Comparison of Methylome profiles between closely related clones of the bacterial plant pathogen *Ralstonia solanacearum*

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DNA methylation in bacteria

- DNA methylation is the most common form of Epigenetic modifications in prokaryotic and eukaryotic genomes
- Epigenetic modifications contribute to alter gene expression without modifying genomic DNA sequences
- DNA methylation has widespread biological roles in eukaryotic genomes but the extent to which similar processes exist in prokaryotes is largely unknown
- In bacteria, DNA methylation has been demonstrated to be essential in the control of chromosome replication, DNA repair, phenotypic switching and virulence

DNA methylation in bacteria

- **DNA methylation is catalyzed by DNA methyltransferase (MTase) enzymes. They transfer a methyl group at a specific DNA motif.**

  Examples:
  - **the Dam Mtase in *E. coli* targets the 5'-GATC-3' motif**
  - **The CcrM Mtase in *Caulobacter* targets the 5'-GANTC-3' motif**

- **A very large diversity of Mtases with their DNA binding specificity exists in the bacterial kingdom**

  Blow *et al.* (PLOS Genet 2016) *The Epigenomic Landscape of Prokaryotes*

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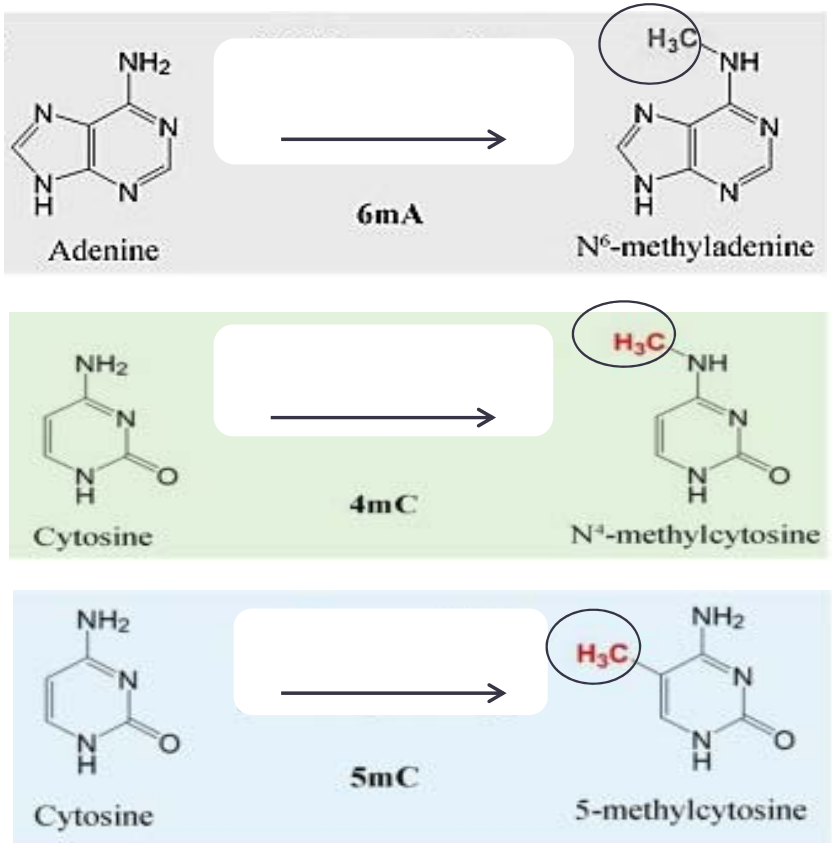
DNA methylation in bacteria

- **Methylated DNA in prokaryotic genomes is usually found in the forms of 6mA (6-methyladenine), 4mC (4-methylcytosine) and 5mC (5-methylcytosine)**



Murray *et al.* (Nucleic Acids Res 2012)
The methylomes of six bacteria

Exocyclic Mtases

methylates exocyclic amino nitrogen
6mA, 4mC

Endocyclic Mtases

methylates pyrimidine ring carbon
5mC

Detection of DNA methylation patterns

- **Restriction pattern**
  - Based on the capacity of some restriction enzymes to digest or not DNA according to the methylation state of the restriction site.

    However, a cumbersome methodology

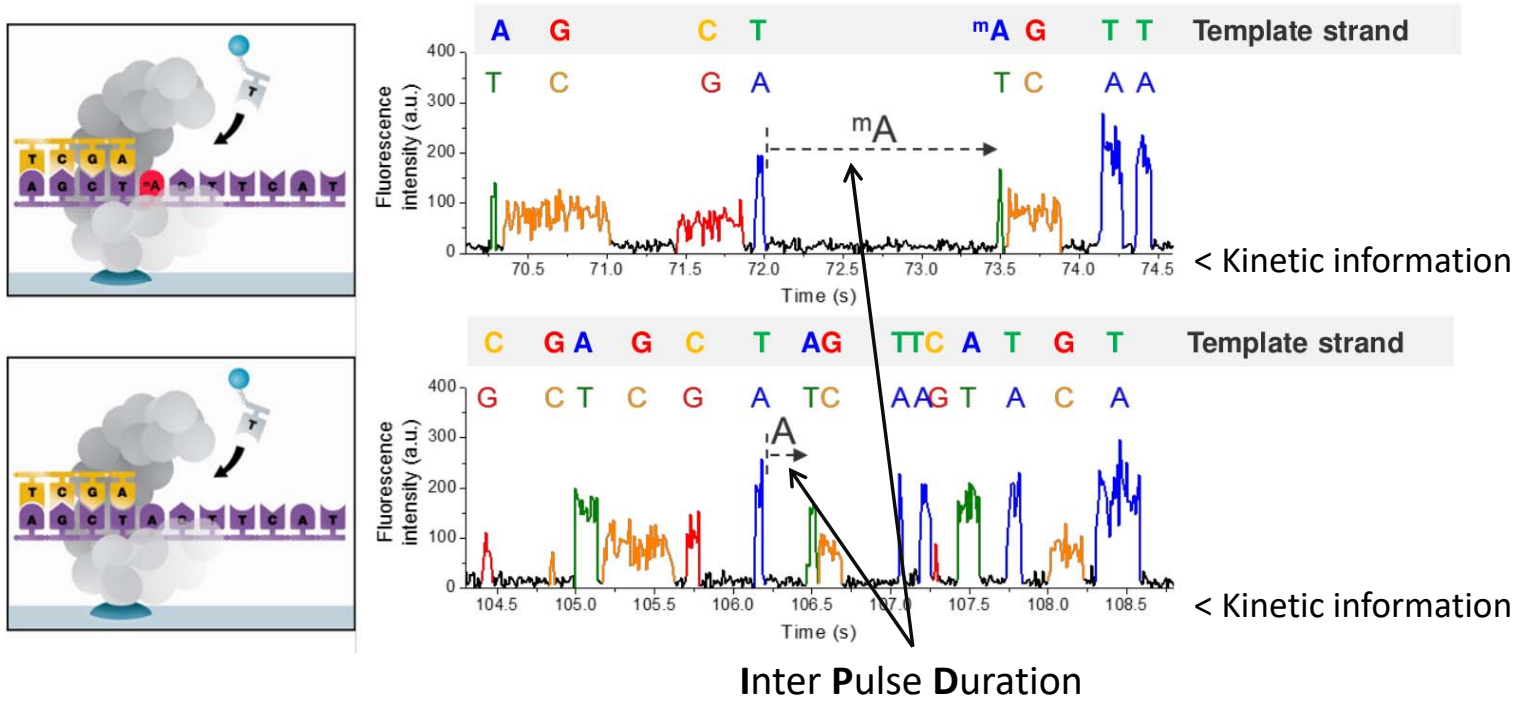
- **Bisulfite treatment**
  - Treatment of DNA samples with bisulfite, which converts unmodified cytosine to uracil, and various sequencing technologies.

    However, detect only 5mC modifications

- **Single Molecule Real Time (SMRT)**
  - A sensitive methodology allowing the detection of several methylation types (6mA, 4mC, 5mC), without previous reaction.
  - Technologies : PacBio RSII (PacBio Sequel?)

Detection of DNA base modifications with PacBio RSII

Example: N^6-methyladenine



IPD ratio = obs IPD / unmodified IPD

Flusberg et al. (2010) Nature Methods 7: 461-465

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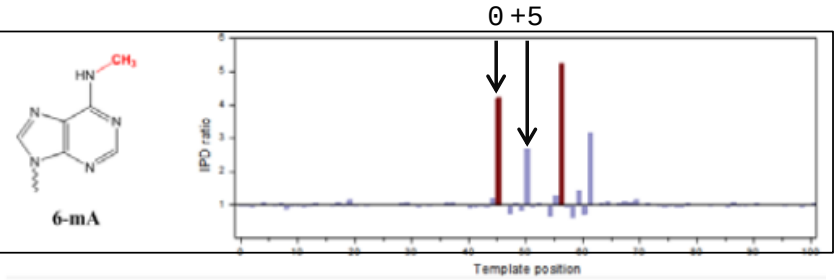
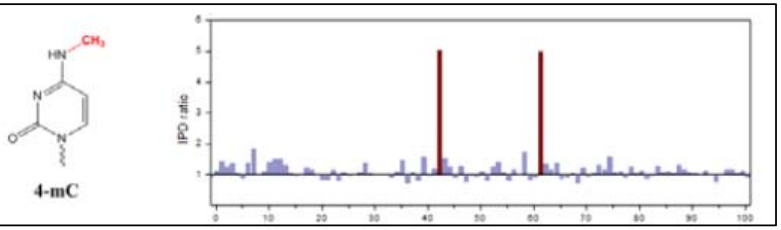
DNA base modifications signal

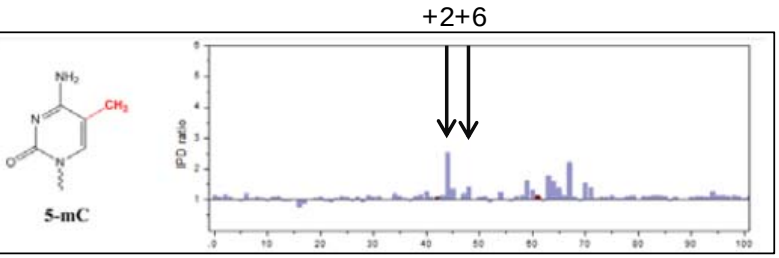
6mA
Signature positions: 0, +5
Strong kinetic signal
Min coverage by strand: **25X**



4mC
Signature position: 0
Weak to strong kinetic signal
Min coverage by strand : **25X**



5mC
Signature positions: +2, +6
Very weak kinetic signal
Min coverage by strand: **250X**

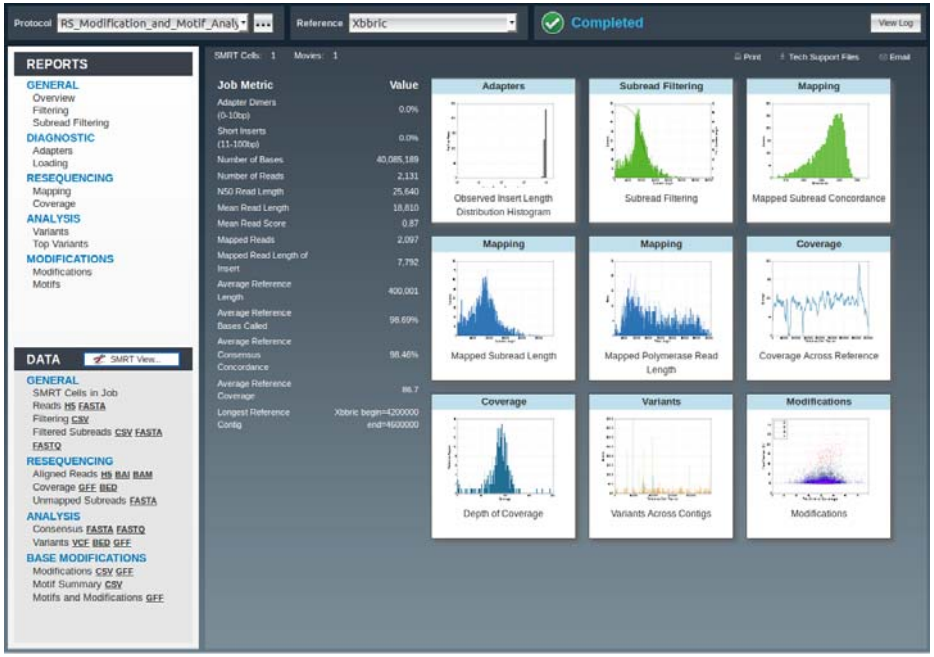


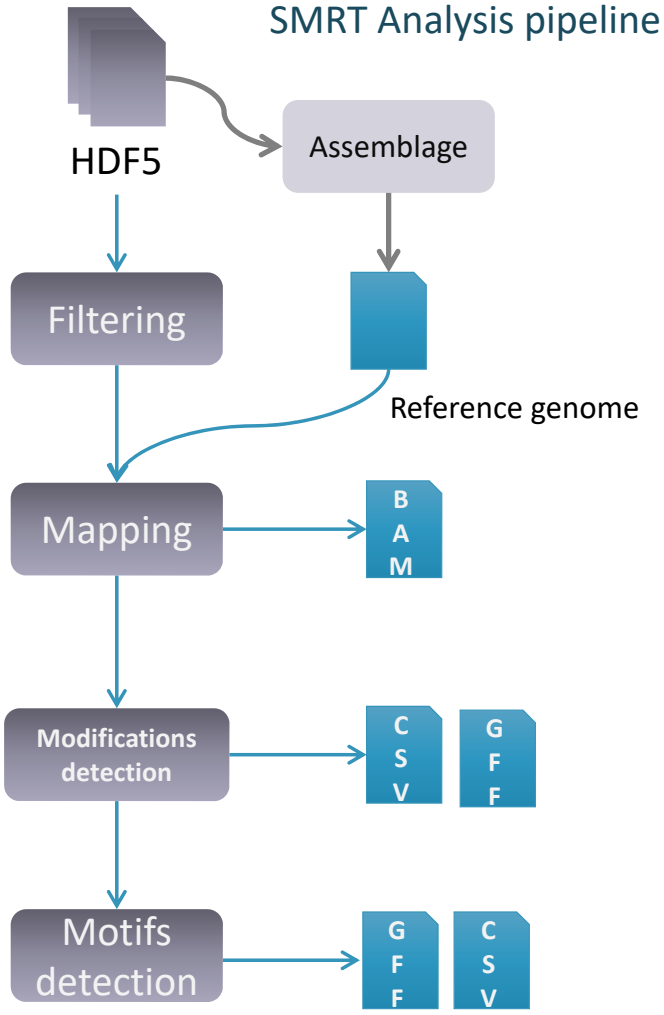
Recommendations

- Good and close genome reference or de novo assembly with PacBio
- >=100x average coverage
- No PCR, amplification remove methylations

Bioinformatics

- **User friendly interface “SMRT Analysis”**
- **Pipeline freely provided by PacBio**
- **Fast , ~8h on Ralstonia**





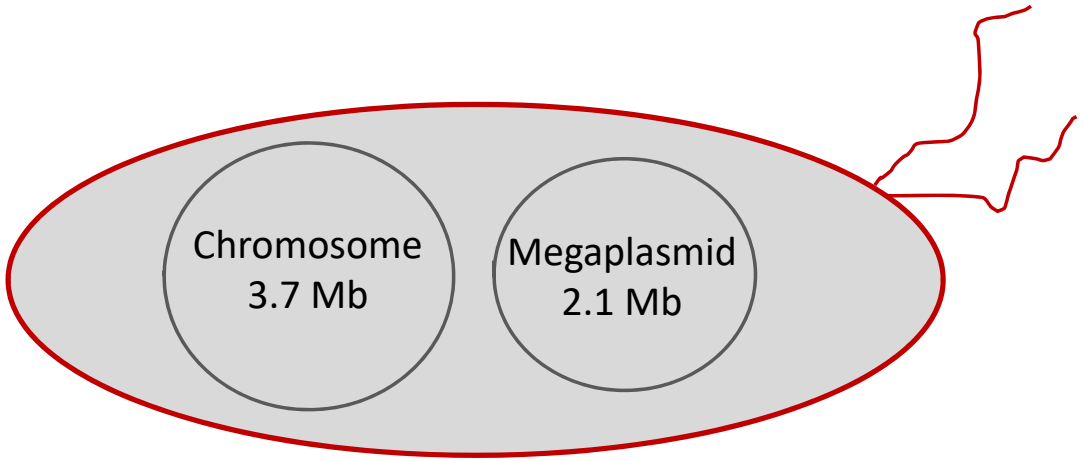
PROJECT: Compare the methylome profiles between strains of the plant pathogenic bacteria *Ralstonia solanacearum*

Using the PacBio RSII machine

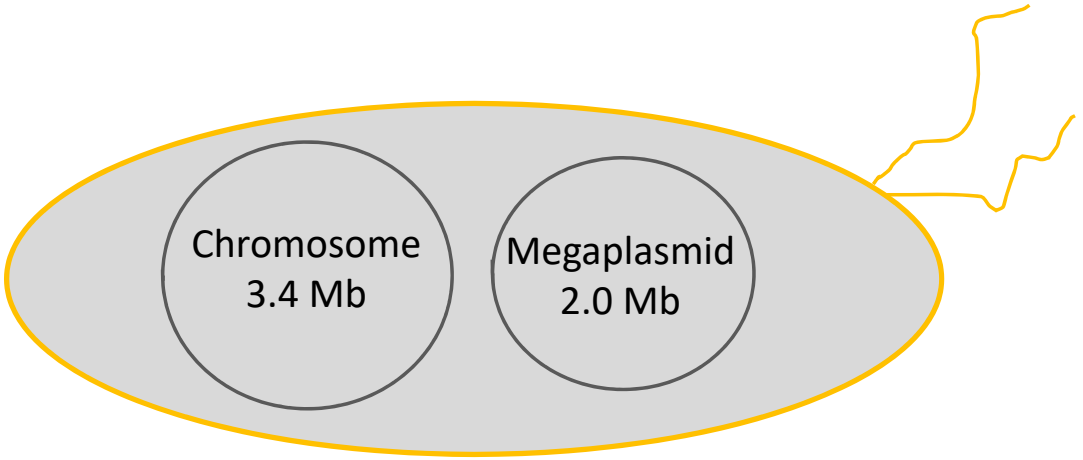
- *R. solanacearum* is the agent of the bacterial wilt disease on more than 200 plant species
- The emergence of new strains, more agressive or virulent on novel hosts, is continuously reported in the field
- **Is there differential methylation marks between strains adapted to different host plants?**

1. Comparison of the methylomes of two wild-type strains

- **GMI1000**
  - **Isolated from Tomato**
  - **Genome: 5.8 Mb**
  - **G+C content: 67%**

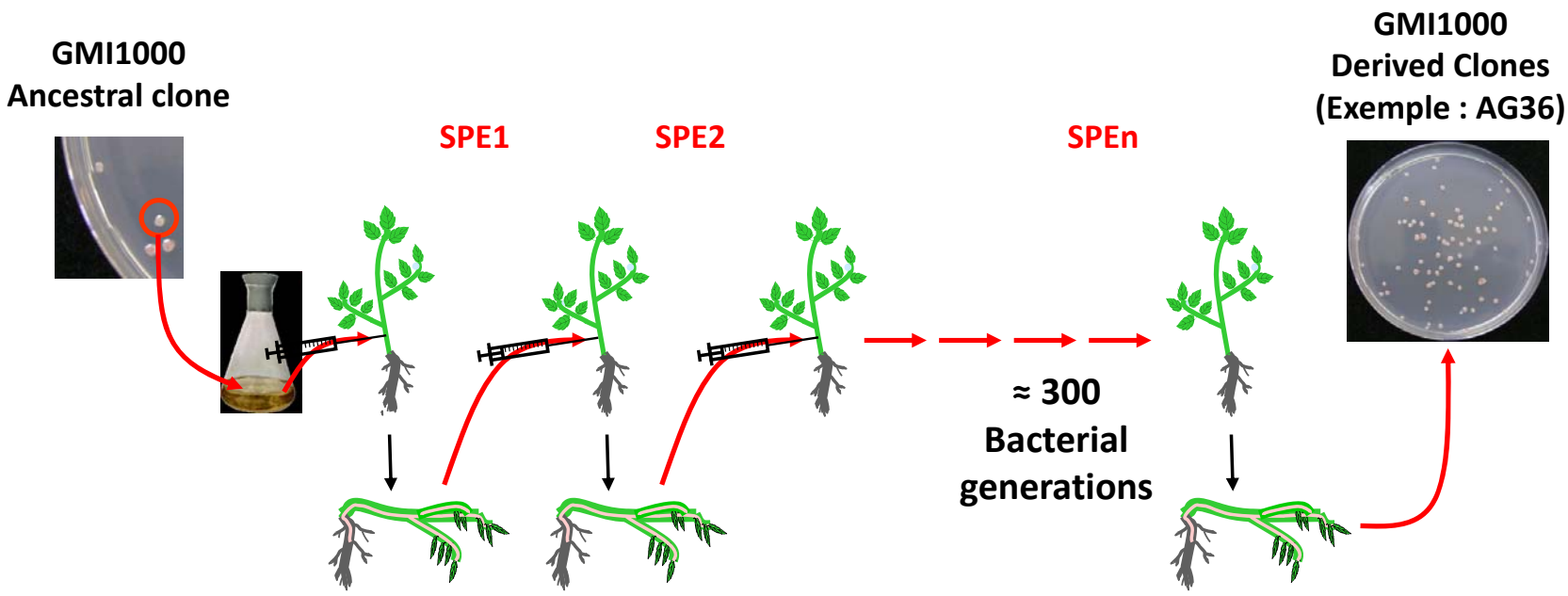


- **UY031**
  - **Isolated from Potato**
  - **Genome: 5.4 Mb**
  - **G+C content: 67%**



2. Comparison of the methylomes of an ancestral clone and its experimentally derived clone

Serial Passage Experiment (SPE) on a given host

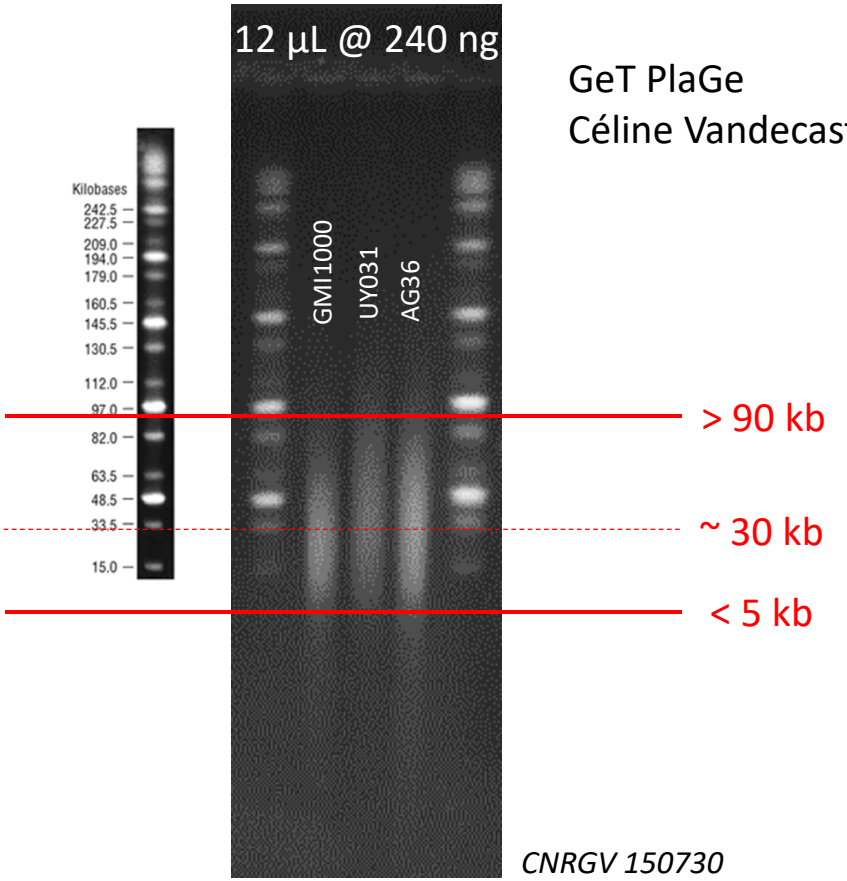


DNA preparation for PacBio sequencing

- **Extraction of high-molecular-weight genomic DNA for long-read sequencing of single molecules**
- **Use of a specific DNA extraction Protocol optimized for PacBio sequencing**

(Mayjonade et al., 2016)

Input gDNA pattern- Pulsed field



GeT PlaGe
Céline Vandecasteele

CNRGV 150730

PacBio sequencing output for three *R. solanacearum* strains

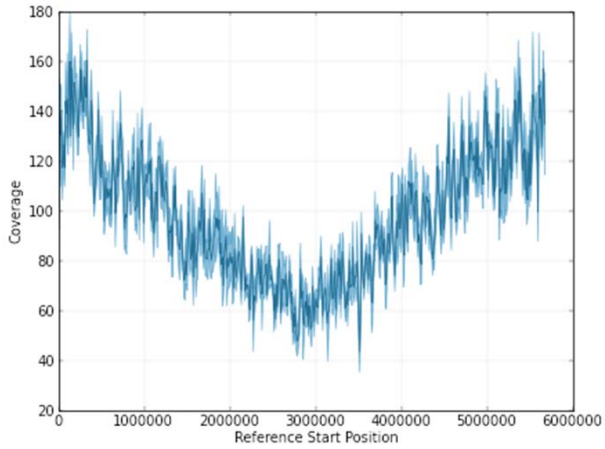
- **UY031 strain : 1 SMRT cell**
  - genome coverage **130x**

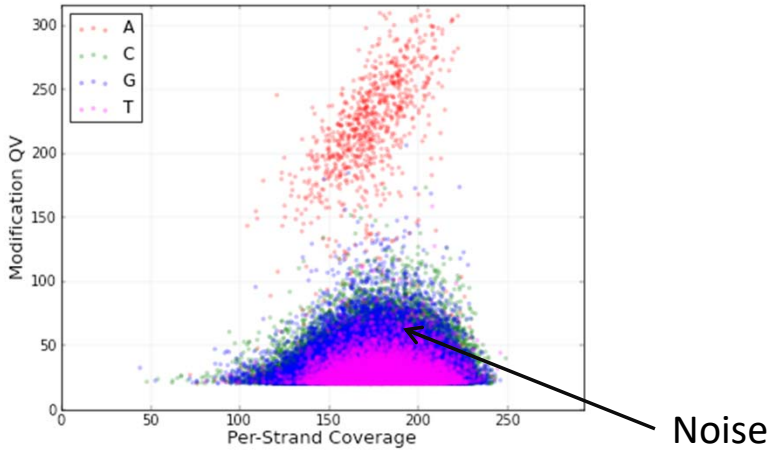
  GMI1000 : 3 SMRT cells
  - genome coverage **416x**

  GMI1000-derived (AG36) : 2 SMRT cells
  - genome coverage **321x**

- **No homogeneous coverage**
  (DNA extracted from bacterial dividing cells)

- **Cytosine bases drowned in noise (G and T)**
- **Good evidences for Adenine bases**





Total numbers of methylated marks in *R. solanacearum* genomes

Modification Type	UY031	GMI1000
4mC	1,293	18,915
6mA	3,162	1,659
Not determined	18,094	205,005
All	**22,732**	**229,207**

- **The different numbers of methylated marks between both strains correlate with the difference in average sequencing coverage**
- **5mC and 4mC methylated marks cannot be distinguished**

Identification of methylation motifs in *R. solanacearum*

- **Use REBASE to validate methyltransferase motifs**

  http://rebase.neb.com/rebase/rebase.html

- **Methylated DNA Motifs identified:**
  - Two 4mC motifs (C**C**CAKNAVCR and YG**C**CGGCRY) only in GMI1000 genome
  - One 6mA motif (CAACR**A**C) only in UY031 genome
  - One 6mA motif (GTWW**A**C) in both GMI1000 and UY031 genomes

- **Bisulfite treatment / Illumina sequencing revealed that the YG**C**CGGCRY is a 5mC modification!**

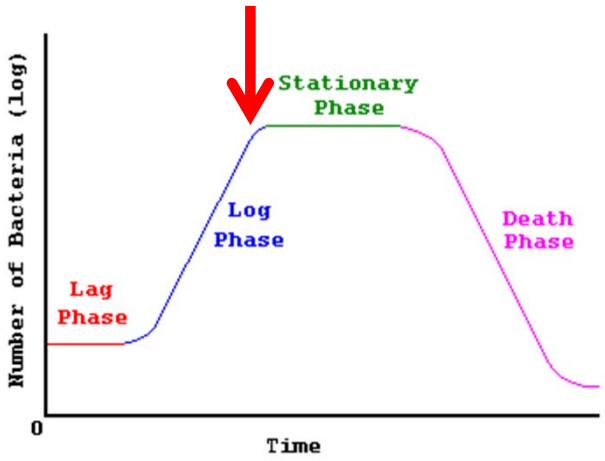
Comparison of the methylomes of GMI1000 ancestral and derived clones

- **GMI1000 ancestral clone:**
- ✓ **6mA:** 186 specific sites
- ✓ **4mC:** 3,864 specific sites

- **GMI1000 derived clone:**
- ✓ **6mA:** 56 specific sites
- ✓ **4mC:** 1,214 specific sites

CONCLUSIONS

- PacBio RSII is a powerful technology to detect 6mA and 4mC modifications in bacteral genomes
- However, for 5mC modification, bisulfite treatment / Illumina sequencing is more appropriate
- Differential methylation marks exist between strains of *R. solanacearum*
- To ensure an homogeneous coverage, DNA must be extracted from bacterial cultures at the end of the exponential growth phase (non-dividing cells)



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