

## Background

Bacteria can utilize DNA methylation to regulate gene expression, yet this phenomenon is poorly understood due to a lack of tools for determining genome-wide methylation status with single nucleotide resolution. Advances in single molecule real time sequencing are now enabling simultaneous detection of sequence and methylation state of DNA, providing the opportunity to explore methylation and gene expression dynamics in parallel. Here we harness the power of Pacific Biosciences' sequencing technology to uncover N6-methyladenosine patterns in *Shewanella oneidensis* MR-1. When grown in minimal media, we found approximately 18,000 sites of N6-methylated adenosine, of which 99.8% were found in one of five motifs. The two most abundant motifs were GmATC and ATCGmAT, which are likely targeted by predicted methylases in the chromosome and plasmid, respectively. The remaining motifs have no matches to any known methylase or restriction enzymes recognition sequences. Interestingly, *S. oneidensis* does encode two putative Type I and three putative Type II methylases with no known target sequences. We are now attempting to determine the target motifs of these putative methylases with additional methylation-detection sequencing of knockout mutants. To study the dynamics of methylation in *S. oneidensis*, we have generated additional data from cells grown under rich media conditions (LB). Preliminary analyses reveal multiple sites that are differentially methylated under minimal and rich media. The exploration of the relationship between differential methylation and gene expression under these conditions is on-going.

## Study Organism

*Shewanella oneidensis* as a test bed for methylome investigations

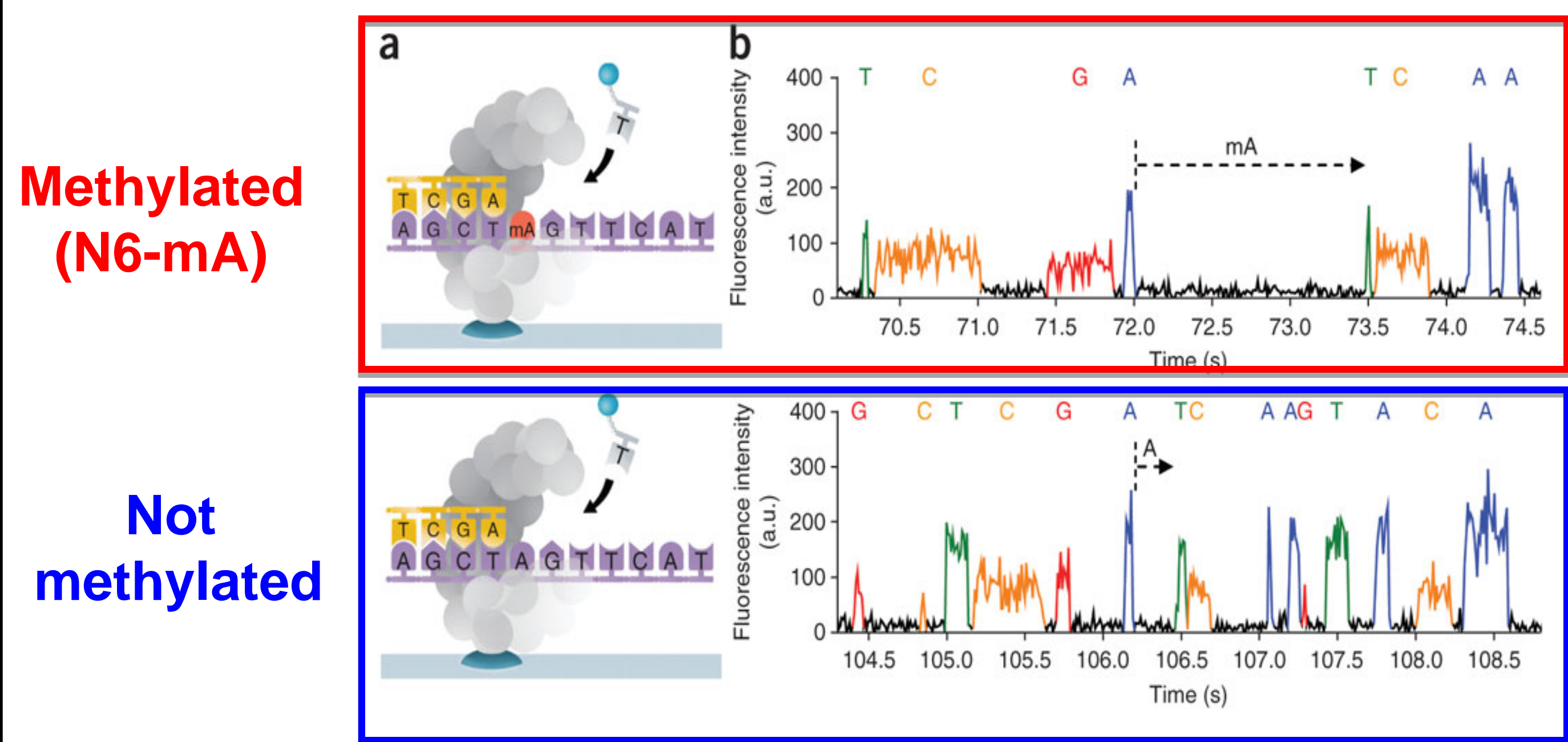


PNNL

- Y-Proteobacterium in sediments
- Utilizes variety of e- acceptors: O<sub>2</sub>, NO<sub>3</sub>, SO<sub>3</sub>, Fe, Mn, U, Cr, organics, etc.
- Contributes to bioremediation

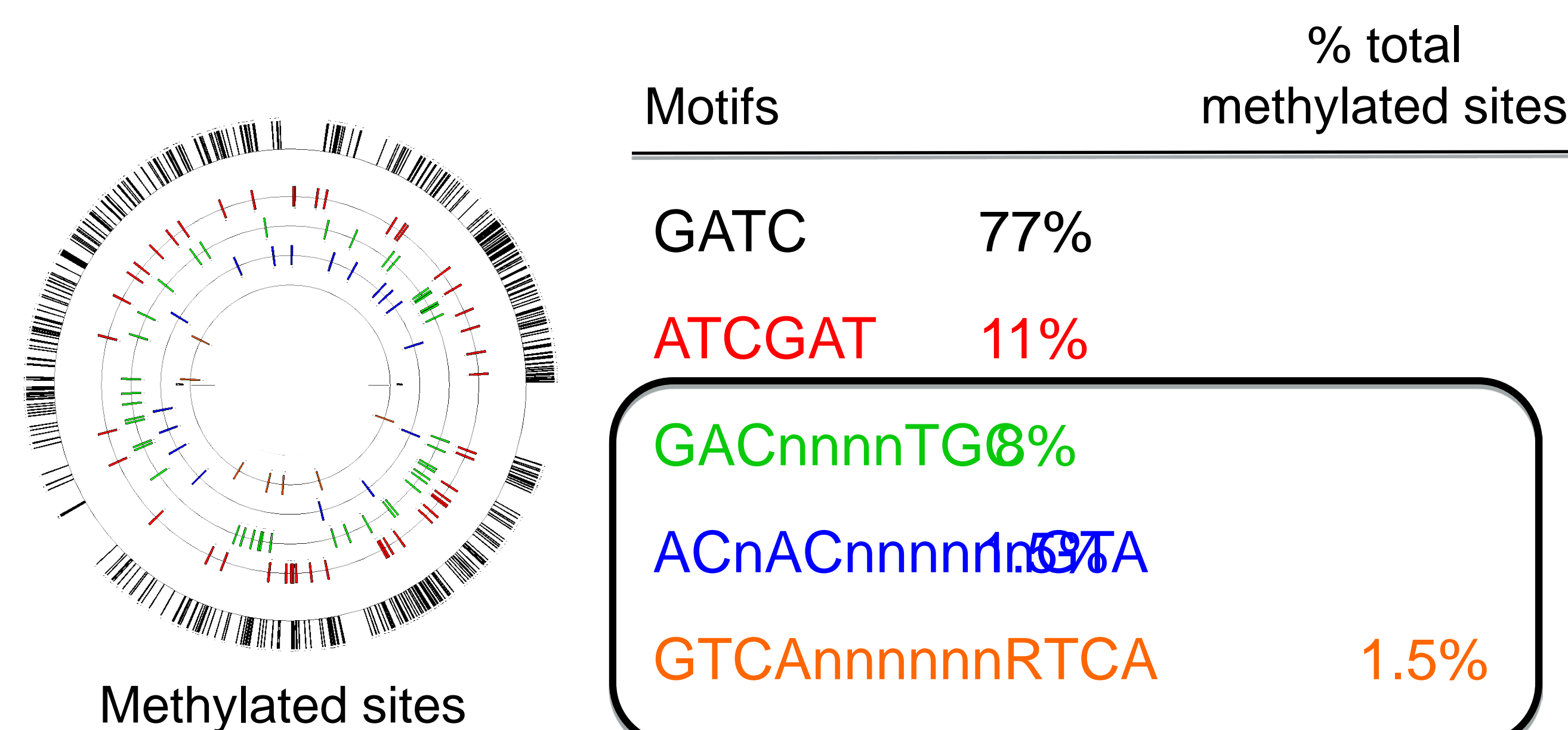
## Methylation Detection

PacBio can identify methylated bases in DNA



Flusberg et al. 2010

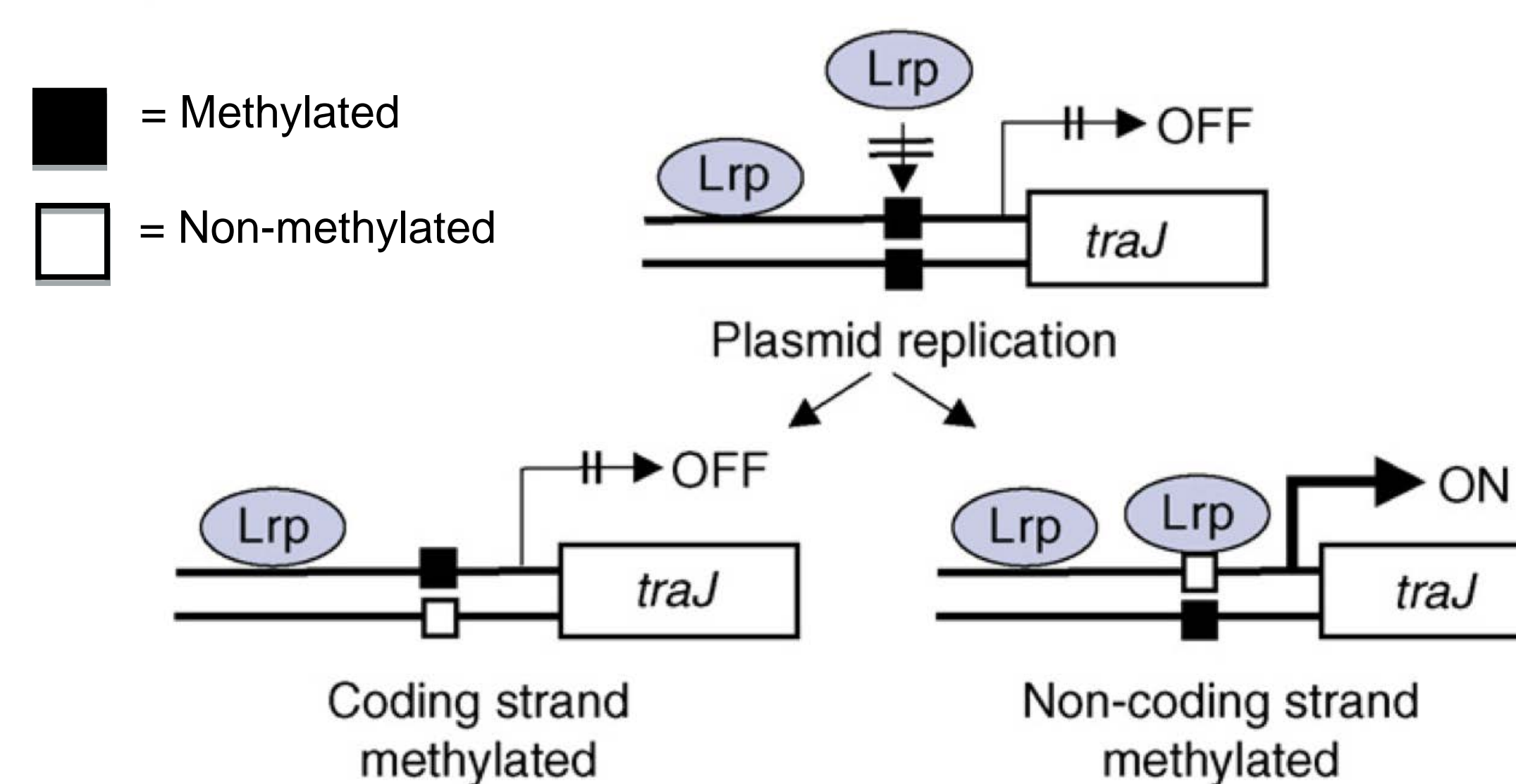
~18,000 methylated A's ... all belong to one of five motifs



Methylated sites

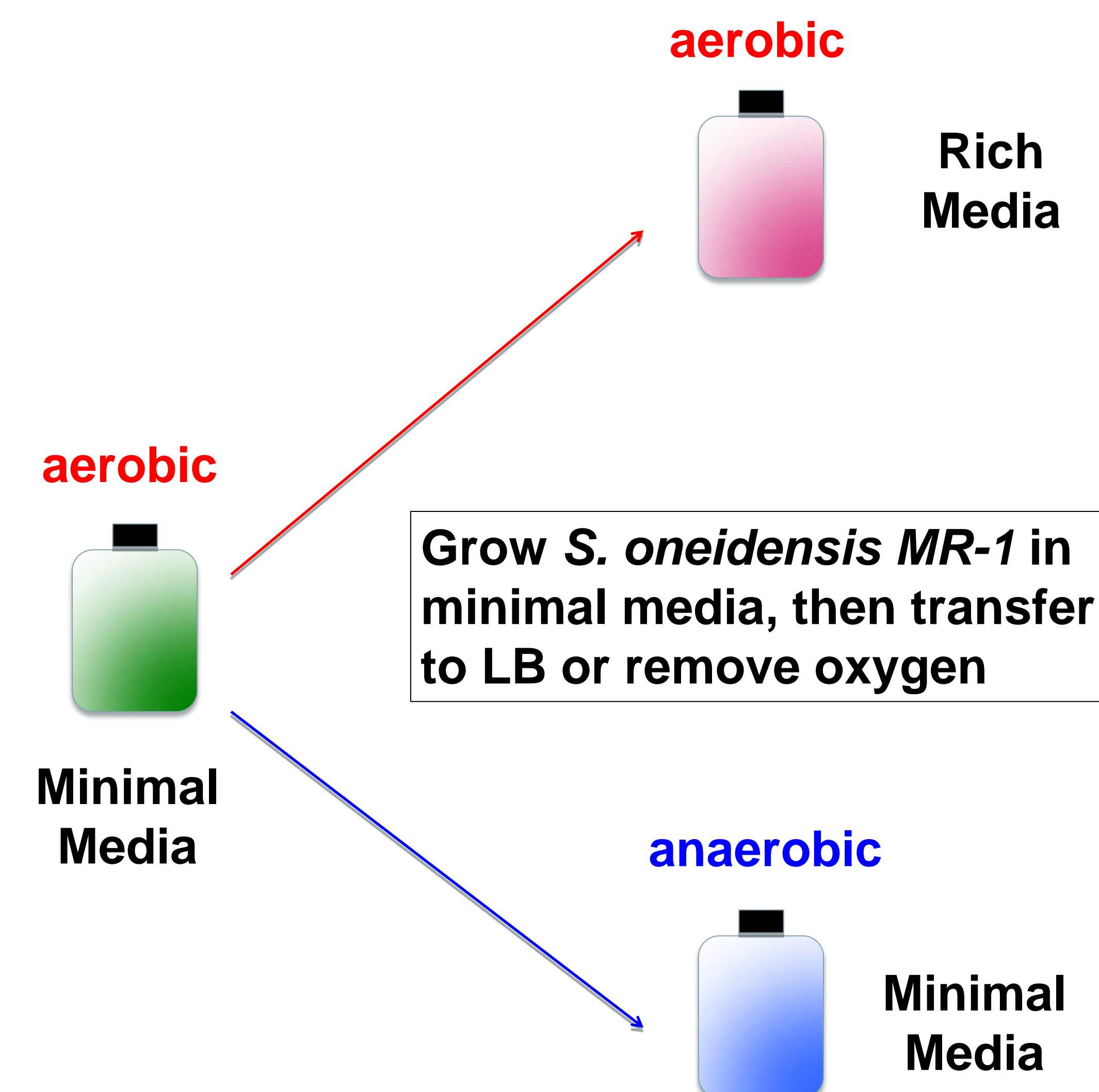
Discovery of previously unknown methylation motifs

Methylation can regulate gene expression

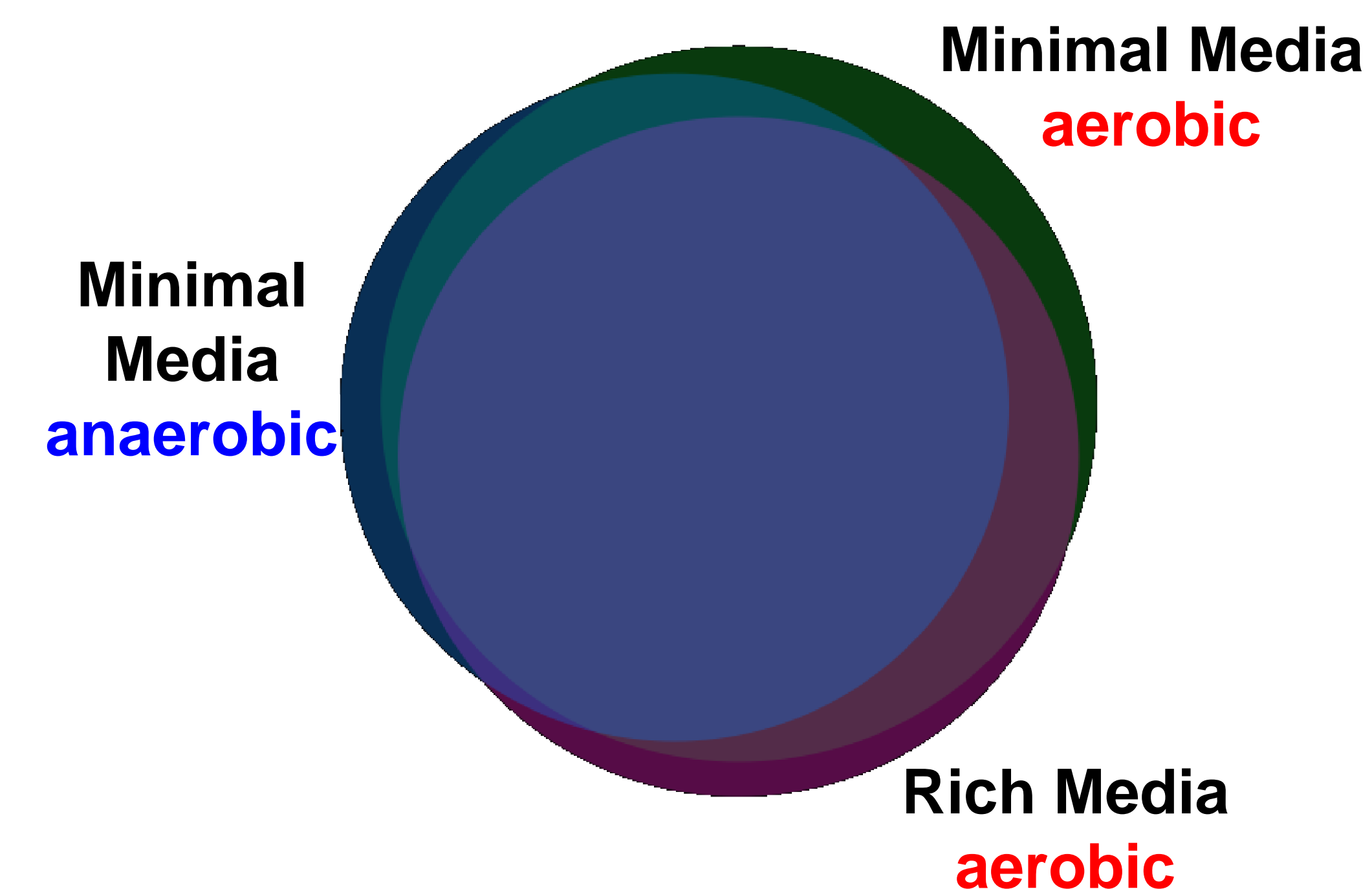


Low & Casadesus 2008

## Methylation Dynamics



Methylation patterns vary under different culture conditions



How does gene expression correlate with methylation dynamics?

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- 2) Pacific Biosciences; Menlo Park, CA
- 3) Lawrence Berkeley National Laboratory; Berkeley, CA