

Methylation Pattern Detection in the Genome of *Bacillus pumilus* Strain SAFR-032

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Bacillus pumilus

SAFR-032



Photo by: USRA Innovation Laboratory, Joey Varelas and Jordan McKaig

- Model Organism
- Endospore-forming bacteria
- Spores are extremely resistant to extreme conditions:
 - Radiation
 - Desiccation
 - Hydrogen peroxide
 - Extreme temperatures
 - Low pressure
 - Simulated Mars environment conditions

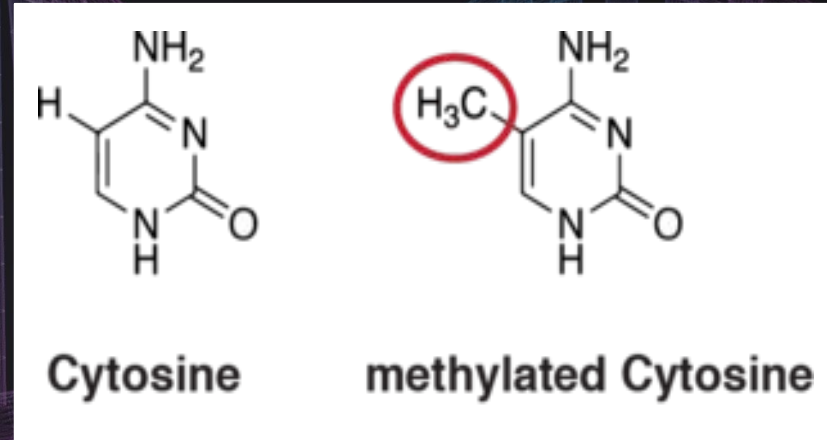
EXPOSE-E Mission: *B. pumilus* SAFR-032



Berger, T., Hajek, M., Bilski, P., Koerner, C., Vanhavere, F., & Reitz, G.S. (2012). Cosmic radiation exposure of biological test systems during the EXPOSE-E mission. *Astrobiology*, 12 5, 387-92 .

Methylation

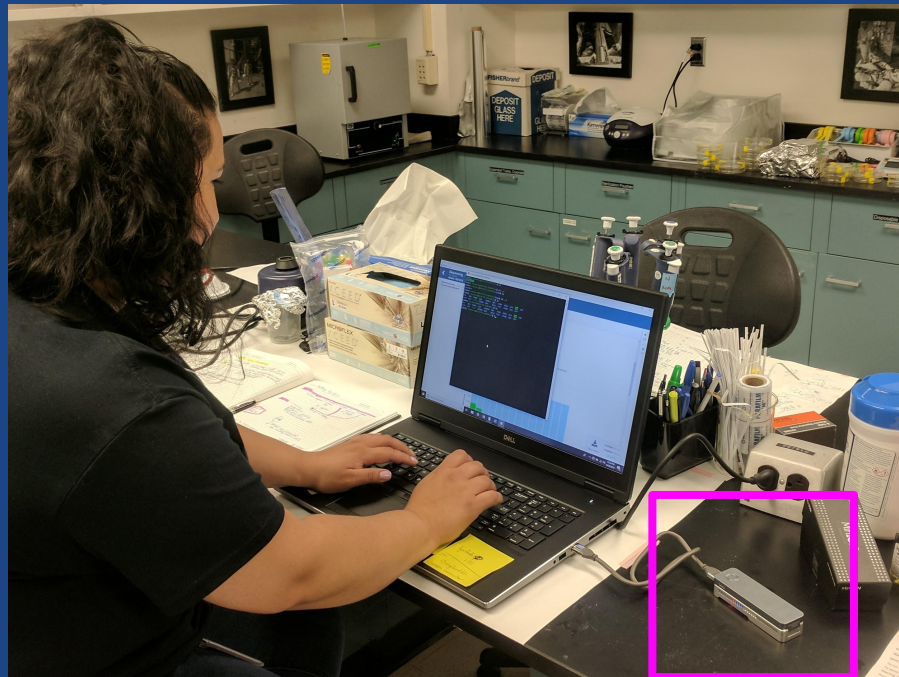
- Addition of methyl groups to DNA
- Heritable changes without changing the primary sequence
- Adenosine (A) and Cytosine (C) are commonly methylated nucleotides
 - *Bacillus* spp. have Dcm and Dam: methyltransferases that modify C's and A's, respectively



Google Image: https://en.wikipedia.org/wiki/DNA_methylation

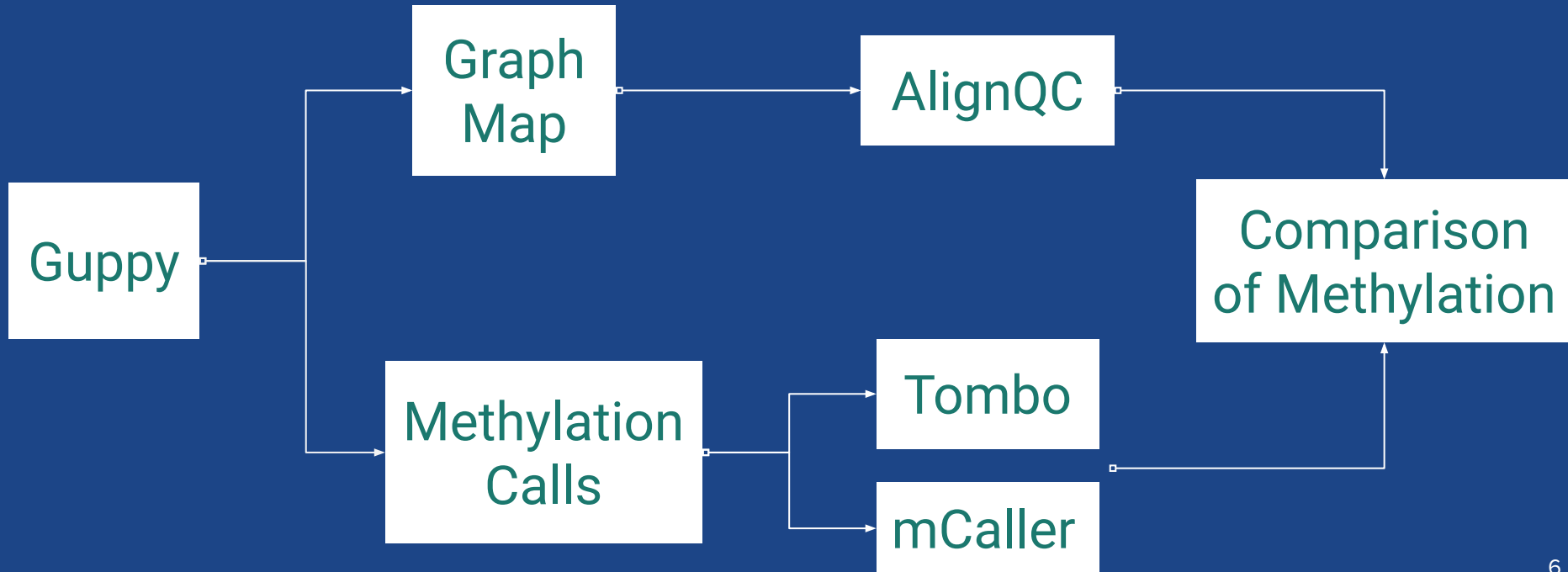
Nanopore MinION

- Portable protein nanopore sequencing USB device for DNA and RNA sequences
- Real-time device
- 10-30 Gbp of DNA sequence data
- Long read lengths of DNA



Picture taken by: Dr. Waters

Goals: Implementation of MinION Processing Pipeline



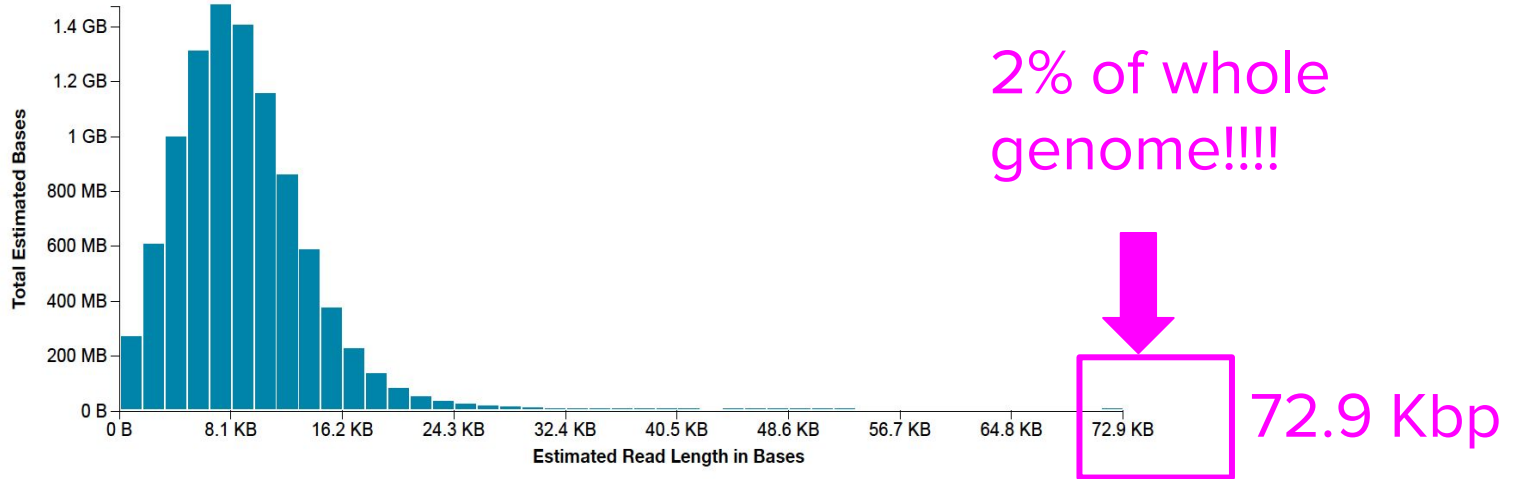
Sequences Generated with MinION

| ISS-Flown | Non-ISS-Flown |
|-----------|---------------|
| Run 1 | Run 1 |
| Run 2 | Run 2 |
| Run 3 | Run 3 |

MinION Reads

Read Length Histogram

Summary read length distribution



Bacillus pumilus SAFR-032 whole genome is about 4 Mbp

BLAST

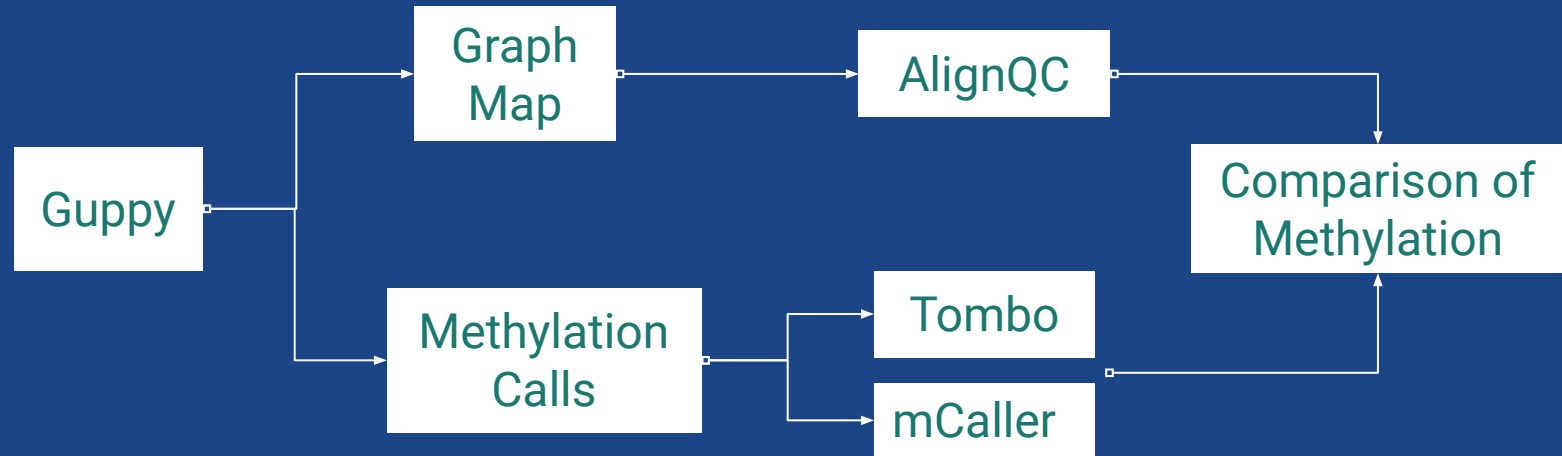
| Organism | Blast Name | Score | Number of Hits | Description |
|---|----------------------------|-------|-------------------|--|
| Bacillus | firmicutes | | 4 | |
| .Bacillus pumilus | firmicutes | | 3 | |
| ..Bacillus pumilus SAFR-032 | firmicutes | 31527 | 1 | Bacillus pumilus SAFR-032 hits |
| .Bacillus pumilus | firmicutes | 24554 | 3 | Bacillus pumilus hits |

Objectives Completed

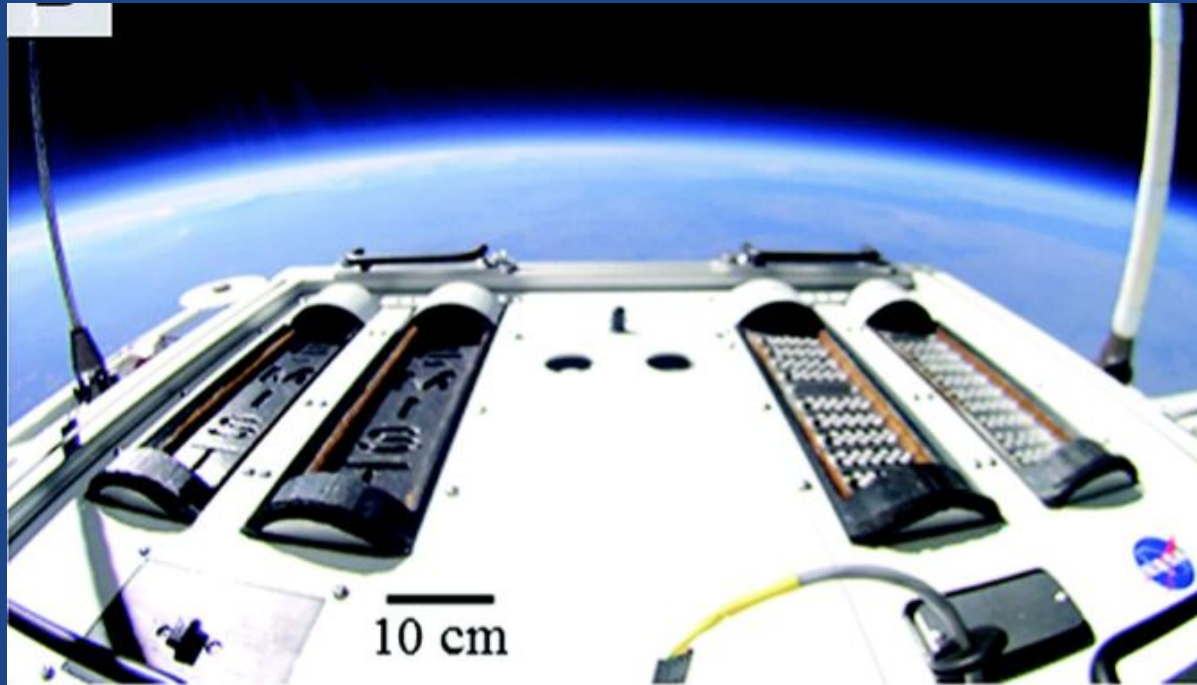
- Creating a bioinformatic pipeline for analyzing MinION reads and determining methylation points
 - Installed and tested with raw reads:
 - Guppy
 - Tombo
 - Graphmap (JM)
 - Samtools(JM)
 - AlignQC(JM)
 - Still resolving: nanopolish and mCaller

Future Work

Running all of the data through the full pipeline for detecting methylation patterns of the *B. pumilus* SAFR-032 genome.



It is important to look at the genomic changes of organisms as a result of spaceflight stressors.



Khodadad, C. L., Wong, G. M., James, L. M., Thakrar, P. J., Lane, M. A., Catechis, J. A., & Smith, D. J. (2017). Stratosphere conditions inactivate bacterial endospores from a Mars spacecraft assembly facility. *Astrobiology*, 17(4), 337-350.

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Venkateswaran



Questions??

References:

- Berger, T., Hajek, M., Bilski, P., Koerner, C., Vanhavere, F., & Reitz, G.S. (2012). Cosmic radiation exposure of biological test systems during the EXPOSE-E mission. *Astrobiology*, 12 5, 387-92 .
- Khodadad, C. L., Wong, G. M., James, L. M., Thakrar, P. J., Lane, M. A., Catechis, J. A., & Smith, D. J. (2017). Stratosphere conditions inactivate bacterial endospores from a Mars spacecraft assembly facility. *Astrobiology*, 17(4), 337-350.
- Tirumalai, M. R., Rastogi, R., Zamani, N., Williams, E. O., Allen, S., Diouf, F., . . . Fox, G. E. (2013). Candidate Genes That May Be Responsible for the Unusual Resistances Exhibited by *Bacillus pumilus* SAFR-032 Spores. *PLoS ONE*,8(6). doi:10.1371/journal.pone.0066012

Image References:

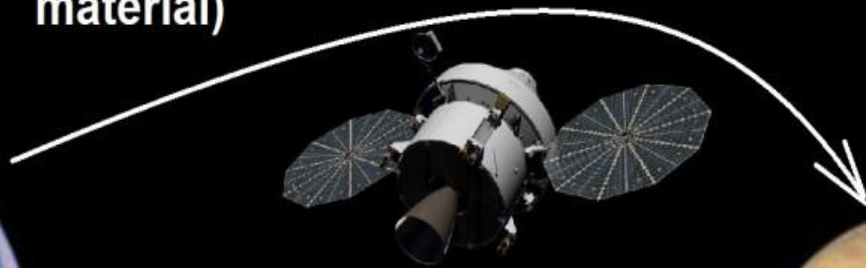
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- <https://www.inverse.com/article/34978-nasa-planetary-protection-officer-is-not-defense-against-aliens>
- <http://www.spacesafetymagazine.com/space-exploration/extraterrestrial-life/>
- http://www.esa.int/Our_Activities/Space_Engineering_Technology/Planetary_protection_preventing_microbes_hitchhiking_to_space
- https://en.wikipedia.org/wiki/DNA_methylation
- <https://time.com/5569057/three-parent-baby-dna/>

Planetary Protection

- Office of Safety and Mission Assurance (OSMA)
- Preventing any contamination or transfer of life from Earth to other planets and vice-versa during space exploration
- False Positives
- Need to accommodate regulations for space explorations



Forward contamination (terrestrial material)



Backward contamination (extraterrestrial material)



Pipeline

MinION

Fast5

Guppy

Tombo

AlignQC

- DNA sequencing device
- Raw reads
- Fast5 Multiple files
- Multiple files to single files
- Raw information
- Voltage
- Base calls
- Converts Single Fast5 files into Fastq files
- Annotate
- Resquiggle
- Methylation detection
- Graphs and Plots
- Quality control
- Filtering
- Comparing to Reference genome