A New Deployment of the Burrows-Wheeler Transform to Improve DNA Methylation Analysis

Ricardo Olanda Rodríguez
Mariano Pérez Martínez
Juan M. Orduña Huertas
César González Segura

Dpto. de Informática, Universidad de Valencia
Juan.orduna@uv.es

GOBIERNO DE ESPAÑA
MINISTERIO DE ECONOMÍA, INDUSTRIA Y COMPETITIVIDAD

Escola Tècnica Superior d'Enginyeria

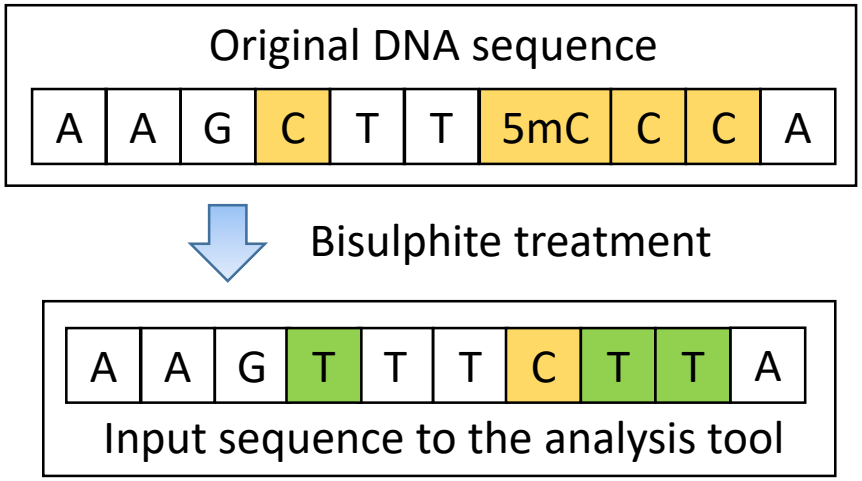
Introduction

- The introduction of Next Generation Sequencing (NGS) has increased the size and length of DNA samples.
- Current sequencers produce billions of short DNA samples, with lengths usually surpassing 400 nt[1].
- Upcoming sequencers will be able to generate samples with lengths up to thousands of nts, producing huge datasets as a result [2].

* [Illumina NextSeq 500 (https://research.ncsu.edu/gsl/files/2015/08/Illumina_NextSeq_500.jpg)]

DNA Methylation

- Methylation is a particular topic of DNA analysis, relevant in the study of cancer, diabetes and other diseases.
- Through bisulfite-treatment, the methylation status can be retrieved with base-pair resolution.



- Several methylation analysis tools have been developed[3,4,5,6], however their performance decreases with read lengths over 150 nts.

Methylation Analysis Pipeline

- Our approach is based on using two mapping algorithms, the Burrows Wheeler Transform[7] and the Smith & Waterman Algorithm[8].

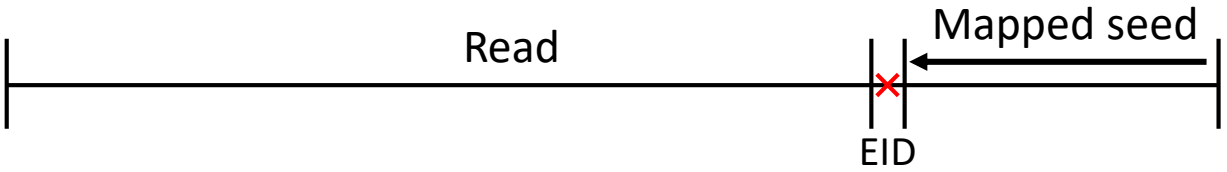
- In HPG-Methyl 2, we propose a new strategy to reduce the execution time and increase sensitivity even for long reads using two techniques:

  - A bidirectional BWT, which can discard wrong alignments more efficiently.

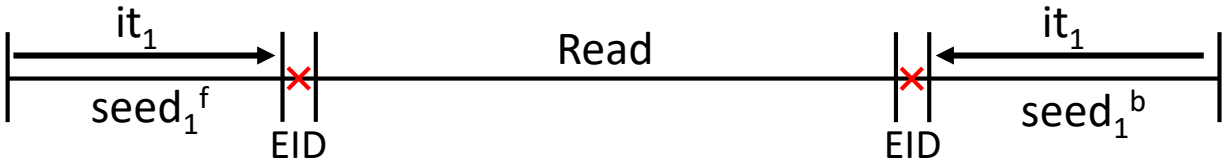
  - A new parallel pipeline scheme, reducing the number of invalid candidate regions and the execution time of the SWA.

New Implementation of the Burrows-Wheeler Transform

- The Burrows-Wheeler transform can be used as a backward search method, allowing errors, insertions or deletions (EIDs)[7].
- Unidirectional BWT performs a backward search, mapping a read segment to the reference genome base by base, until an EID is found.



- Bidirectional BWT performs the search from both ends simultaneously, doubling the maximum number of allowed EIDs.

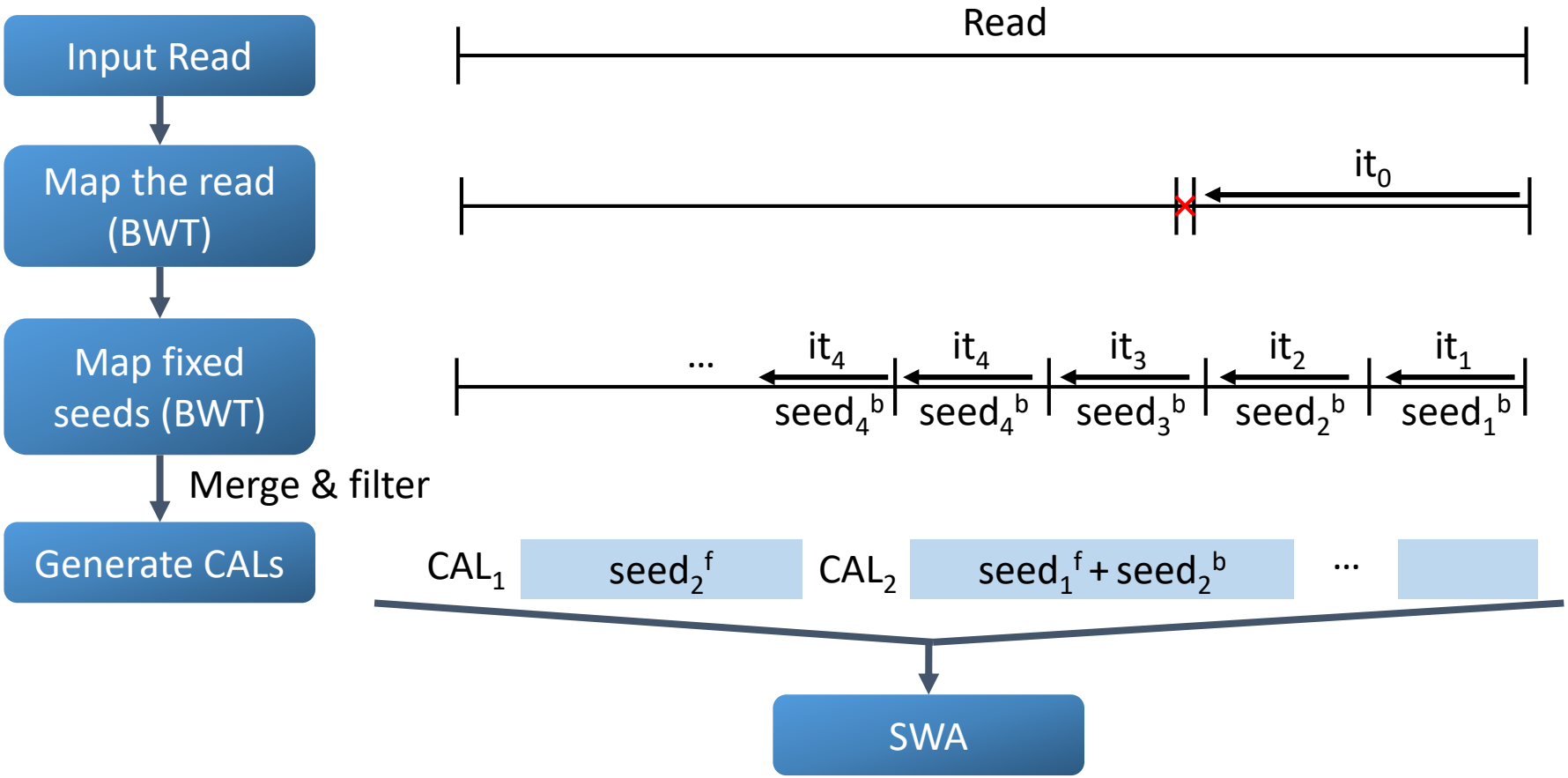


Implementation in a New Parallel Pipeline

- We have used HPG-Methyl[9], a parallel methylation analysis tool, as the basis to implement these new strategies.
- To take full advantage of the new BWT implementation, HPG-Methyl features several changes to the pipeline:
  - Several stages are merged into a single, more flexible stage.
  - This new stage produces fewer candidate regions (CALs), but much more effective.
  - As a result, use of the SWA algorithm is greatly reduced, improving execution times.

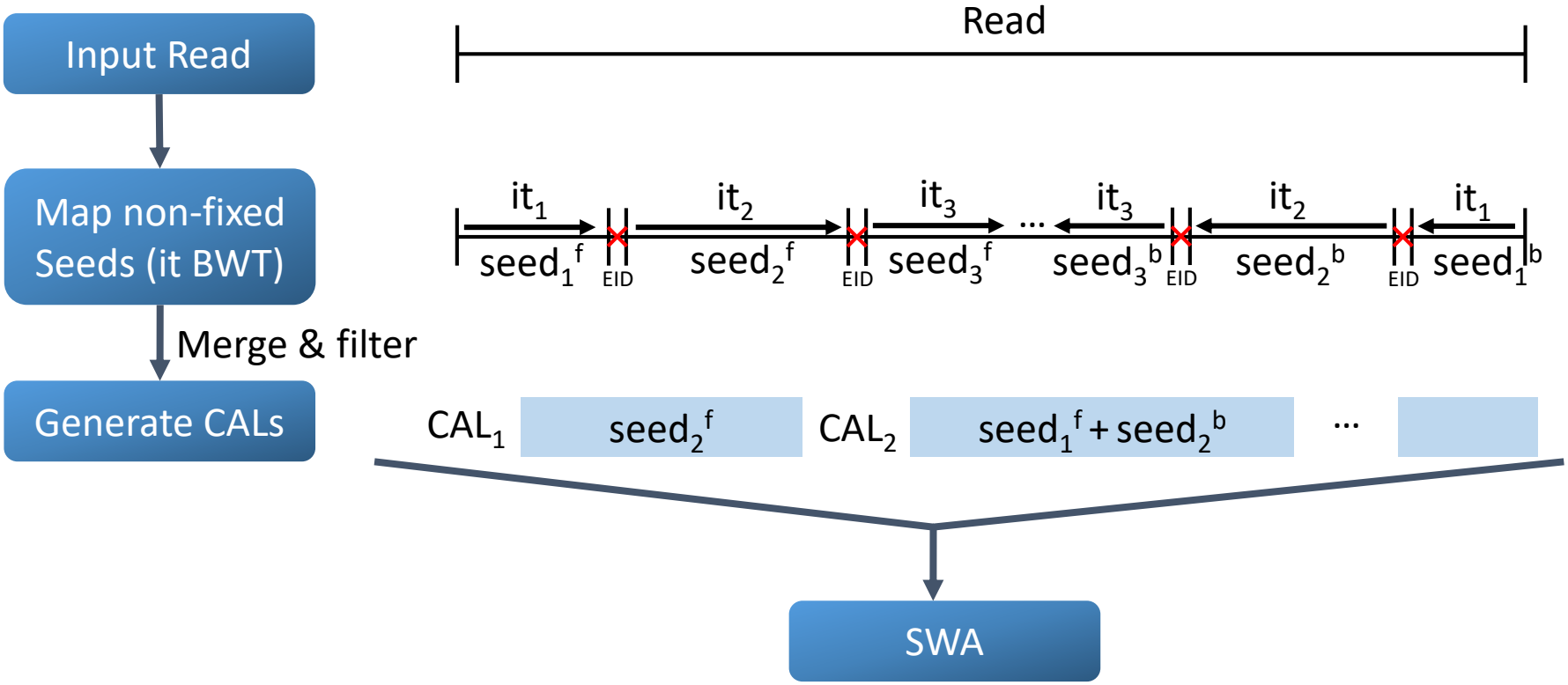
Implementation in a New Parallel Pipeline

- Stages of the original pipeline in HPG-Methyl.



Implementation in a New Parallel Pipeline

- Modified stages of the parallel pipeline in HPG-Methyl 2.



Performance Evaluation

The performance of HPG-Methyl 2 was compared with HPG-Methyl[9] and Bismark[3], using:

- Synthetic methylation datasets with read lengths between 75 nt and 3200 nt, with 4 million reads.
- Real methylation datasets from the European Nucleotide Archive[10].

Tests were conducted in a computer platform as in the table, measuring:

- Sensitivity.
- Execution time.

Computer Platform
Intel i7 3930K (6 cores @ 3,8 GHz)
48 GB RAM
SSD Storage
Ubuntu 14.04 LTS

Performance Evaluation: sensitivity

- HPG-Methyl 2 yields the most accurate results, with sensitivity rates over 99% in all cases.

Length (nt)	HPG-Methyl 2		HPG-Methyl		Bismark	
	Right	Wrong	Right	Wrong	Right	Wrong
75	**99,50%**	0,69%	93,37%	0,62%	88,30%	0,10%
150	**99,01%**	0,46%	96,87%	0,80%	94,59%	0,08%
400	**99,75%**	0,18%	97,55%	0,48%	97,55%	0,10%
800	**99,93%**	0,06%	96,94%	0,48%	98,45%	0,08%
1600	**99,75%**	0,06%	96,94%	0,48%	—*	—*
3200	**99,68%**	0,08%	96,42%	0,49%	—*	—*

* Aborted after waiting for 3 days (4320 minutes)

Performance Evaluation: execution time

- Our new implementation allows for a significant reduction in the execution time.
- Now HPG-Methyl scales with the increasing sequence lengths of NGS.

Length (nt)	HPG-Methyl 2	HPG-Methyl	Bismark
75	**1,288**	1,366	69,579
150	**1,550**	1,950	106,173
400	**5,041**	10,85	248,107
800	**11,26**	50,60	1246,89
1600	**34,44**	996,567	—*
3200	**164,593**	7733,38	—*

* Aborted after waiting for 3 days (4320 minutes)

Conclusions

- We have proposed a new strategy for methylation analysis, using a bidirectional BWT implementation and a new parallel pipeline.
- Our implementation reduces execution times by an order of magnitude and achieves sensitivity rates over 99%, even for very long reads.
- The software is open source and available in Github: `https://github.com/grev-uv/hpg-methyl`

Future Work

- This strategy can be exported to other methylation analysis tools, and to DNA and RNA analysis tools.
- We plan on applying the same strategy of bidirectional BWT to similar tools, such as HPG-Aligner.
- Also, HPG-Methyl will keep being updated with performance improvements and new features.

References

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