



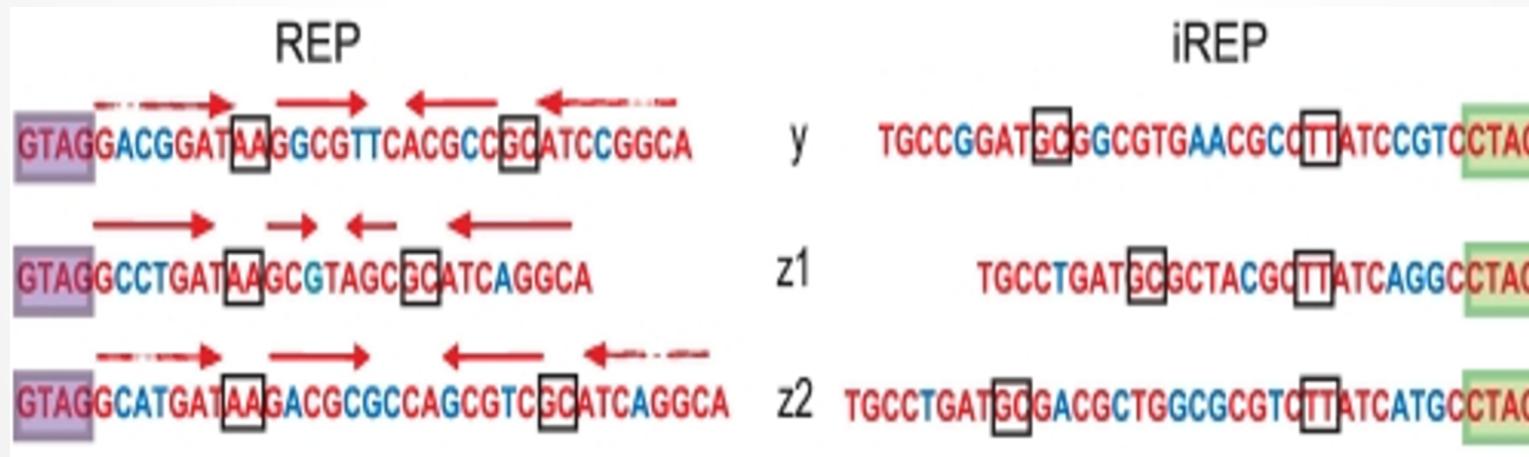
REP involved in transcription regulation?

REP features

- Repeated Extragenic Palindrome.
- 20 - 40 nucleotides.
- 3 classes depending on length and secondary structure : Y, Z¹ and Z².

REP features

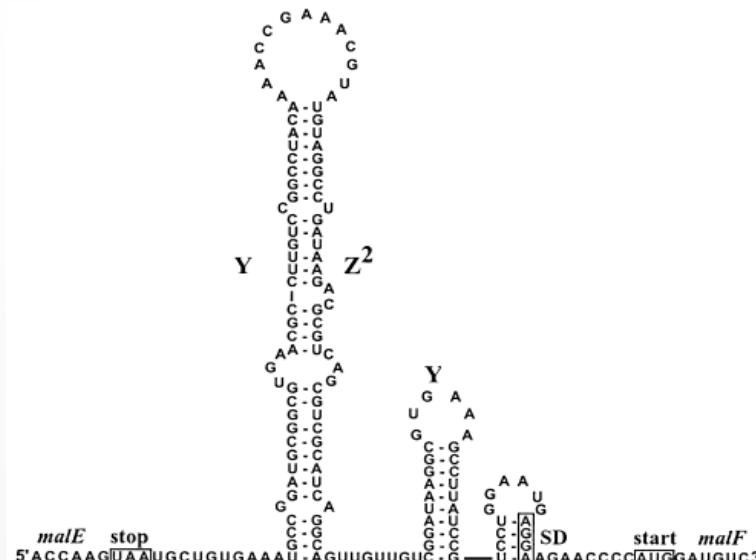
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Ton-Hoang et al. 2012

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- Stem-loop secondary structure with a bulge.

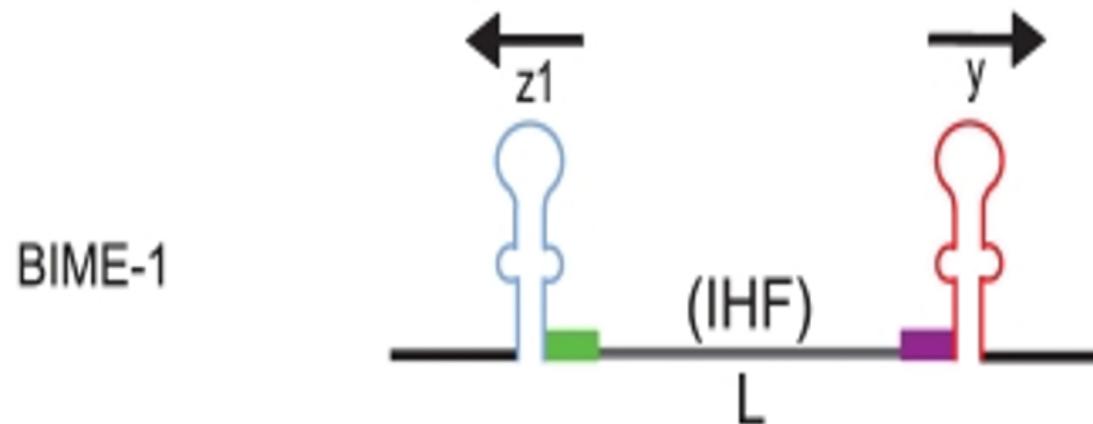


REP features

- Repeated Extragenic Palindrome.
- 20 - 40 nucleotides.
- 3 classes depending on length and secondary structure : Y, Z¹ and Z².
- Stem-loop secondary structure with a bulge.
- Numerous bacterial genomes, ≈ 1% for E. coli.
- BIME organization.

BIME features

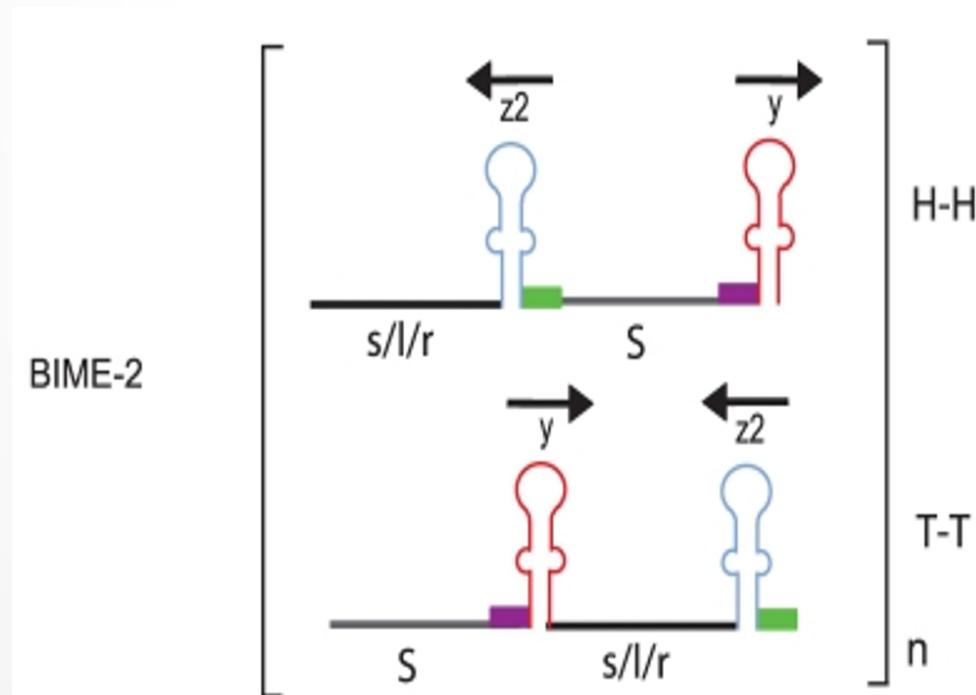
- Bacterial Interspesed Mosaic Element.
- 3 classes depending on REP composition :
Bime-1, Bime-2, atypical.



Ton-Hoang et al. 2012

BIME features

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Objectives

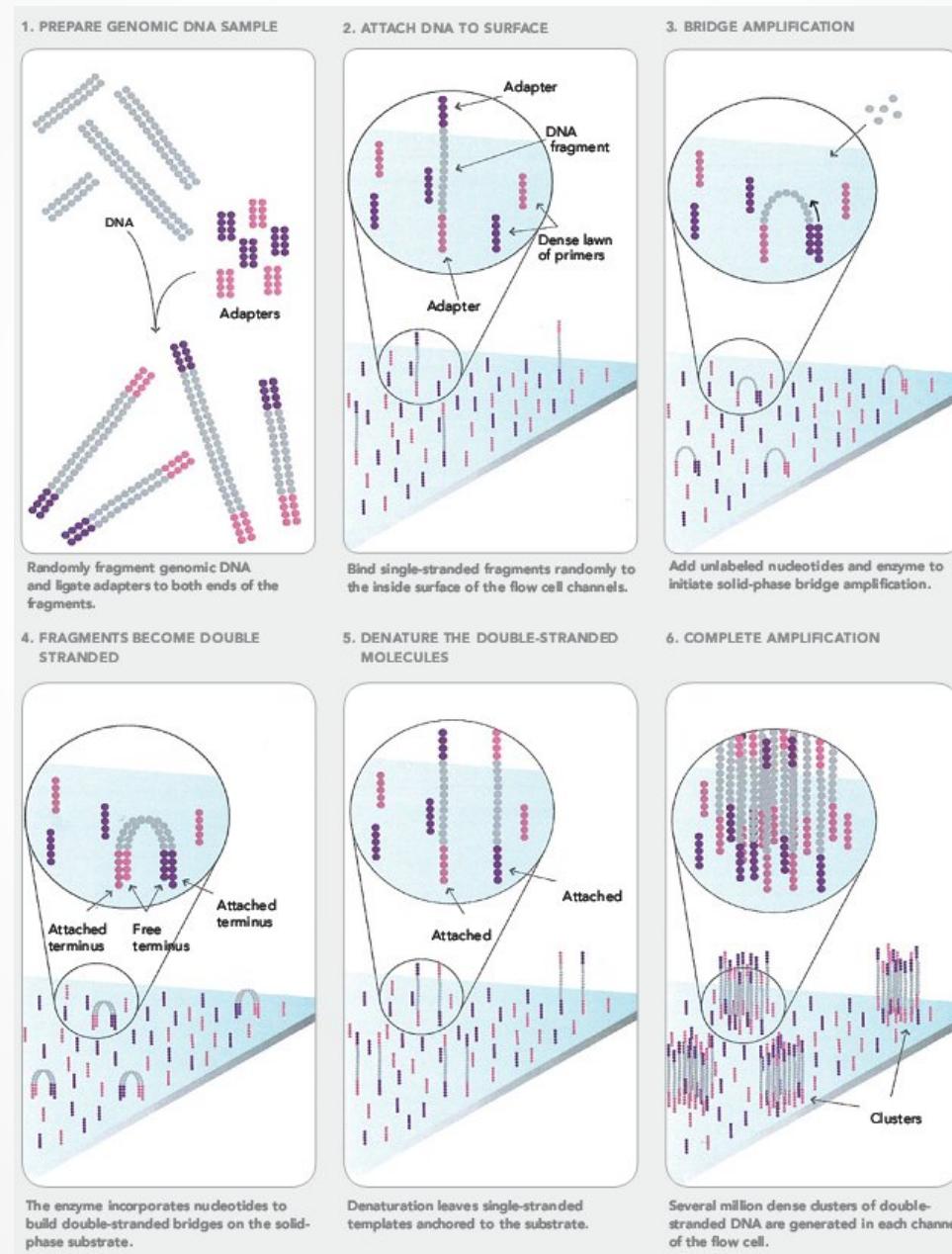
Does REP are involved in the transcription regulation in E. coli genome :

- as protection from RNA degradosome ?
- as transcription terminator ?
- as recognition site for proteic factors ?

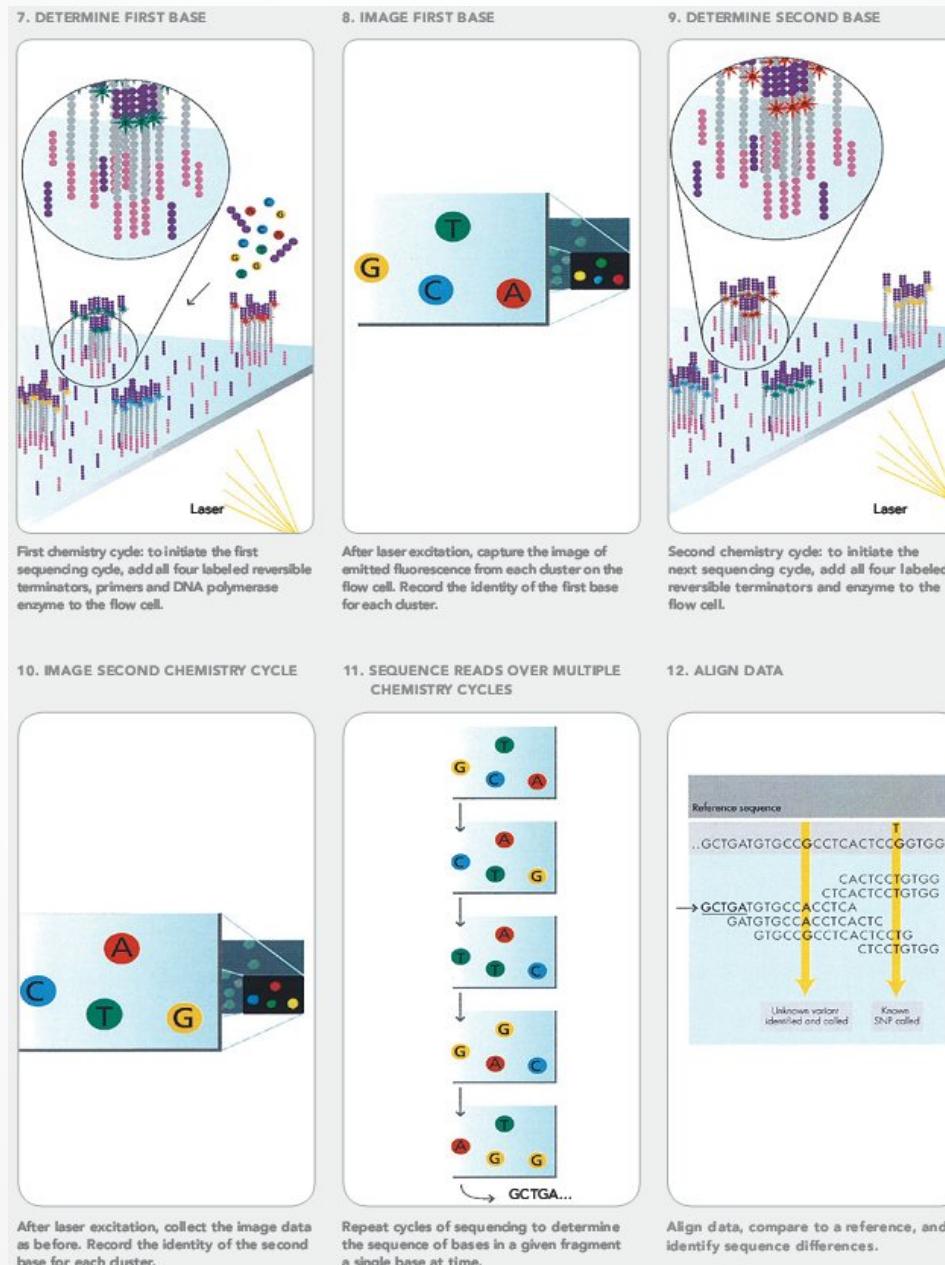
Data choice

- Escherichia coli K-12 MG1655 on Glucose Minimal Medium ([LaCroix et al. 2014](#)).
- Why ?
 - RNA-Seq experiment.
 - Large number of replicates :
 - ✓ 9 for Glucose minimal medium (ALE)
 - ✓ 2 for the wild type (WT)
 - Paired-end and specific strand library preparation.

Illumina technology



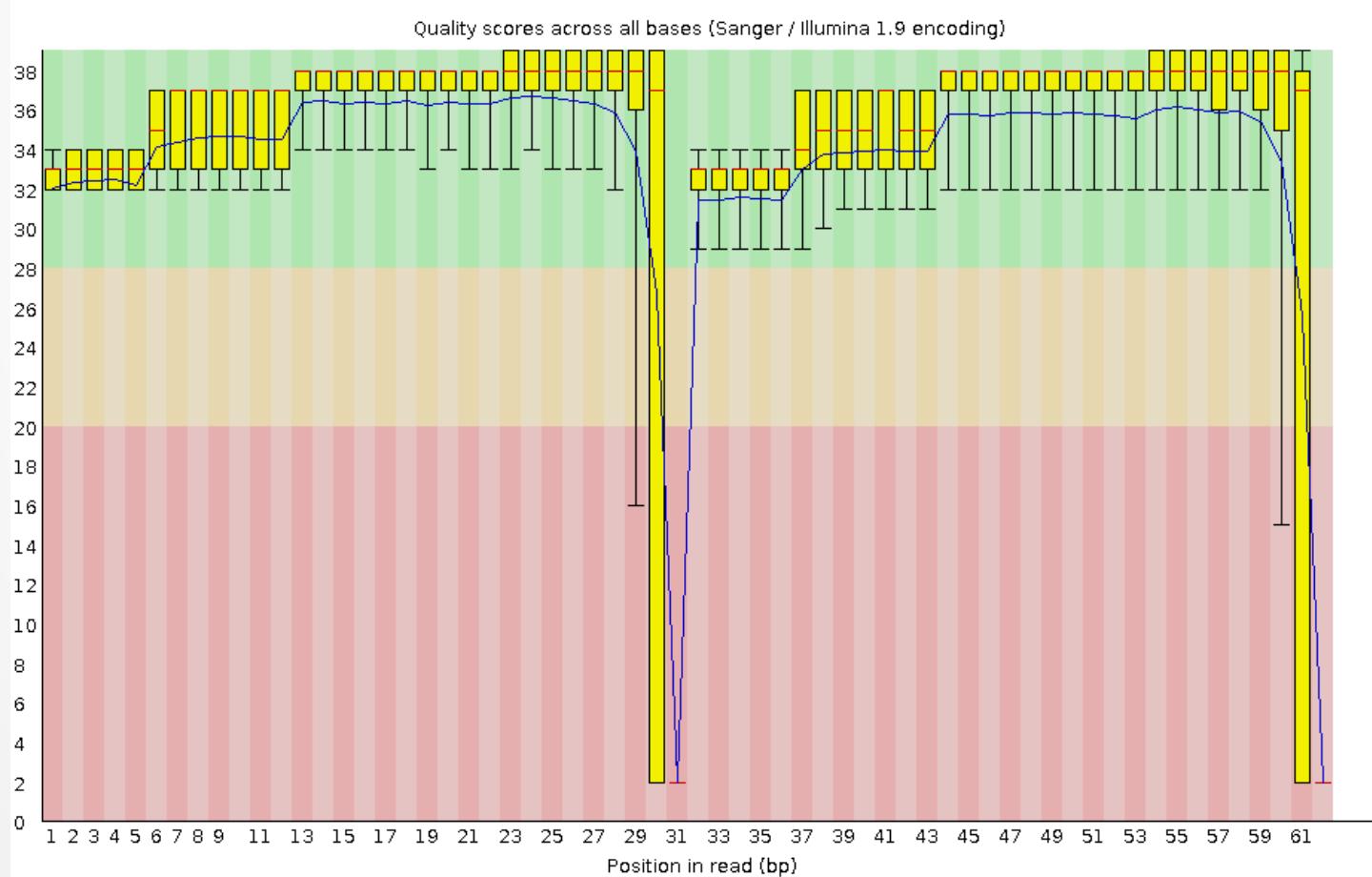
Illumina technology



Quality control

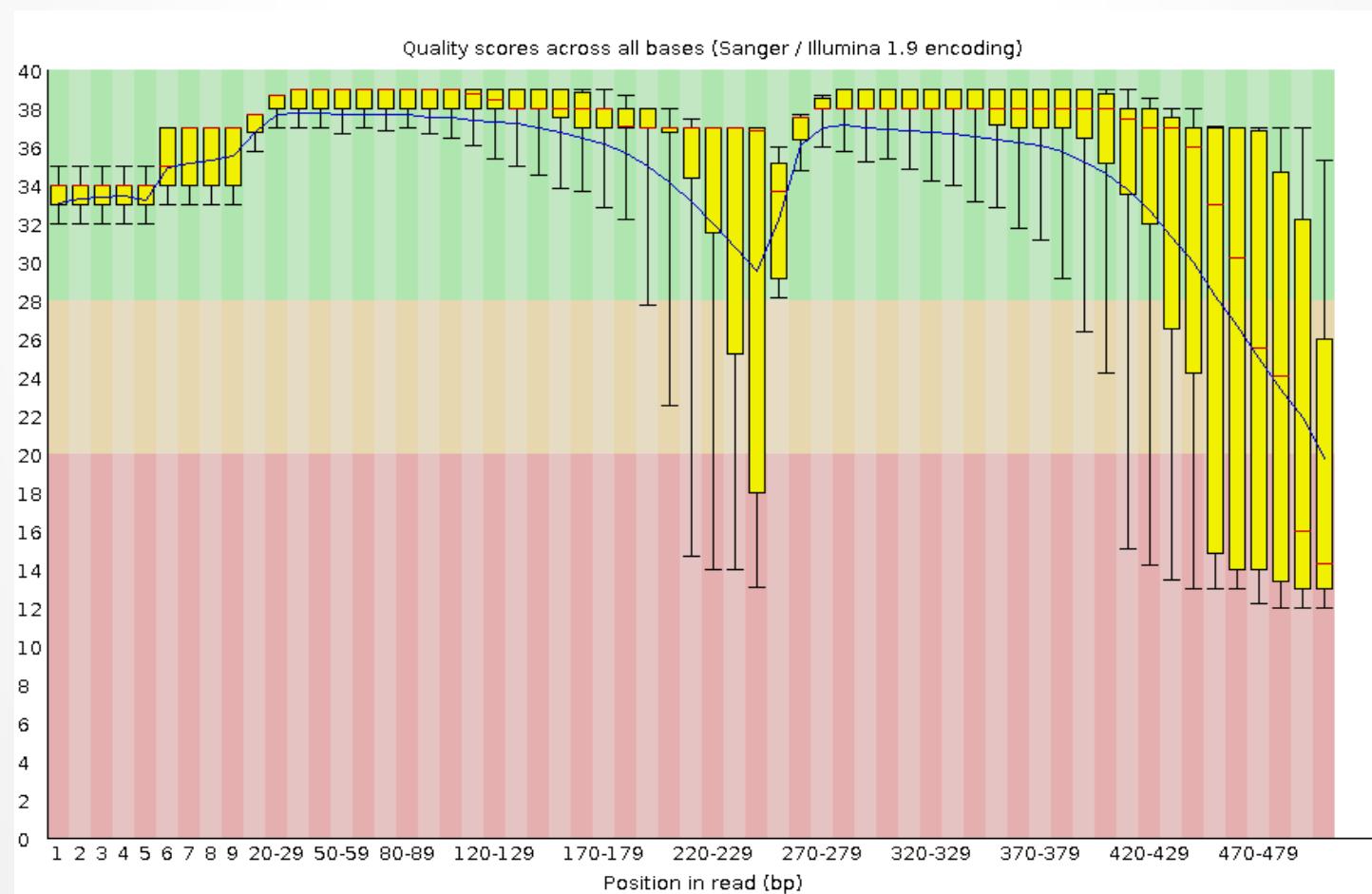
Quality control
with fastqc :

Measure	Value
Total sequences	7 772 244
Sequences flagged as poor quality	0
Sequence length	62
% GC	53



Quality control

Quality control
with fastqc : 1 ALE sample rejected (35-502 bp)



Alignments

- Alignment on NC_000913.2 E.coli genome (**BWA**) :
 - Index of the reference genome
 - Alignment (SAM file)

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 - Index of the reference genome
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- Post-alignment process (**Samtools**) :
 - Convert to binaries (BAM file)
 - Sort the reads by genomic position
 - Remove PCR duplicates
 - Merge files
 - Index alignment merged file

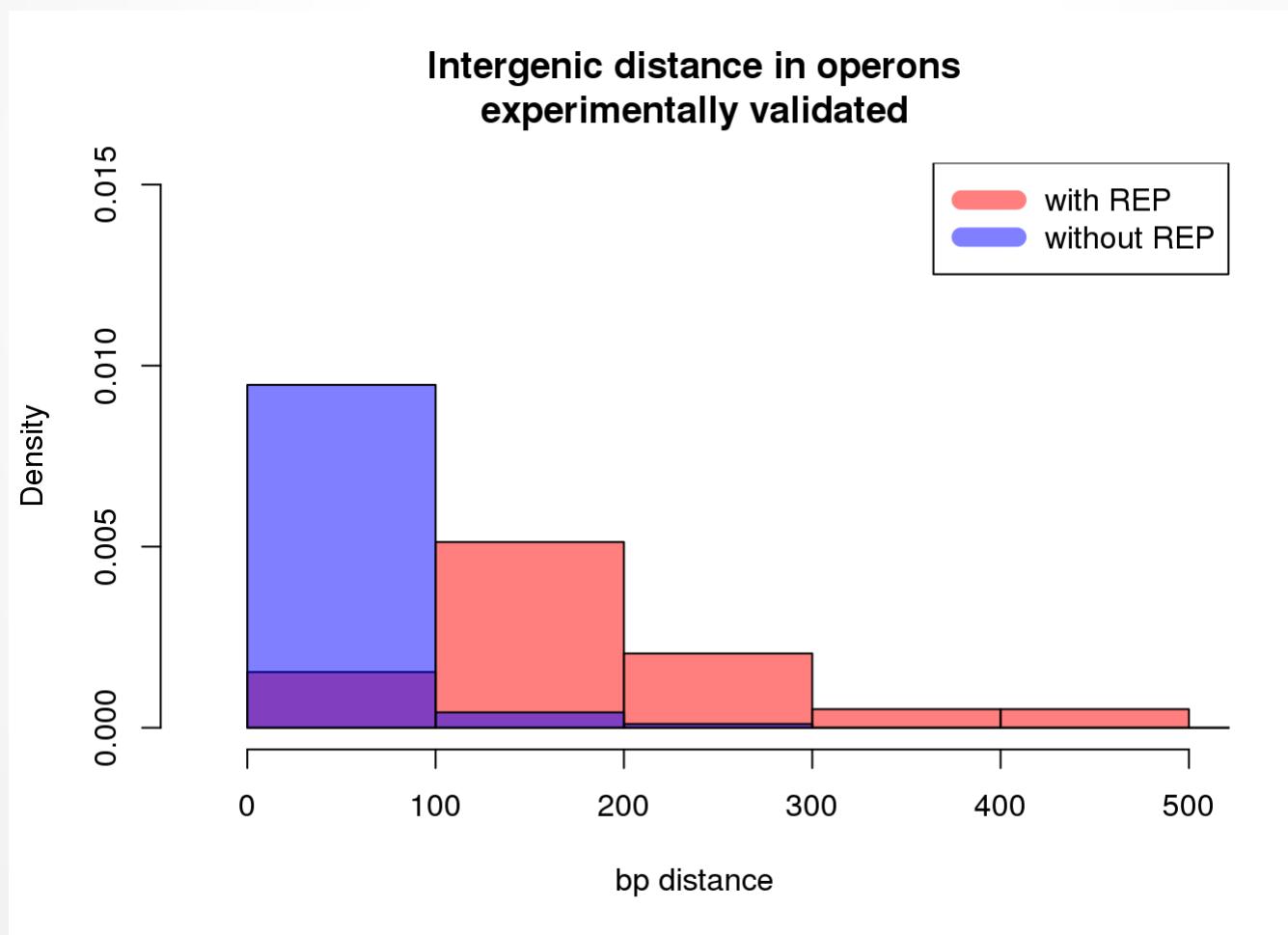
Genomic informations

From GFF, laboratory database, RegulonDB and DOOR
design of reference files (**Bedtools, Python scripts**)

- Operons
- REP in operons
- Genes surrounding REP in operon
- Terminators...

Genomic informations

For operons from RegulonDB :



Differential expression in operon with REP

**Detect Differential Expression (DE)
between genes surrounding a REP in an
operon.**

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Keep in mind :

- Not looking for a gene DE in different conditions.
- Focus on minimal Glucose medium condition (ALE).
- Not focus on mRNA, transform the GFF to get the expression of tRNA, rRNA and ncRNA.

Differential expression in operon with REP

- Reads count table for expressed features
(Bioconductor package easyRnaSeq)

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- Correct reads count with RPKM (Reads Per Kilobase Per Millions mapped reads) :

$$RPKM = \frac{nb\,mapped\,reads \times 1000\,bases \times 10^6}{length\,of\,transcript \times total\,nb\,of\,mapped\,reads}$$

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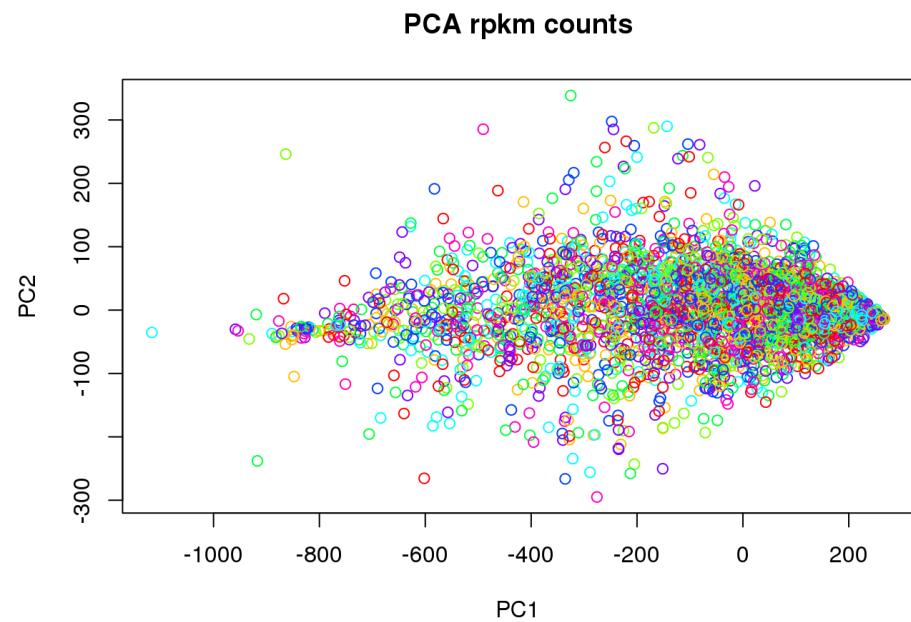
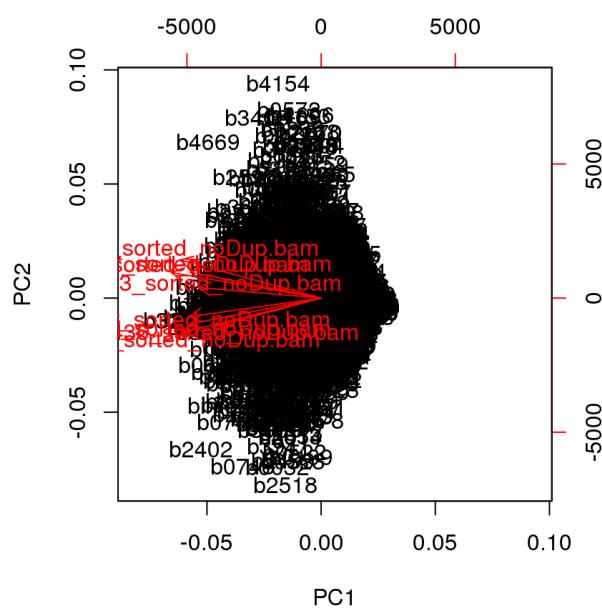
Molar concentration of a transcript :

- RNA length
- Total number of reads in the measurement

Facilitates transparent comparison of transcript levels within and between.

Differential expression in operon with REP

- PCA check for RPKM corrected data :

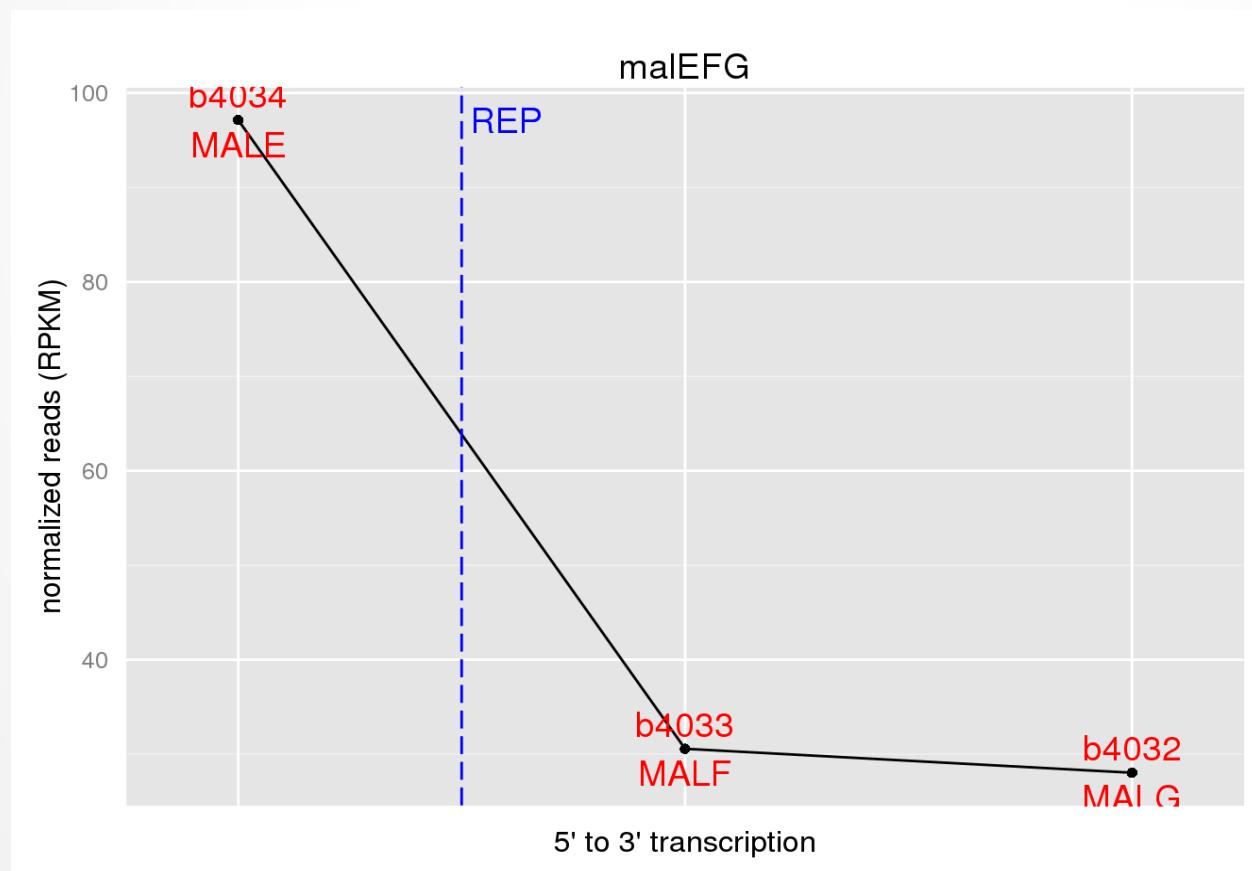


Differential expression in operon with REP

- For genes surrounding a REP, Wilcoxon test to detect DE :
 - P-value : < 0.01
 - RPKM counts > 10 for at least one gene

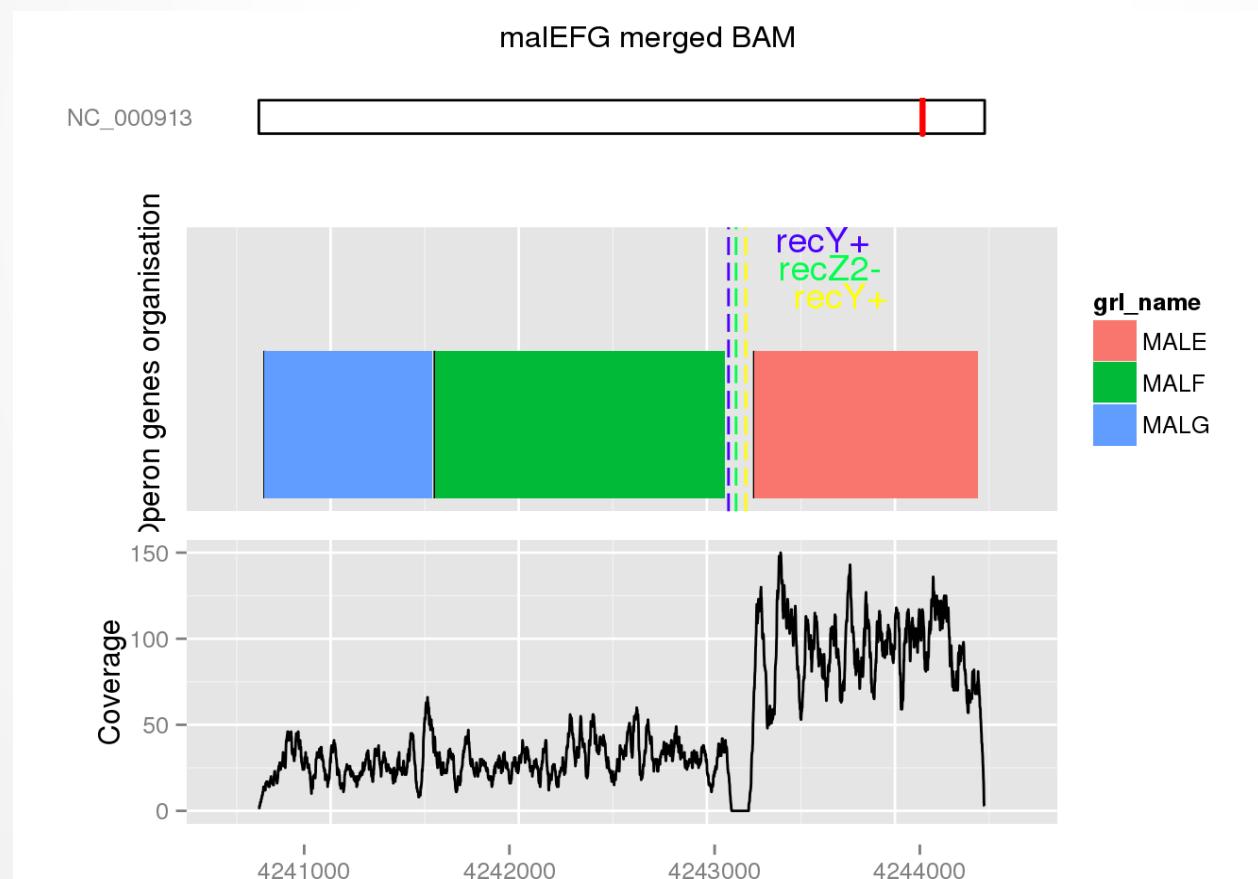
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Differential expression in operon with REP

- 17 over 36 operons with DE around the REP.

Function	Number of operons	DE change feature
ABC transporter	7	↗
	2	↘
Multipurpose conversions 4-aminobutyrate aminotransferase, PLP-dependent	1	↘
IF2 : membrane protein/conserved protein	1	↗
Amino acid biosynthesis : Glutamine synthetase	1	↘
Inner membrane protein, DUF485 family	1	↗
Anaerobic respiration carbamoyl phosphate phosphatase and maturation protein for [NiFe] hydrogenases	1	↗
Degradation of small molecules: Carbon compounds mannitol-1-phosphate dehydrogenase, NAD-dependent	1	↗
Central intermediary metabolism : Pool, multipurpose conversions glycerol kinase	1	↗

Differential expression in operon with REP

- 9 ABC transporter operons ([abcDB source](#)) :

DE for genes surrounding a REP in ABC transporter :

Number of ABC transporter operons	DE change feature	More expressed gene function
7	⬇	Solute Binding Protein
2	↗	Nucleotide Binding Domain

Search for transcription change events on BiME

- GFF modifications to convert BiME regions as mRNA.
- Extraction of genomic positions for genes bounded regions with BiME like :

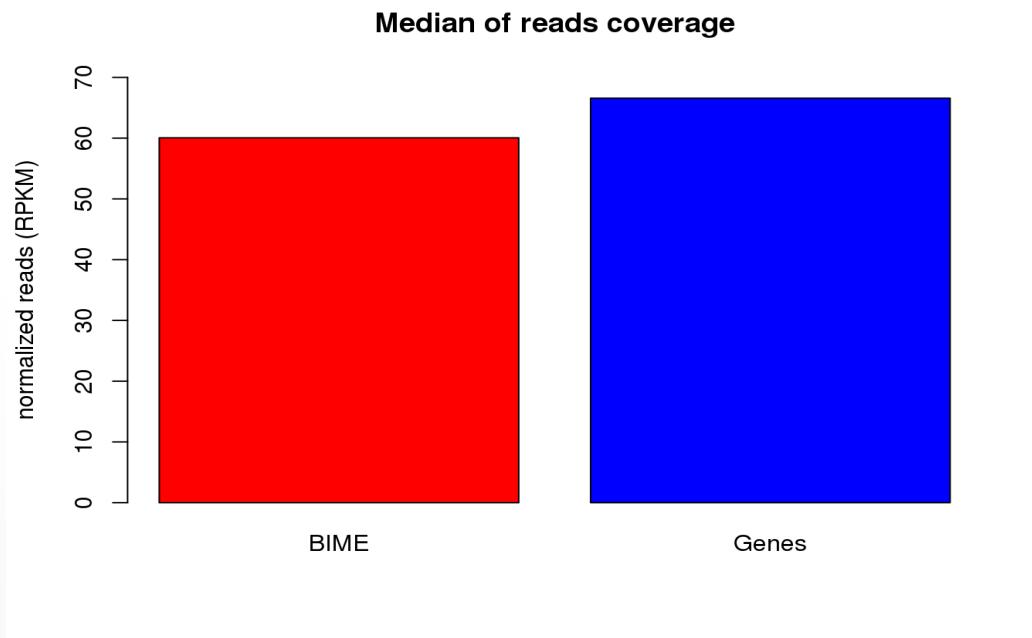

```
{Gene} {IntraGenic-BIME-IntraGenic} ... {Gene}
```
- Coverage per base calculation on these regions
(Bedtools)
- RPKM correction.

Search for transcription change events on BIME

- GFF modifications to convert BIME regions as mRNA.
- Extraction of genomic positions for genes bounded regions with BIME like :

{Gene} {IntraGenic-BIME-IntraGenic} ... {Gene}

- Coverage per base calculation on these regions (**Bedtools**)
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Search for transcription change events on BiME

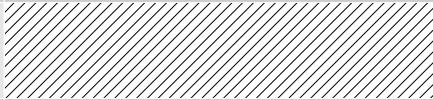
- Looking for correlation between (Fortino et al. 2014) :
____|^__ or __|^____ profiles and coverage.
- Profiles simulated with 0 or 1.
- Sliding window (100 bp) on the BiME region.

Search for transcription change events on BiME

- Looking for correlation between (Fortino et al. 2014) :
____|^__ or __|____ profiles and coverage.
- Profiles simulated with 0 or 1.
- Sliding window (100 bp) on the BiME region.
- Correlation test :
 - same strand :
 - Wilcoxon test for DE
 - one gene with RPKM count > 10
 - 2 fold change
 - Correlation test P-value < 10^{-7}
 - Correlation coefficient > 0.7

Search for transcription change events on BIME

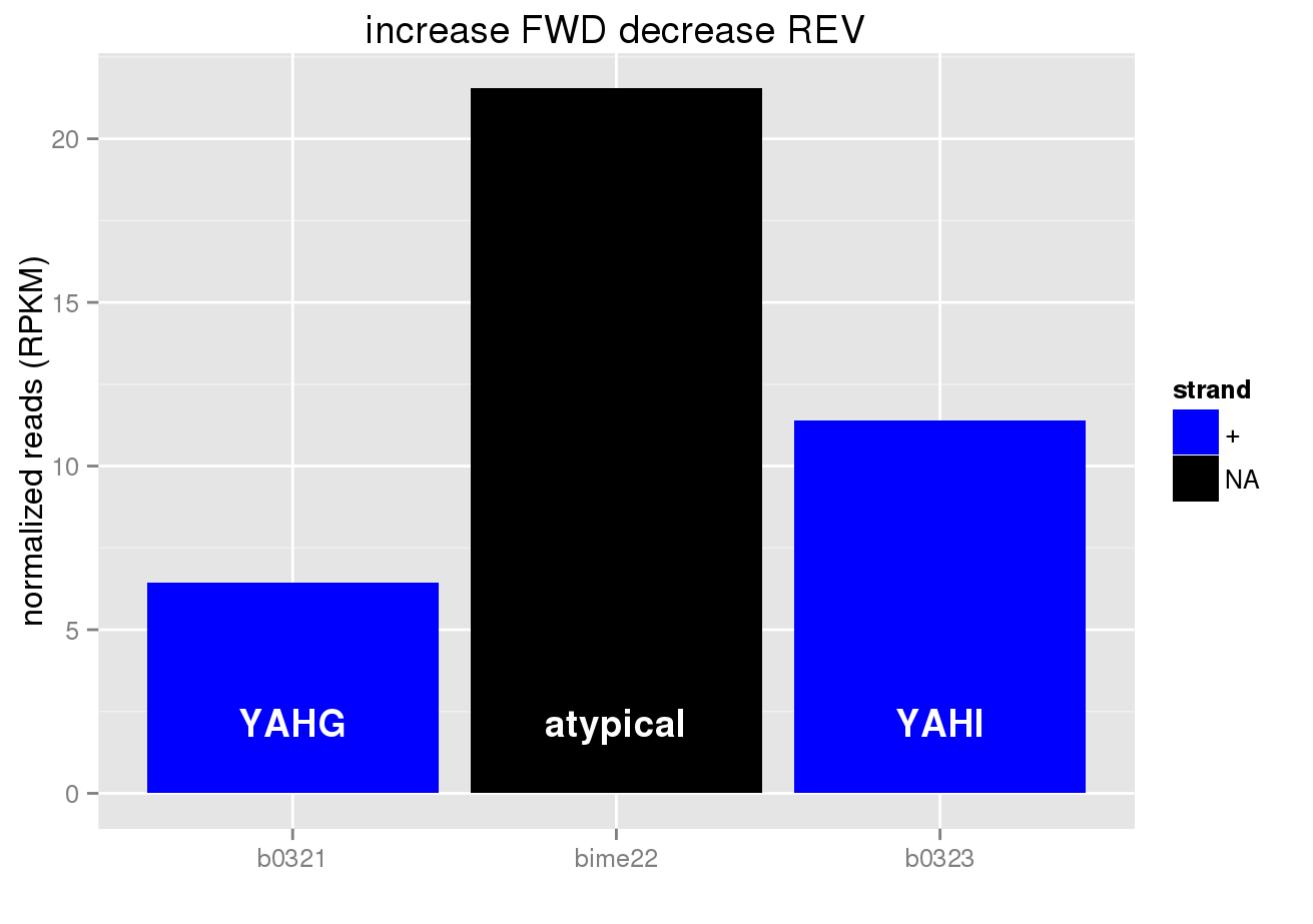
Results overview :

Number of regions	Number of regions without unique REP	Event	on BIME region
29	25	__ ^__	YES
316		__ ^__	NO
43	29	^__ __	YES
308		^__ __	NO

On BIME __|^^\^ : stabilisation

1 case :

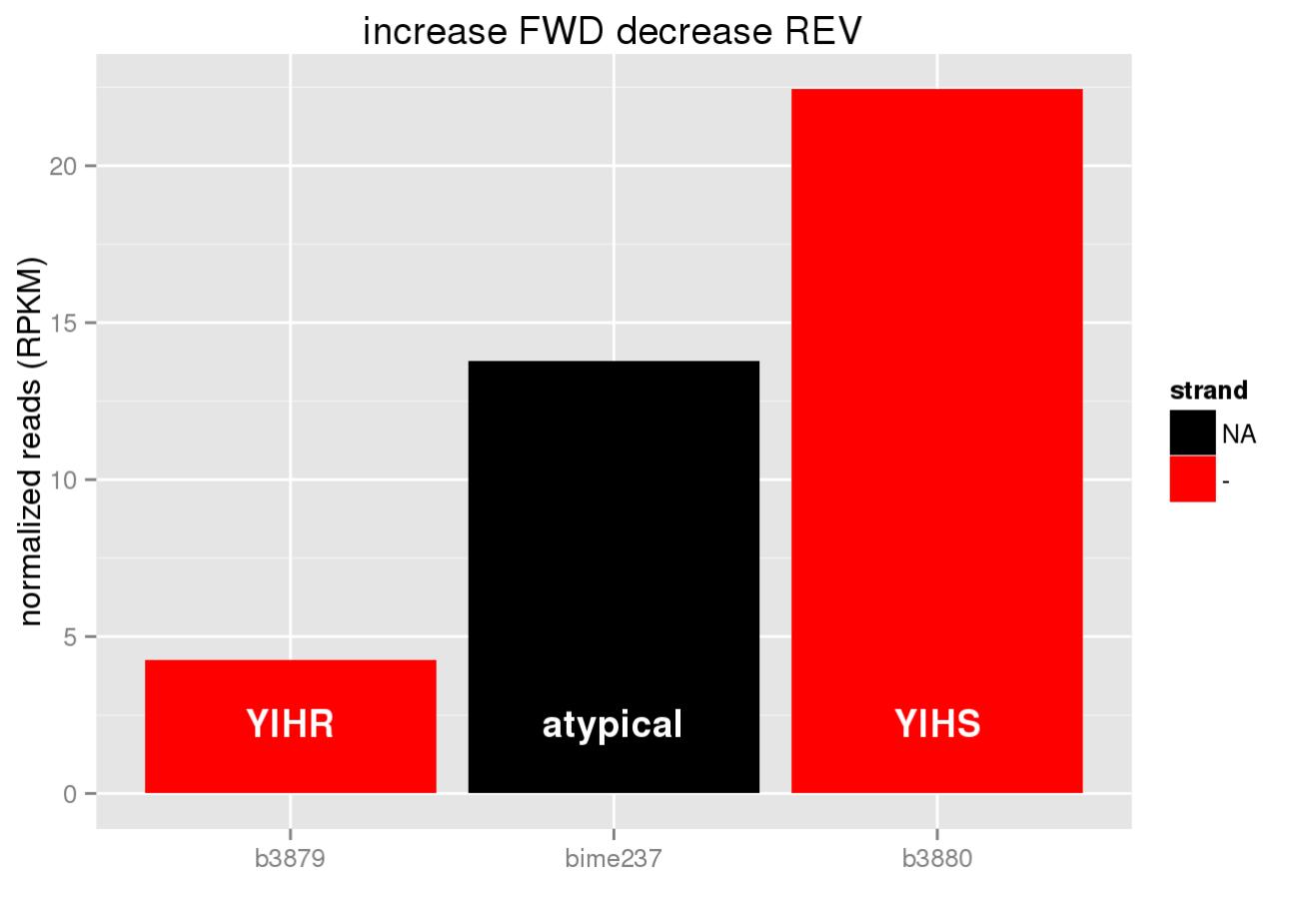
BIME-1	BIME-2	atypical BIME	unique REP
0	0	1	0



On BIME ___|^^\^ : stabilisation

5 cases :

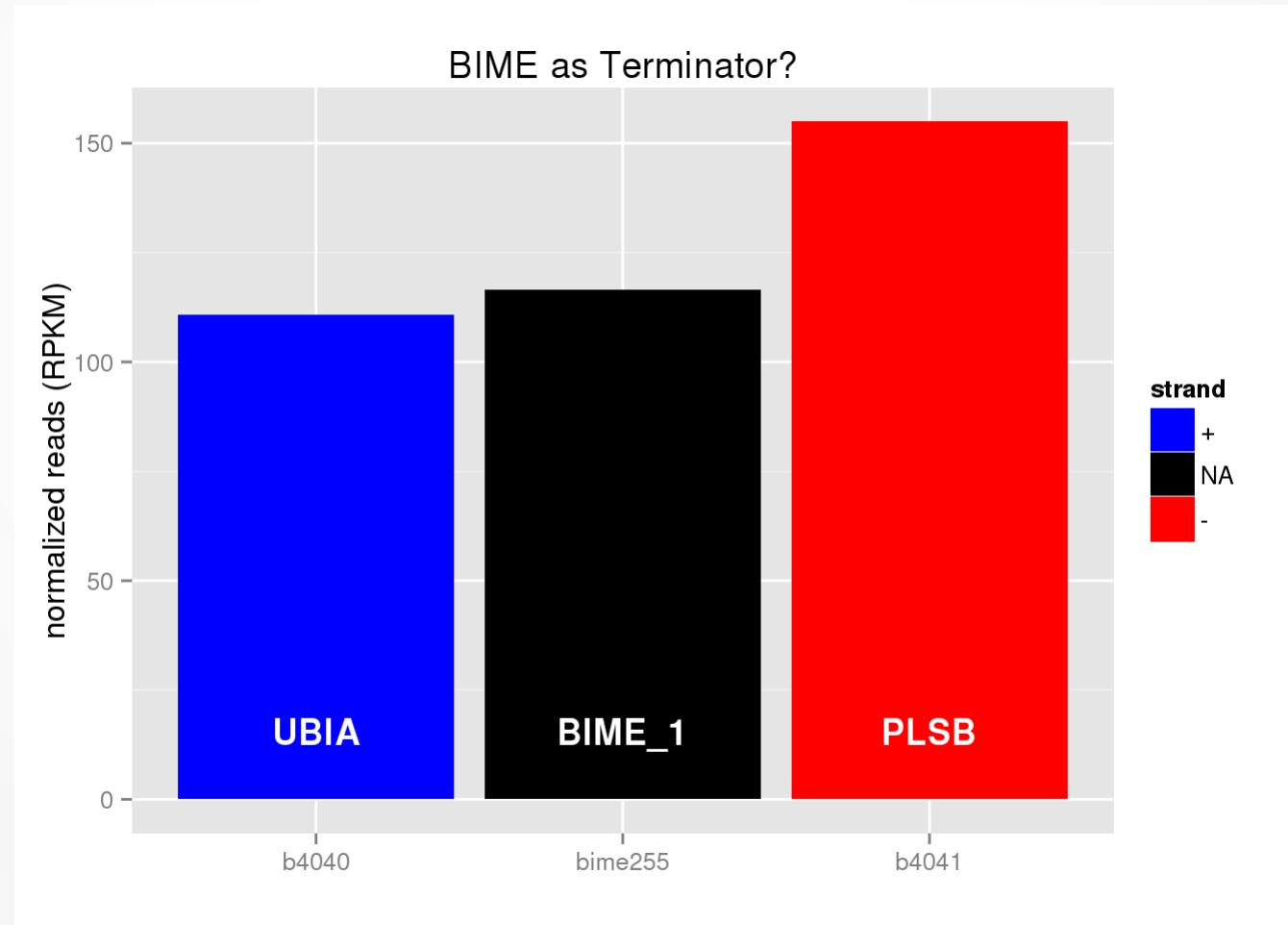
BIME-1	BIME-2	atypical BIME	unique REP
3	0	1	1



On BIME ____|^^\^ : terminator

23 cases :

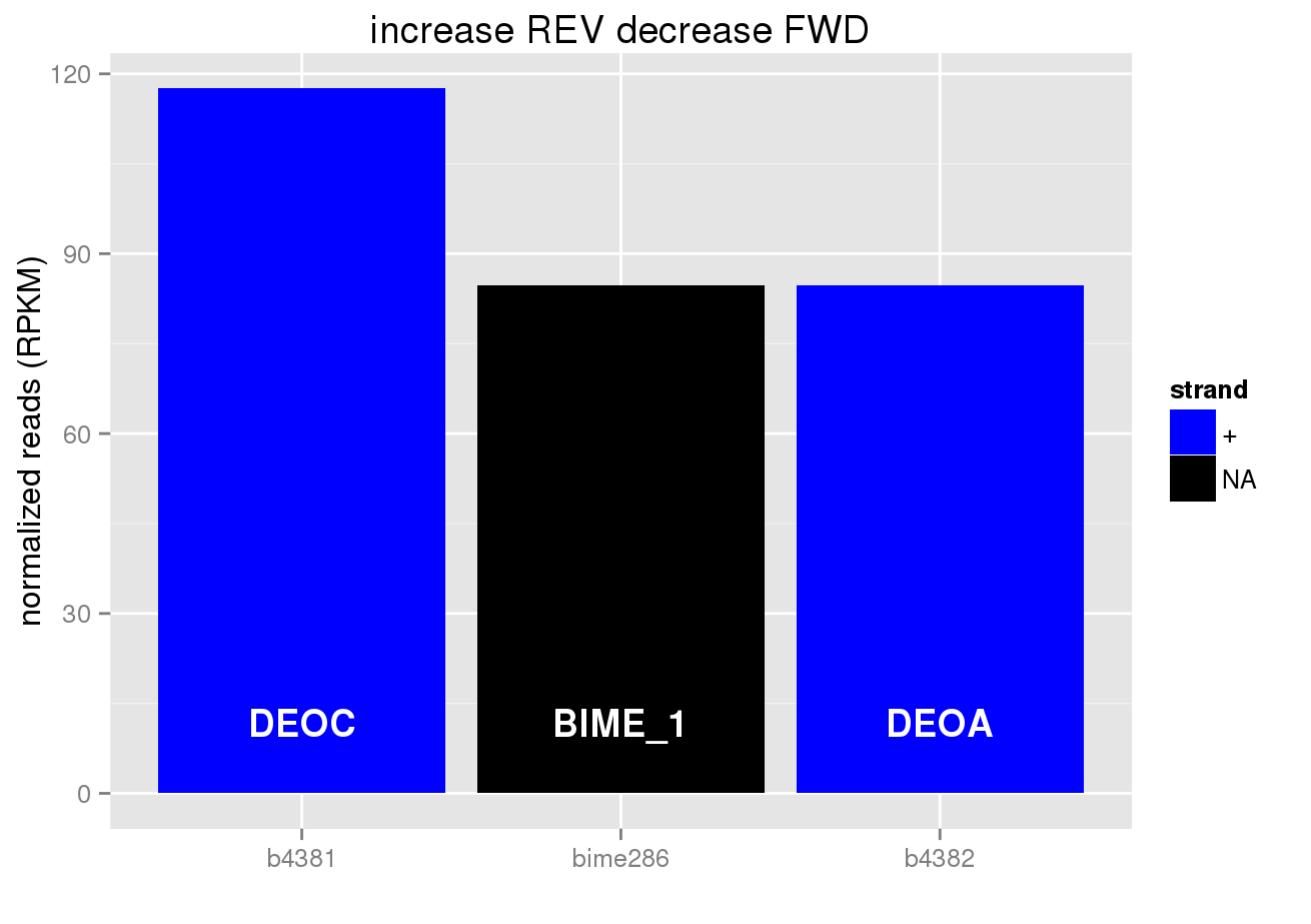
BIME-1	BIME-2	atypical BIME	unique REP
11	2	3	7



On BIME ^^^|_____ : stabilisation

9 cases :

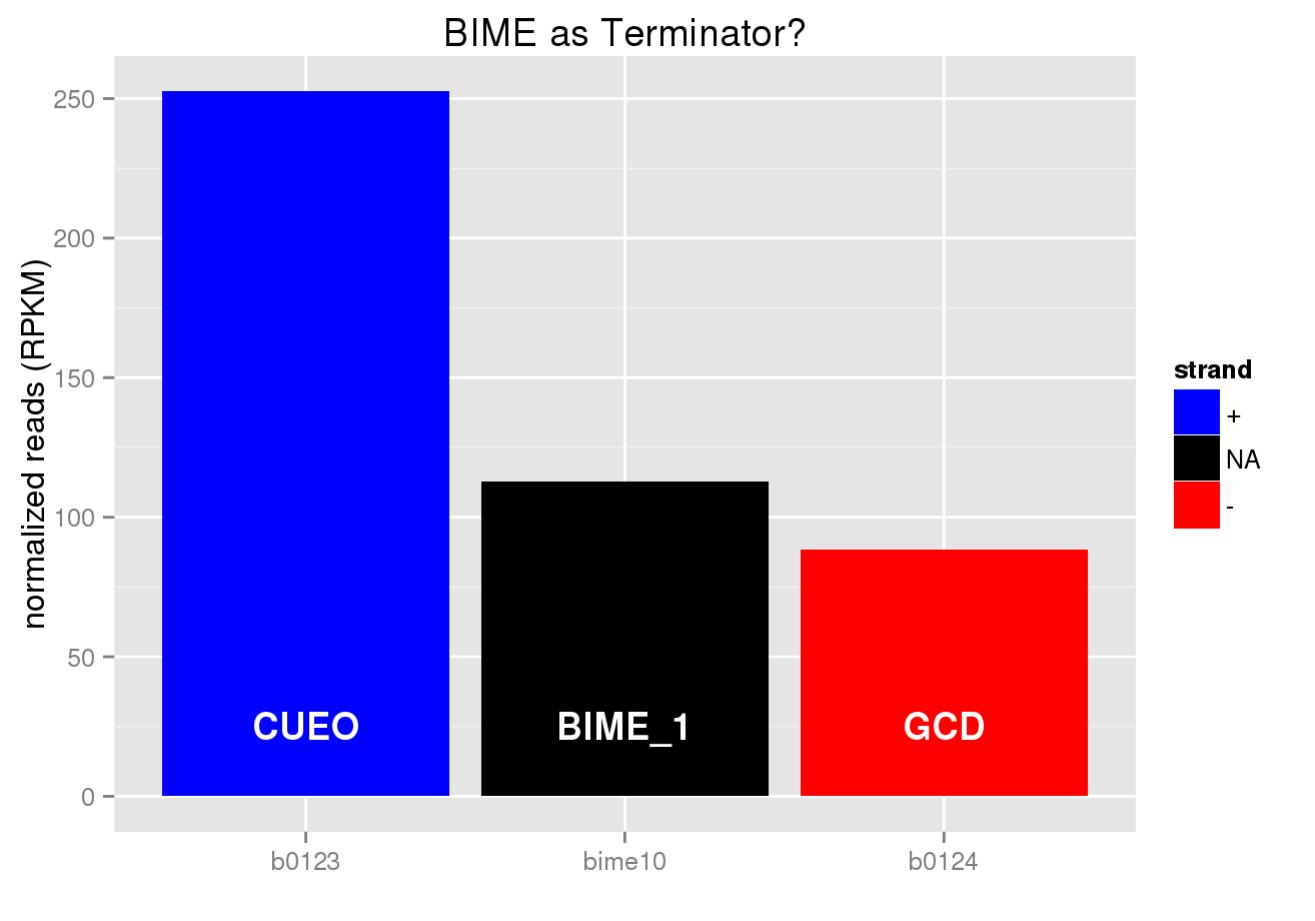
BIME-1	BIME-2	atypical BIME	unique REP
5	2	1	1



On BIME ^^^|____ : terminator

34 cases :

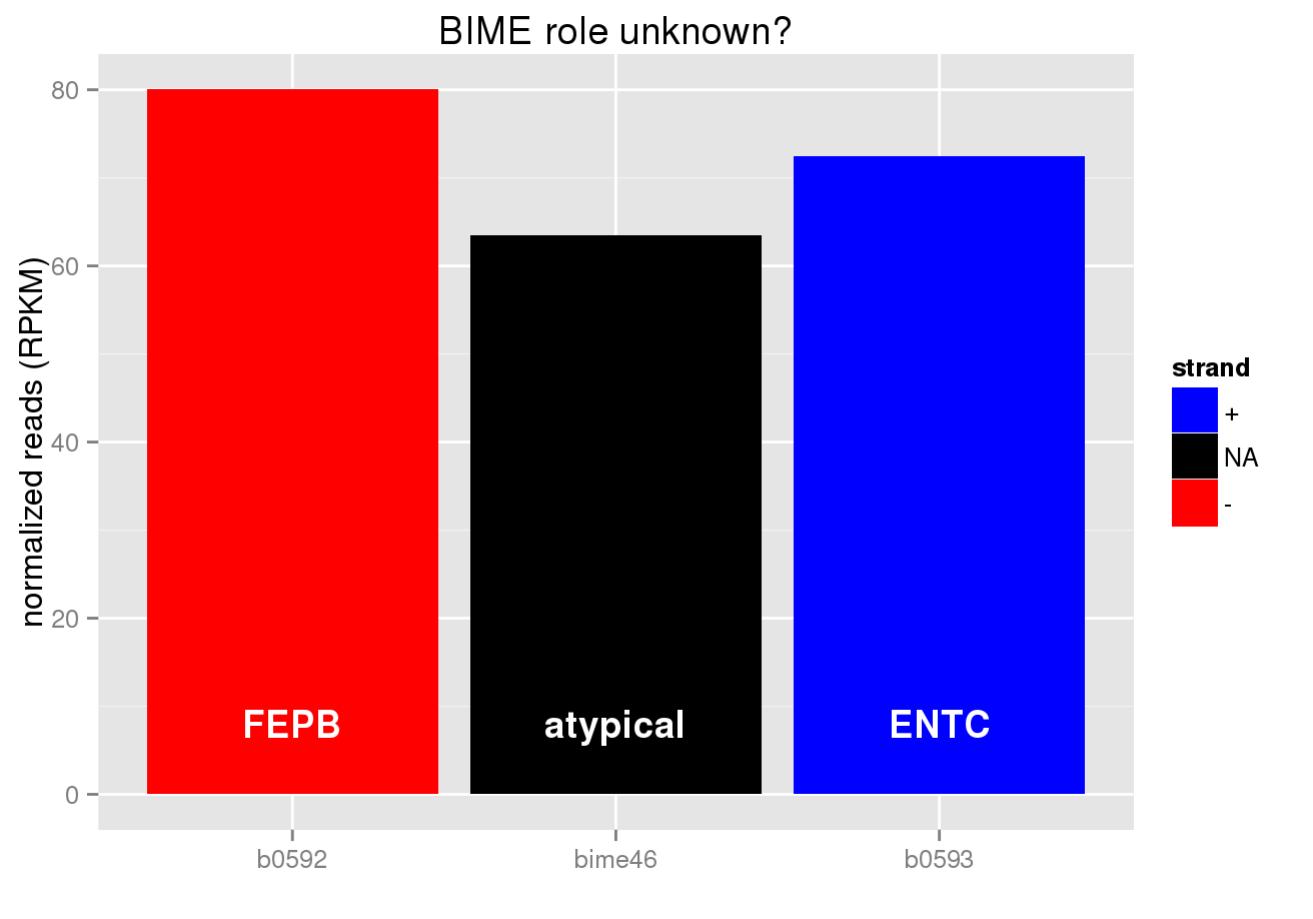
BIME-1	BIME-2	atypical BIME	unique REP
10	8	3	13



On BIME ^^^|____ : enhancing

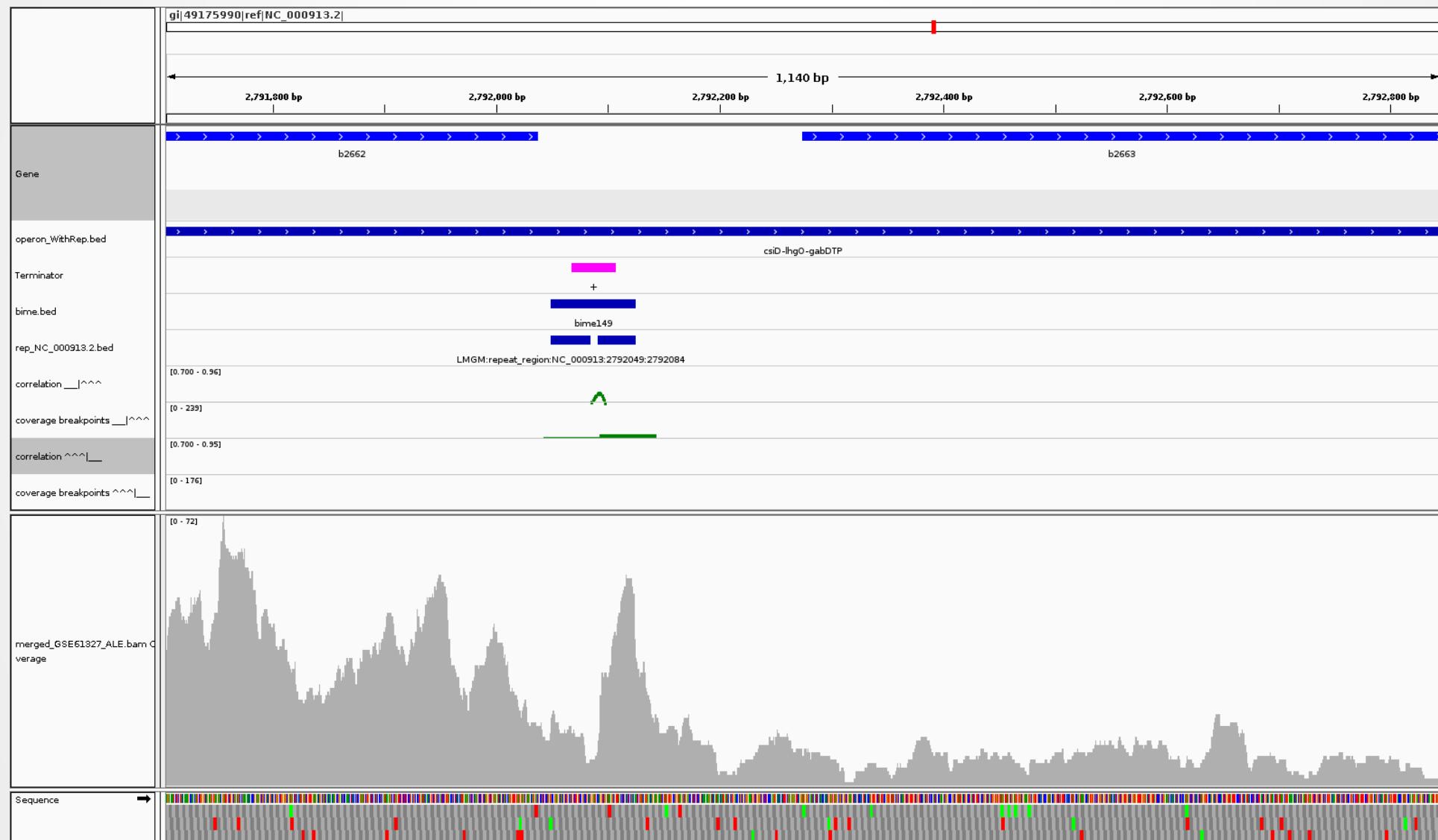
1 case :

BIME-1	BIME-2	atypical BIME	unique REP
0	0	1	0



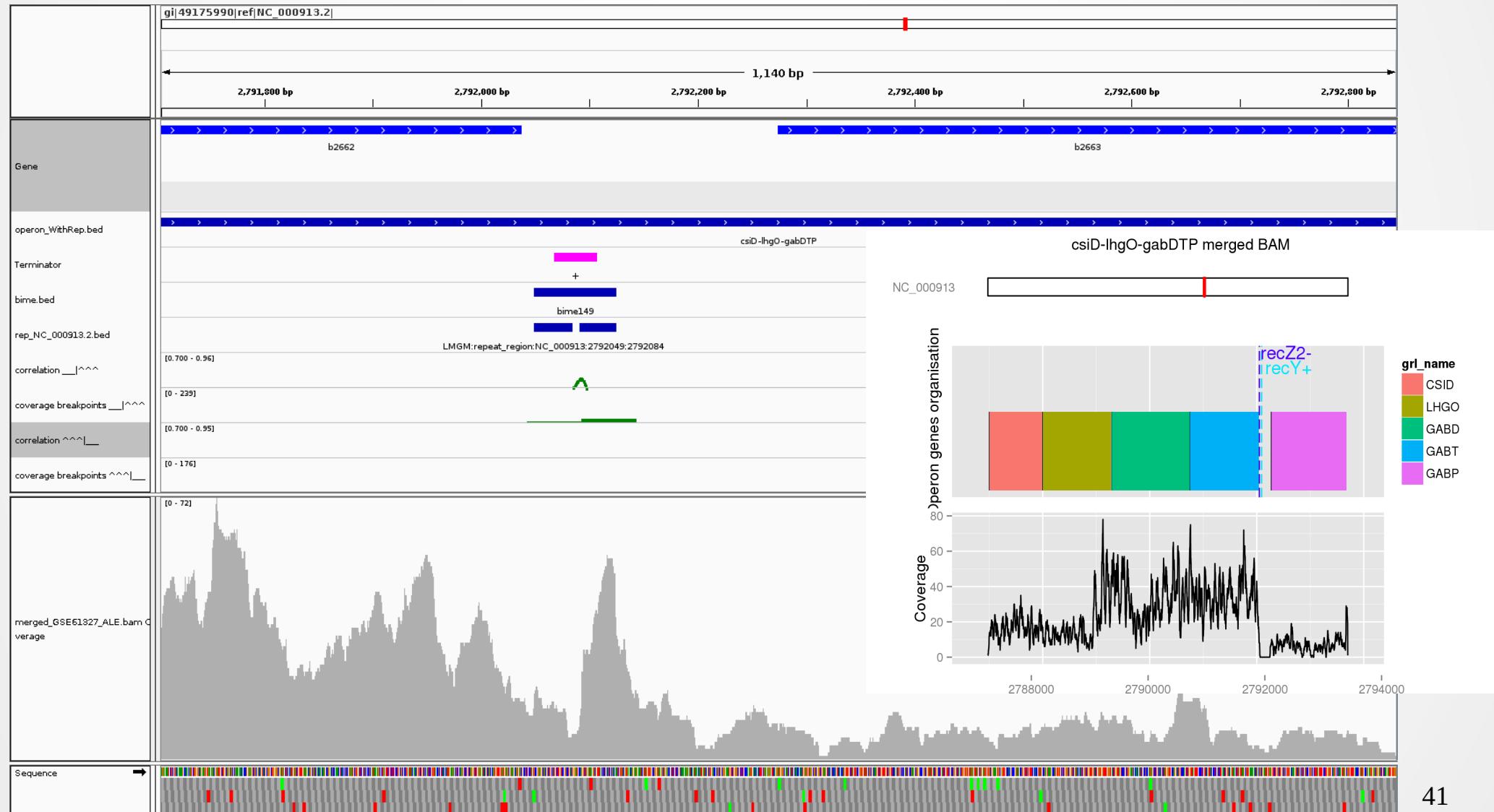
Links with REP results

Operon [csiD-lhgO-gabDTP](#) (Multipurpose conversions 4-aminobutyrate aminotransferase, PLP-dependent)



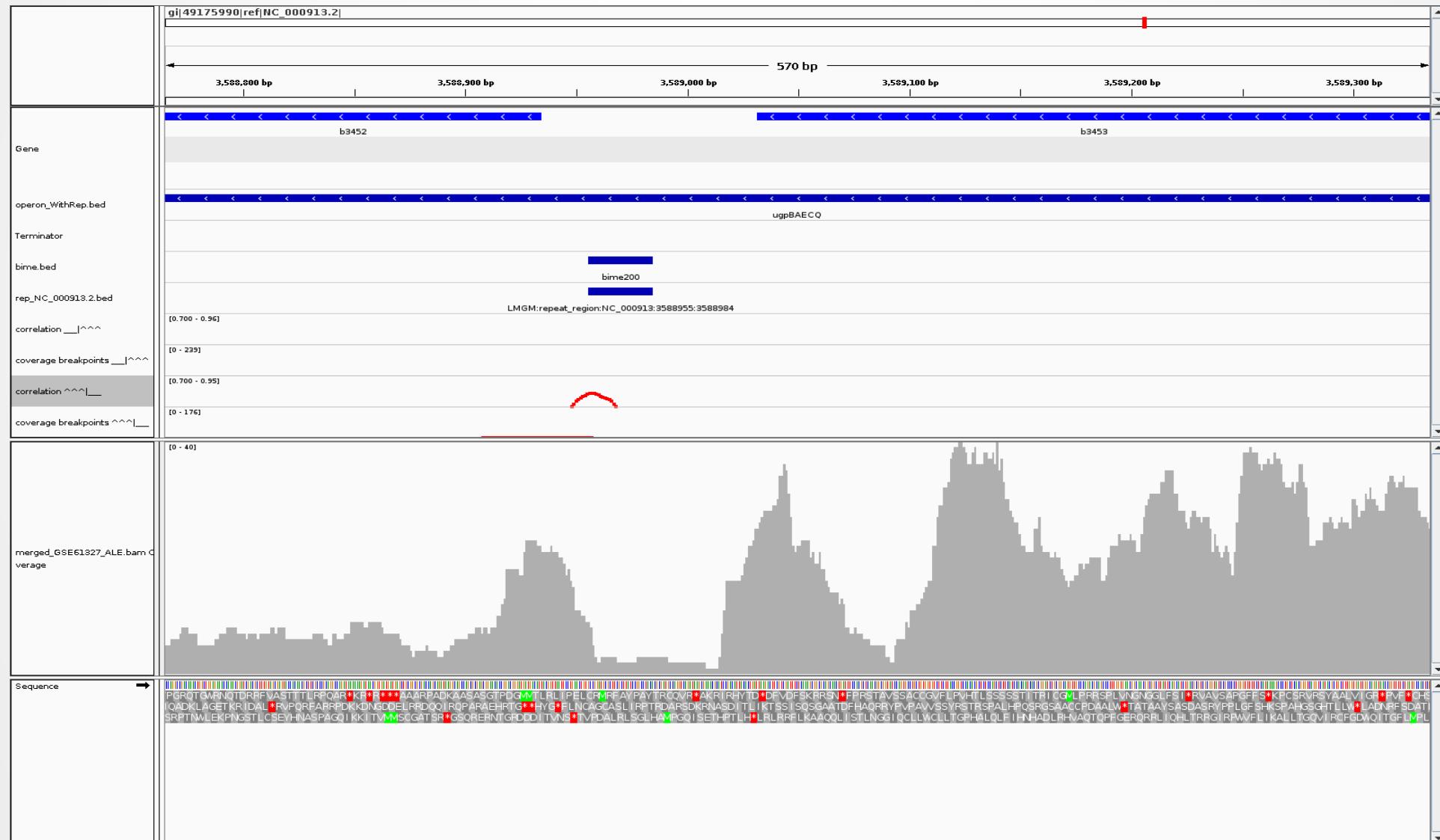
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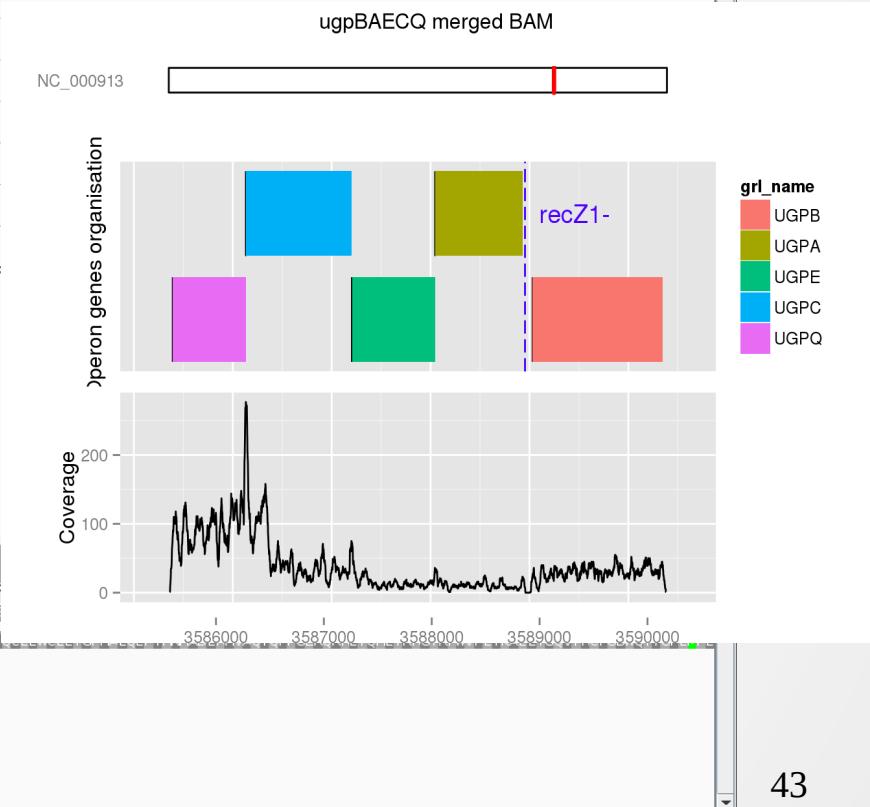
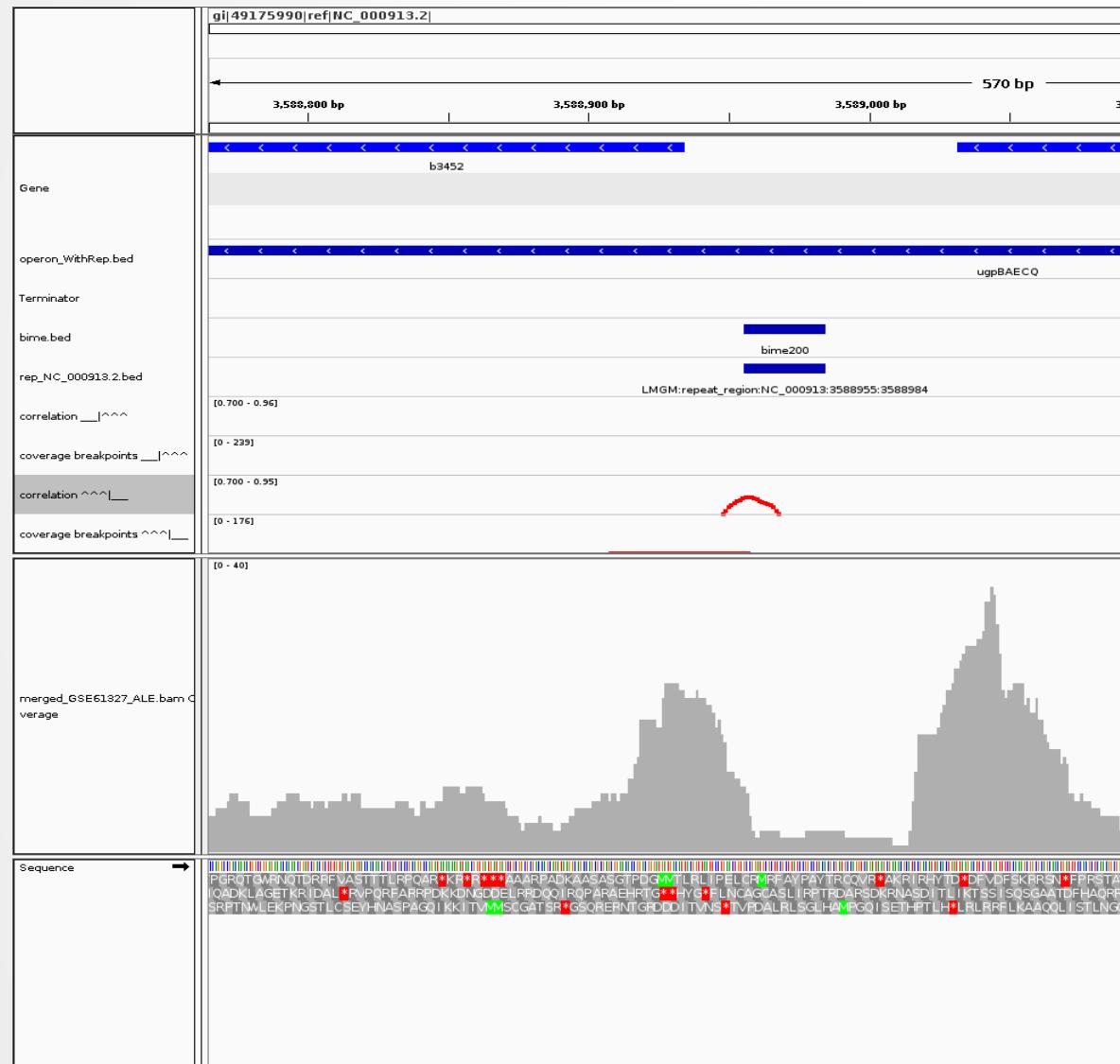
Links with REP results

Operon upgBAECQ (ABC transporter)



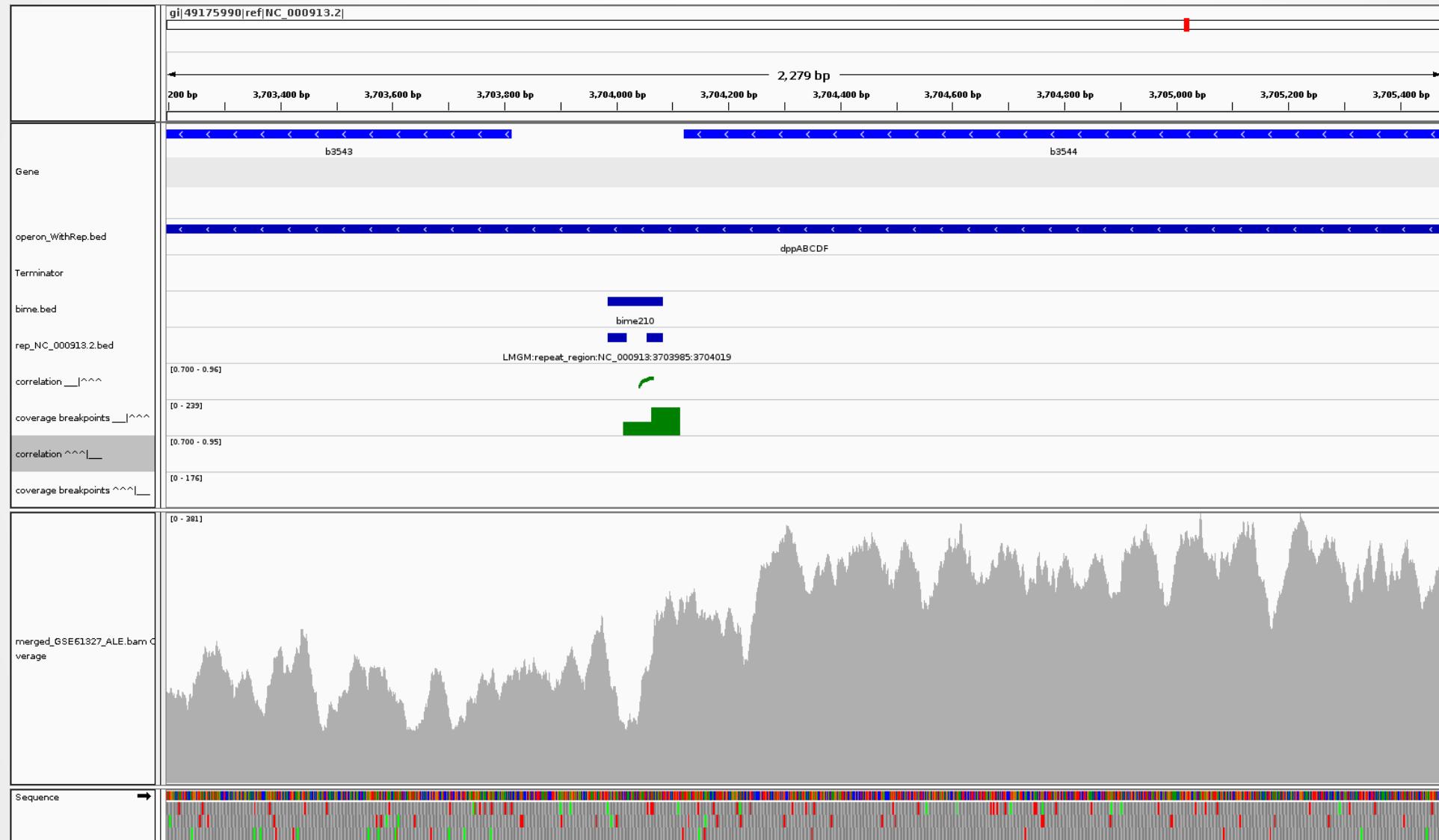
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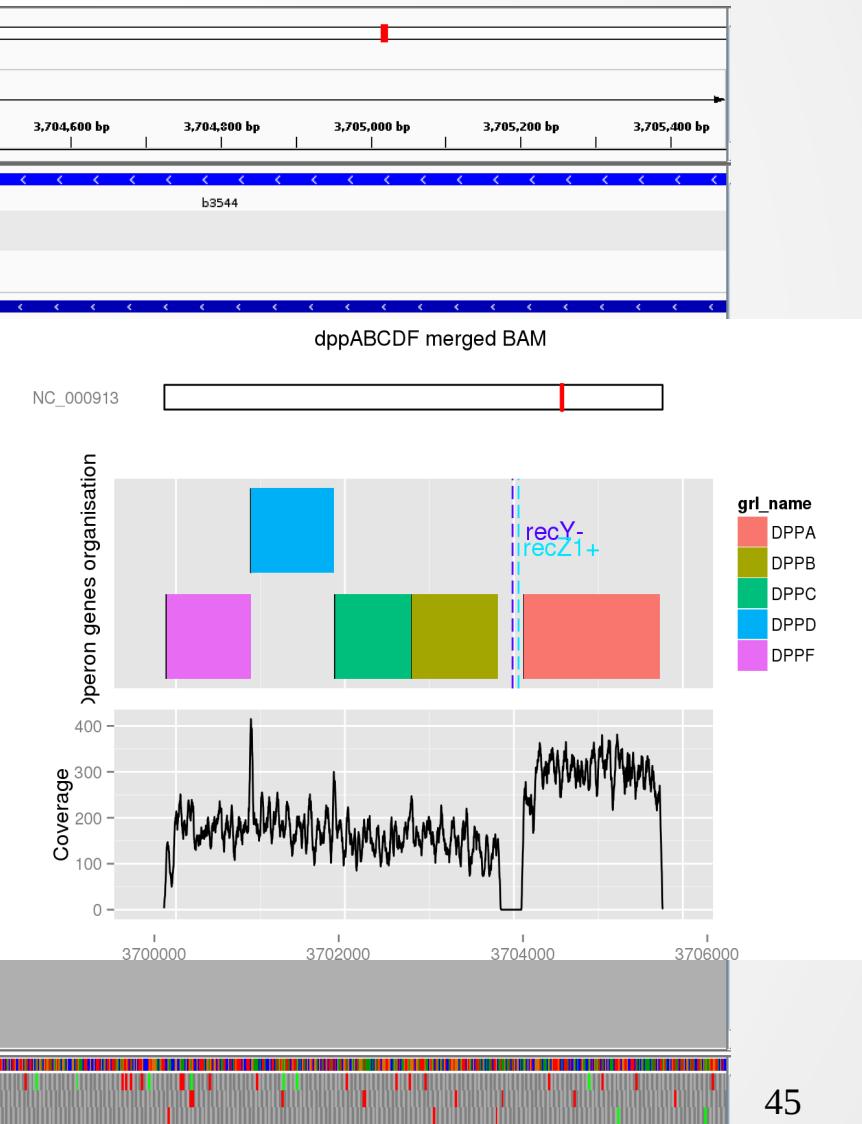
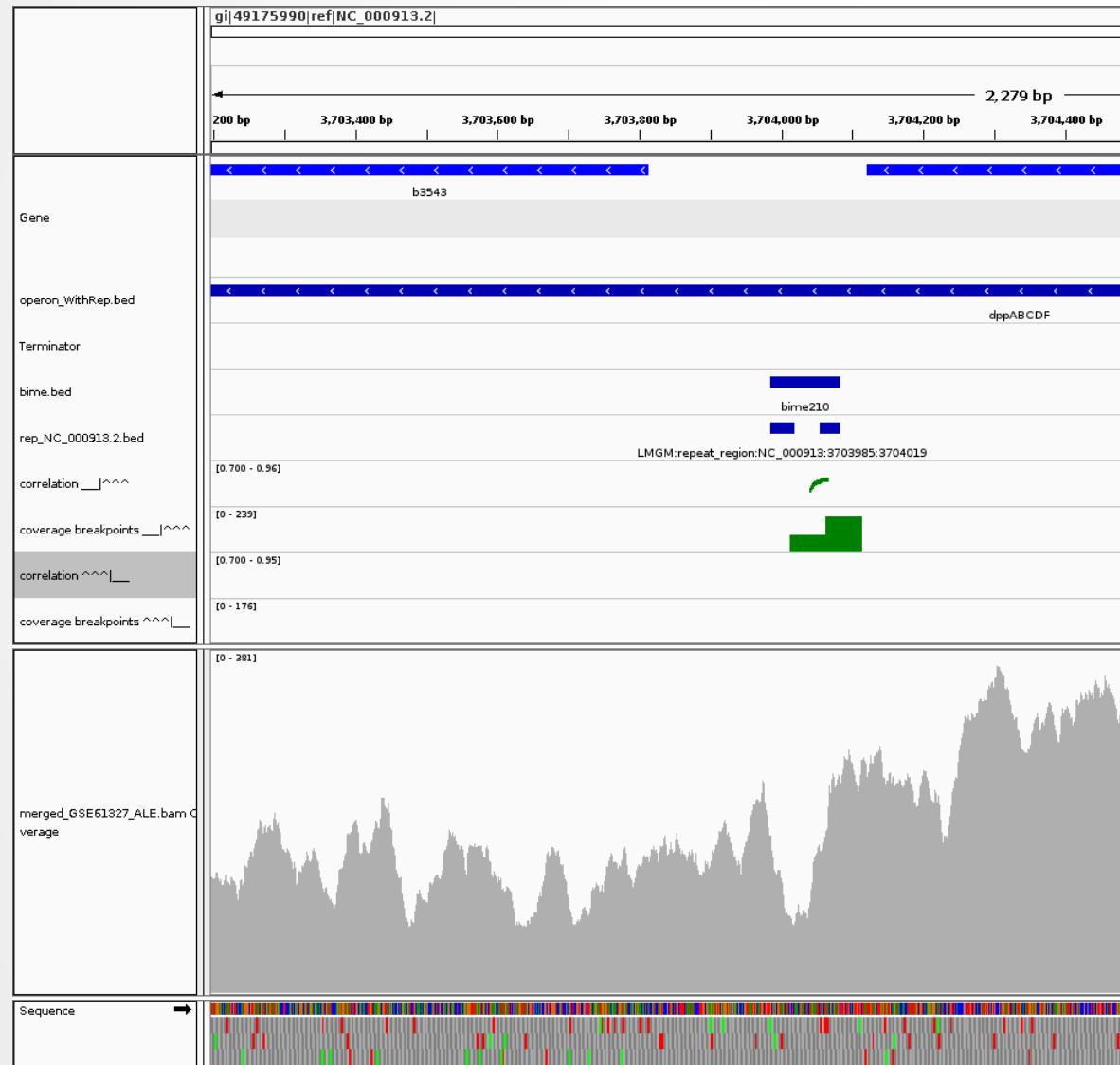
Links with REP results

Operon dppABCDF (ABC transporter)



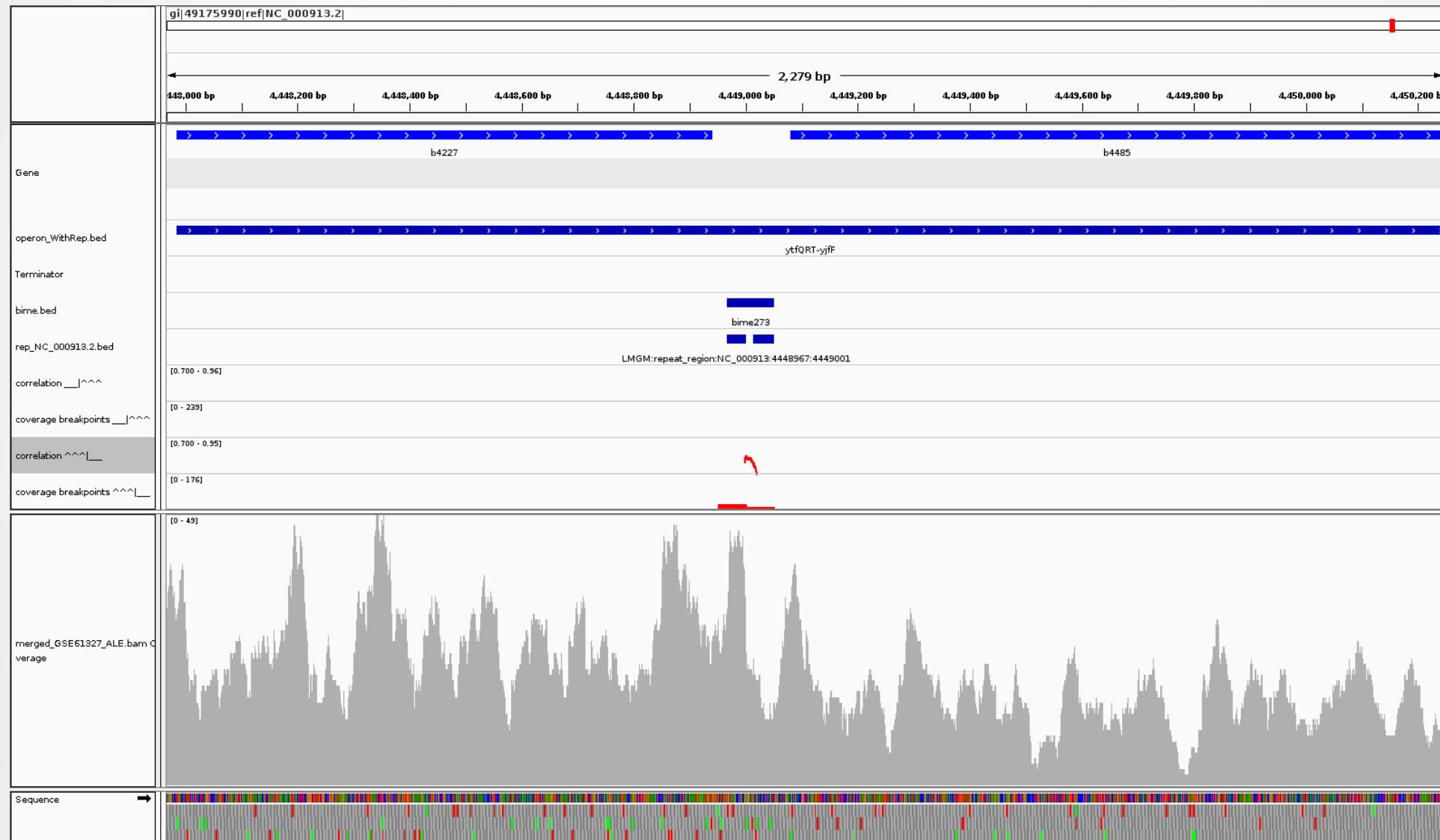
Links with REP results

Operon dppABCDF (ABC transporter)



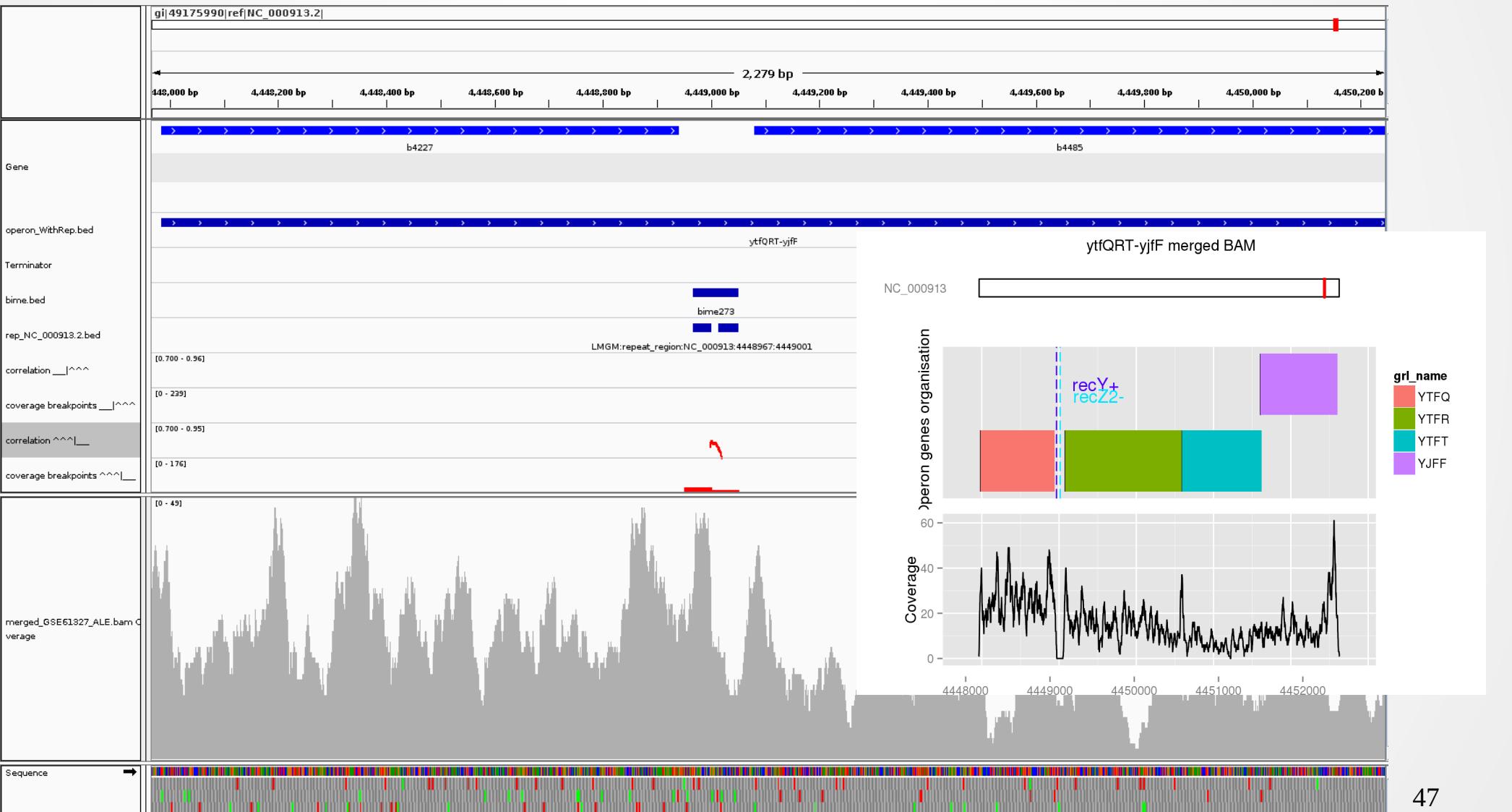
Links with REP results

Operon *ytfQRT-yjfF* (ABC transporter)



Links with REP results

Operon *ytfQRT-yjfF* (ABC transporter)



Perspectives

- Improve transcription change discovery ?
- Alignment of REP or BIME expression profiles to look for a pattern.
- Kolmogorov test on expression profiles for BIME classes to search differences.
- RIP-Seq or RIP-Chip analysis to check interactions RNA-proteins (data?, methodology?)