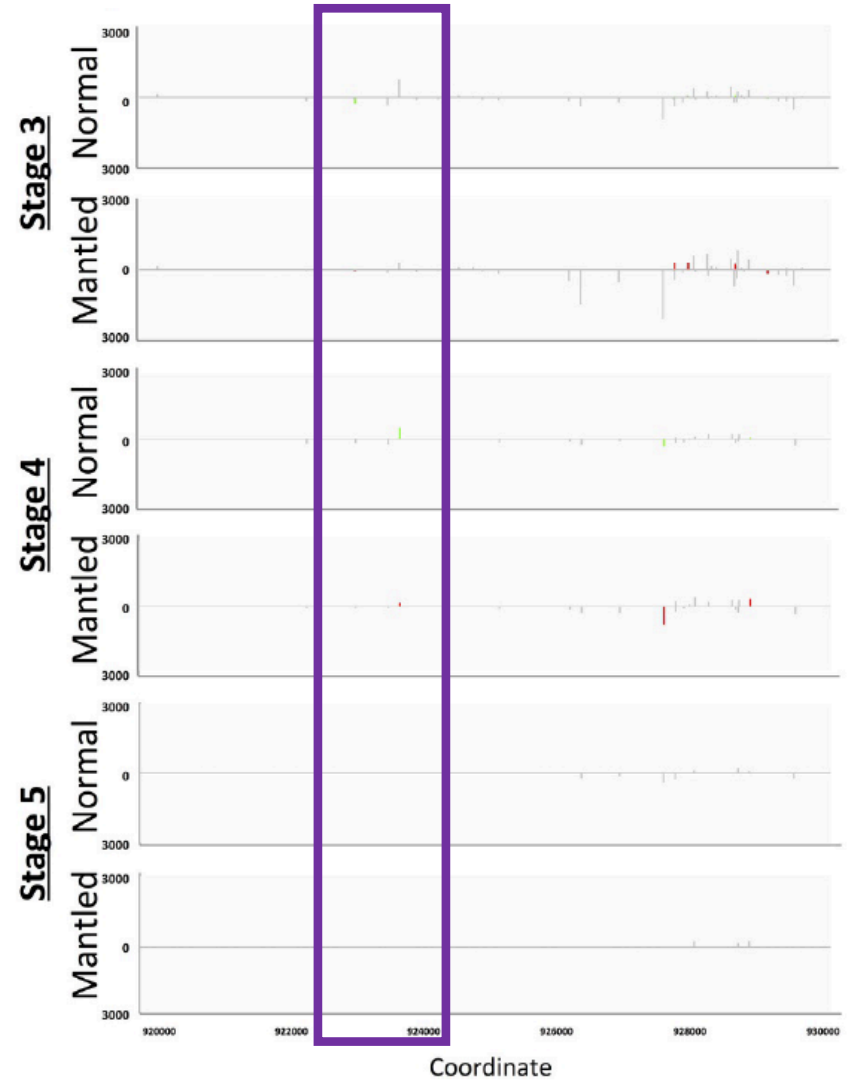
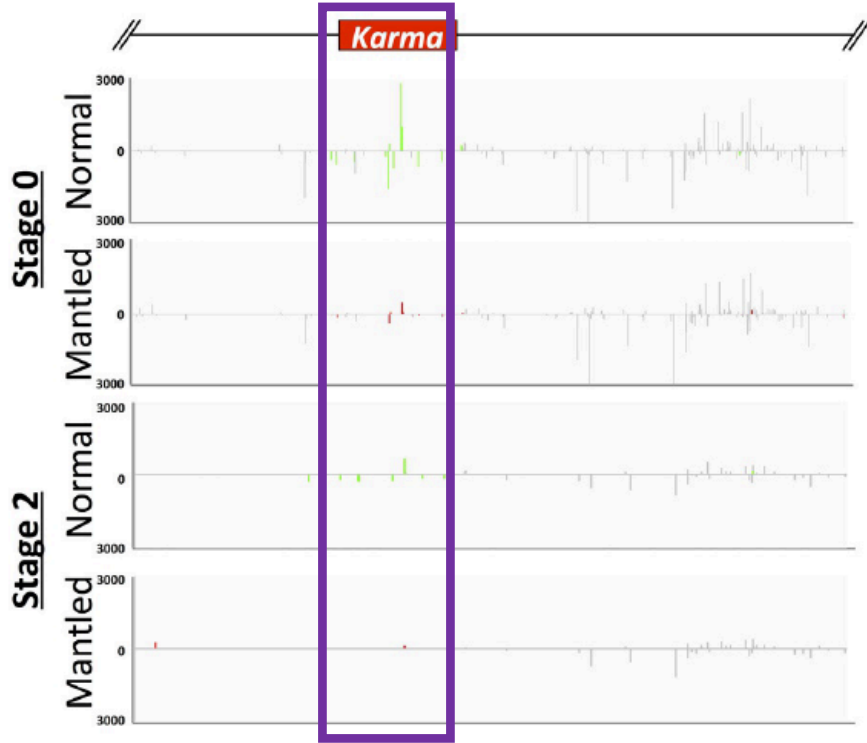
Why is *Karma* not methylated in the mantled phenotype?



Reduction in small RNAs at *Karma* observed in tissue cultured or mantled samples



Loss of small RNAs at *Karma* observed in mantled and later stages of inflorescence development

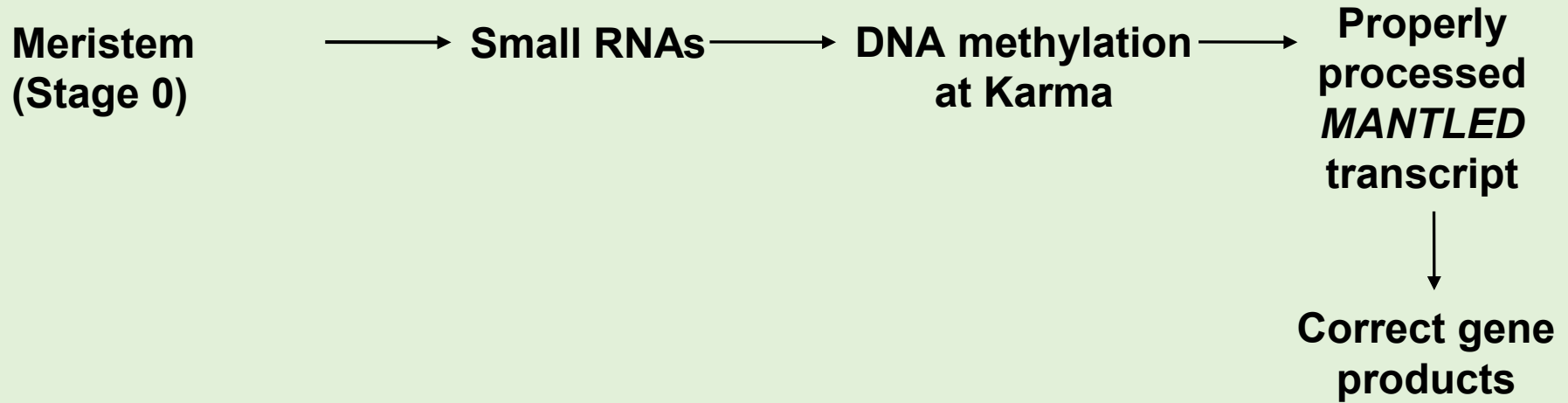


Summary

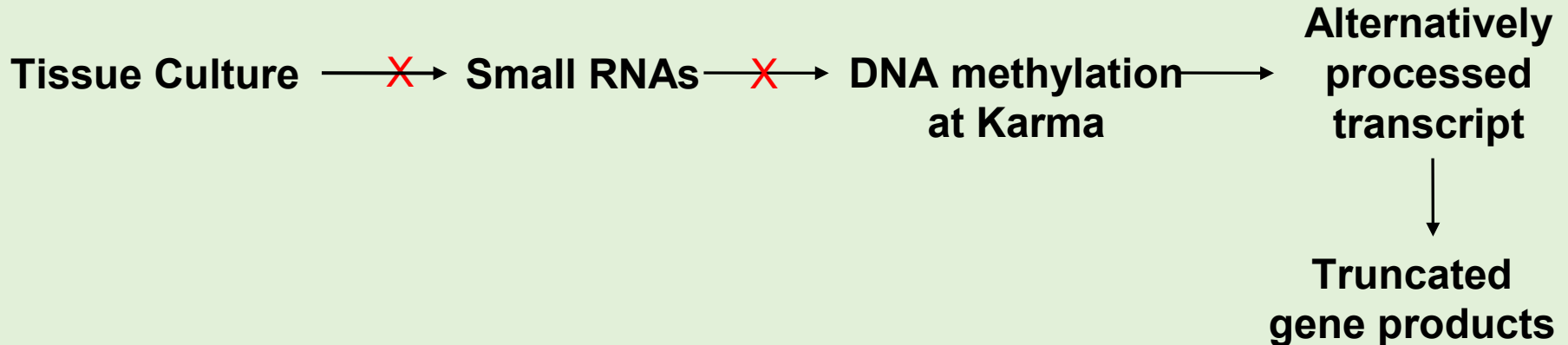
1. DNA hypomethylation at Karma is the underlying cause of mantled phenotype.
2. DNA hypomethylation at Karma effects alternative splicing of the *MANTLED* gene
3. The spliced product may have a role in the mantled phenotype
4. Small RNAs specific to Karma may also play a role in DNA hypomethylation at Karma
5. Absence or presence of DNA methylation at Karma is a strong predictor of the mantle phenotype: *Good Karma or Bad Karma*

Model for mantled phenotype

Normal fruit development



Mantled fruit development

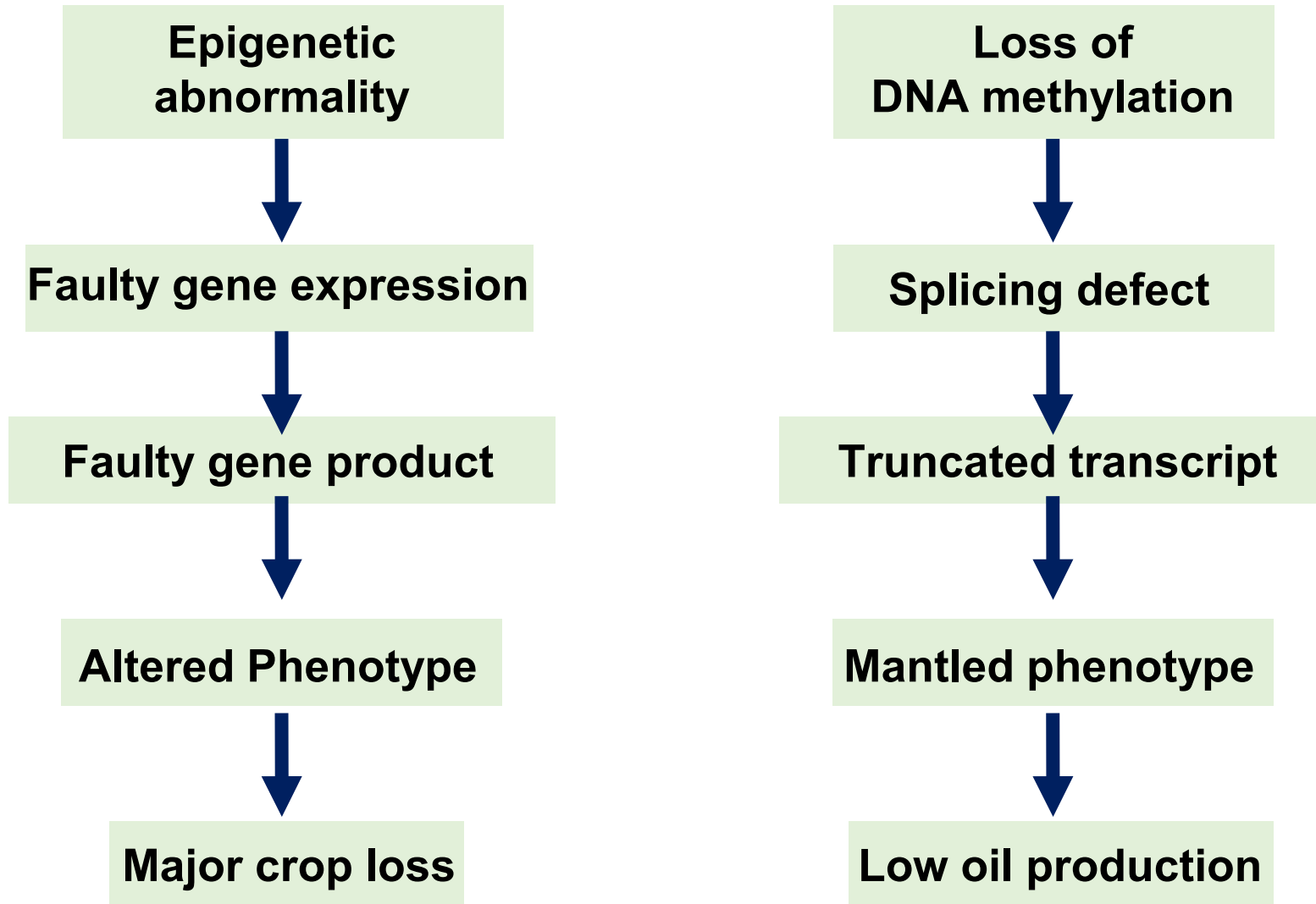


Future Experiments

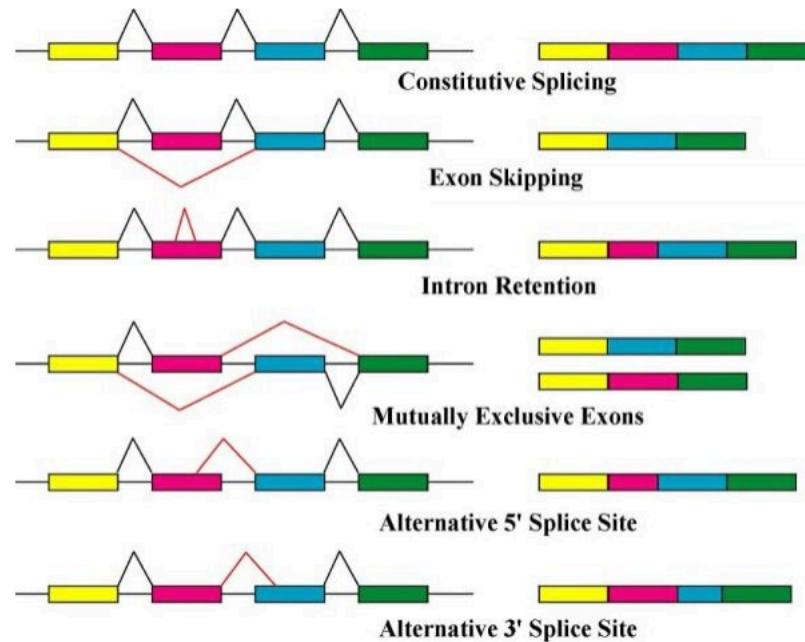
1. Targeted epigenetics to cure the mantled phenotype using CRISPR based tools
2. Develop PCR based assay to identify mantled phenotype at early stages of tissue culture (Orion genomics)
3. DNA Methylation is mostly present in the promoters and in the exons of a gene. Interesting case to study the role of methylation in the intron of a gene and its role in splicing

Questions?

Broad overview of the story of the paper

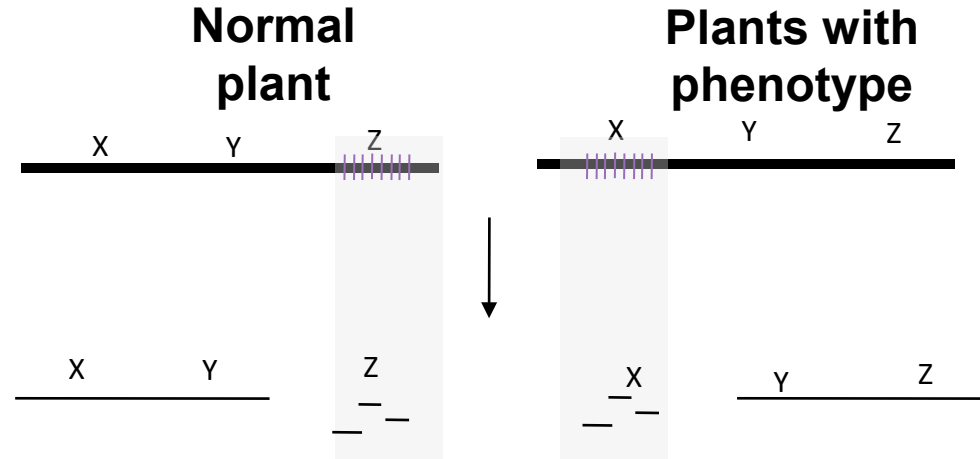


Alternative Splicing

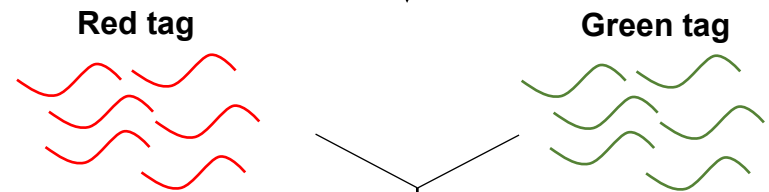


Methylation sensitive restriction enzyme-based assay followed by hybridization to DNA microarray

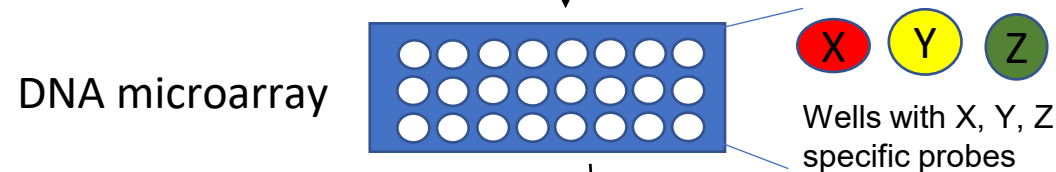
1. Methylation sensitive restriction enzyme (McrBC) : Digests DNA with methylated cytosines



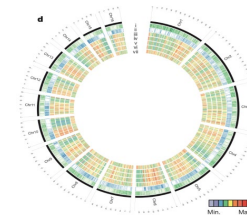
2. Label digested DNA using fluorescent tags



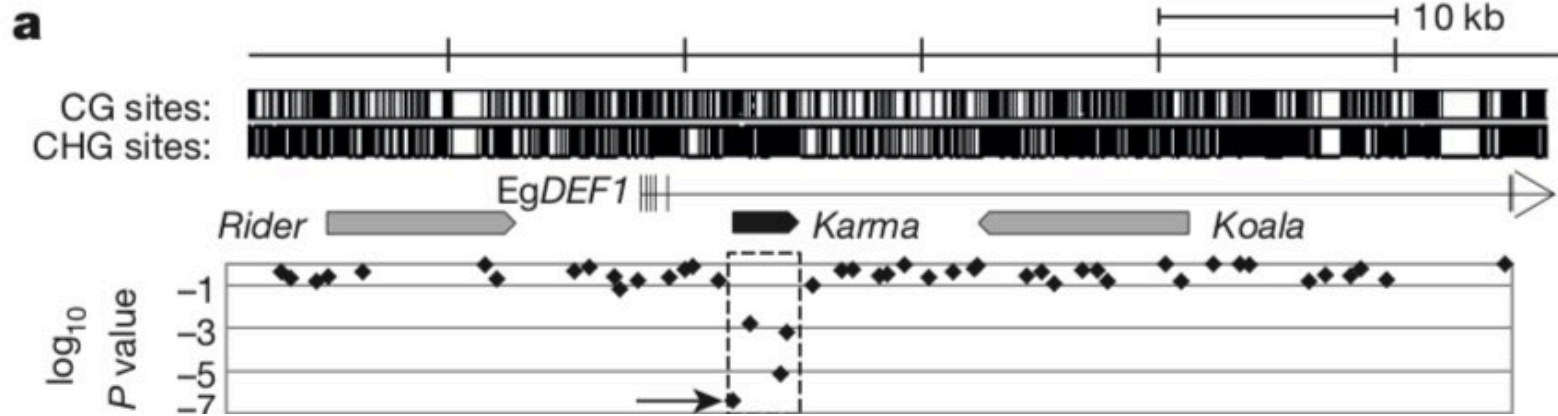
3. Mixing and hybridization



4. Assemble data to form DNA methylation maps and compare



The DMR lies within the 35 kb intron 5 of *EgDEF1* gene



**Unreported repetitive element:
Karma line element (3.2kb)**

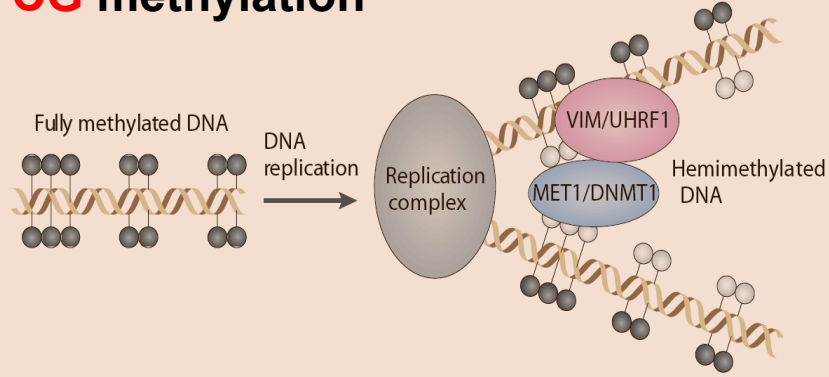
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1 : TTCAAATGATGAGGATGATCTCTCAAGTCAGCACTAGACCATCTTCTAAATAGGAAGAT : 62
63 : CTATGGAAGCAACTCTCCATATGCAGTGGCTTCAAAATGGGAAATGCCAATCGAAGTTAT : 124
125 : CCATGTTGGCAAGTAAACAGGAAAAAAGAATACTATCAGCACTAGCAGGCGGATCA : 186
187 : GAAGATTGCAATAGCAGCAATCCAATCCACTCTCAACTTTTTTCTACCTACTAG : 248
249 : GCTCCCTGAGGAATGACTCACTCCAGCTATTAGAAGATCTTTATCCGAAAGGACCTCTG : 310
311 : GCTTTGCAATGAGATGAGATTTATGAGCAAGAAATCCGATGACAGTATGAGCT : 372
373 : GCTTTGGAAAAGTCACTCCGATGATAATTTCCCAATCTCCTCTATGACACTCTAGTGT : 434
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497 : TTGTTATCACCCCTATCCAAAAAATTTGGTGTATTCAGTTAGAGACTTAGGCCAA : 558
499 : TAAGCCTGATTAATGGAGTAATAAAAAATTTCAAAAATCTATCGAAAAGCCTCTCAGAG : 620
621 : AAAATGATTTGTTAATTTATCCAGAGACTTCTTCAACAAGGAAGAAATATCTCGA : 682
683 : ATATTTTGTATGACTAGTGAACATATACACTCTCCAAAGCTGAATACACAGGATCTCA : 744
745 : ATATAAAGTCGACTCGAAGAGCTTTGACAATGTGGATGGAGCTTCTAATGAAATTTG : 806
807 : CTATCCAGCAGGGGCTTTGATTCGAGTGGTGTCAATGGATGAAATCTGATTTATAGAG : 868
869 : CTAATTTCTCAGTCTTATTAATGGTGAATAAGTTAACTTTTAAATGGGAAAGATCTC : 930
931 : AGGCAAGGAGTCTCTATTCGCTAGCTTCTTCTTAGTGTGATATAGATGATCAAG : 992
993 : GGAGCAGTAGTTCATCTTTTGTGGAATGGATCATATAATCATGGATTAACCTCA : 1054
1055 : AAGCTTTAGTTCATCGAGACACCTAATTTTCAGATATGATCTAAATGATCAAAA : 1116
1117 : CTCTAAATTTTACTCTATAGTATAGGACTAGTGGGTCTCAAAATTAACCTTGAAAA : 1178
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1241 : AAGCAAGTGGCTACATTTCCATTAATTTGGGTCTCCCACCTCATCTTAAACTGA : 1302
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1737 : TGCATTTGGAGGTTTCAACACACATGAAATATTTACTCTCTAATCCAGCTTCAGGT : 1798
1799 : AGCACTAGTATTTTGGAAAGATGTTGGTACAPAAATATCCACAGGATGATGATTT : 1860
1861 : CCTCACCTTACACAAATGACATTTGAAGTCAACAACCTCAGTGGCAAGGATTAAGCAATC : 1922
1923 : ACTGATAATAGCTCTTTAGTACTCTCTCTCCCAAGATACCAAGAAAGATTTCCAGATC : 1984
1985 : TATAGGAAAGCATTGAACAAATTAACATTAAAGGCAAGCCTGACTACTATCAATGGAAATGG : 2046
2047 : TTTAGTGAATATTTTGGCATGAAGATCTACTATTTCTGCAAGATGGAGGATTTG : 2108
2109 : CCCTCTACTGATATATATATAAATCTCTATACCAAGAAAGCCAGATTTTCCT : 2170
2171 : GGCTAAGTCTCACAAATAATCCCAATGAAGACTAATCTCTTAATAGAGGAATAATTGGA : 2232
2233 : ACTGATTAAGTACTTACACTTTGCGATGACTATACAGAAATTAATGATCTAATGCTCATCTA : 2294
2295 : TACTTTTCAAAGCAATTTGGAATCAAGTACTTTCAGACCTGCAATTTGCGAACTTTTAT : 2356
2357 : GCATGCTTAACACCCTATGGGATCTGGAGACTCATCAATATGCAACACGATAGAGACCT : 2418
2419 : AAATAGCTCTCTATGCTAATTTGGTCAATGGTCTTTTGGAAAGAAATAAAGATTT : 2480
2481 : ATGCACTCTAATCTTTTACAGGATTTTACAGGATTTTACAGGATTTTACAGGATTTTACAGG : 2542
2543 : GGGCATCACACCTAACACGGGCAACTAAGATGTTAGCTCTCTGAGAAATTTCTCTTA : 2604
2605 : TCTAAGAATGAAACACACAATTTTGTAGGAAATACAGATGCTACAGGCGCAGATGAAT : 2666
2667 : GTTTATGAGCATTTTATAGCTGCACCTATATGATCTATGCTGCAAGGATTAATAT : 2728
2729 : AACCATGGATTTAGTTAGTGTGACTATGAGAAATCATCTCCAATACATTTATGTAACAC : 2790
2791 : TGAATCAATGATTTTCACTAGTAGGACCTCTATACAGATGATGCTCCCTGATGTA : 2852
2853 : CTATAGTATACTATATCATAAATAAATAGGAAAGTTTACCTCTCTCTCAAAAAA : 2914
2915 : AGTATCTCATGCTATCCTATATGCTGATCTCCTTTGCTACTCTTTTATTTACTCTTT : 2976
2977 : AAATGGTCTTACCATAATATCAGCCCTTTTAAATTTGCTTTGGATATGCAATCTC : 3038
3039 : CACTCTCAATCACTCAAGCAAGCAAAACATTTATCACACTTGAAGAACCAATATAAGAA : 3100
3101 : TACCAGAAATTTATCATGAAATTCAGAACTTTGGTCTTACTCTCTCCATCAATCA : 3162
3163 : AAAGGTTCAAATGATGA : 3181
    
```

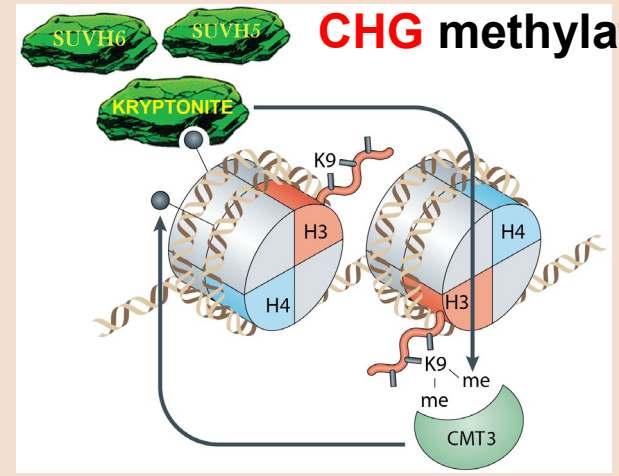
Splice acceptor site
(methylated in all 4 four
populations)

Four interlinked self-reinforcing methylation systems

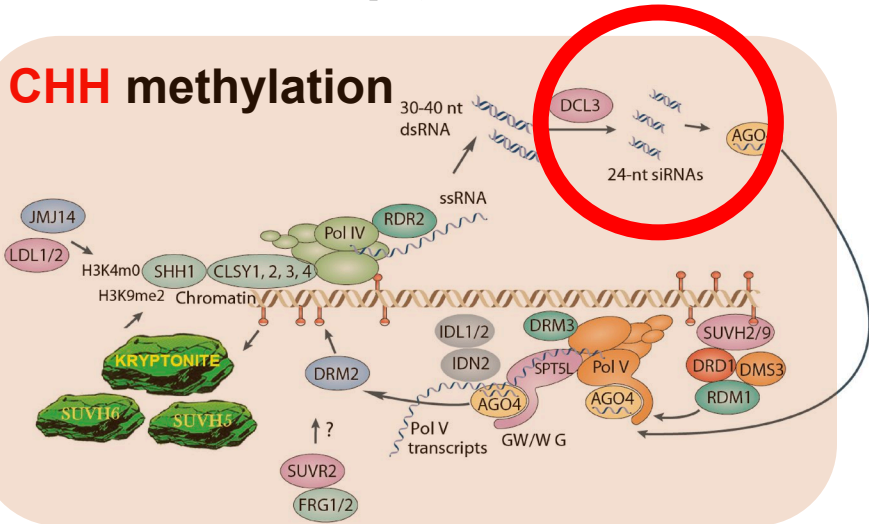
CG methylation



CHG methylation



CHH methylation



CHH methylation

