

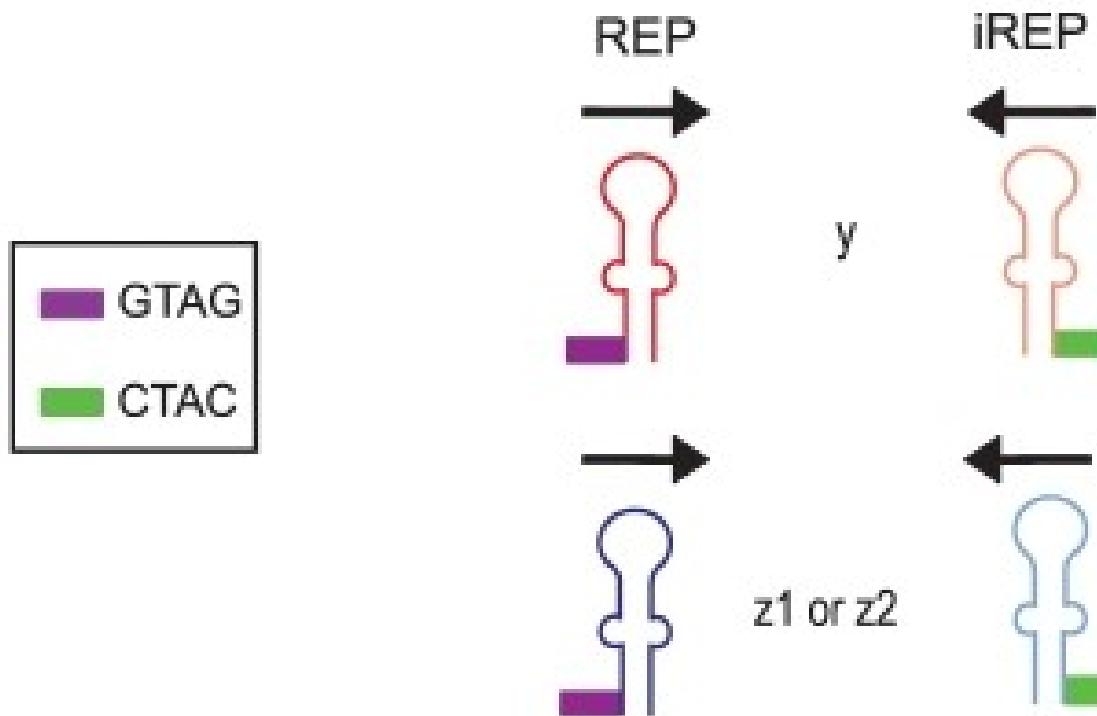
RNA-Seq study of
mRNA metabolism in operons:
role of BIME
in *Escherichia coli*.

Nicolas Jeanne
Juin 2015

Tuteurs : Yves Quentin
Roland Barriot
Rapporteur : Christine Gaspin

REP & BIME features

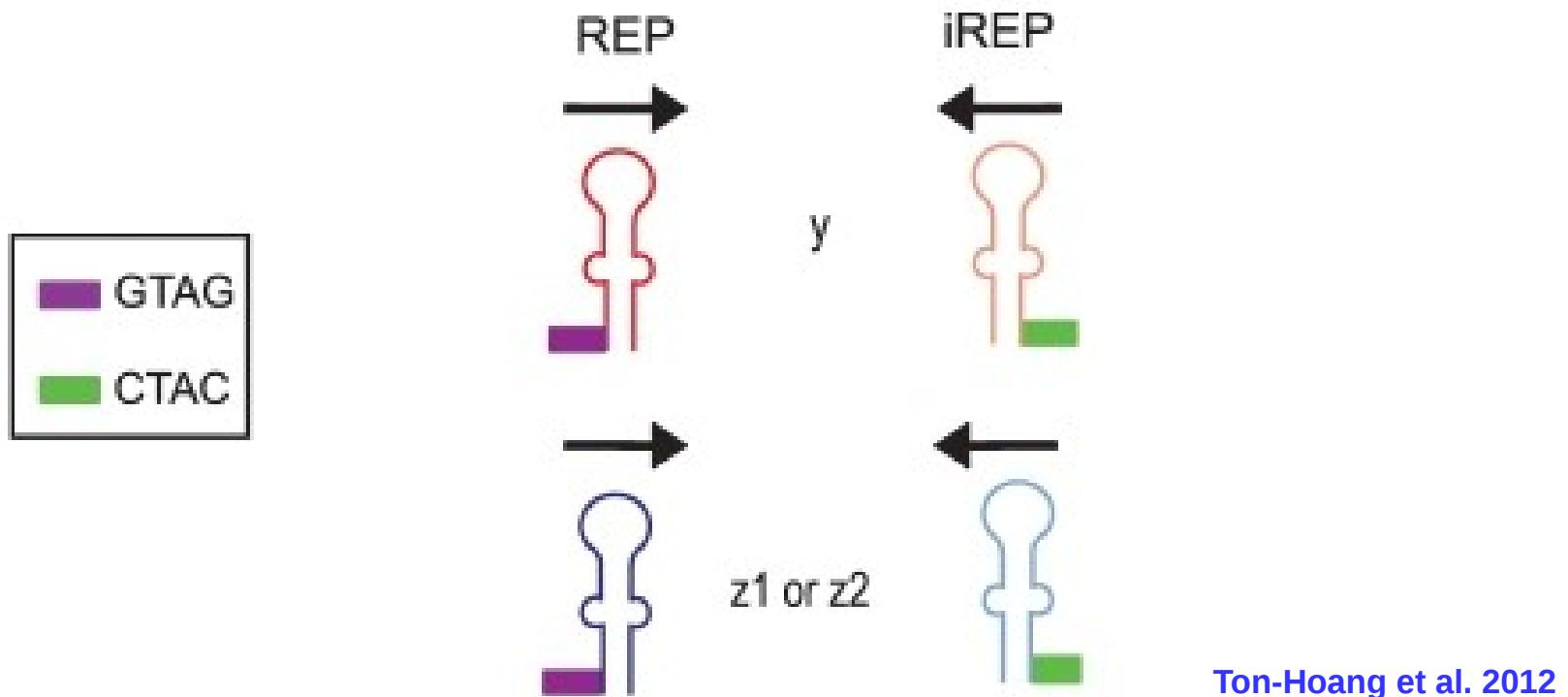
- Repeated Extragenic Palindrome



Ton-Hoang et al. 2012

REP & BIME features

- Repeated Extragenic Palindrome



- BIME set-up (Bacterial Interspesed Mosaic Element)

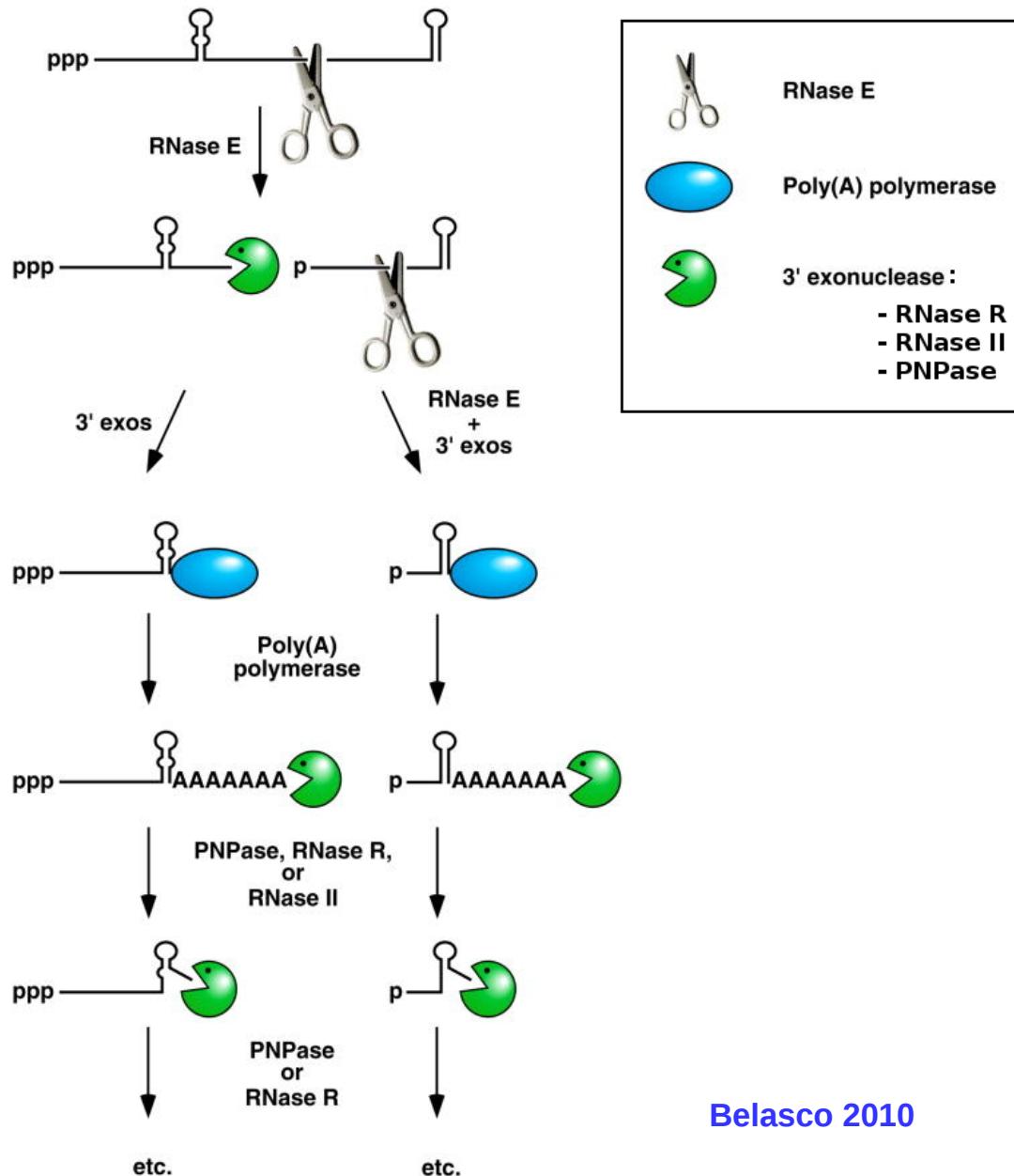
BIME properties?

- Structural roles:
 - ◆ Homologous recombination
 - ◆ Mobile DNA sequence insertion sites
- DNA level:
 - ◆ Protein binding sites
 - ◆ Transcription initiation
 - ◆ Site specific recombination sites
- RNA level:
 - ◆ Control of translation
 - ◆ Transcription termination
 - ◆ 5' end transcript stabilisation

BIME properties?

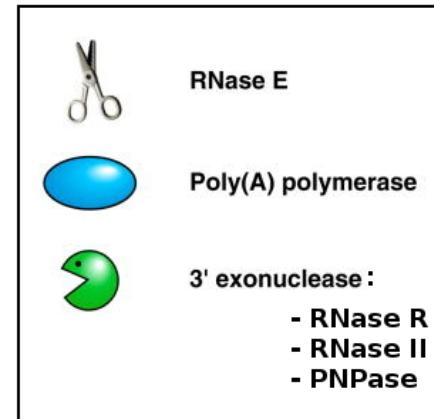
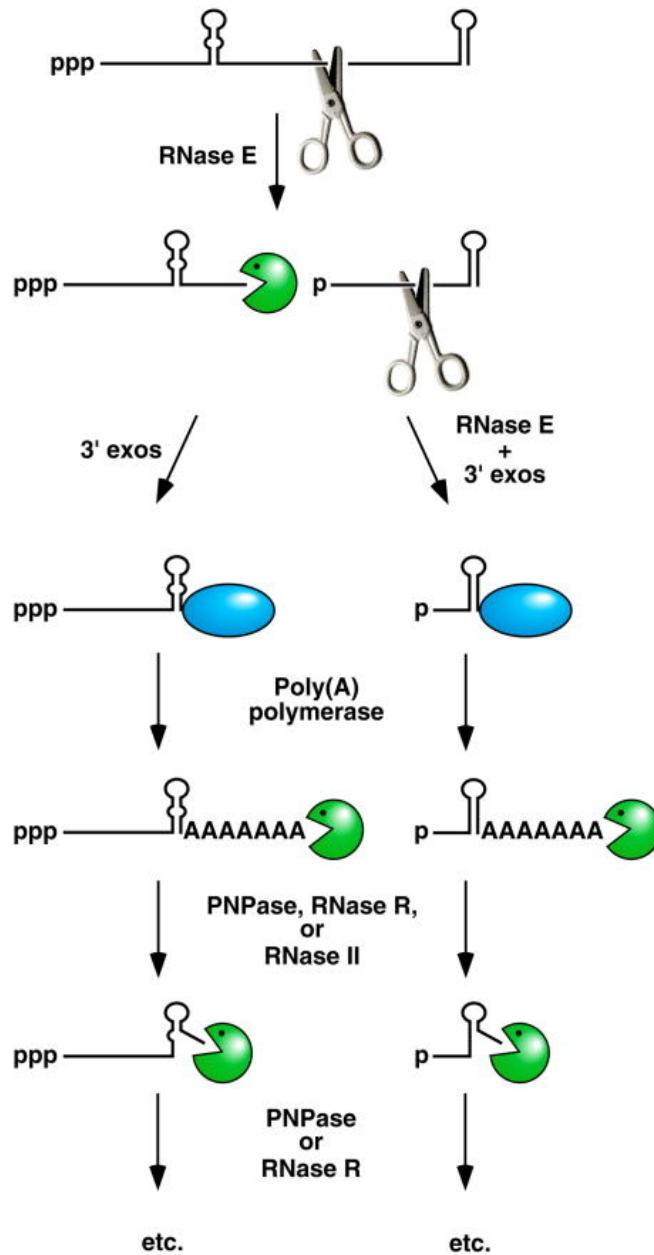
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 - ◆ **Transcription termination?**
 - ◆ **5' end transcript stabilisation?**

mRNA degradosome



Belasco 2010

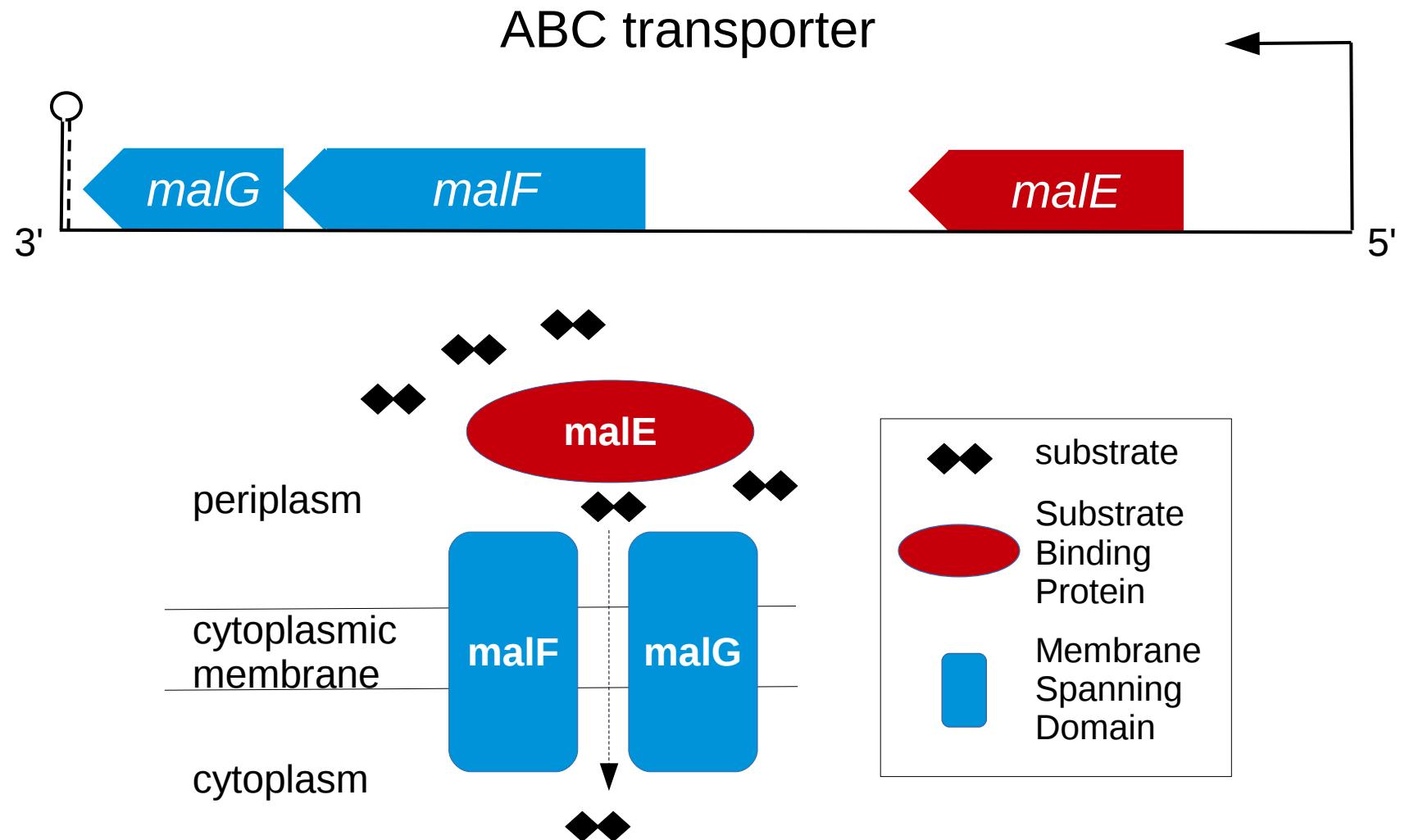
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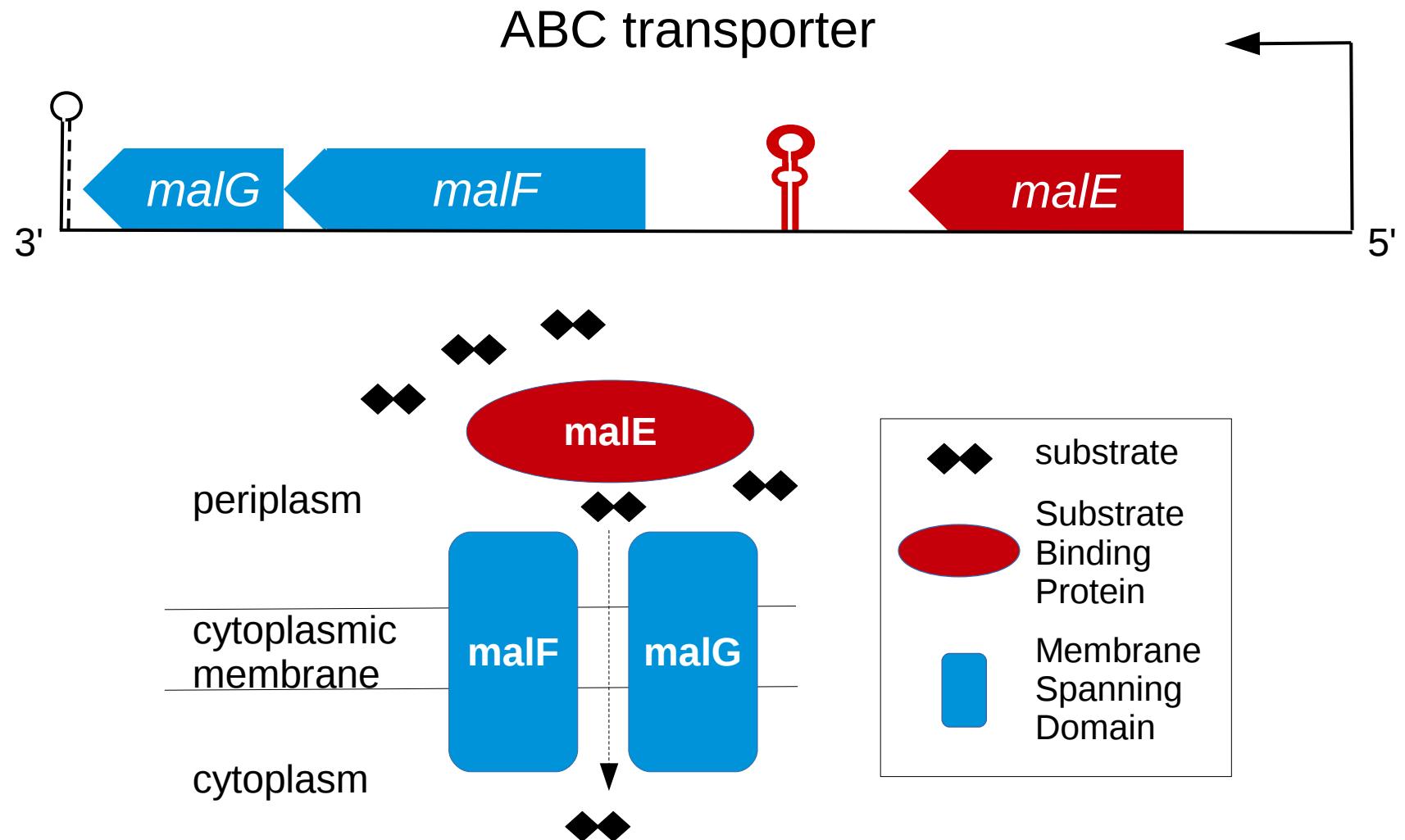
BIME secondary
structures?

Belasco 2010

malEFG operon

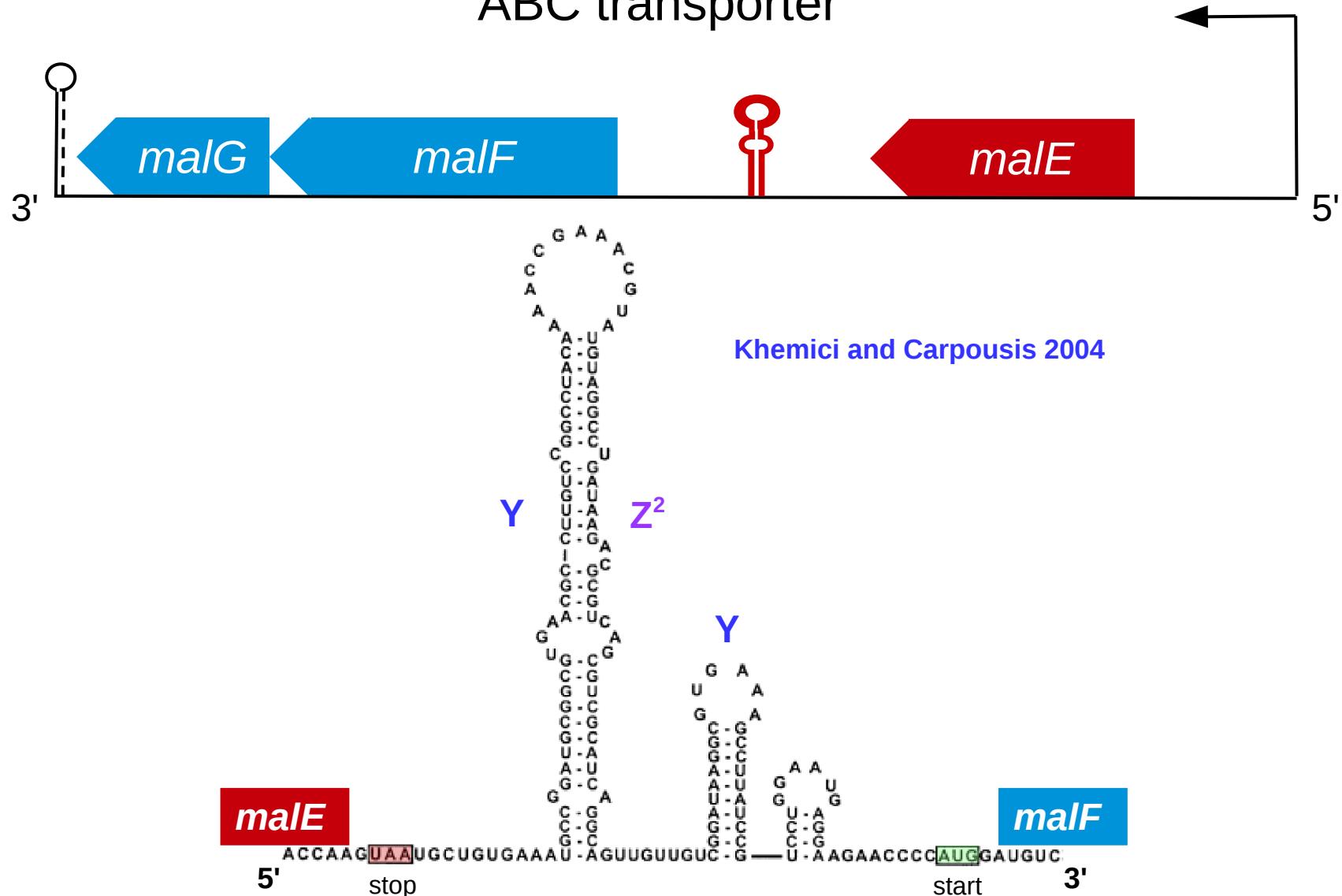


malEFG operon



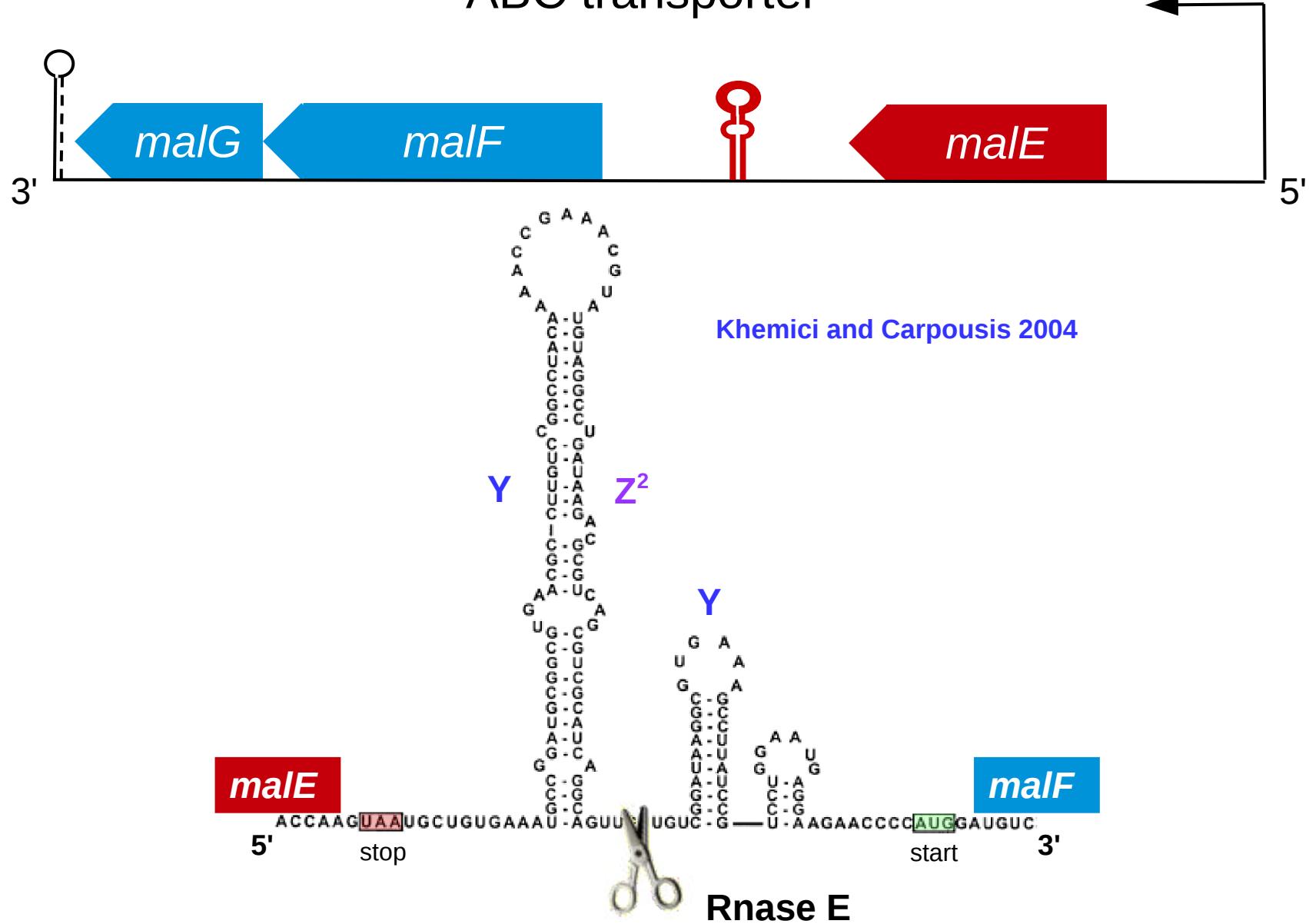
malEFG operon

ABC transporter

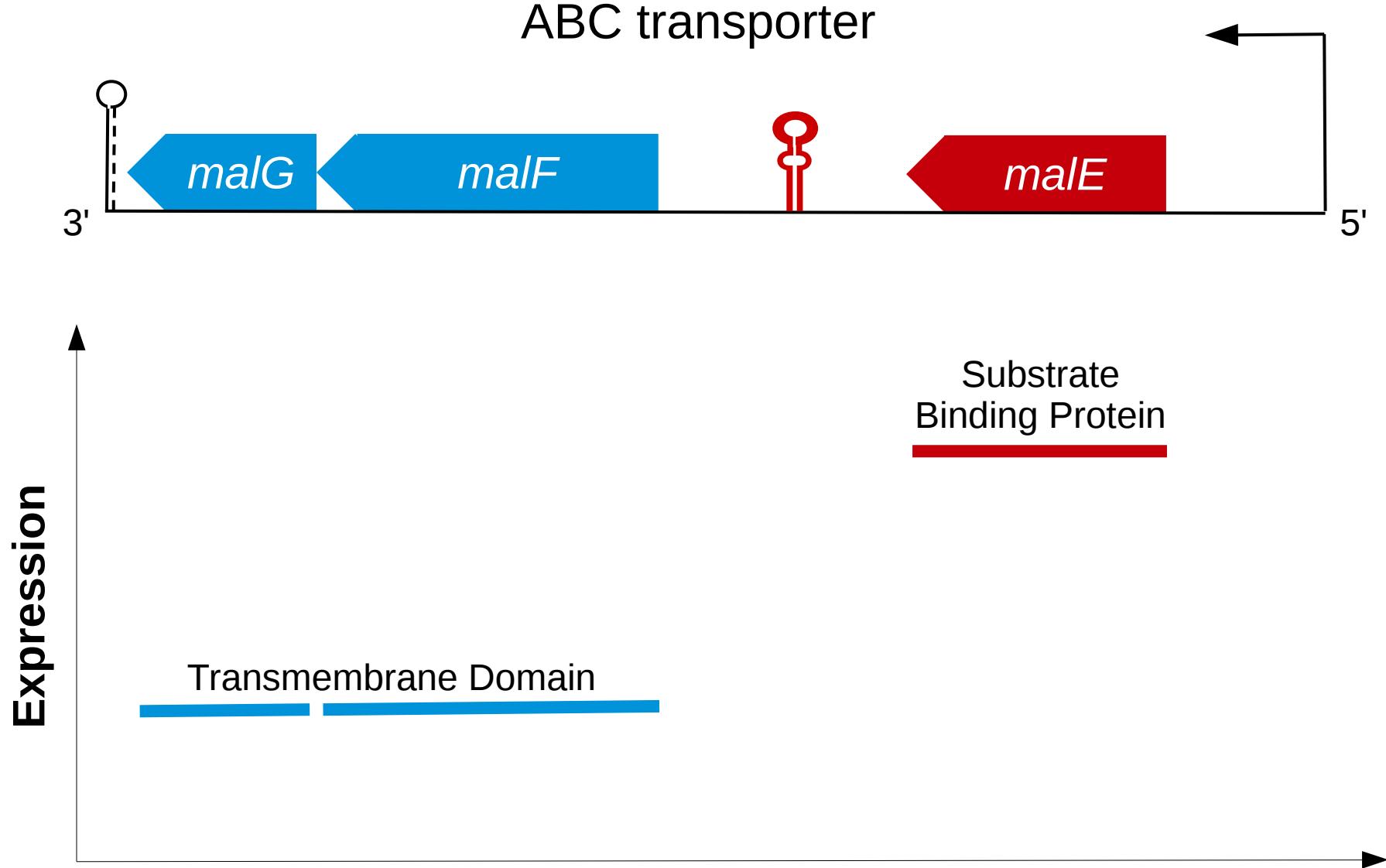


malEFG operon

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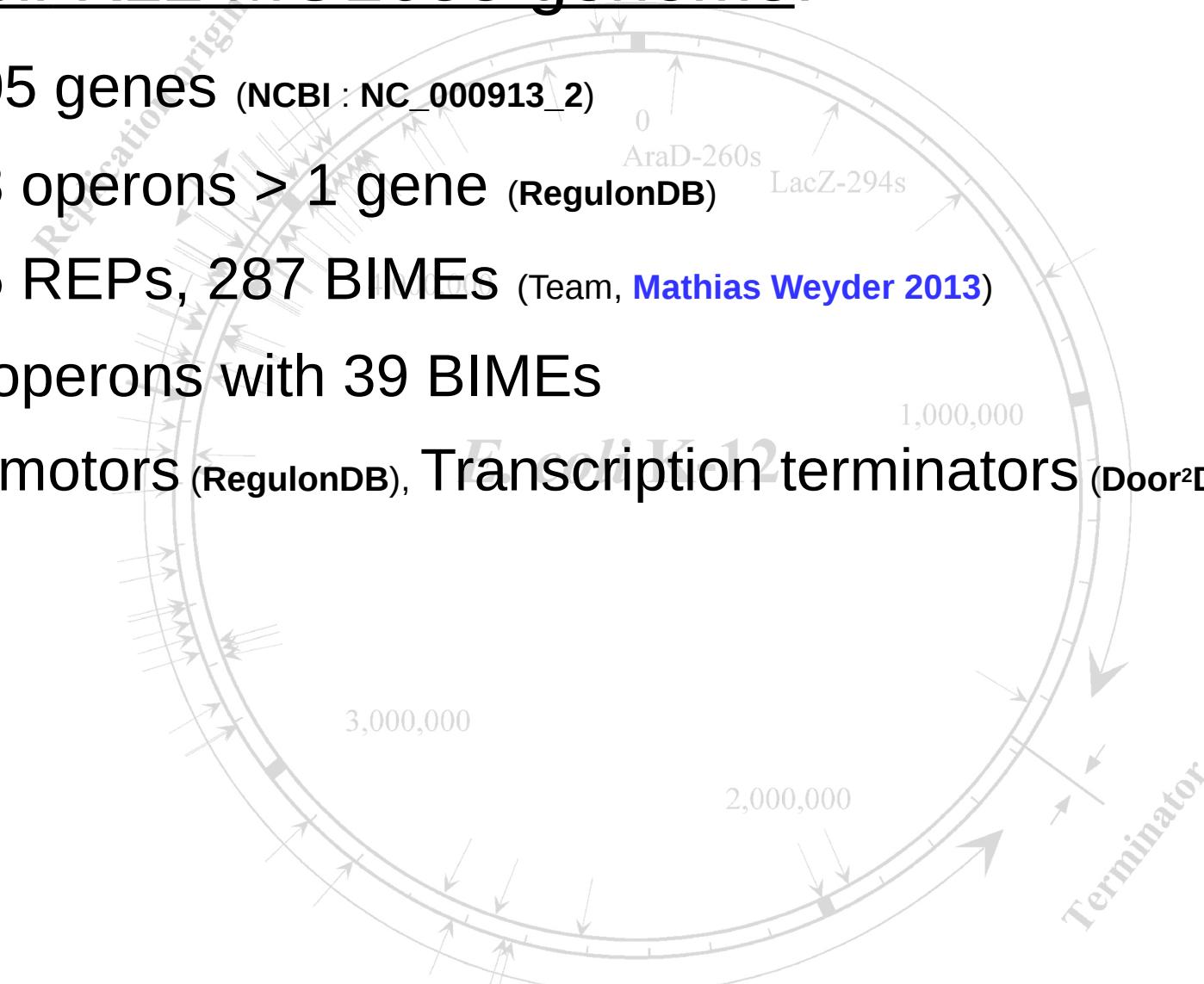
malEFG operon



BIME effect study in whole genome

- *E. coli* K12 MG1655 genome:

- 4605 genes ([NCBI : NC_000913_2](#))
- 848 operons > 1 gene ([RegulonDB](#))
- 605 REPs, 287 BIMEs ([Team, Mathias Weyder 2013](#))
- 36 operons with 39 BIMEs
- Promotors ([RegulonDB](#)), Transcription terminators ([Door²DB](#))



BIME effect study in whole genome

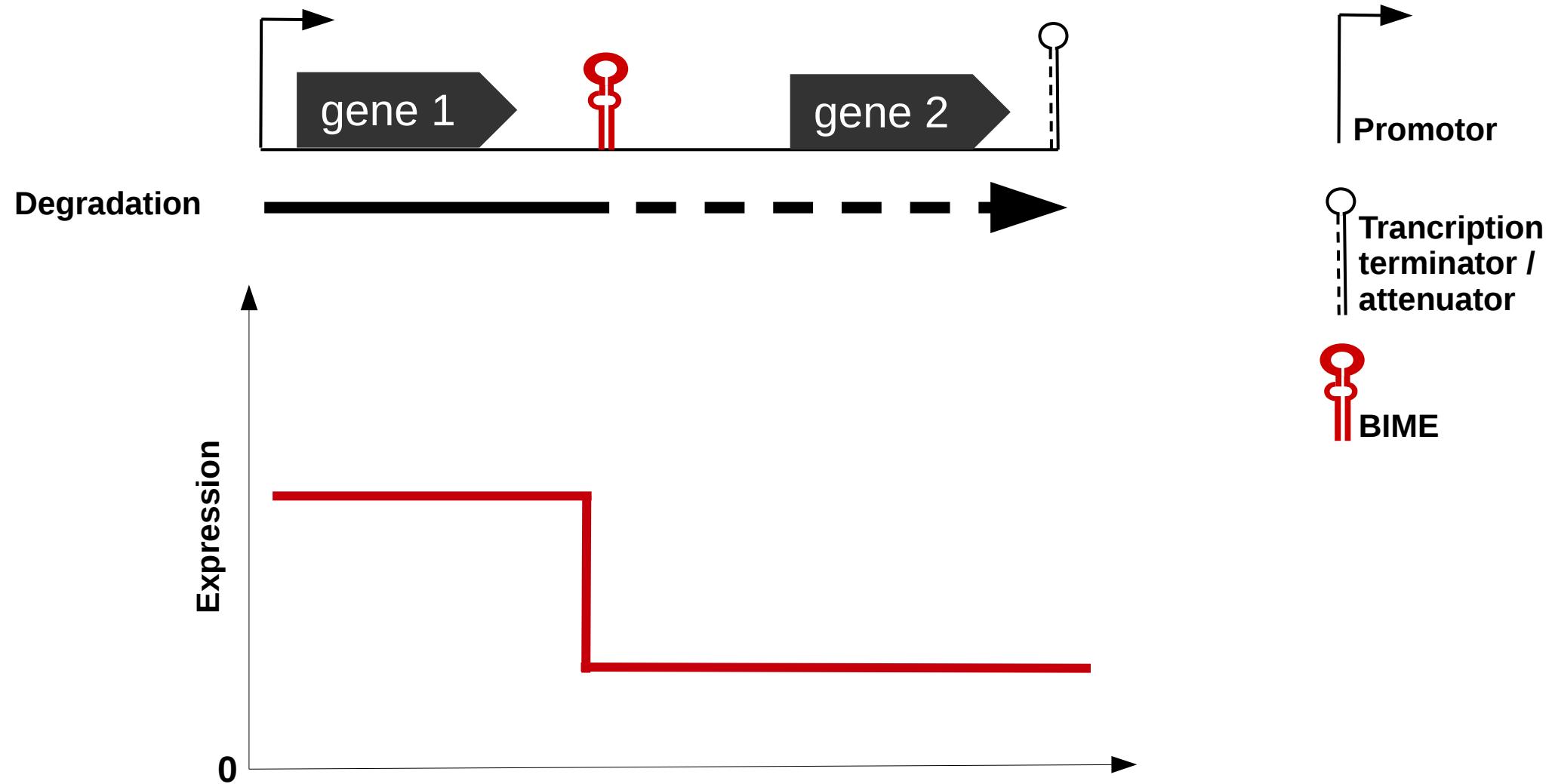
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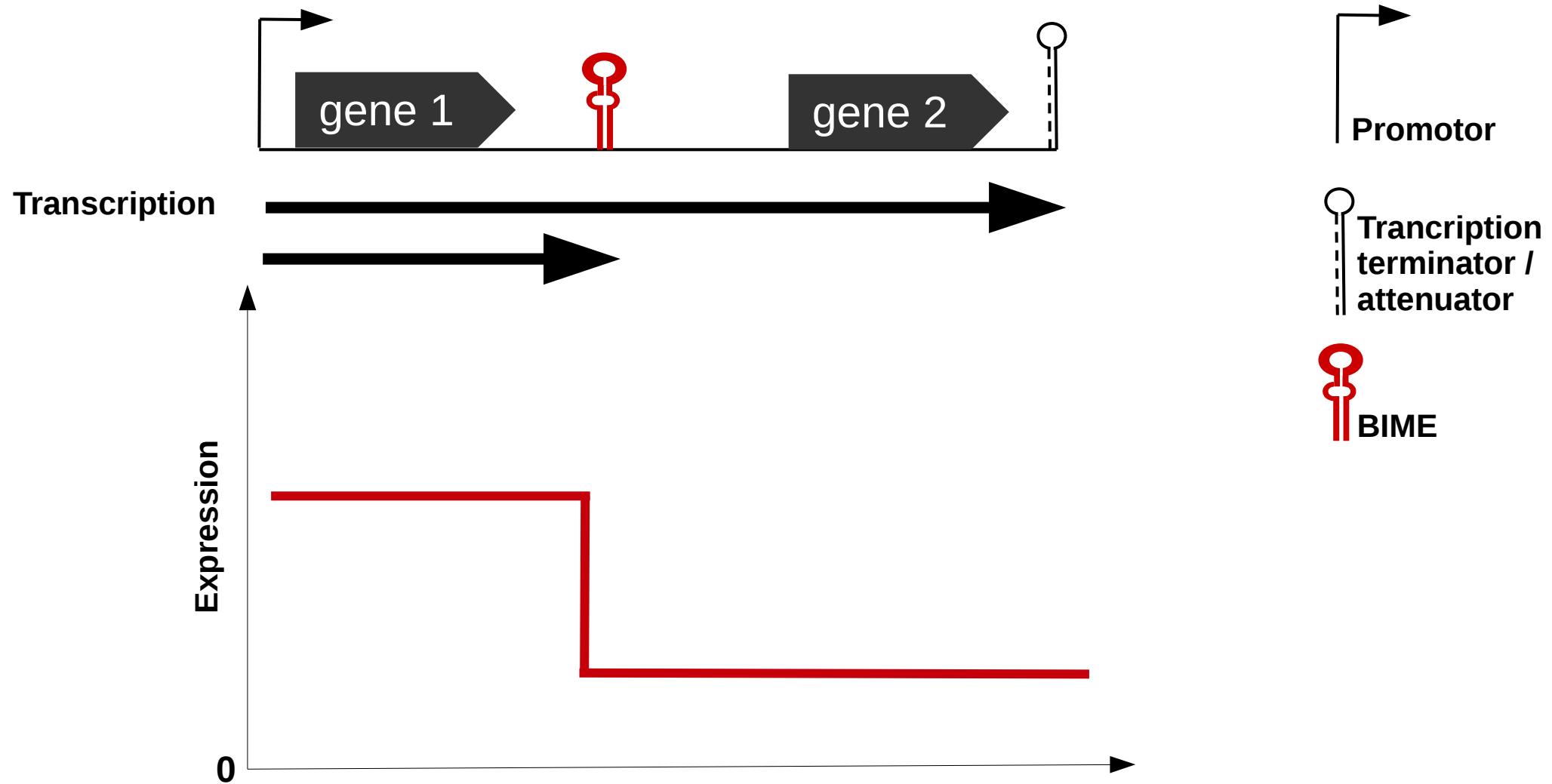
- RNA-Seq:

- Whole transcriptome study
- Snapshot of transcription & degradation
- 3 data sets from 2 experiments ([GEO database](#))
- 8, 3 and 3 replicates

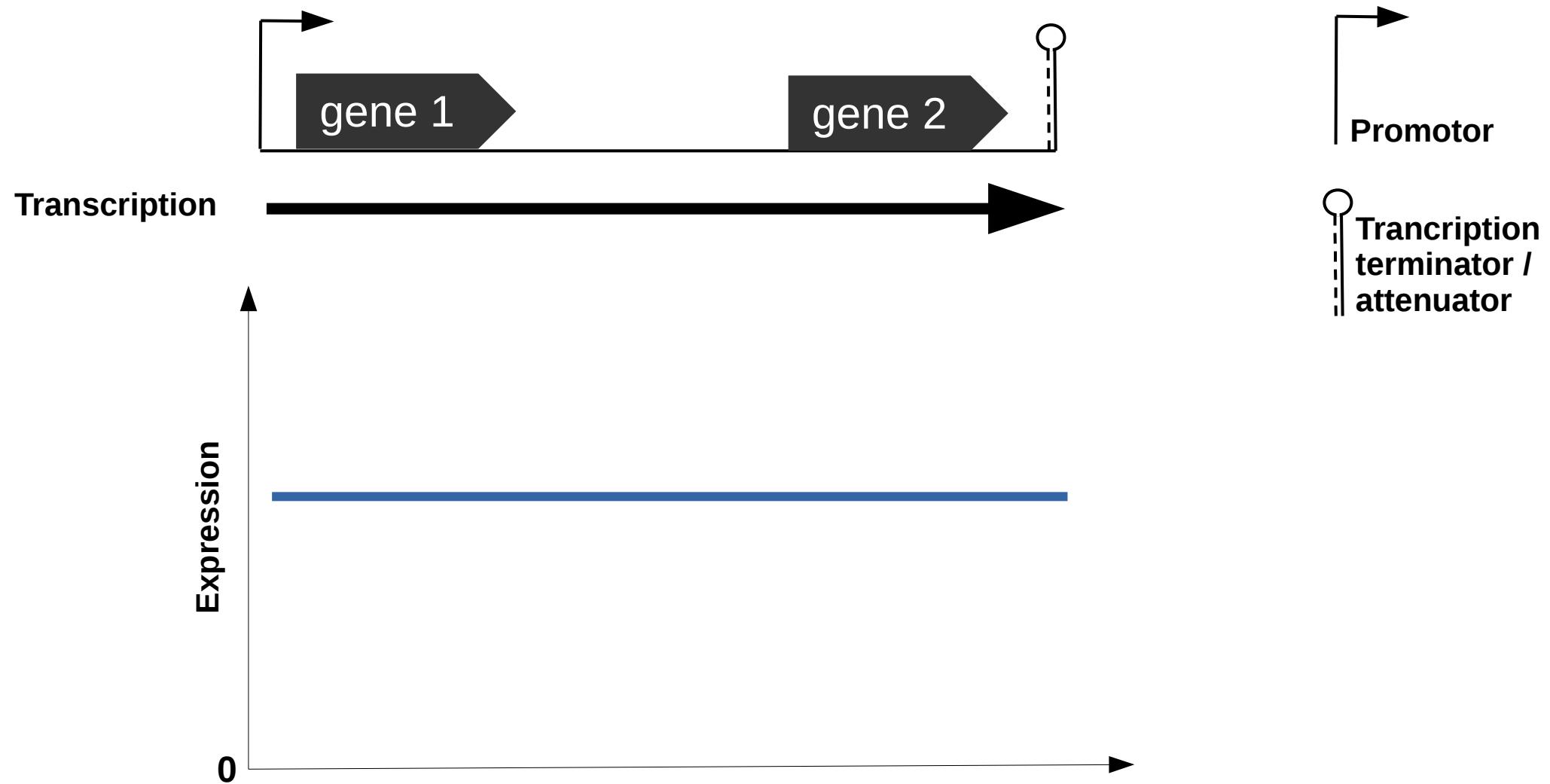
Possible expression patterns



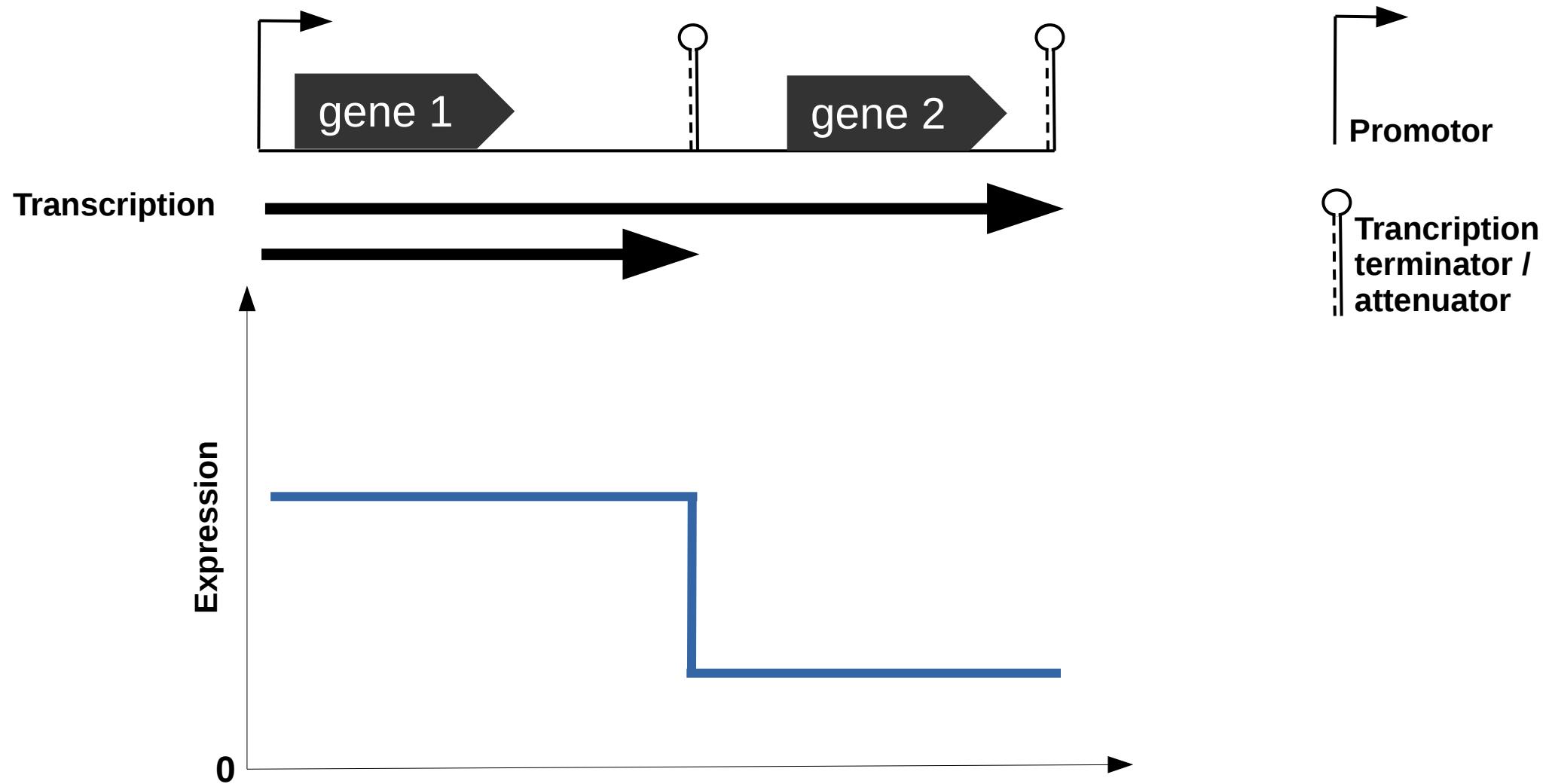
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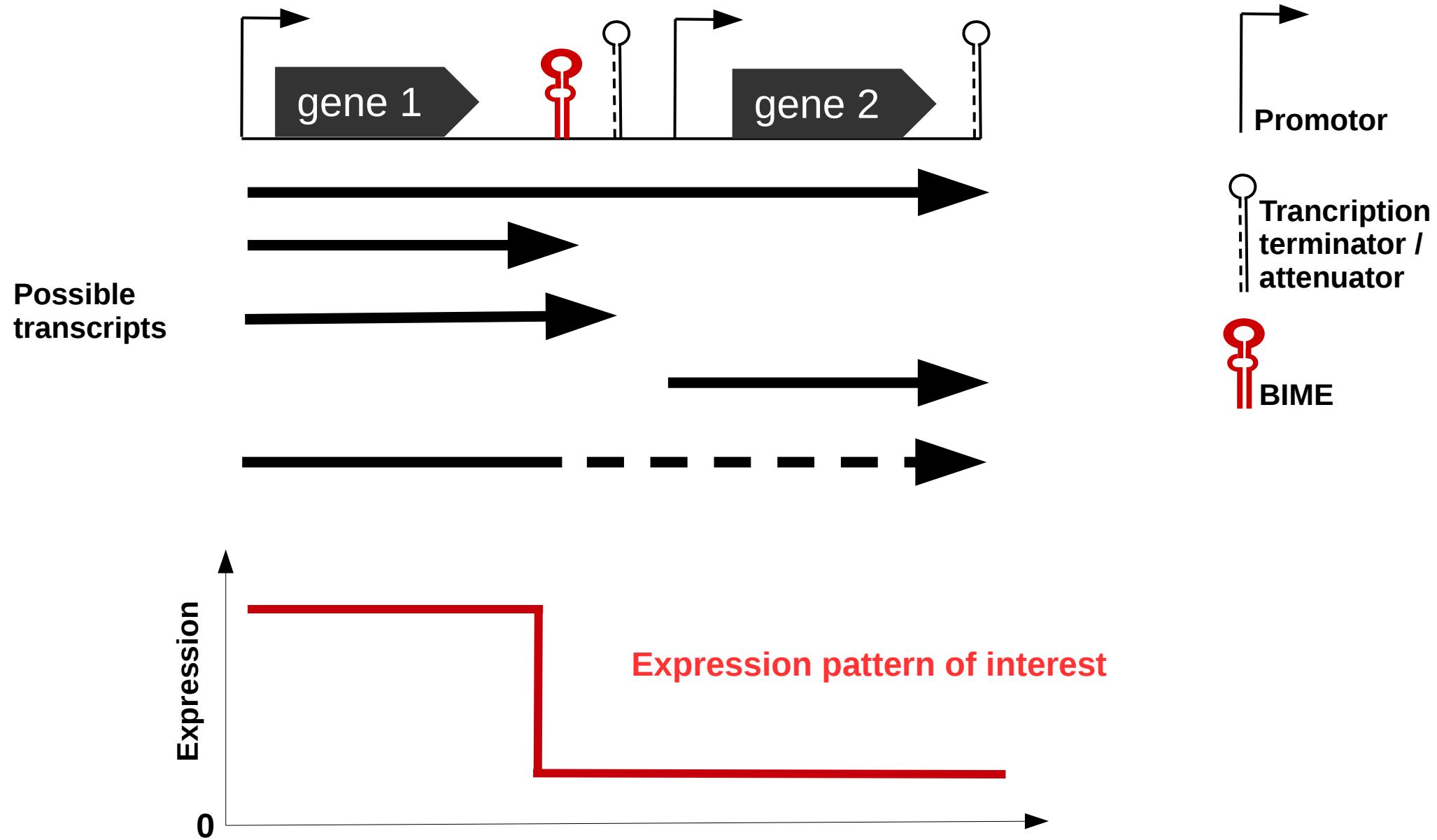
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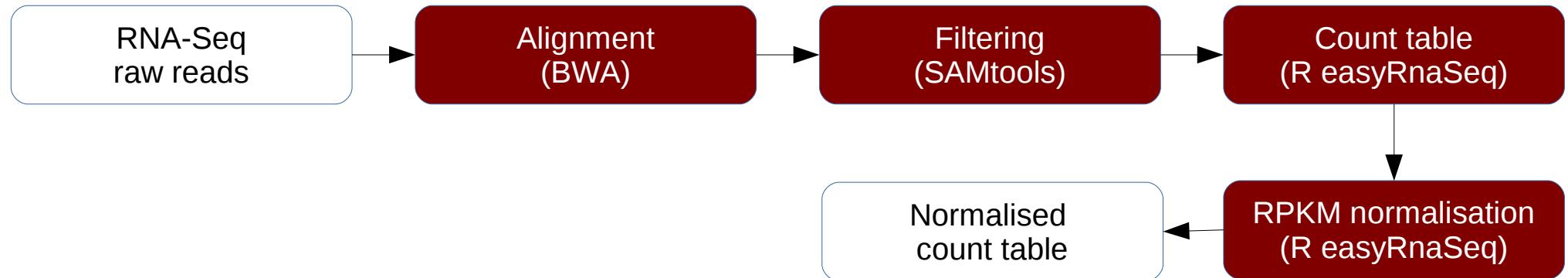
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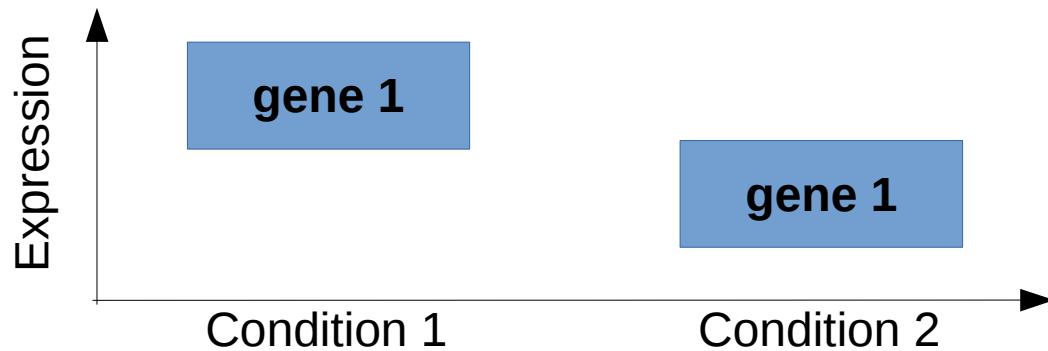
Analysis pipeline



Data pre-processing

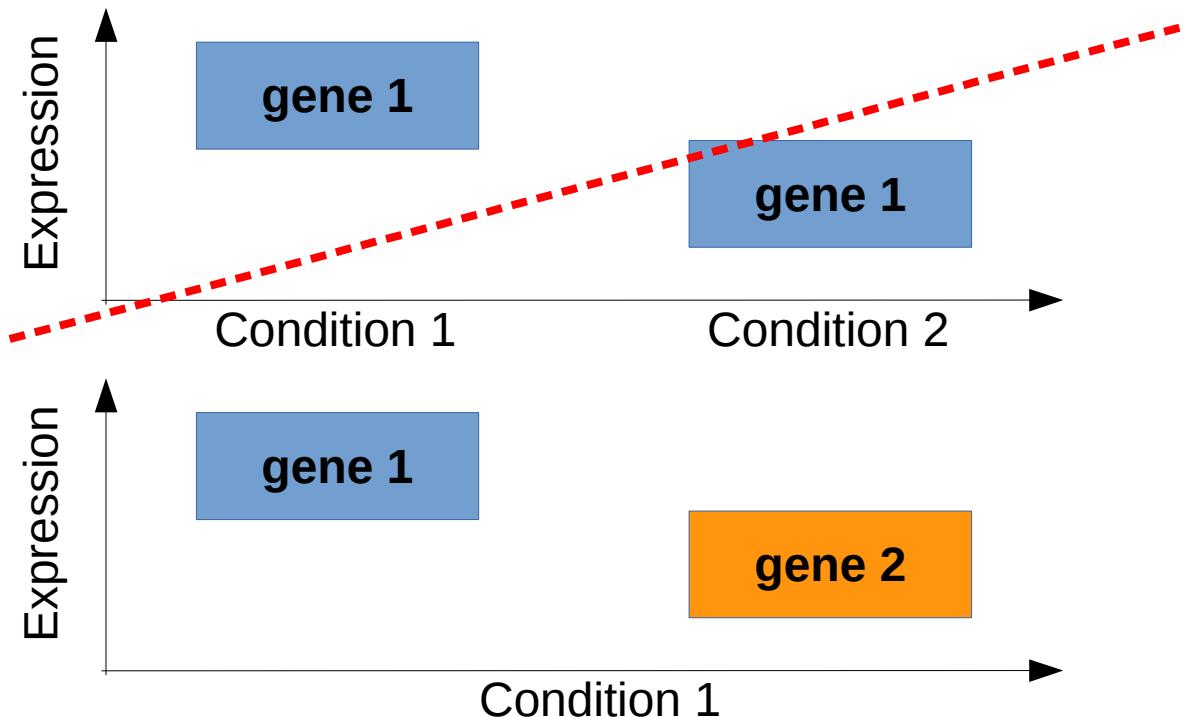
RNA-Seq normalisation

- Keep in mind:



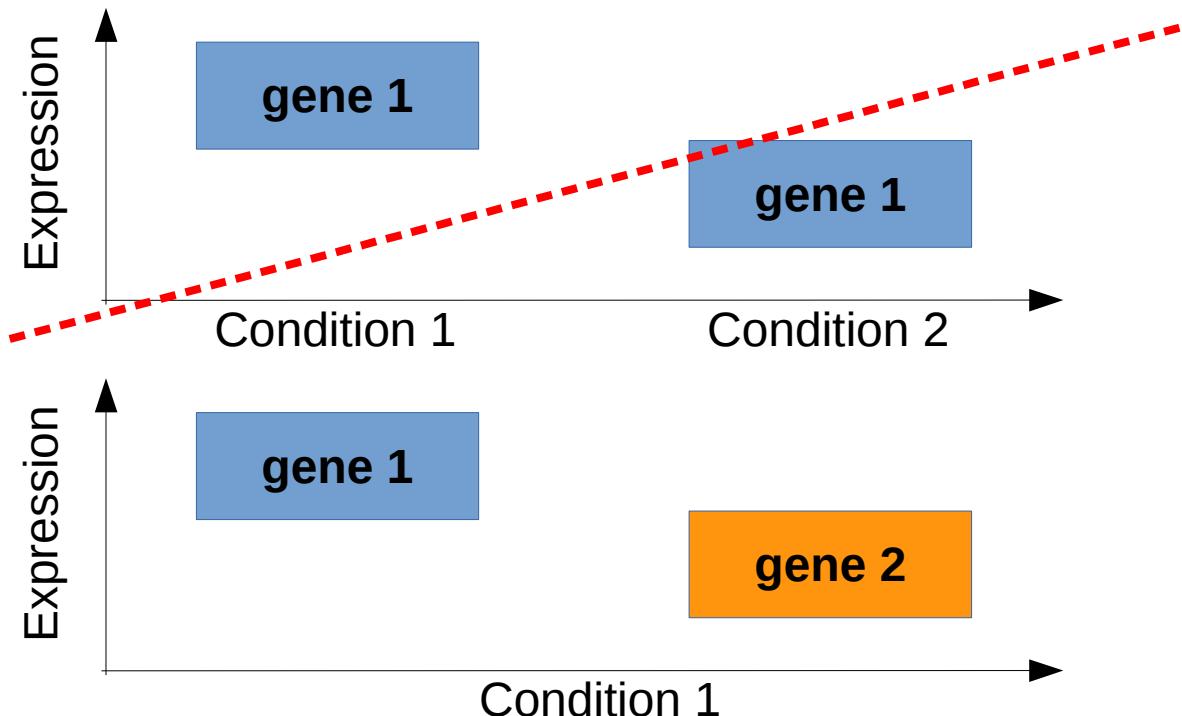
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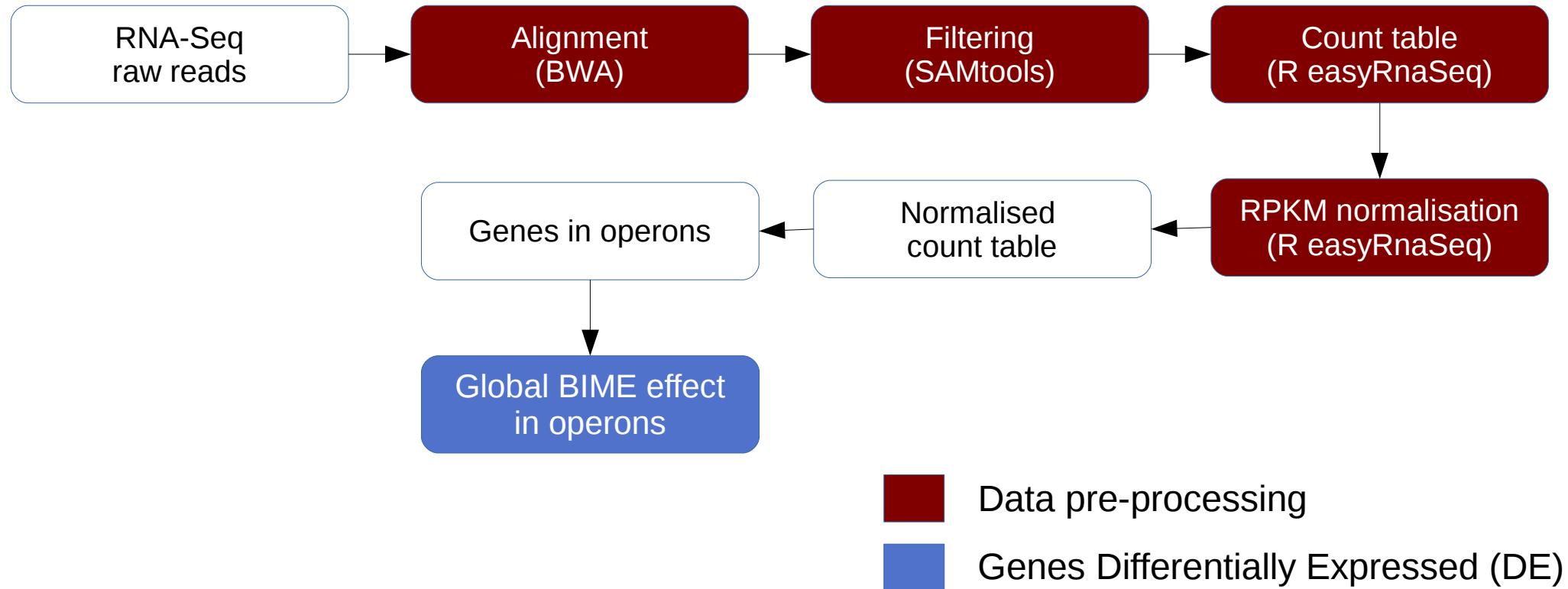
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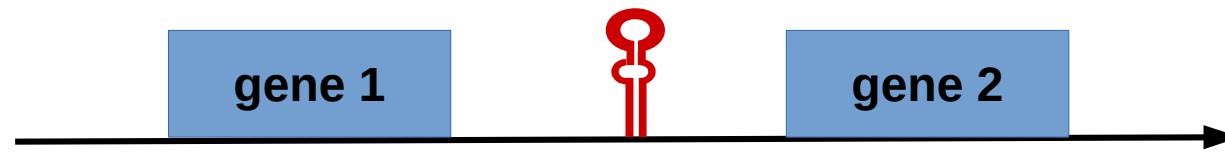
- RPKM normalisation:

- Transcript size
- Library size

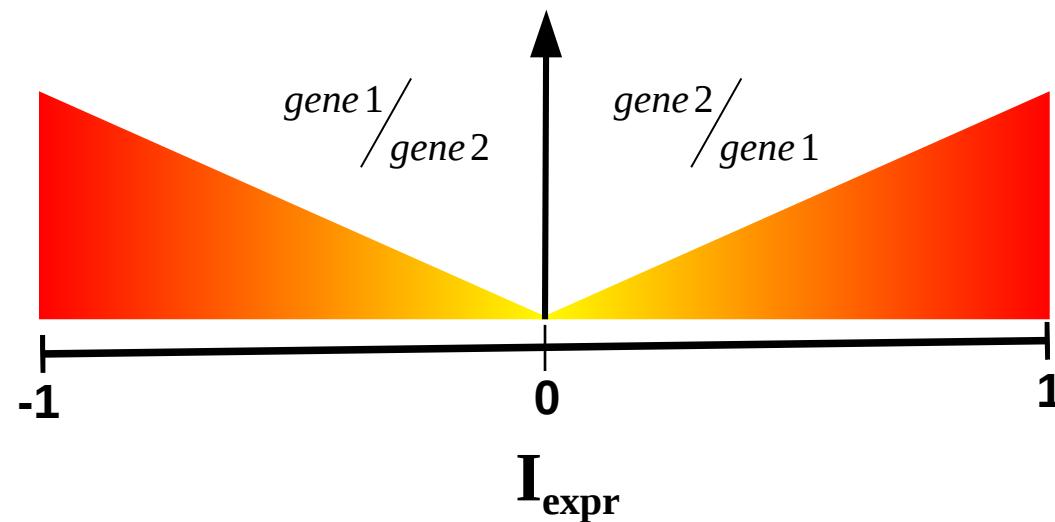
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Global BIME effect in operons

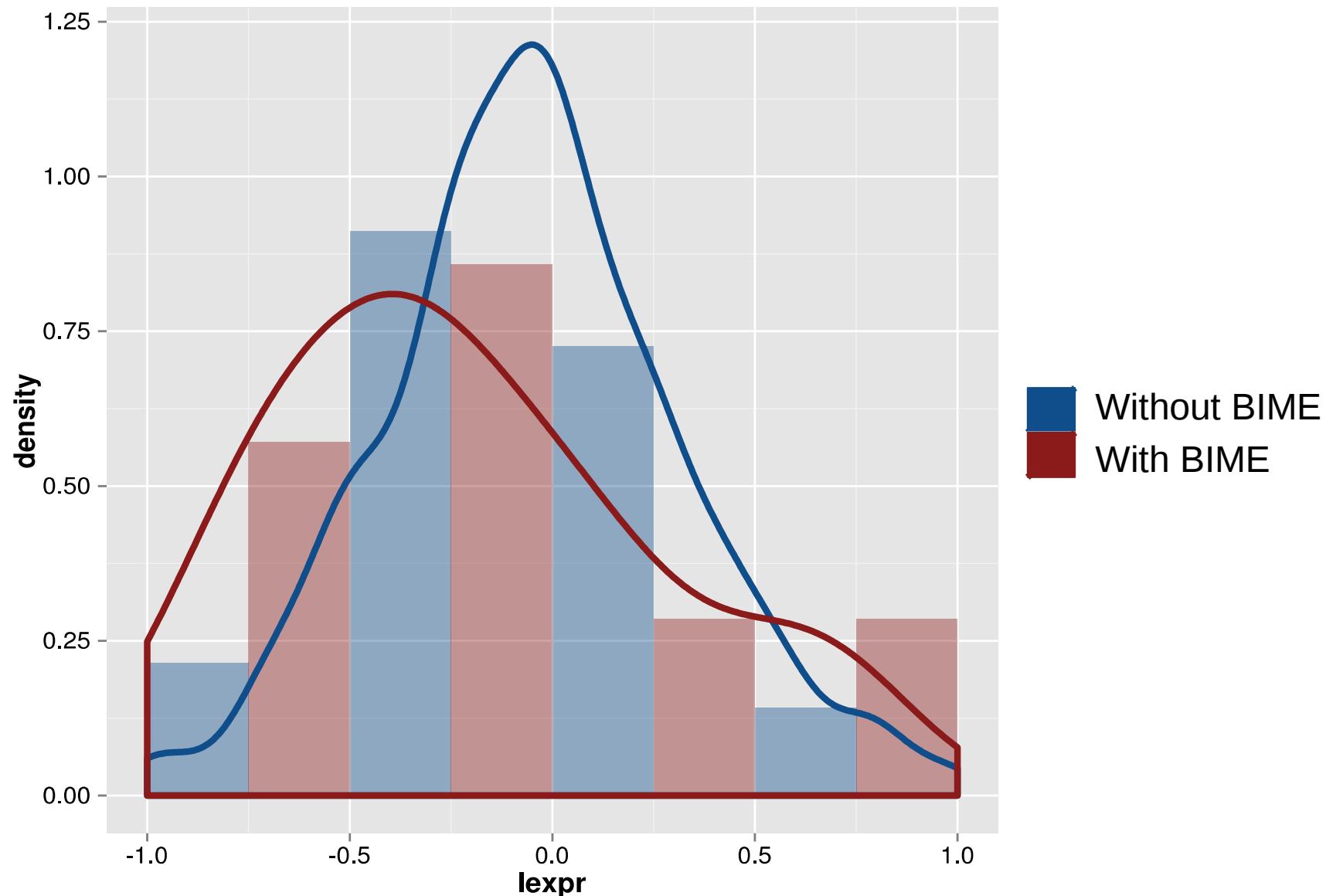


$$I_{expr} = \frac{RPKM_{gene2} - RPKM_{gene1}}{RPKM_{gene2} + RPKM_{gene1}}$$

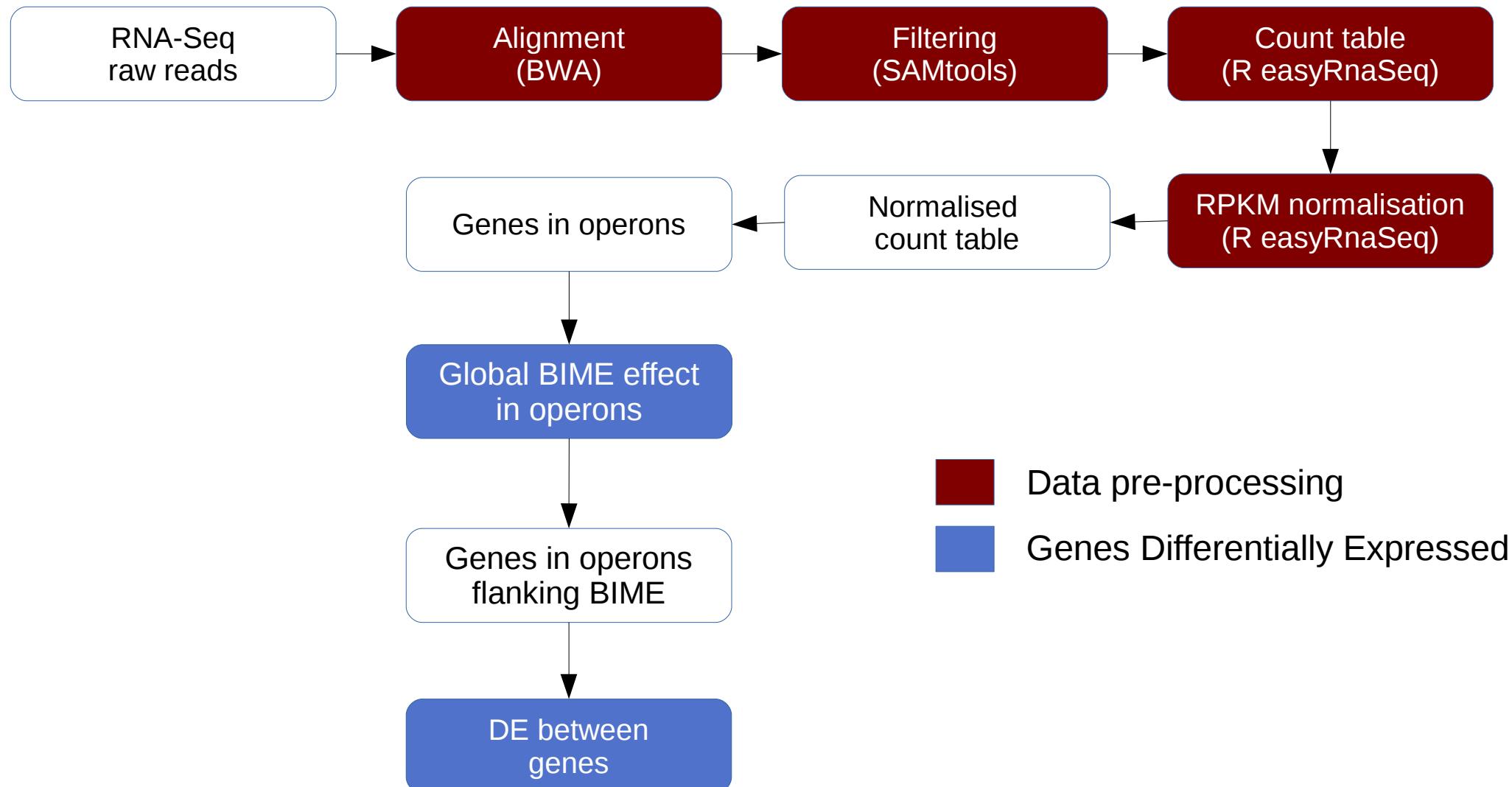


Global BIME effect in operons

Distributions of I_{expr} without annotated promotor and terminator in intergenic region

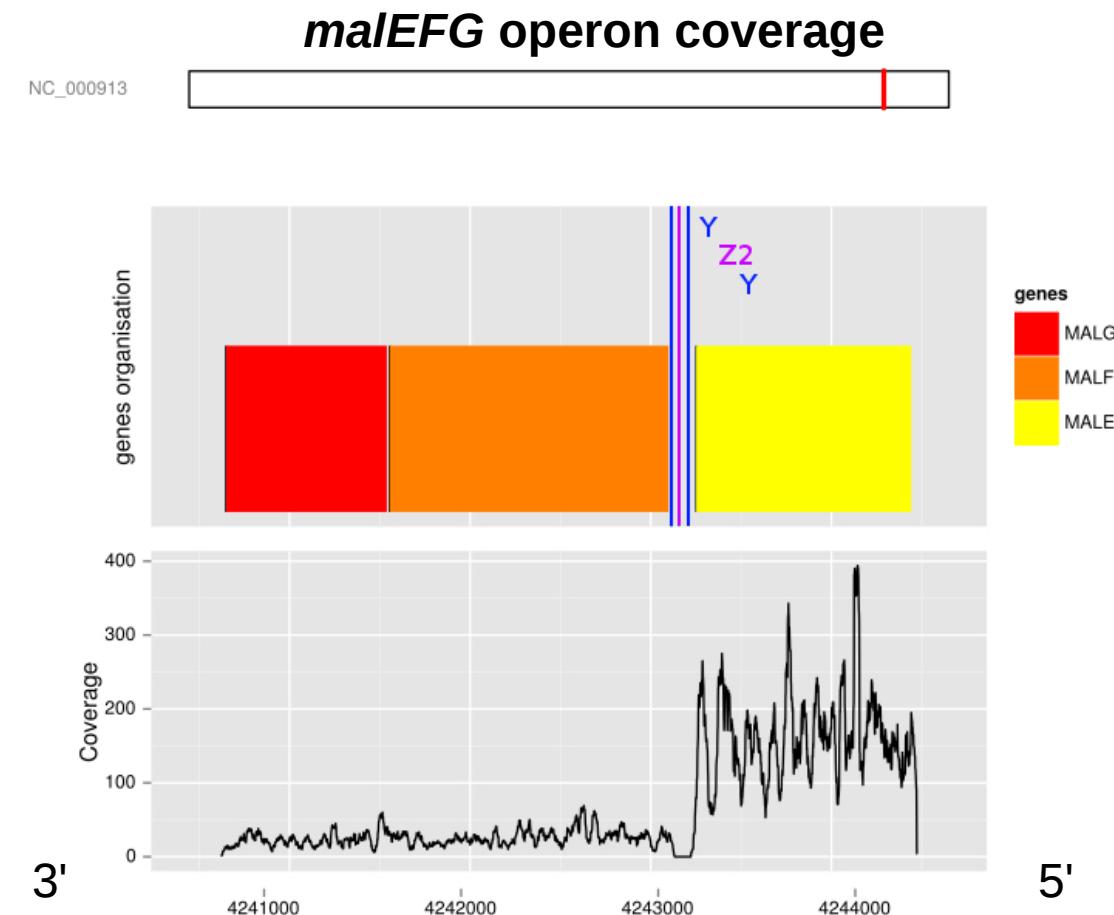


Analysis pipeline



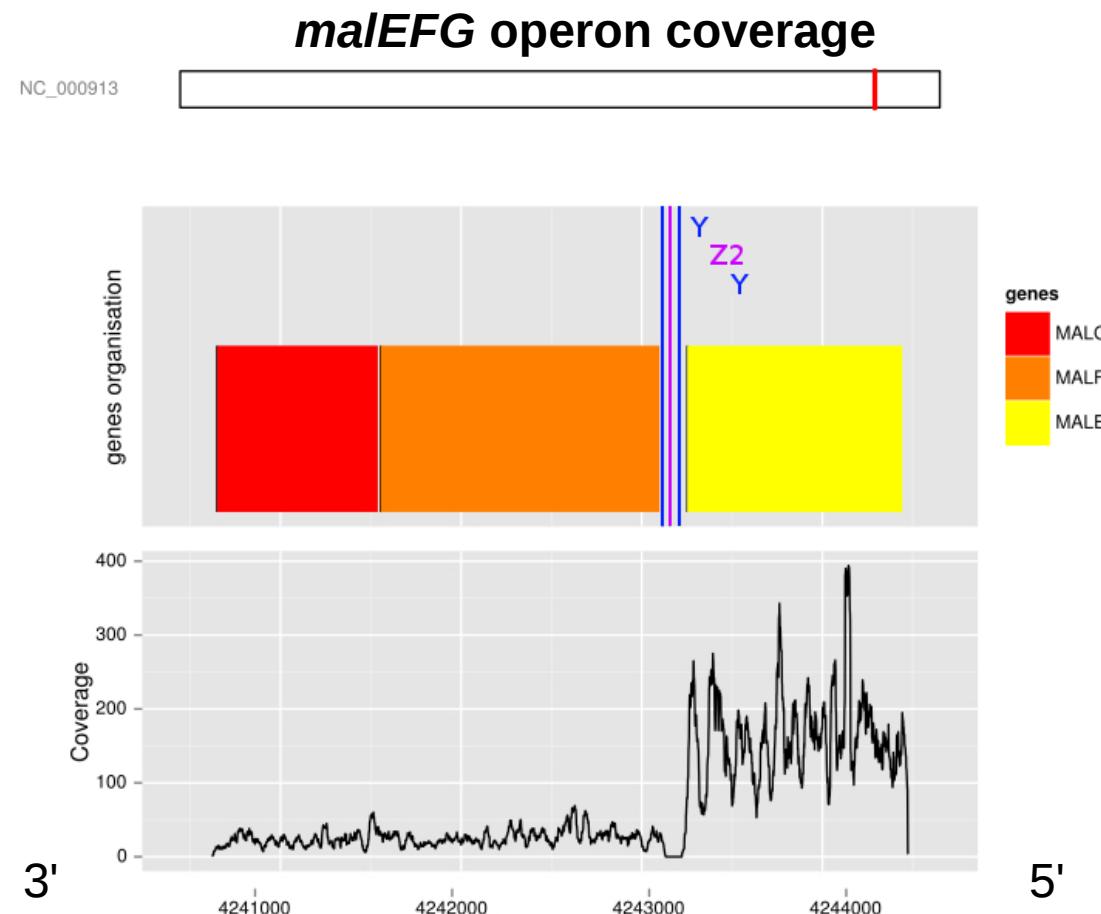
Genes flanking BIME Differential Expression?

- Wilcoxon rank sum test for DE (p-value threshold : 0.01)



Genes flanking BIME Differential Expression?

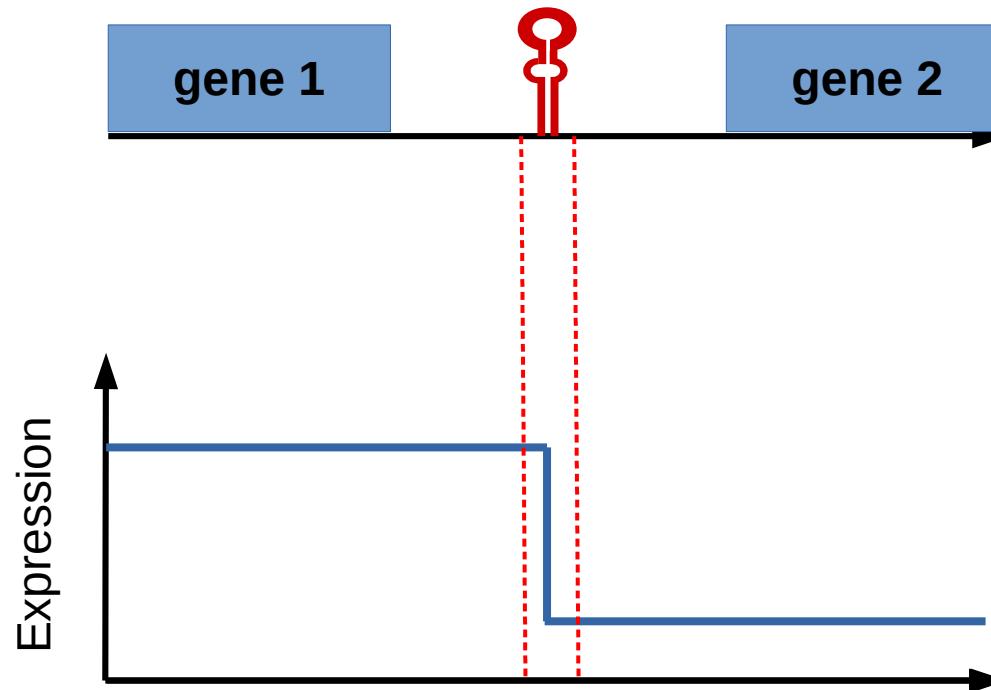
- Wilcoxon rank sum test for DE (p-value threshold : 0.01)



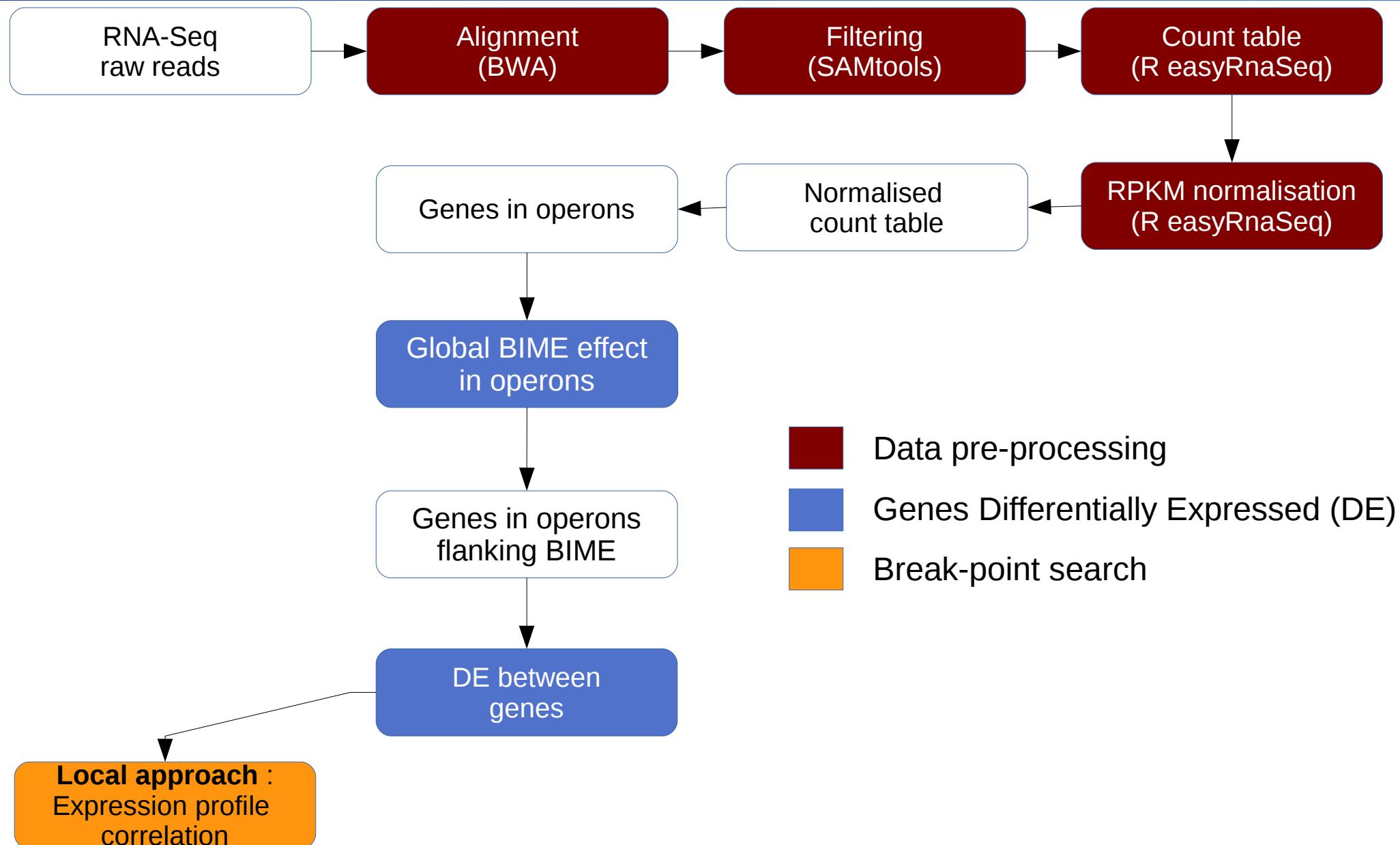
- 25 DE on 39 pairs of gene flanking a BIME (3 data sets)

Genes difference of expression linked to the BIME?

- Coverage break-point on BIME?

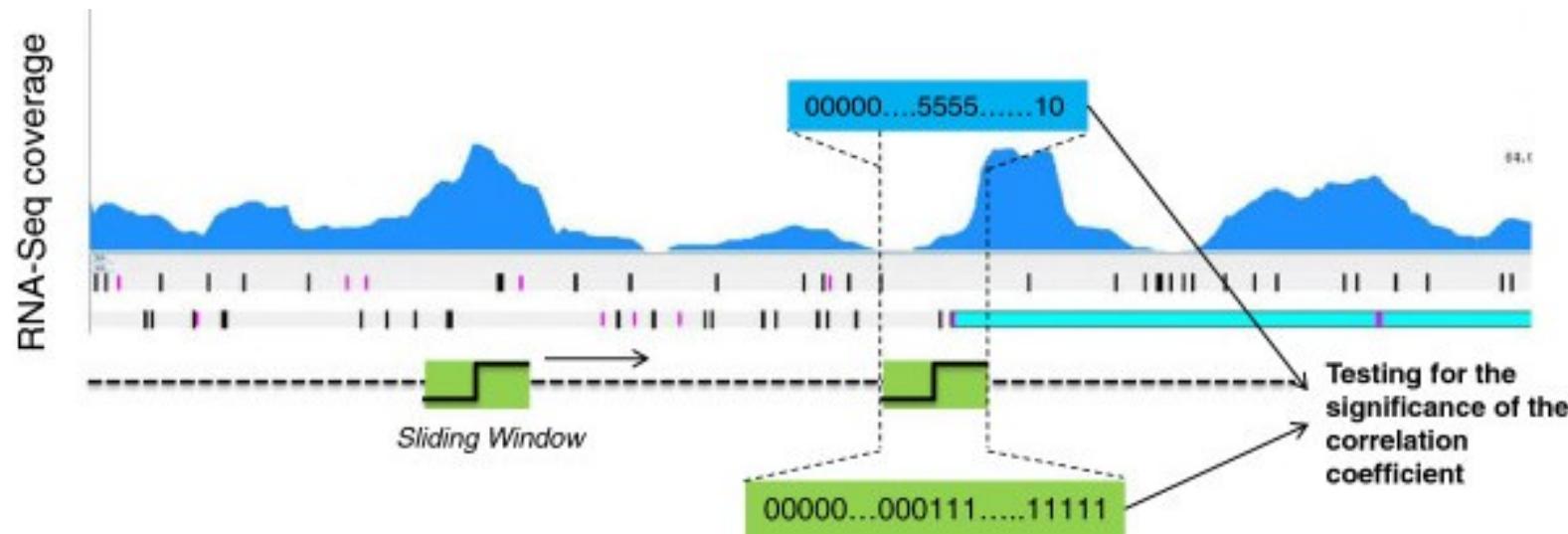


Analysis pipeline



Adaptation of operon detection method (Local approach)

Expression profile correlation

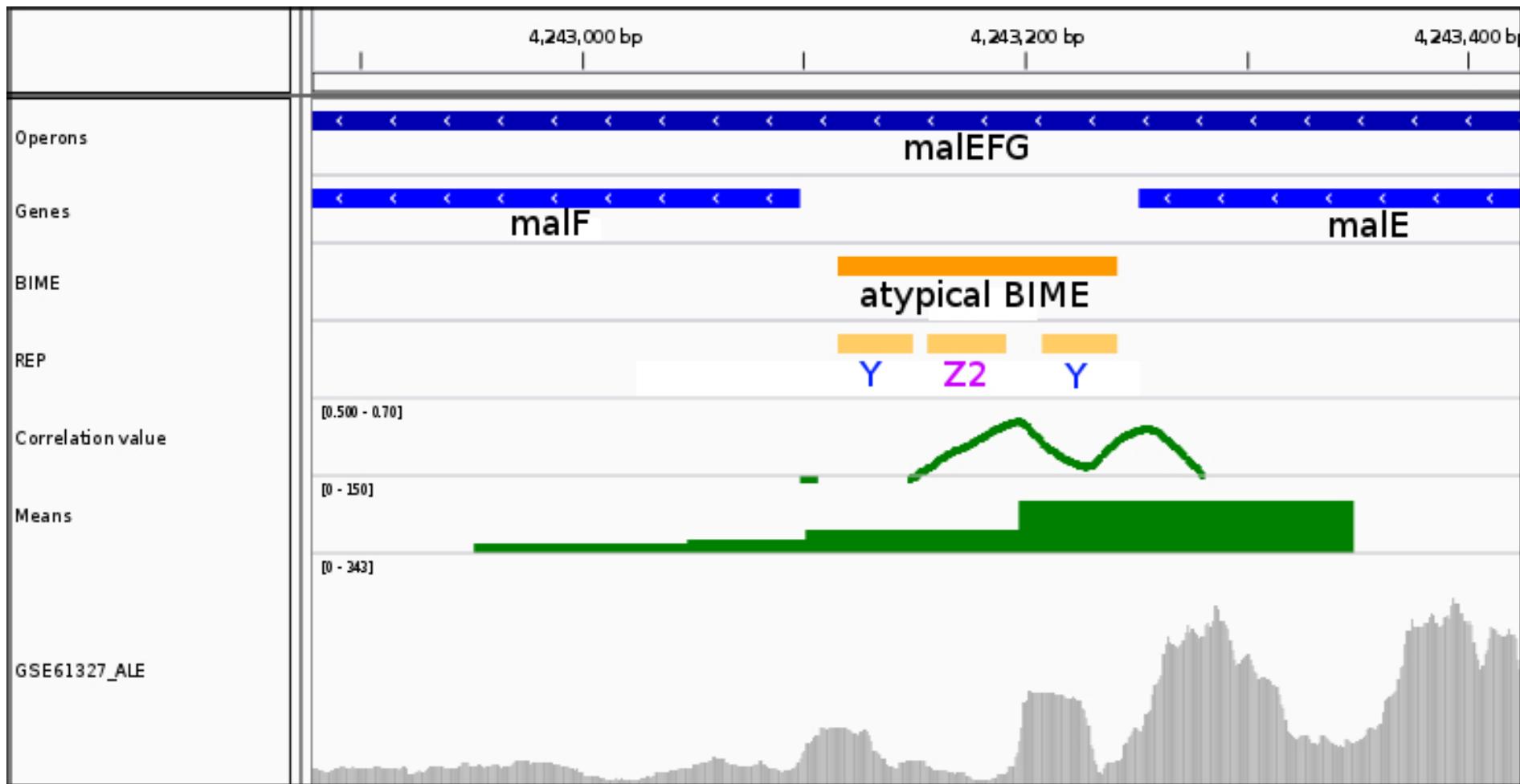


Fortino et al. 2014

- Window size: 300 bp
- 2 fold change of expression
- Correlation > 0.5 & p-value threshold < 10^{-7}

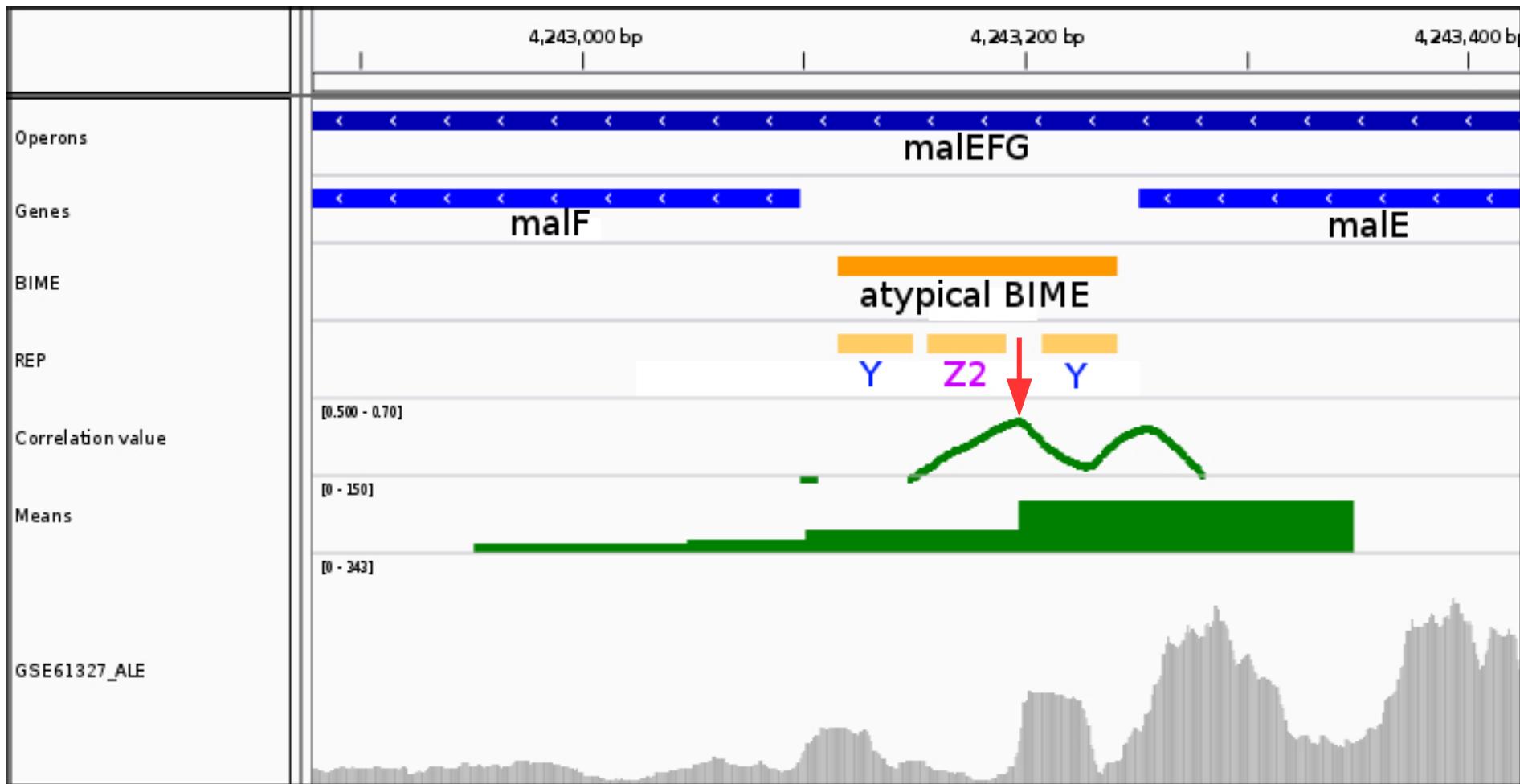
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Expression profile correlation on *malE-malF* BIME



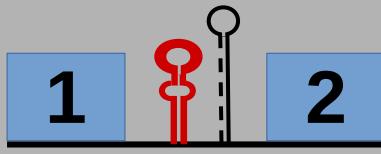
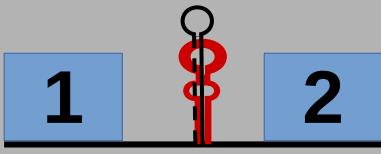
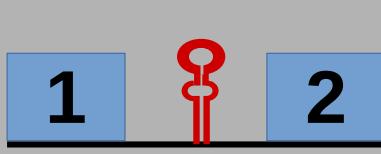
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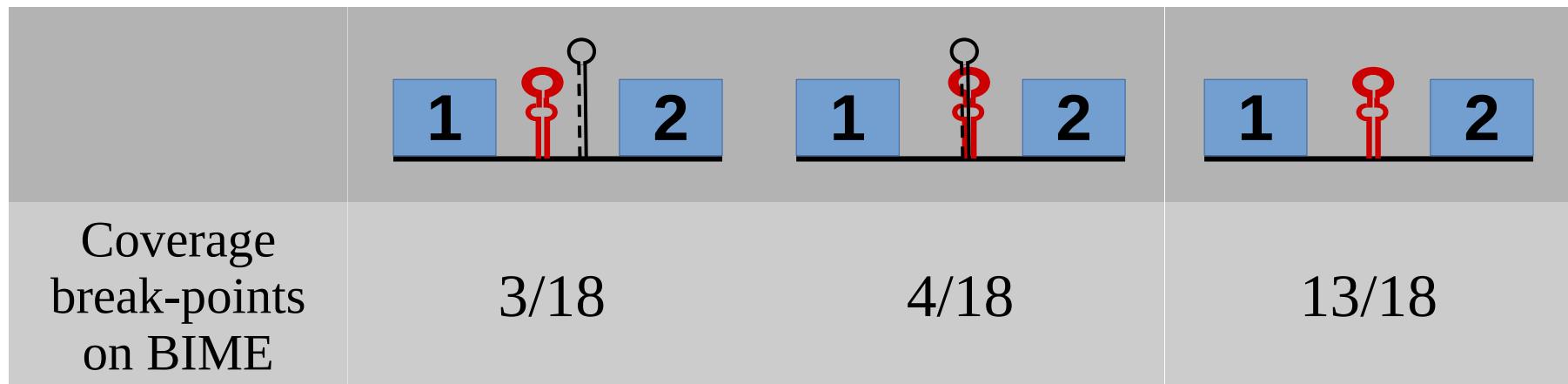
Local approach results

- 18 break-points on BIMEs for 25 DE

			
Coverage break-points on BIME	3/18	4/18	13/18

Local approach results

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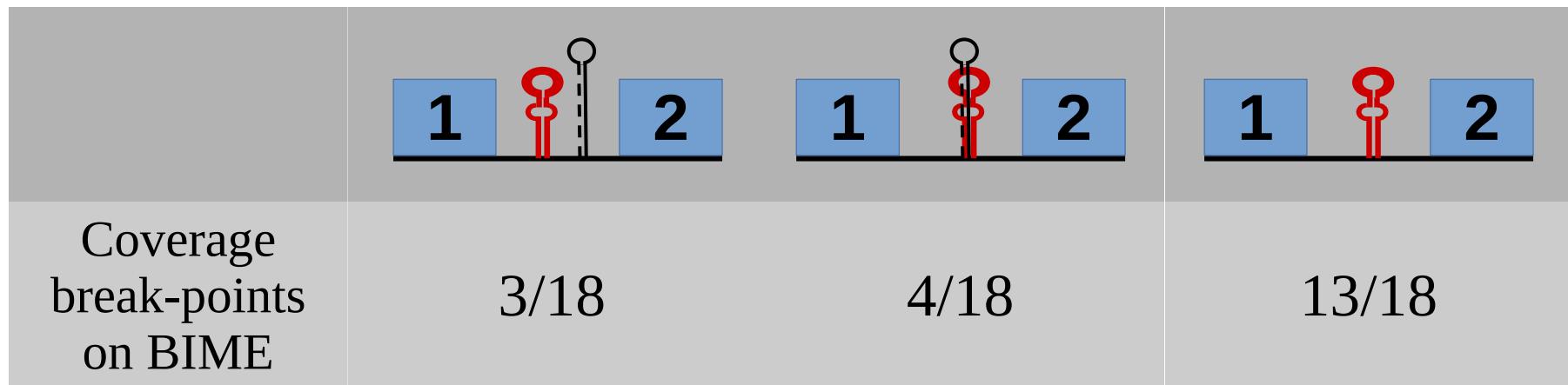
- Weakness:



- RNA-Seq coverage not uniform
- False positive detection

Local approach results

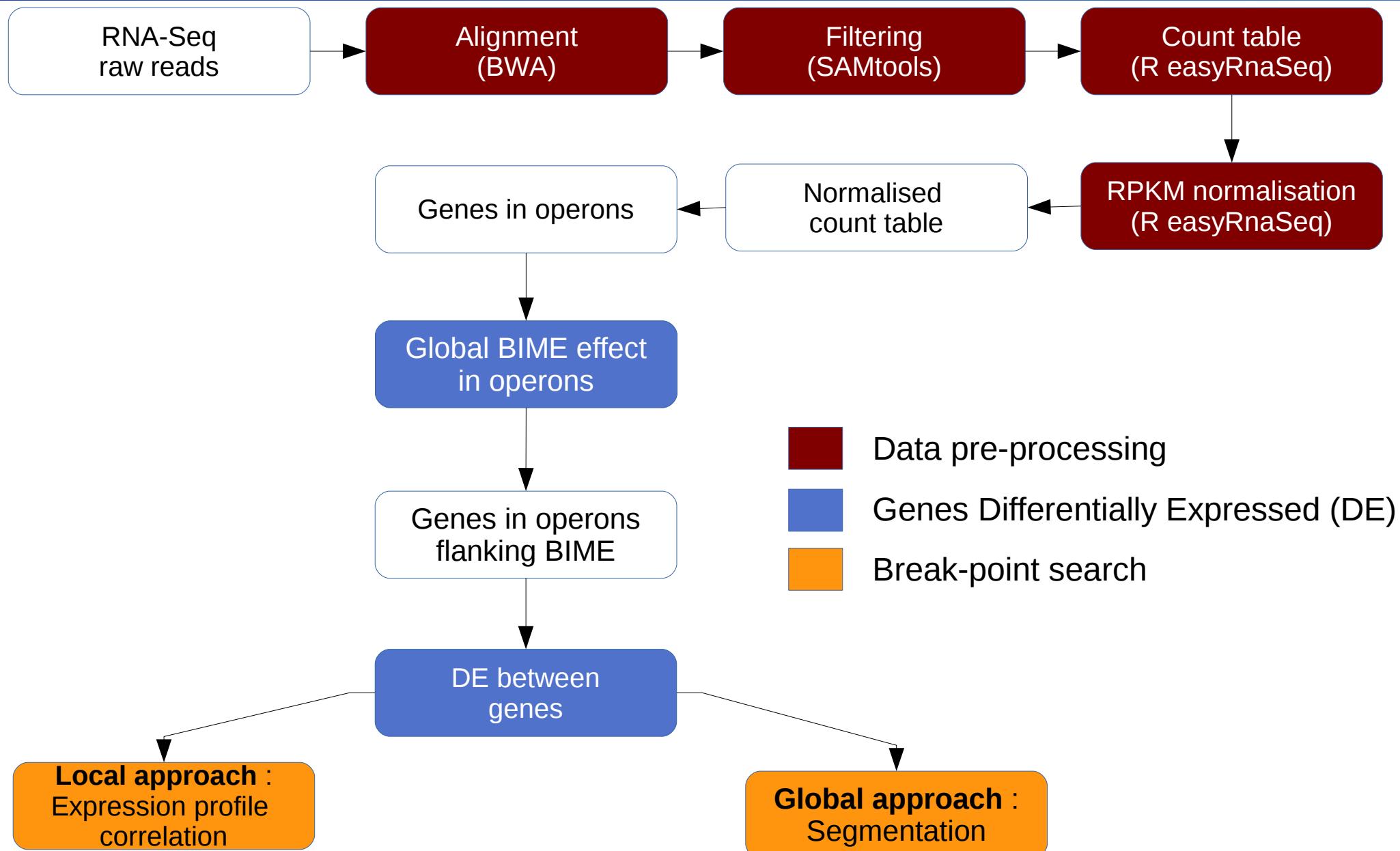
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- Weakness:
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 - False positive detection
- Other approach?

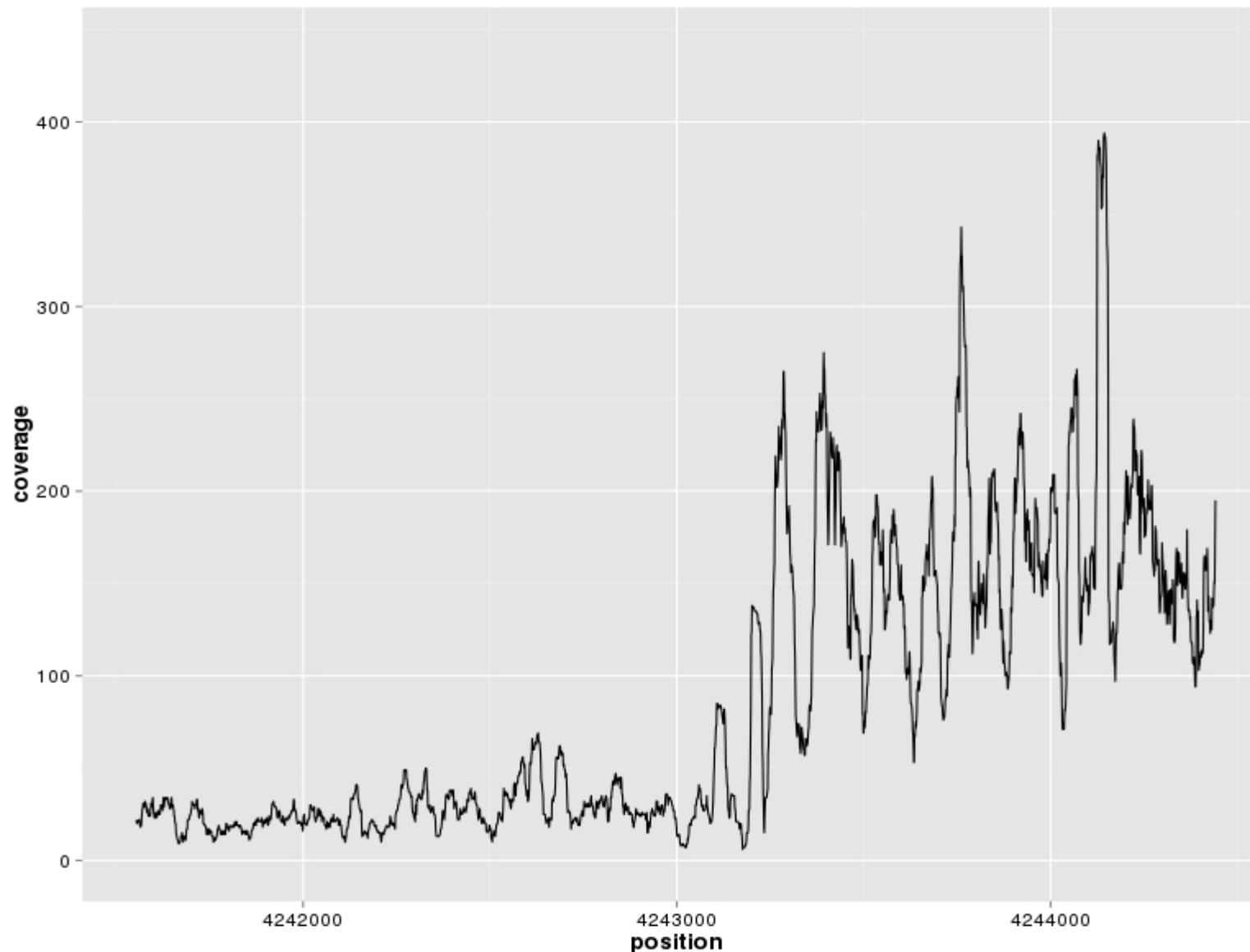


Analysis pipeline



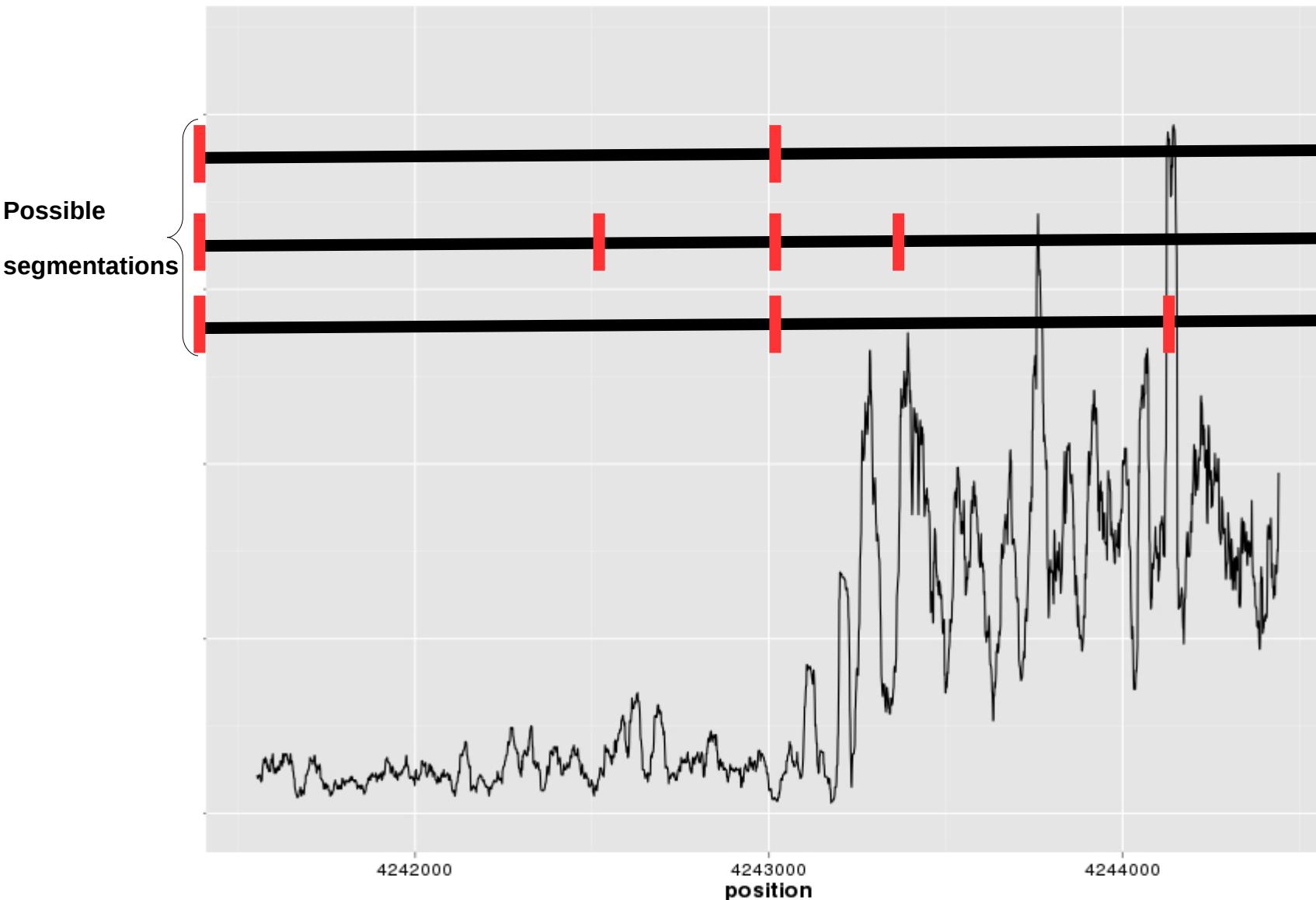
Segmentation (Global approach)

Segmentation on *malE-malF* BIME



Segmentation (Global approach)

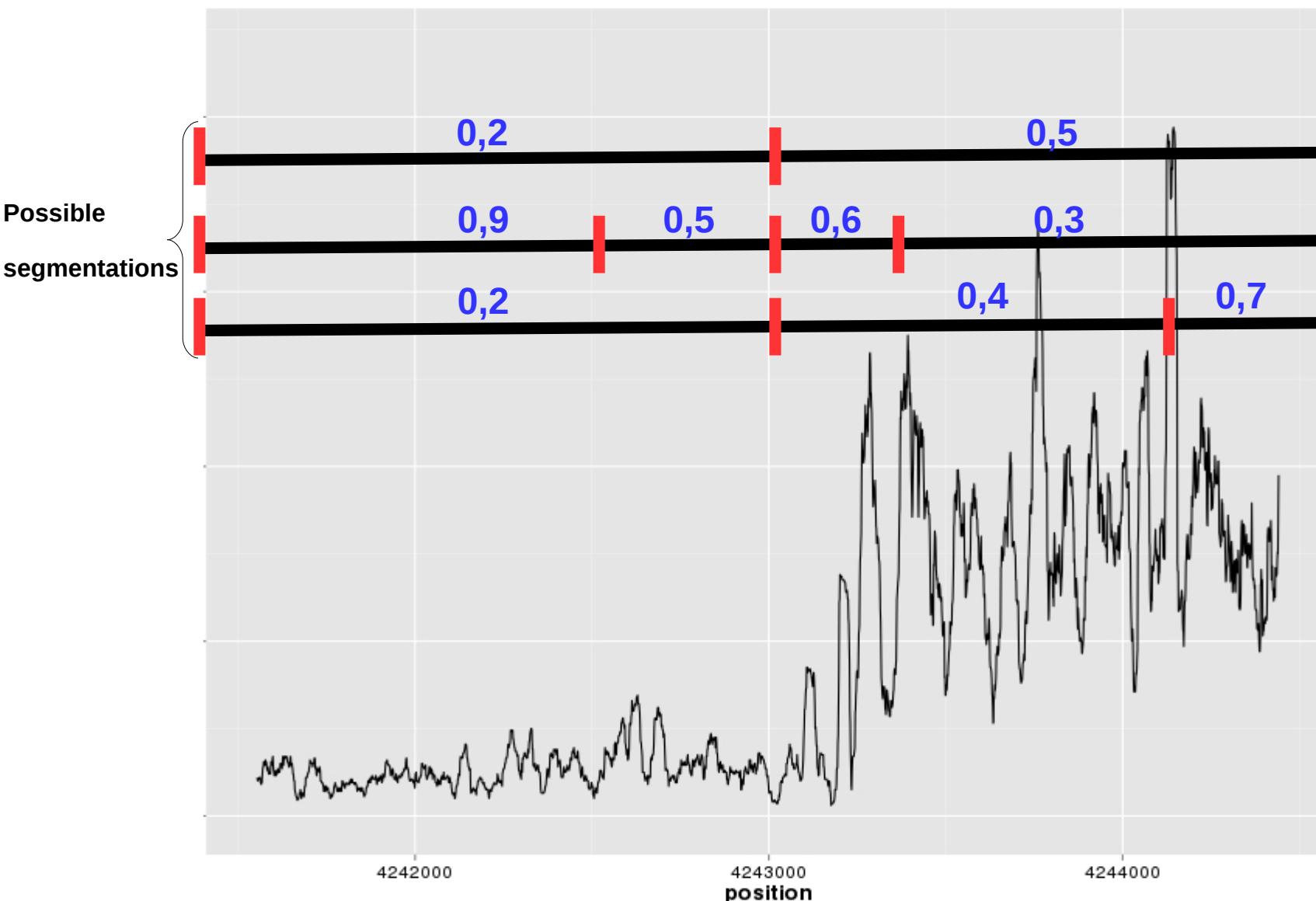
Statistical model : $Y_t \sim G(\theta_r)$



Cleynen et al. 2014

Segmentation (Global approach)

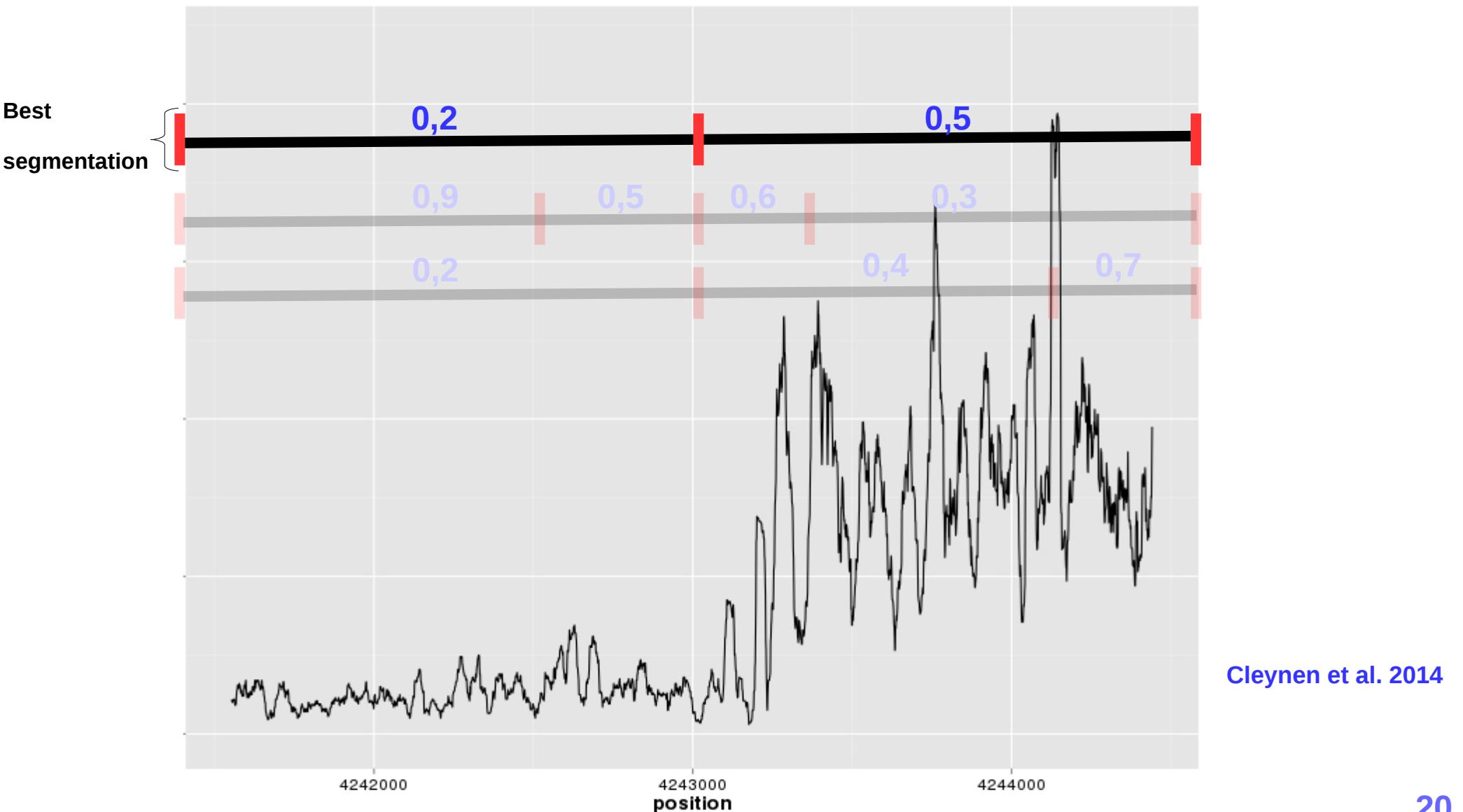
Optimal cost : $c(r) = \min_{\theta} (\sum_{i \in r} y(y_i, \theta))$



Cleynen et al. 2014

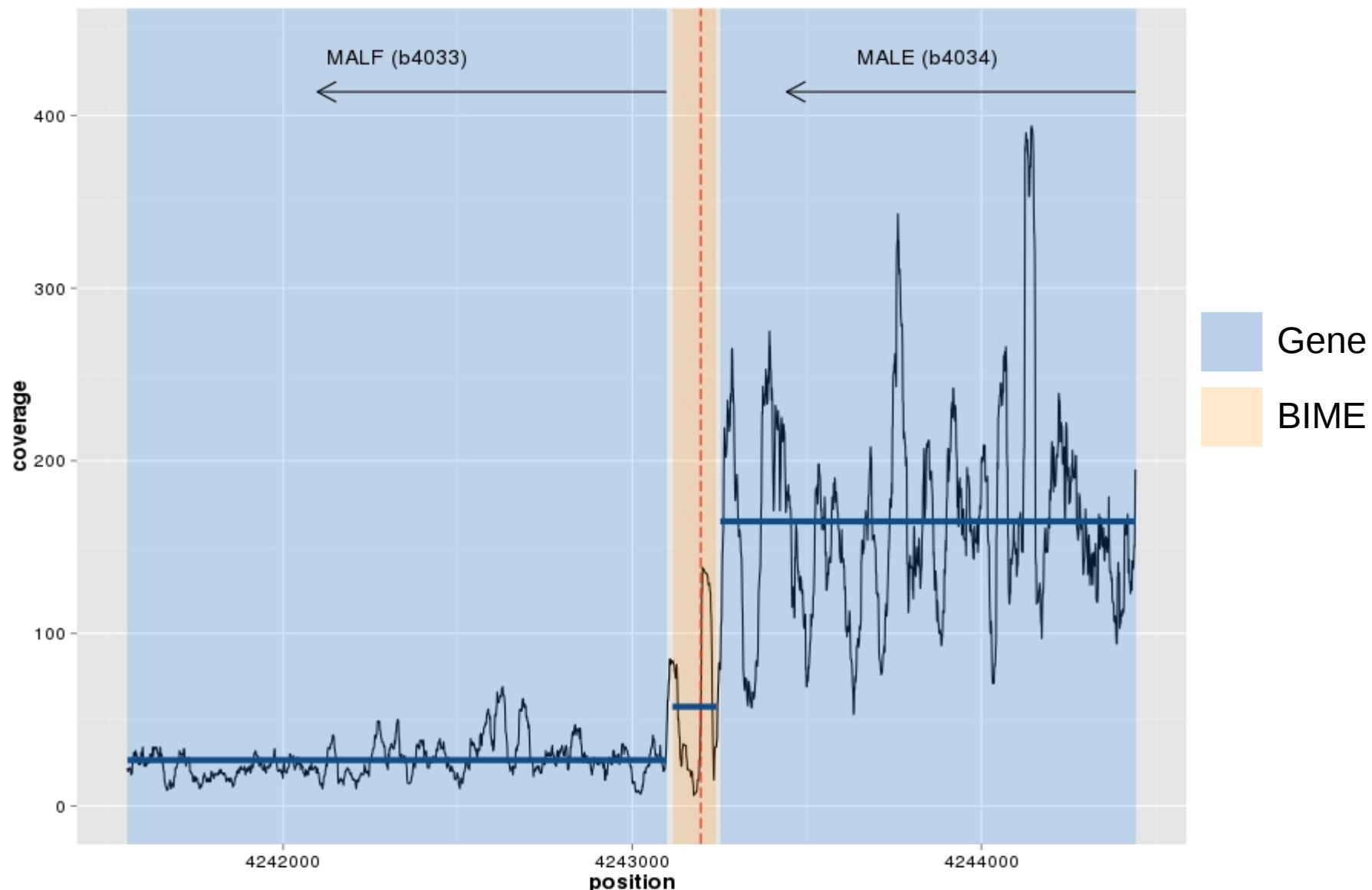
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Pruned Dynamic Programming :



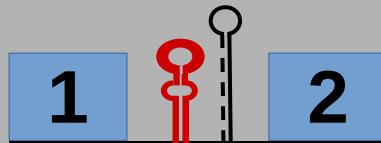
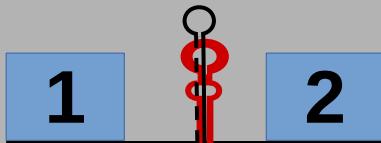
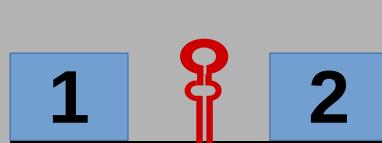
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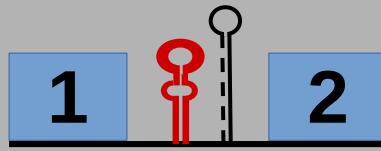
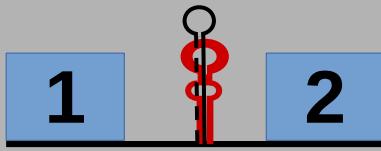
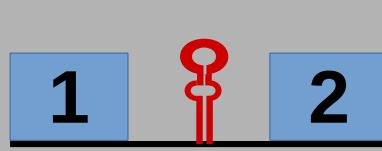
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Coverage break-points on BIME	3/17	3/17	12/17

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- Weakness?

Sensitive to the number of replicates?

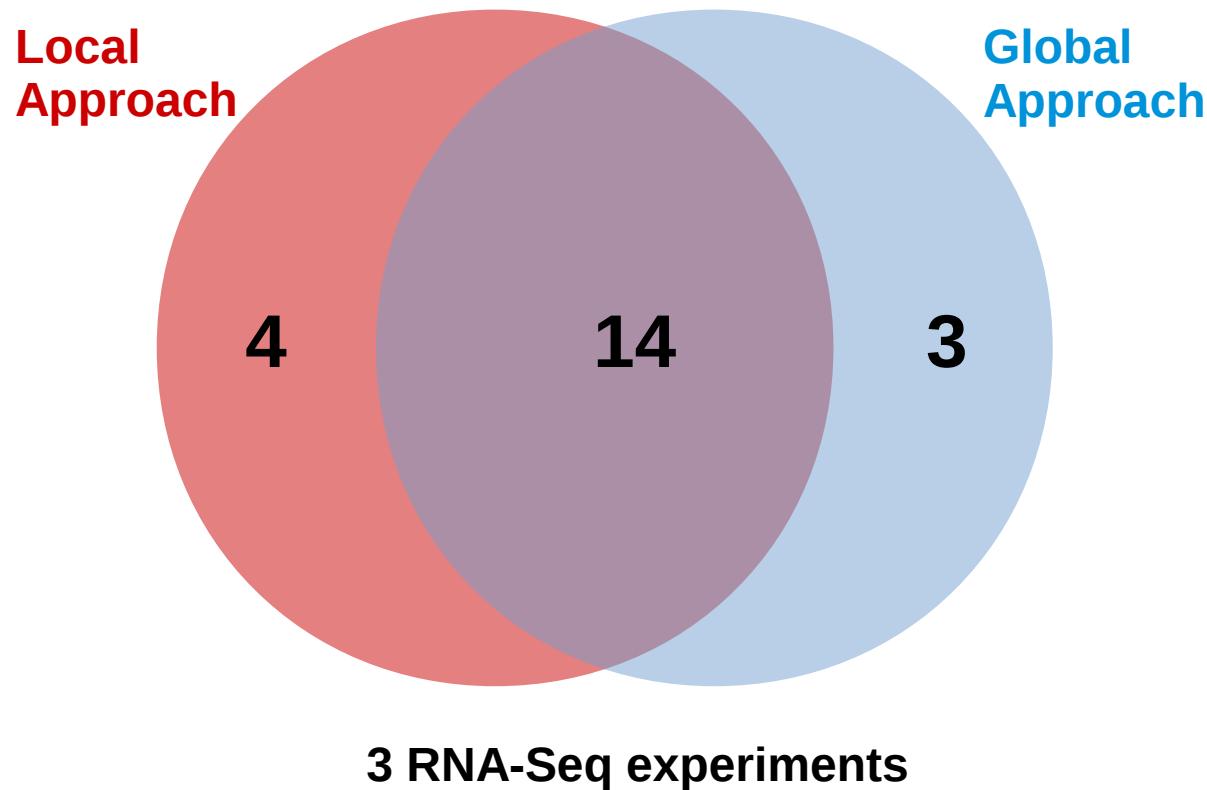
Question

Are BIMEs involved in the mRNA metabolism in operons for *E. coli* genome:

- as transcription terminator?
- as stabiliser of 5' end of transcript?

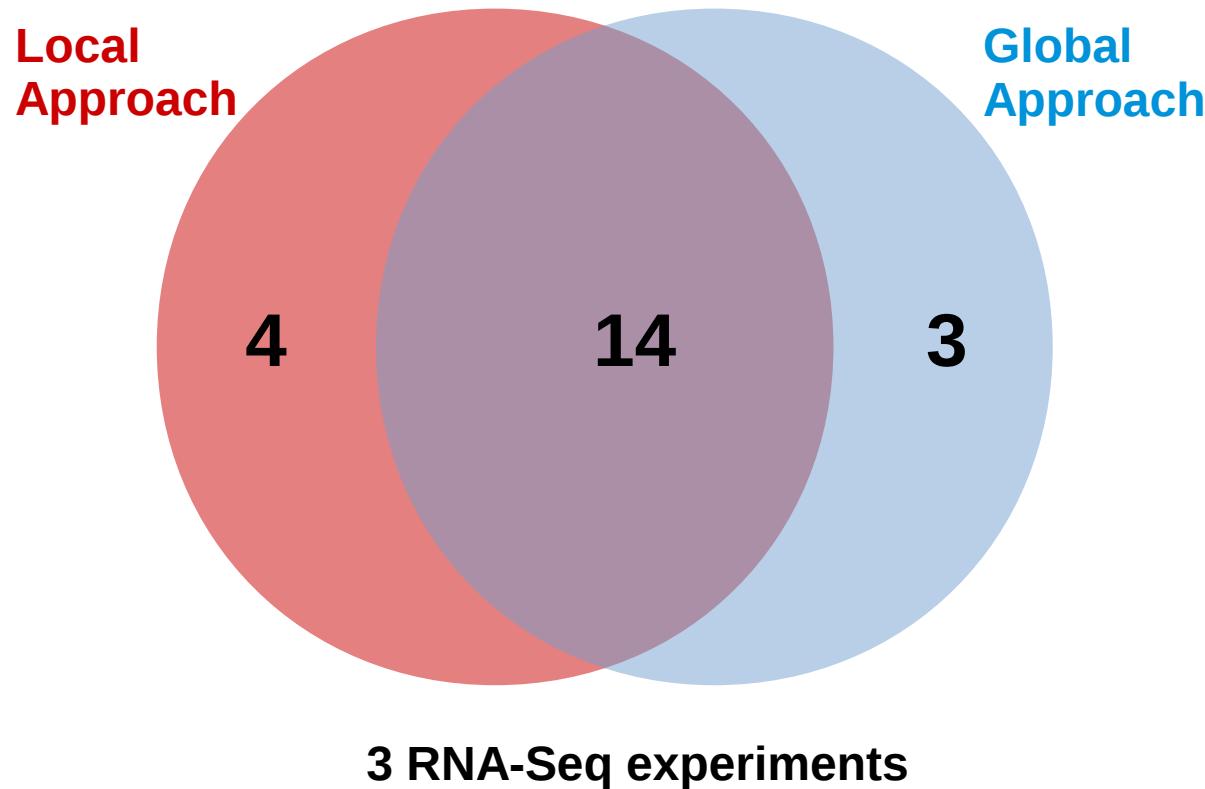
Break-point search result synthesis

- Two methods: 21 break-points



Break-point search result synthesis

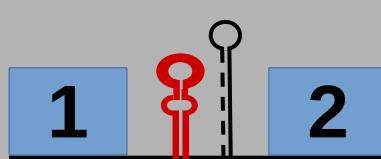
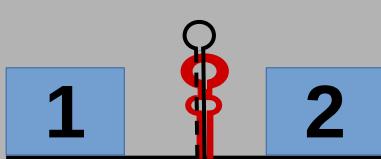
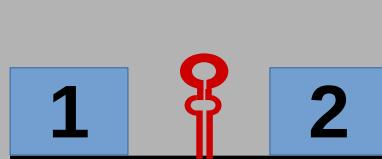
- Two methods: 21 break-points



- 14 common detections

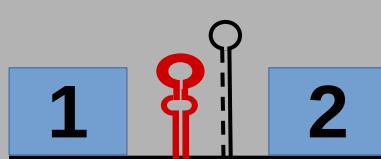
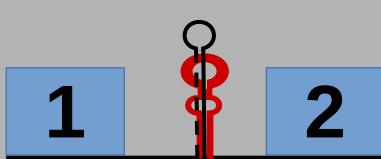
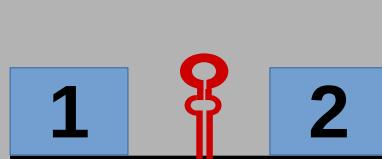
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- Intergenic region structure with respect to BIME coverage break-point:

			
Coverage break-points on BIME	4/21	4/21	15/21

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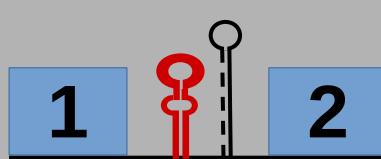
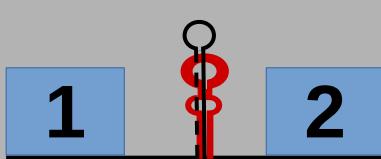
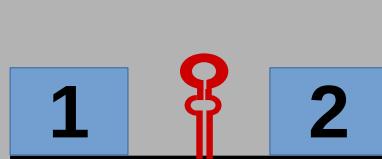
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- Transcription terminator function

Break-point search result synthesis

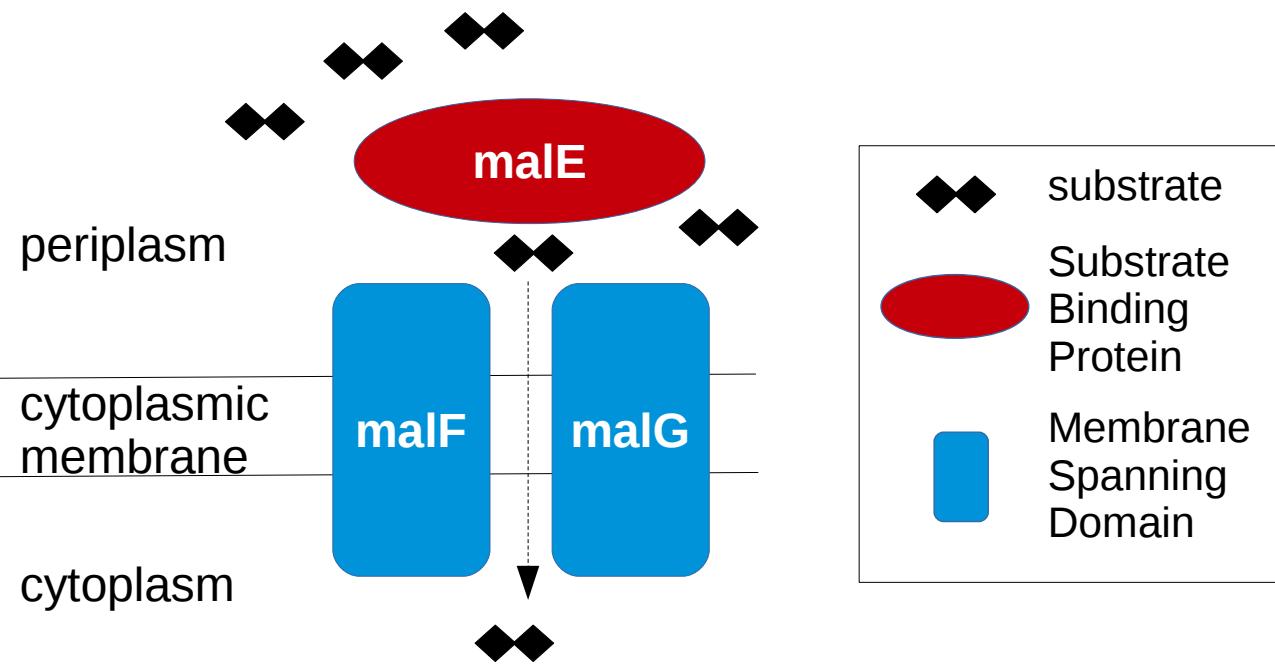
- Intergenic region structure with respect to BIME coverage break-point:

			
Coverage break-points on BIME	4/21	4/21	15/21

- Transcription terminator function
- Stabiliser of 5' end transcript

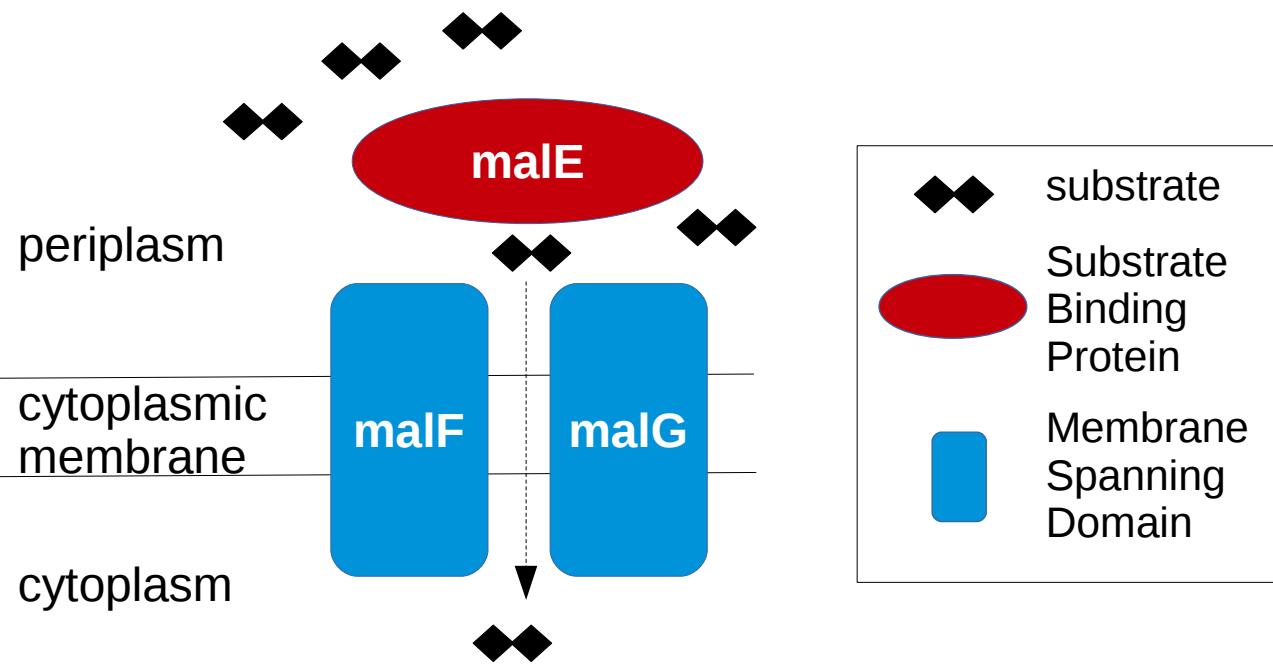
Biological synthesis

- *malEFG* model validated



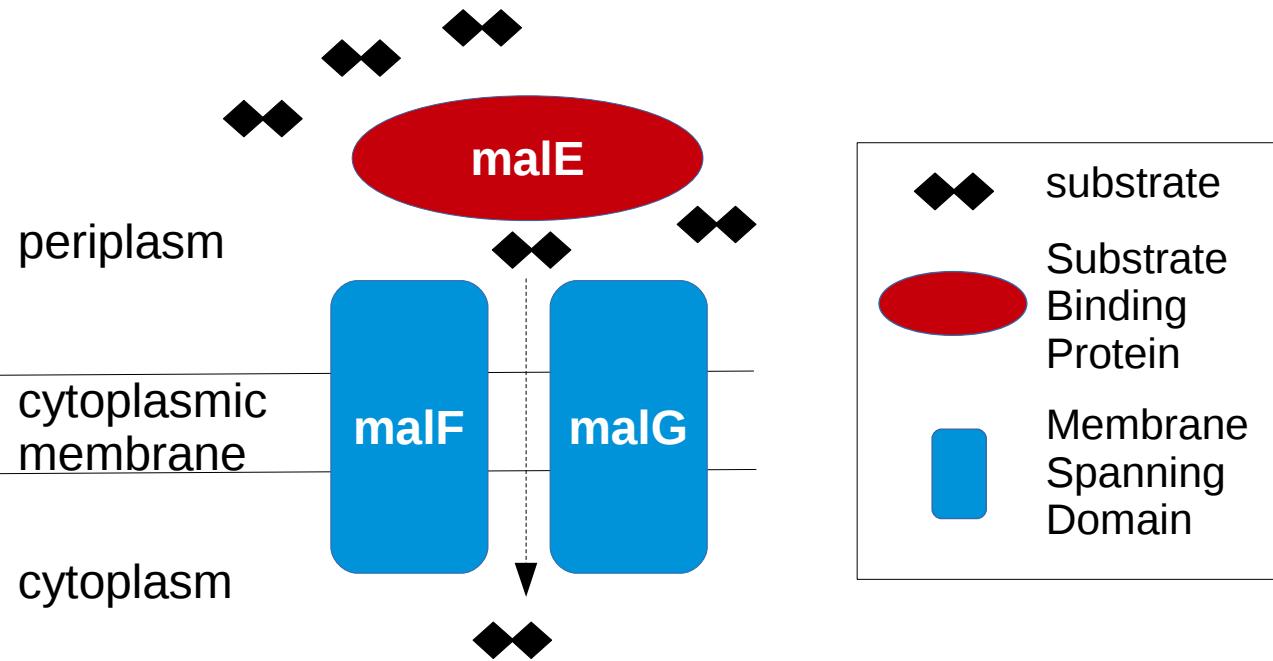
Biological synthesis

- *malEFG* model validated
- 28 ABC transporters in *E.coli* K12



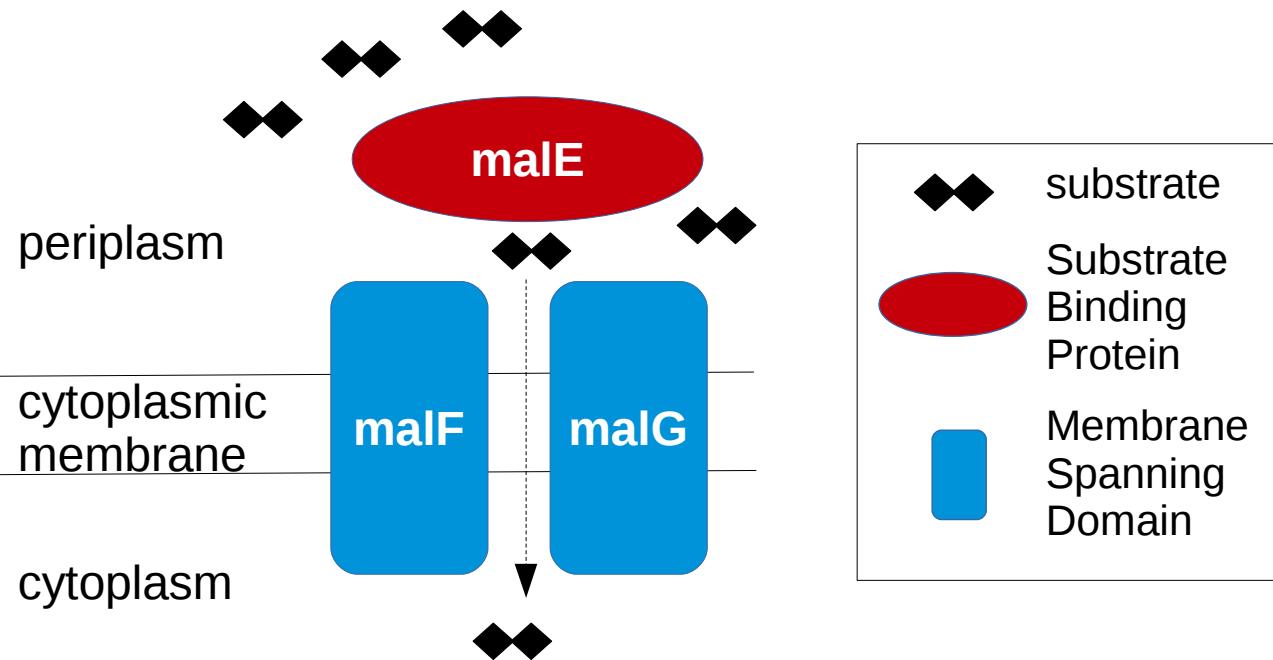
Biological synthesis

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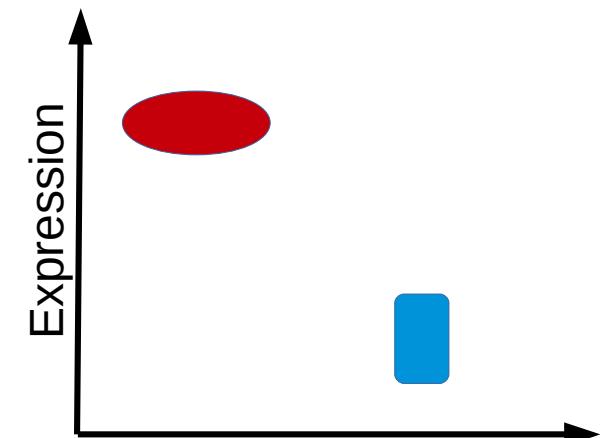
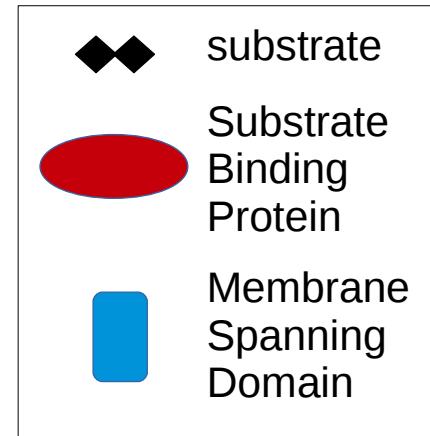
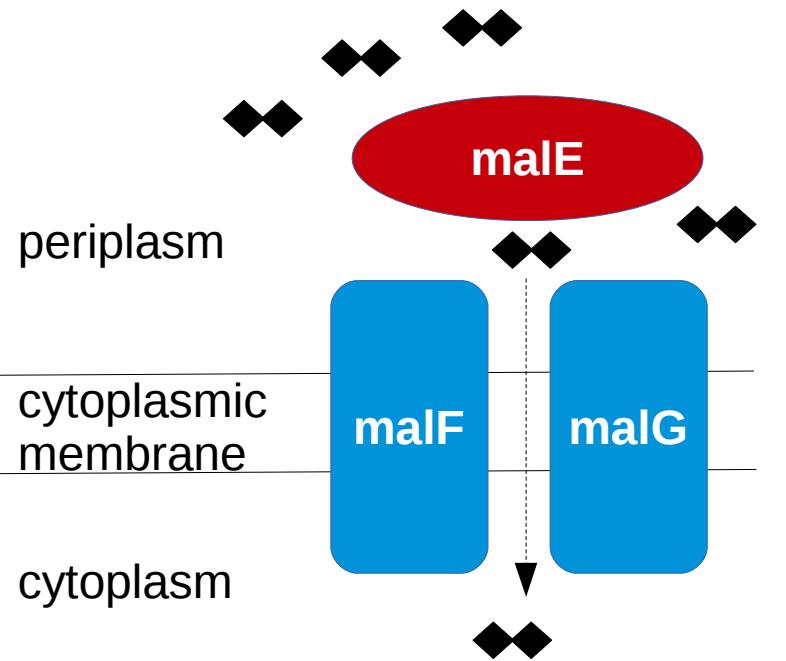
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- 6 without terminator



Biological synthesis

- *malEFG* model validated
- 28 ABC transporters in *E.coli* K12
- 7 ABC transporters with 8 BIMEs on 21 detections
- 6 without terminator → Substrate Binding Proteins



Conclusions

- DE → Segmentation method suited (Global search for coverage break-points)
- Ready to use with other data sets
- Annotation quality?
- For now RNA-Seq data most appropriate for those studies (coverage not uniform)
- BIME effect on upstream gene
- Predominance of 5' end transcript stabiliser function

Perspectives

- RNA-Seq experiment designs
 - ◆ Test transcription termination function:
WT & alteration of degradosome genes
 - ◆ Test transcript 5' end stabiliser function:
Transcription blocking & time sampling for monitoring of the transcript degradation
- Benchmark with segmentation
 - ◆ reduce the number of replicates (same experiment)
- Generalisation to other species

Acknowledgments

LMGM: Genomics of integrated systems

Gwennaele Fichant

Yves Quentin

Roland Barriot

Petra Langendijk-Genevaux

Mathias Weyder

Arnaud Frèche

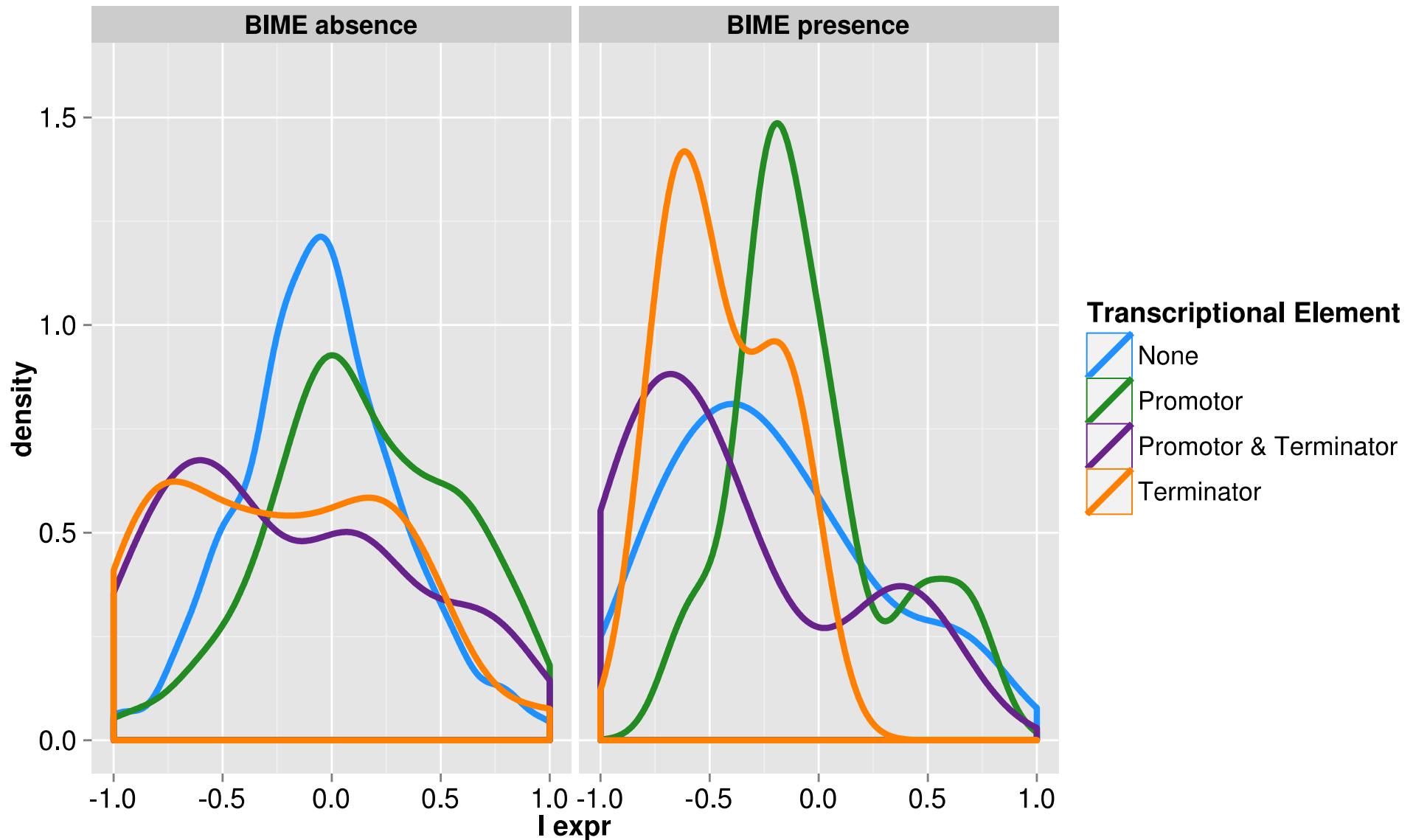
INRA: Applied Mathematics and Informatics

Christine Gaspin



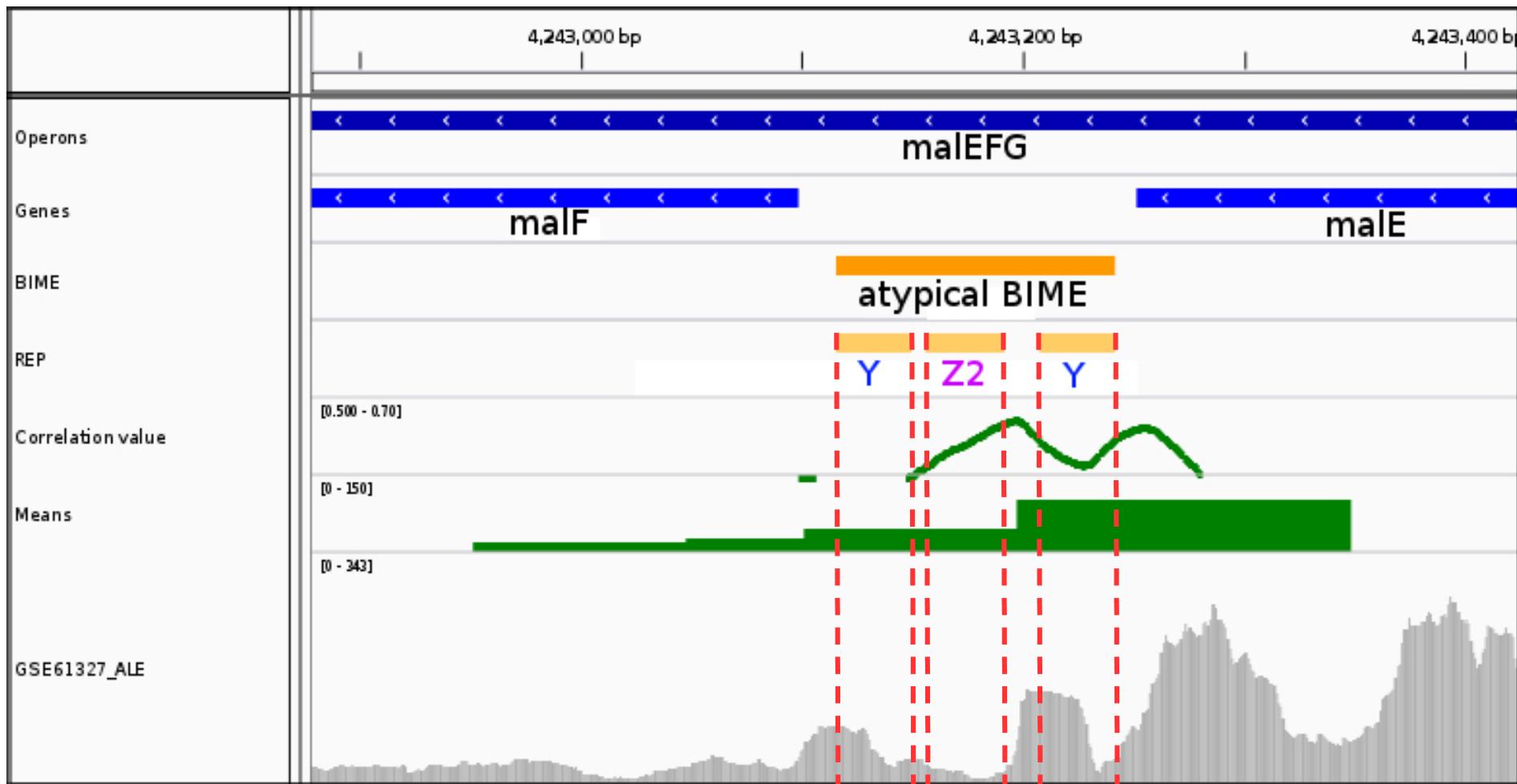
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Distributions of I_{expr} depending on Transcriptional Elements



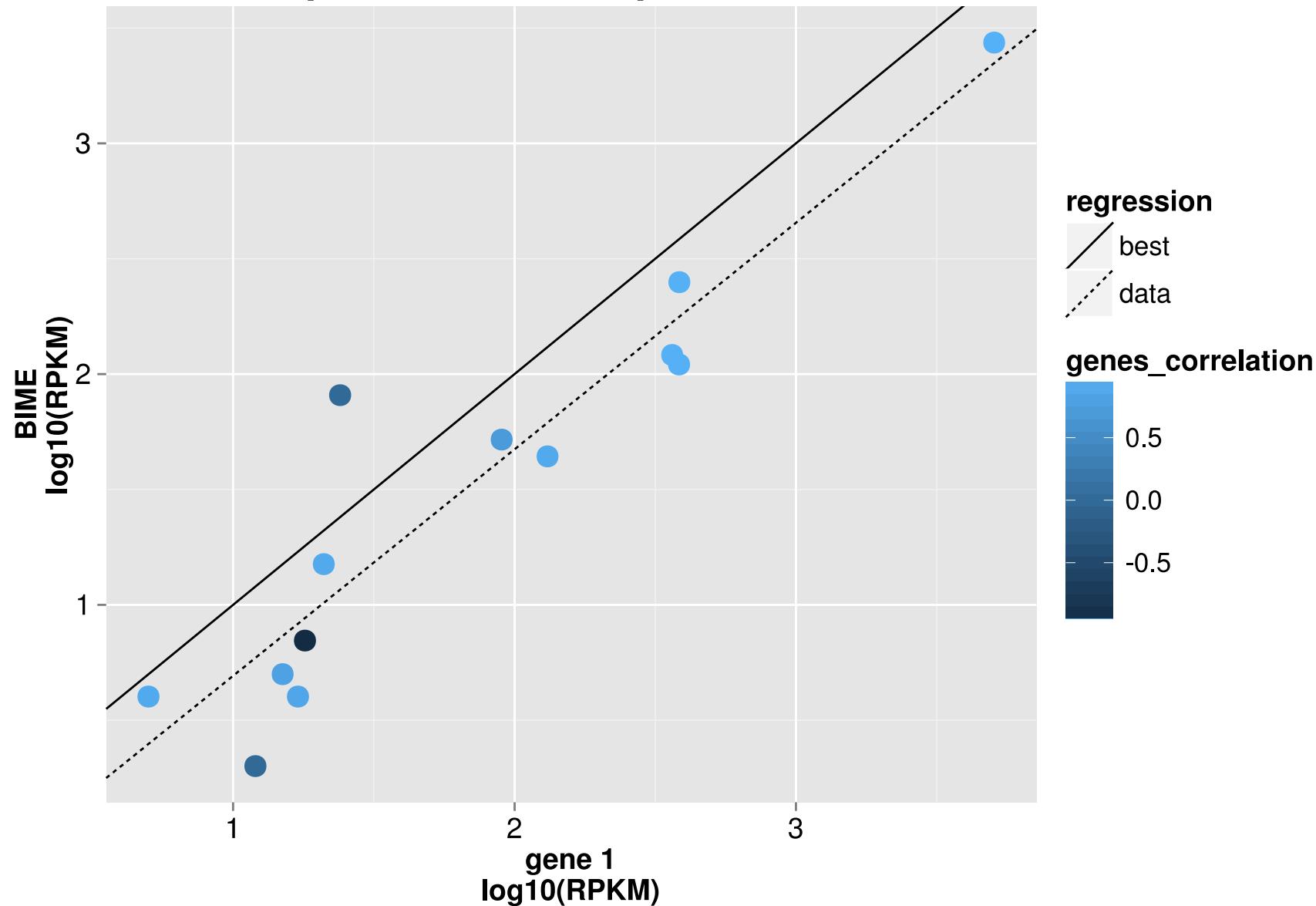
Adaptation of operon detection method (Local approach)

Expression profile correlation on *malE-malF* BIME



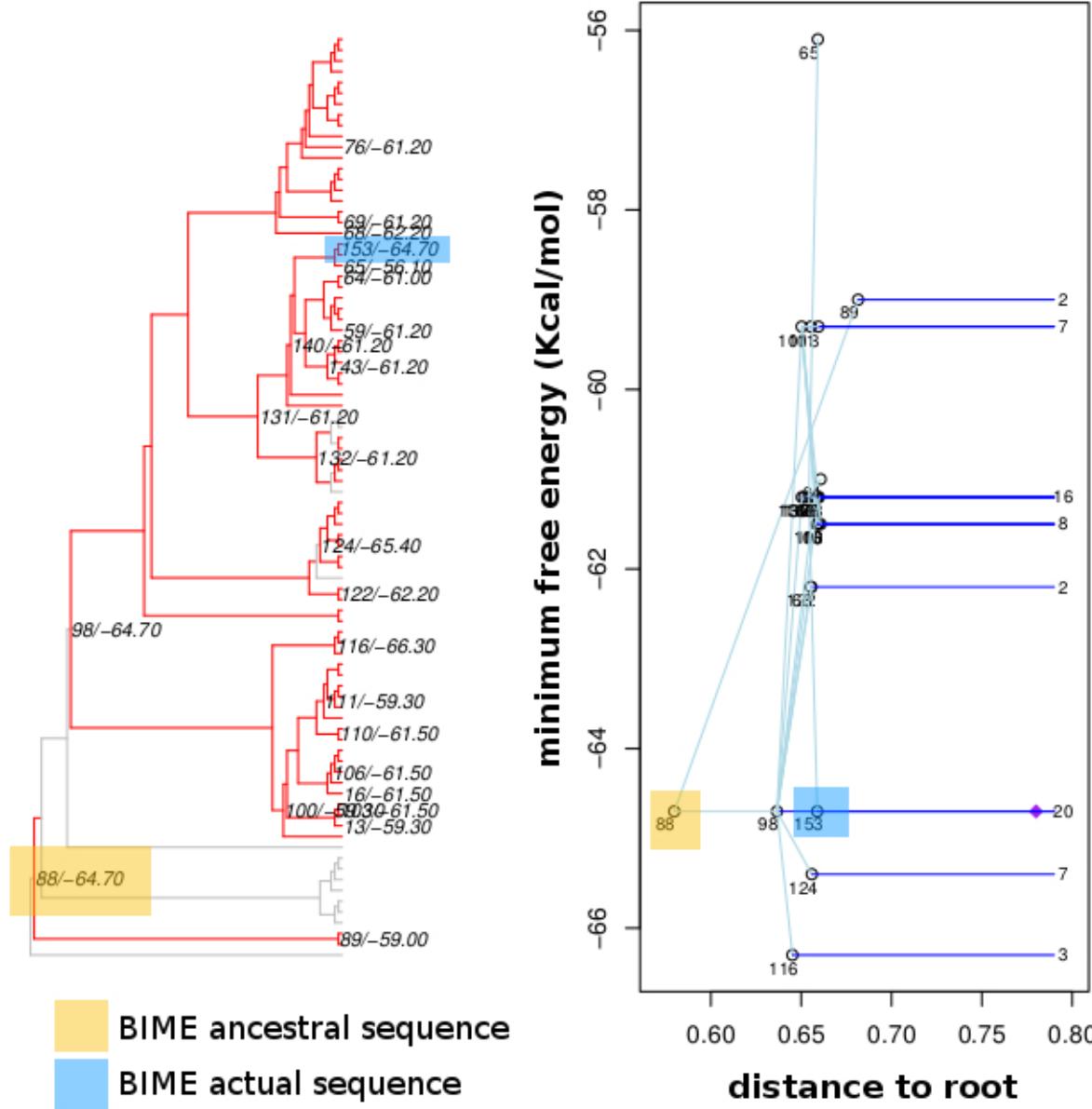
BIME sequencing bias

No DE in operons, no promotor or terminator



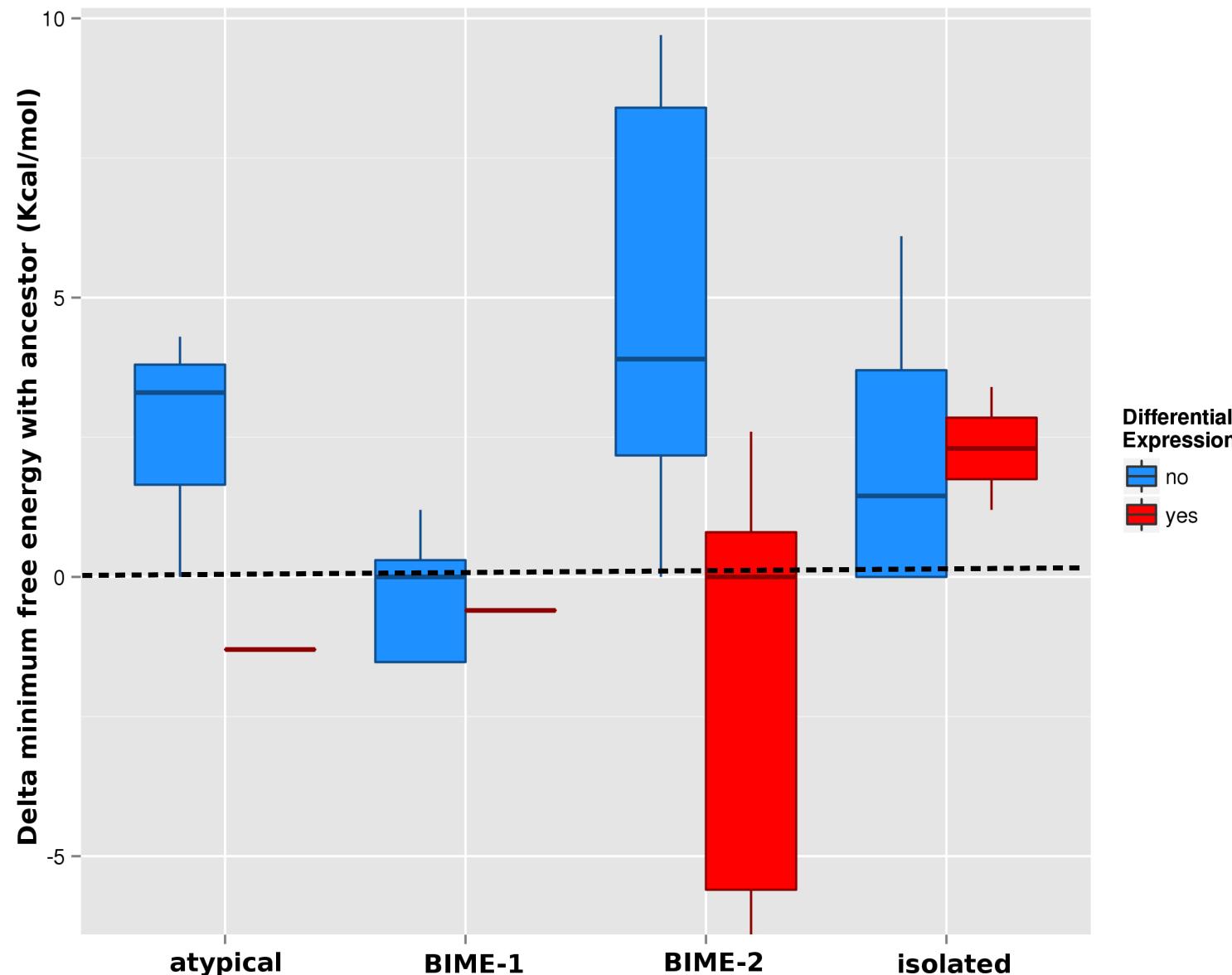
Ancestral sequences and secondary structures

malE-malF BIME 2nd structure energy evolution



Ancestral sequences and secondary structures

Delta of energy between ancestor & actual BIME



Segmentation efficiency & replicates

