

**Figure S2: List of the 10 sequenced regions targeted by PCR after DNA bisulfite conversion.**

## Data contents and legends

List of the 10 sequenced regions targeted by PCR after DNA bisulfite conversion. The CGI number according to genome assembly V9 and the XLOC number according to previous RNA-Seq data [40] is given when present. The number of CpG sites for each amplicon is given. Primers details are given in Table S1. The slide numbers containing detailed information for each sequenced amplicon are indicated.

Region	CGI number	XLOC number	Number of CpG sites	Slide number
Scaffold1255: 365901..366597	cgl_10018082	XLOC_005912	25	2, 3, 4
Scaffold1154:309231..309691	cgl_10026162	XLOC_004001	15	5, 6, 7
Scaffol37178: 4921..5527	cgl_10002304	XLOC_022371	7	8, 9, 10
Scaffold41174: 81270..81829	cgl_10004794	XLOC_025872	20	11, 12, 13
Scaffold33832: 13205..13768			6	14, 15, 16
Scaffold42366: 107142..107725	cgl_10006702	XLOC_027741	20	17, 18, 19
Scaffold42366: 108172..108673	cgl_10006702	XLOC_027741	11	20, 21, 22
Scaffold1720: 210182..210722	cgl_10011947	XLOC_013090	7	23, 24, 25
Scaffold433: 896527..897085			12	26, 27, 28
Scaffold43170: 116309..116863	cgl_10009103	XLOC_029410	4	29, 30, 31

For a targeted region, each data set contains:

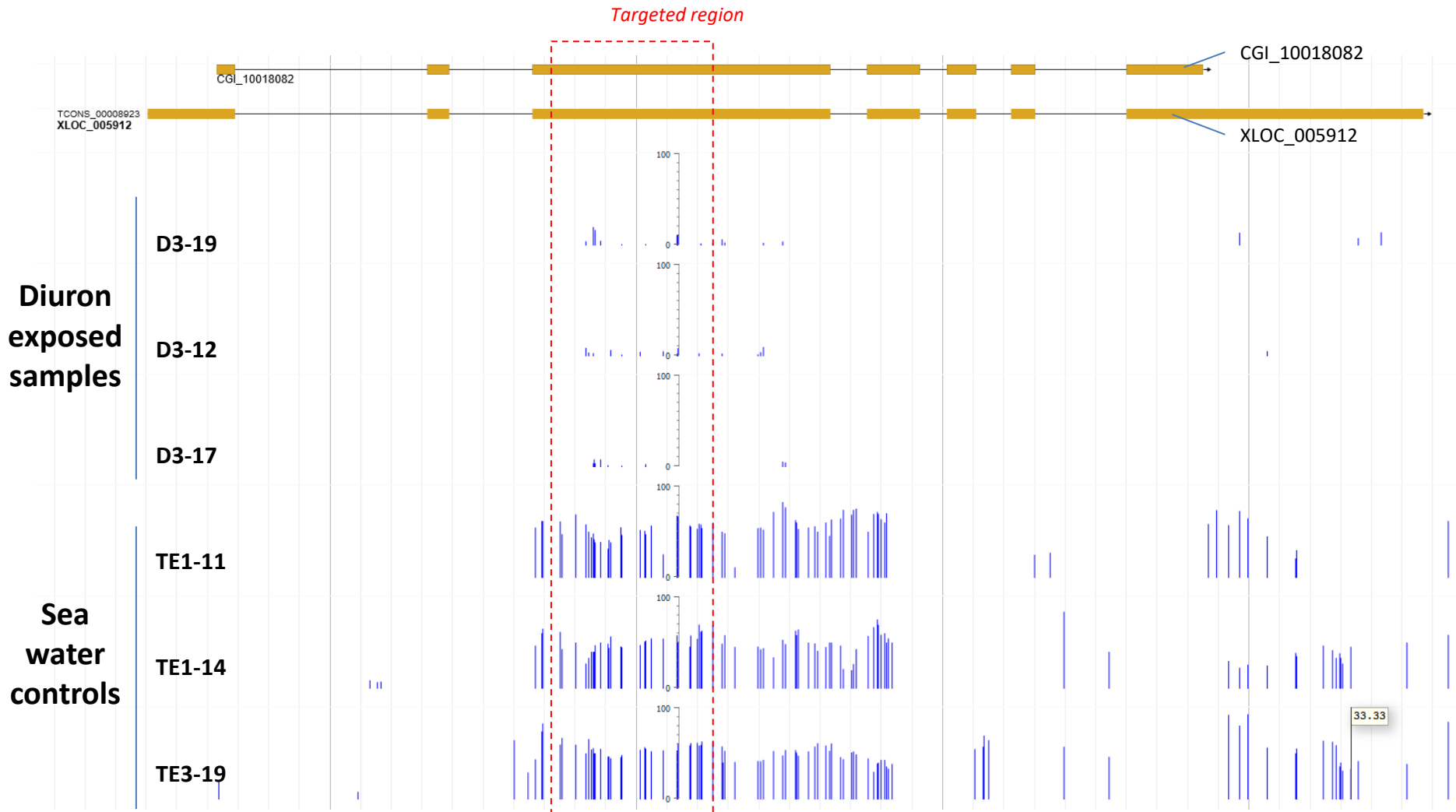
- **A Screenshot of the BS-Seq results for the three individuals per condition.** This screenshot is an extract of the *C. gigas* jbrowse interface which allows visualization of transcriptomic and DNA methylation data. Upper panel represents the annotation of genes in the current genome assembly V9 (CGI number), and the CDS according to previous transcriptomic data [40]. Bottom panel represents the methylated cytosine position. X-axis represents the position of each methylated cytosine. Y axis (bars) represents the % of methylation observed for each methylated cytosine (scale is 0 to 100). The 6 tracks represent the 3 biological replicates of individual spat from genitors treated with diuron or control (from top to bottom).
- **The comparison between the BS-Seq and the target analysis using conventionnal sanger sequencing**
- **The graphic results indicating interindividual variability and treatment effect on each CpG sites:**
  - **DNA methylation interindividual variability in each condition:** This graphe represents the average methylation of all the CpG sites included in the tested region for each of the 10 individual tested per condition
  - **Effect of each treatment on DNA methylation level for each CpG sites:** This graphe represents the average methylation of all the individuals for each of the CpG sites included in the tested region.

The individuals 1 to 10 correspond to sea water controls (SWC - blue color). The individuals 11 to 20 correspond to solvent control (SC - Orange color). The individuals 21 to 30 correspond to diuron exposed samples (D - Gray color).

# Scaffold1255:365901...366597

## – Screen shot of BS-Seq data

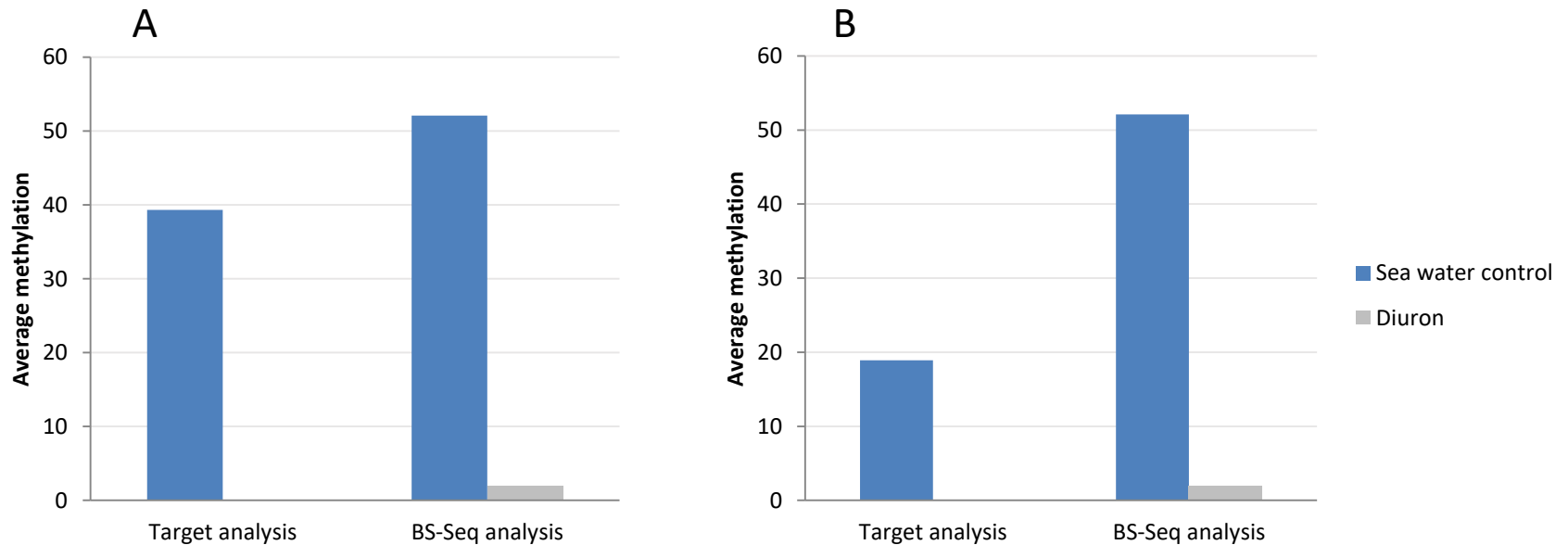
*Jbrowser extract of scaffold1255:363148..369661 (6.51 Kb)*



*Blue bars represent the percentage of methylation at CpG position*

# Scaffold1255: 365901...366597

## Comparison Sanger/BS-Seq

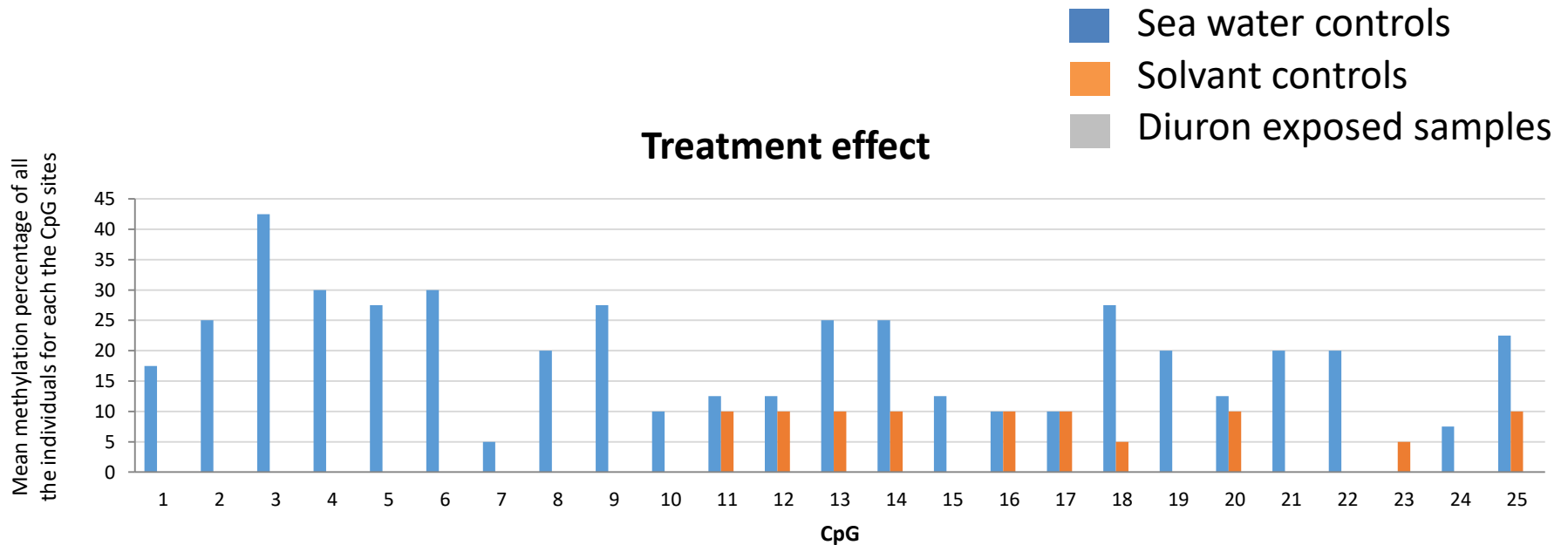
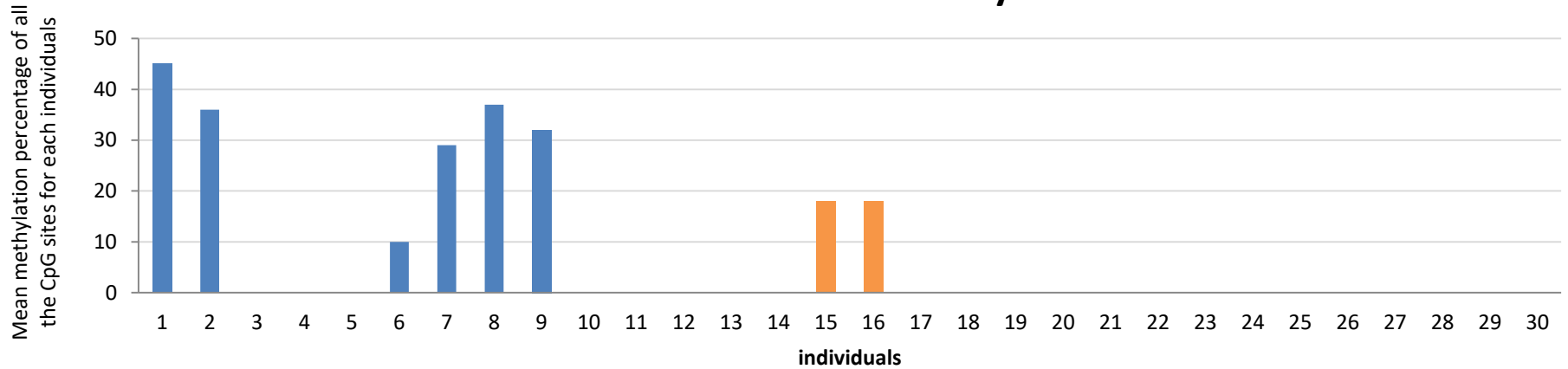


A- Comparison using the average methylation of the same 3 individuals for target analysis and BS-Seq.

B- Comparison using the average methylation of 10 individuals for target analysis and 3 individuals for BS-Seq analysis

# Scaffold1255: 365901...366597

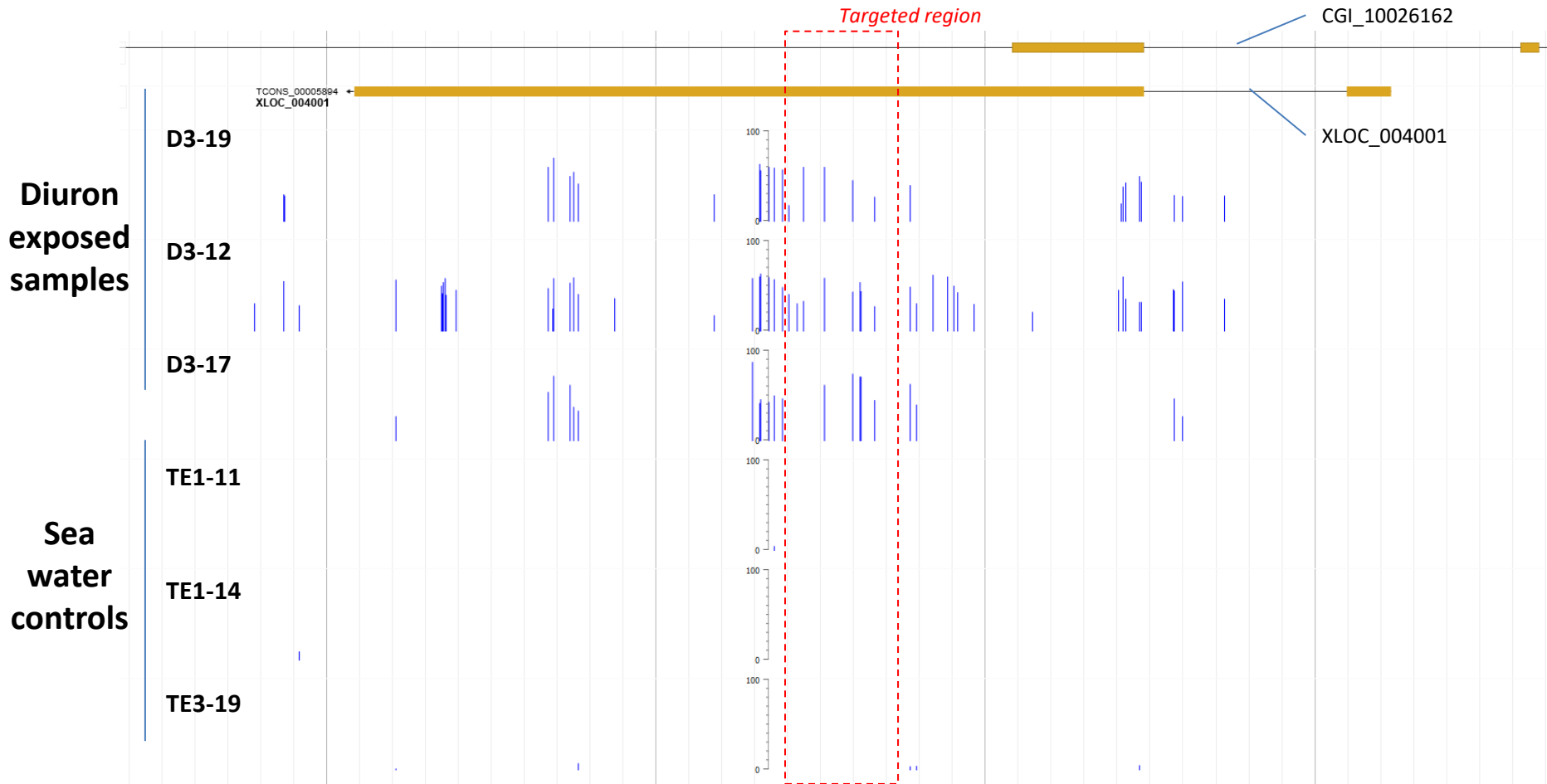
## Interindividual variability



# Scaffold1154:309231..309691

## Screen shot of BS-Seq data

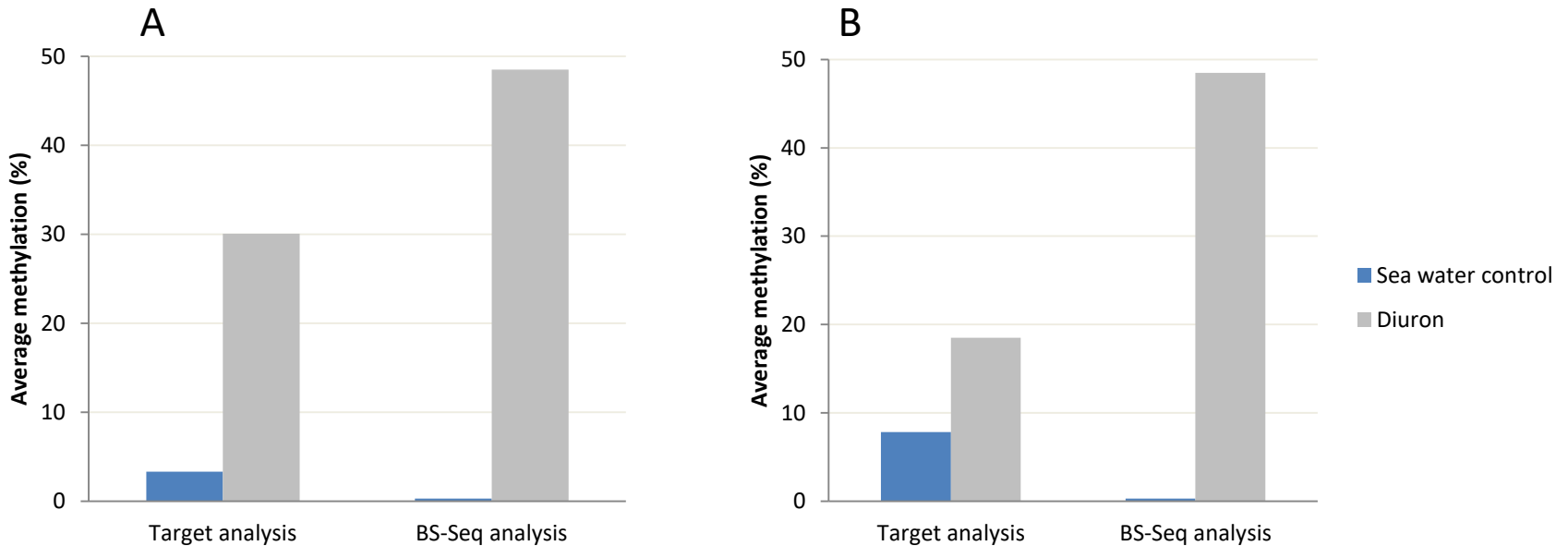
*Jbrowser extract of scaffold1154:306155..312161 (6.01 Kb)*



*Blue bars represent the percentage of methylation at CpG position*

# Scaffold1154:309231..309691

## Comparison Sanger/BS-Seq

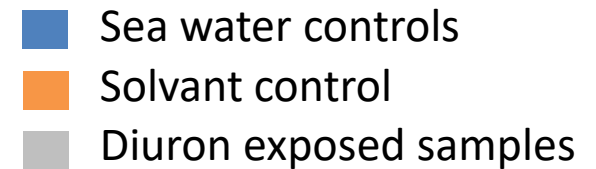
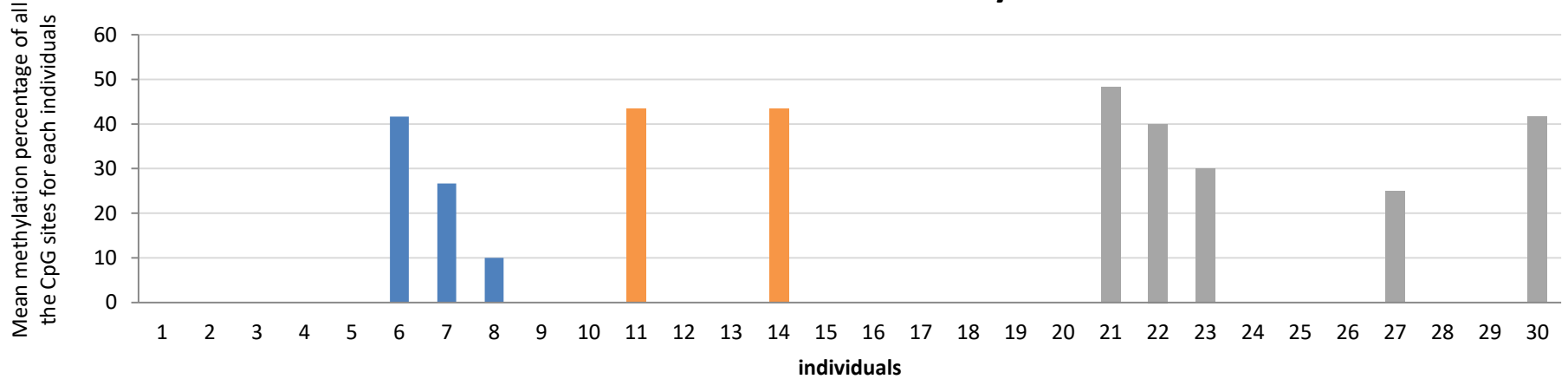


A- Comparison using the average methylation of the same 3 individuals for target analysis and BS-Seq.

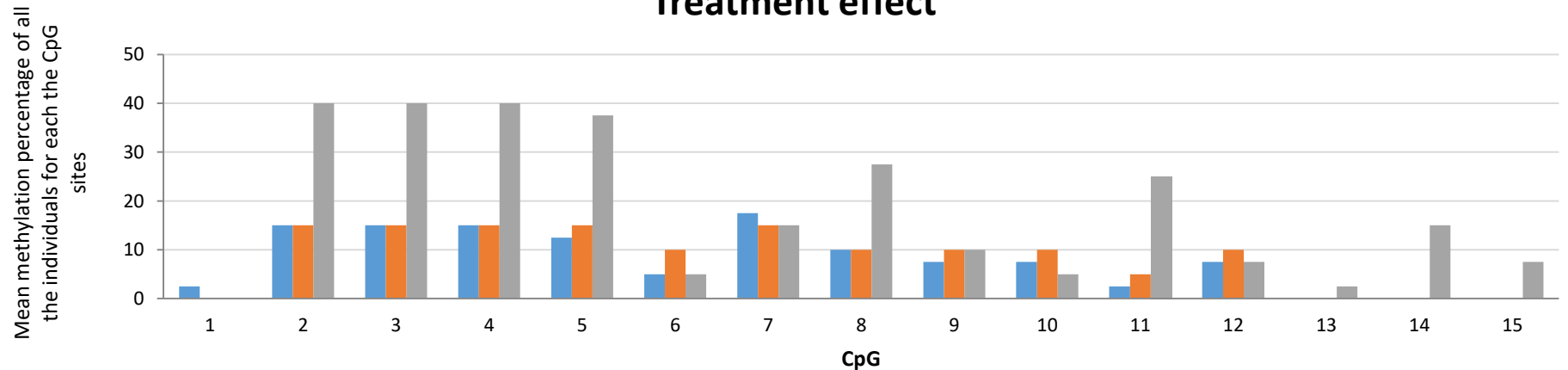
B- Comparison using the average methylation of 10 individuals for target analysis and 3 individuals for BS-Seq analysis

# Scaffold1154:309231..309691

## Interindividual variability



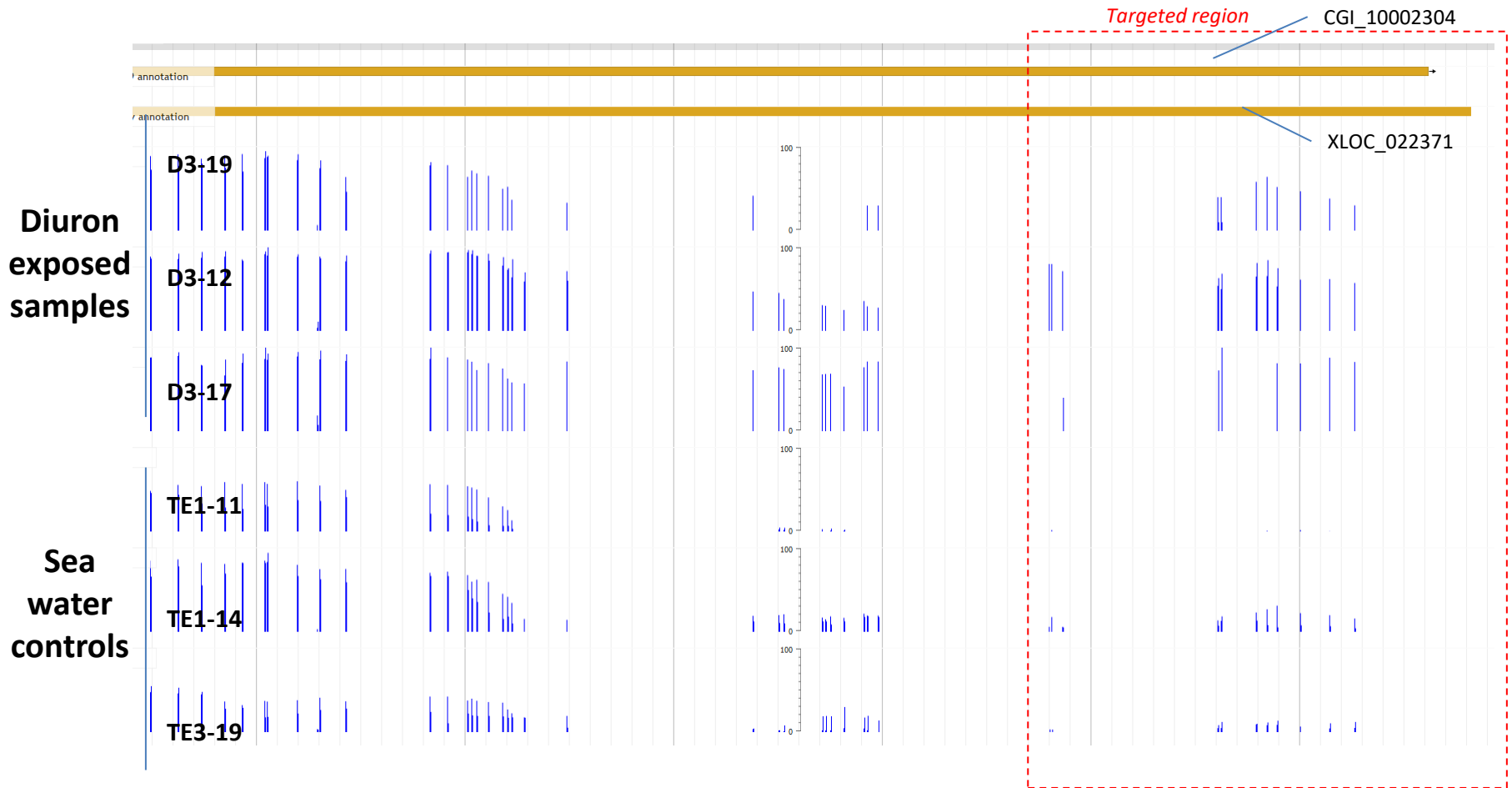
## Treatment effect



# Scaffol37178: 4921..5527

## Screen shot of BS-Seq data

*Genome extract from scaffold37178:3782..5512 (1.73 Kb)*

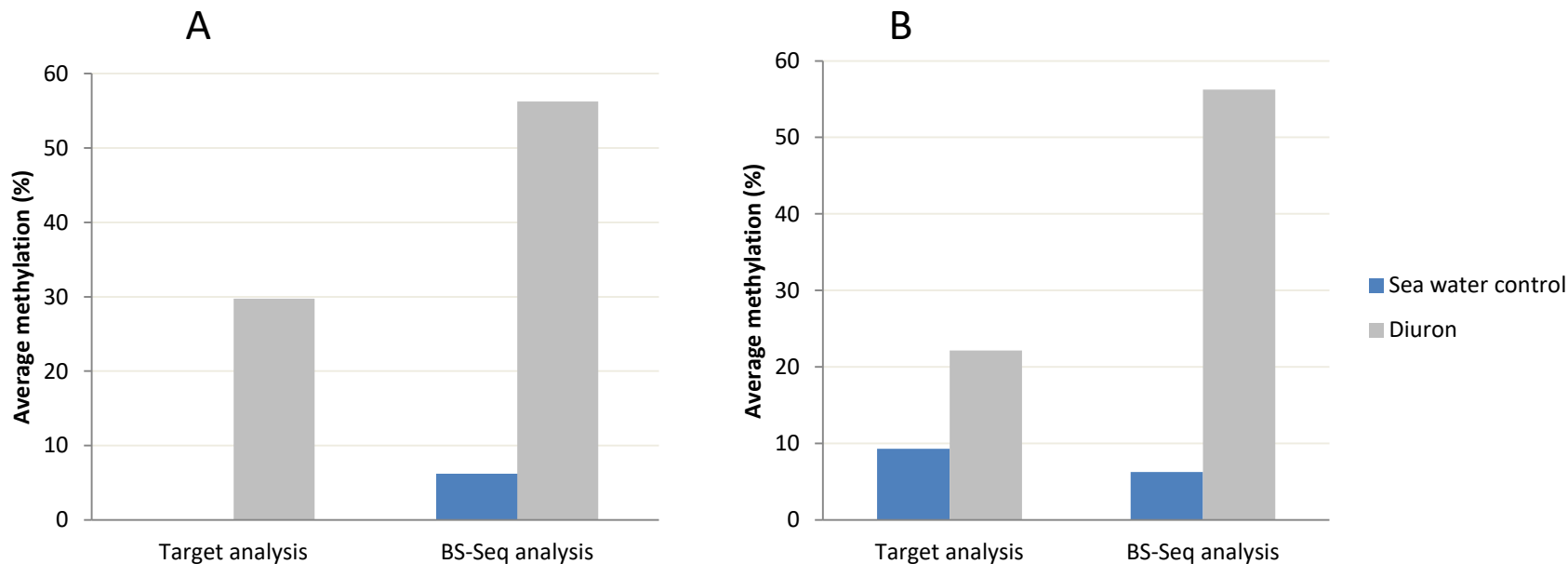


*Blue bars represent the percentage of methylation at CpG position*



# Scaffol37178: 4921..5527

## Comparison of Sanger/BS-Seq

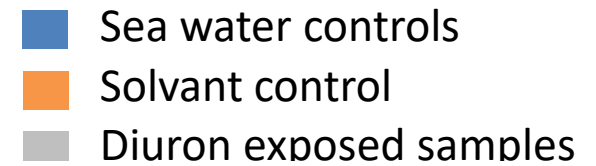
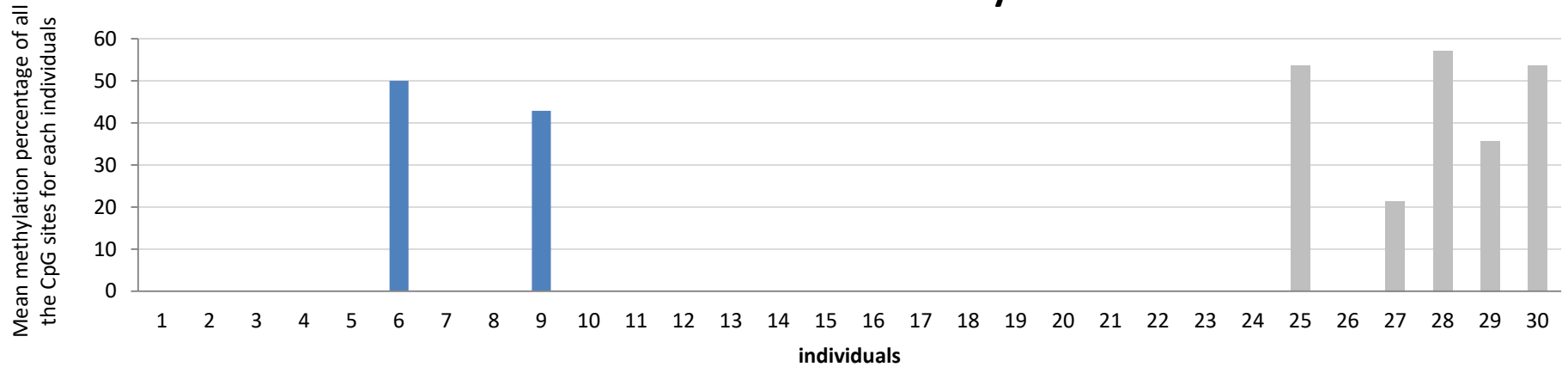


A- Comparison using the average methylation of the same 3 individuals for target analysis and BS-Seq.

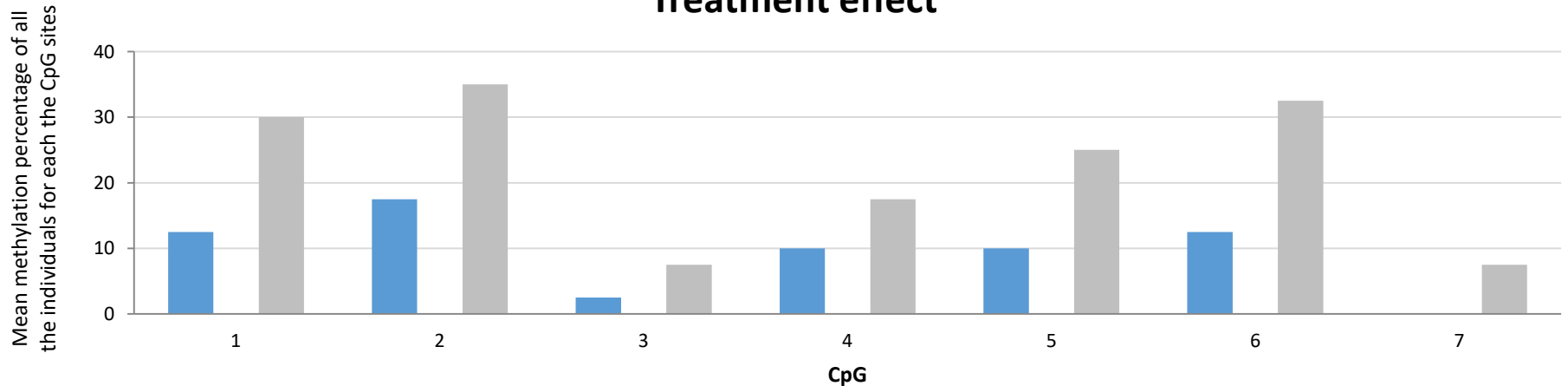
B- Comparison using the average methylation of 10 individuals for target analysis and 3 individuals for BS-Seq analysis

# Scaffol37178: 4921..5527

## Interindividual variability



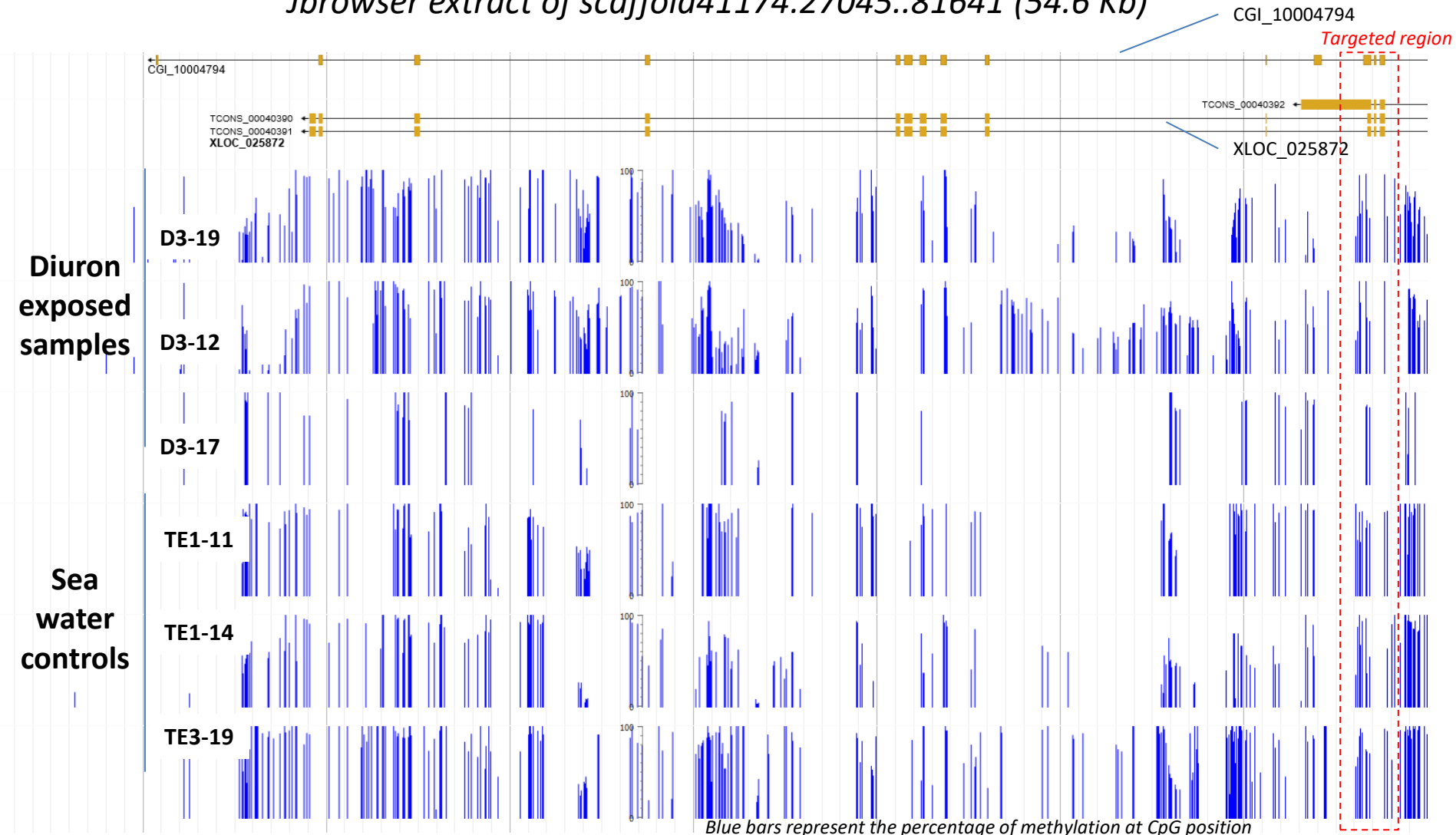
## Treatment effect



# Scaffold41174: 81270..81829

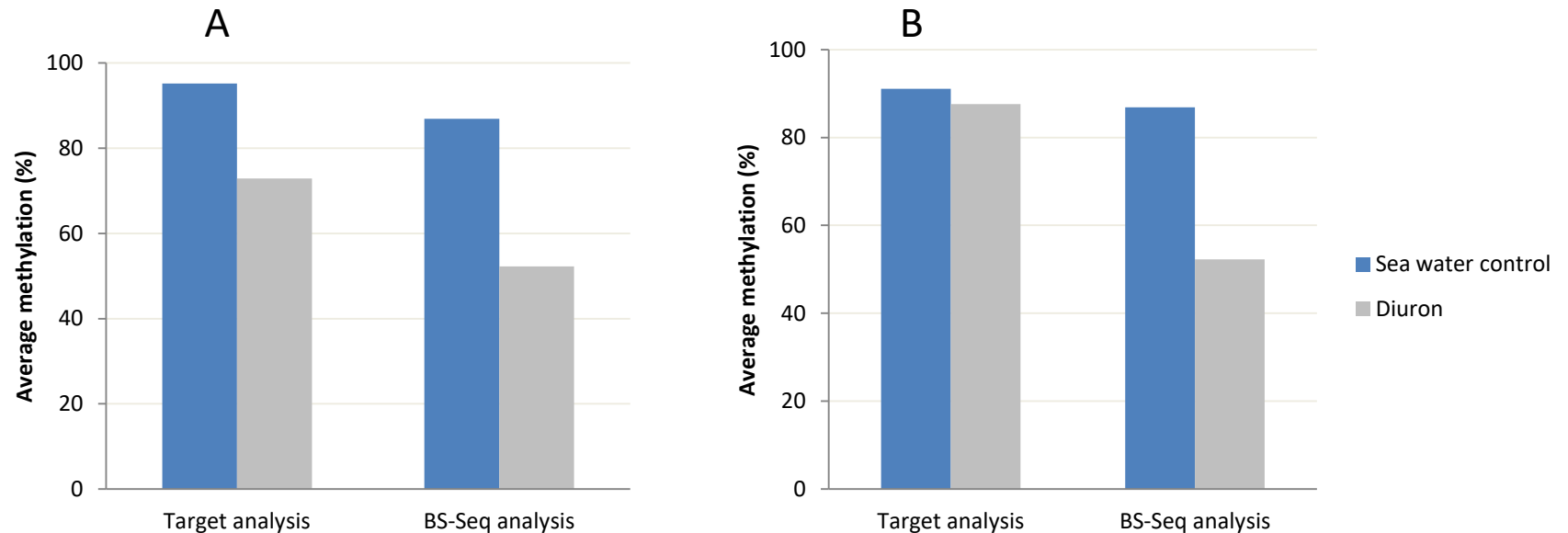
## Screen shot of BS-Seq data

*Jbrowser extract of scaffold41174:27045..81641 (54.6 Kb)*



# Scaffold41174: 81270..81829

## Comparison of Sanger/BS-Seq

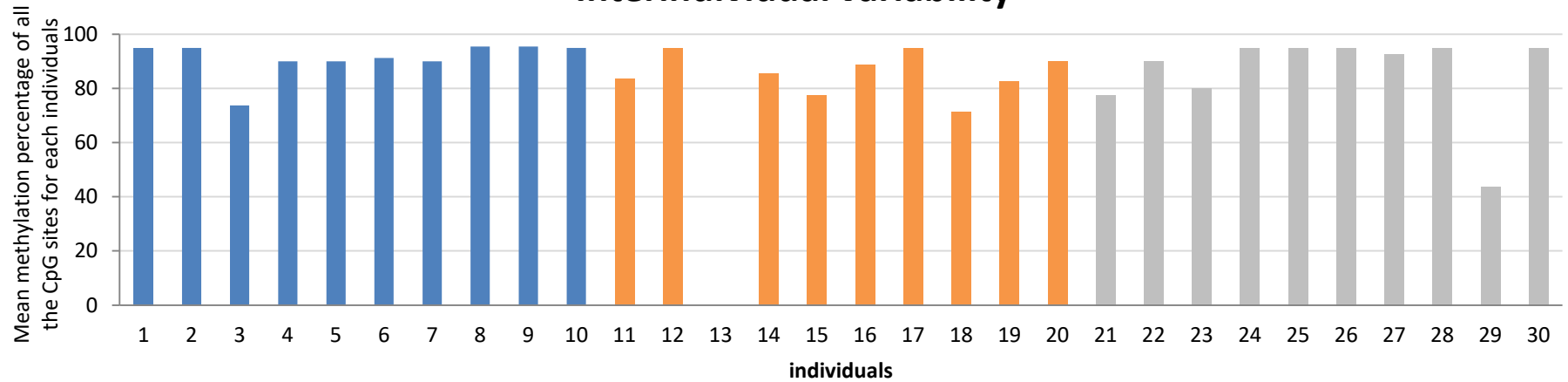


A- Comparison using the average methylation of the same 3 individuals for target analysis and BS-Seq.

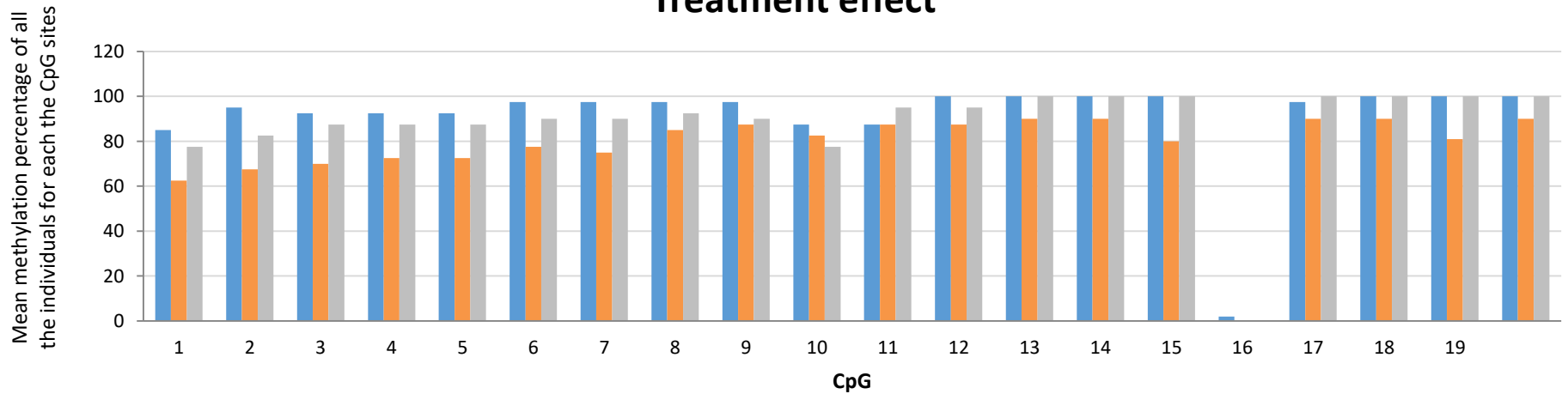
B- Comparison using the average methylation of 10 individuals for target analysis and 3 individuals for BS-Seq analysis

# Scaffold41174: 81270..81829

## Interindividual variability



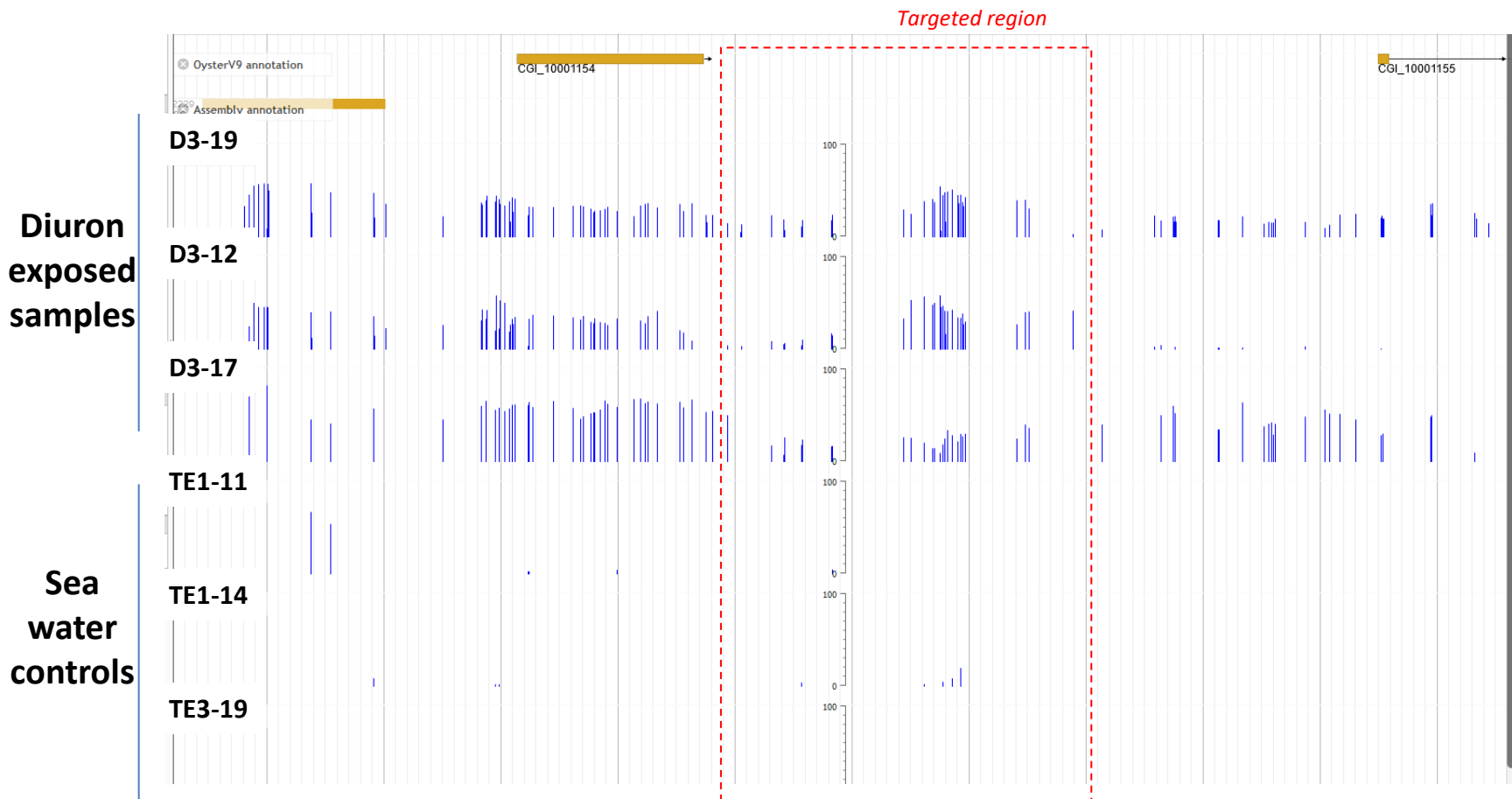
## Treatment effect



# Scaffold33832: 13205..13768

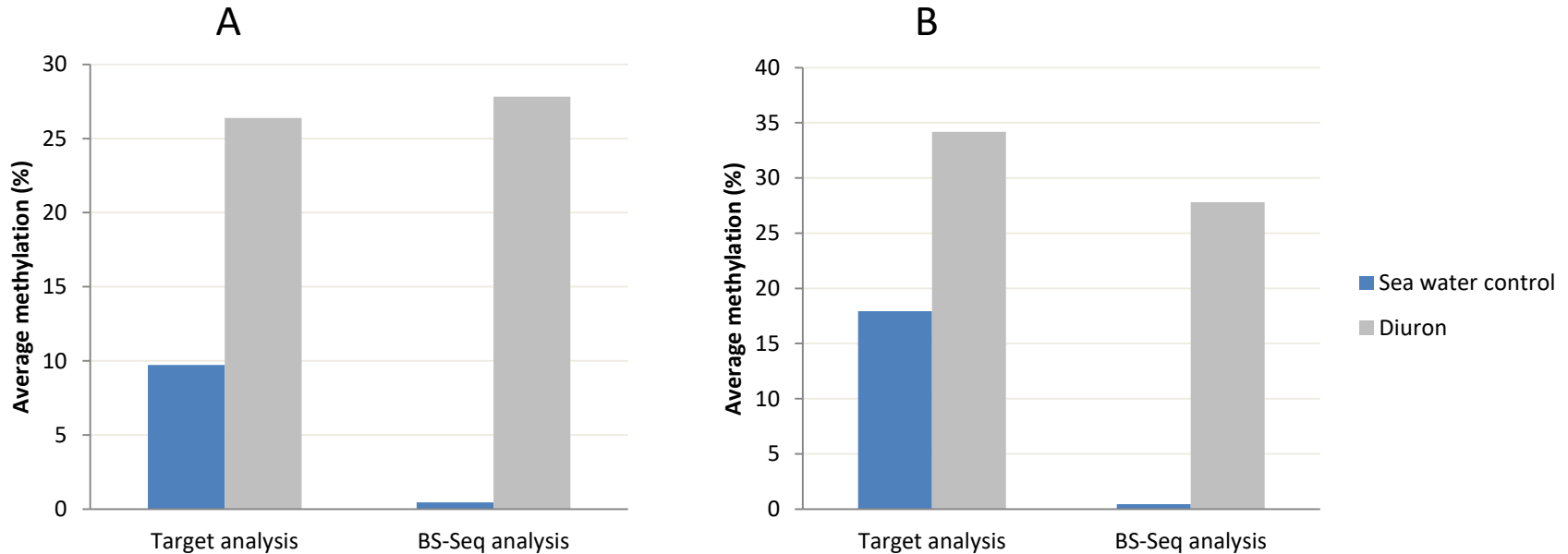
## Screen shot of BS-Seq data

Jbrowser extract of scaffold33832:12053..14898 (2.85 Kb)



# Scaffold33832: 13205..13768

## Comparison of Sanger/BS-Seq

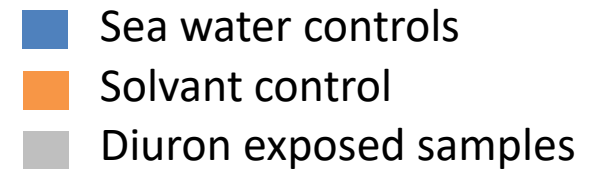
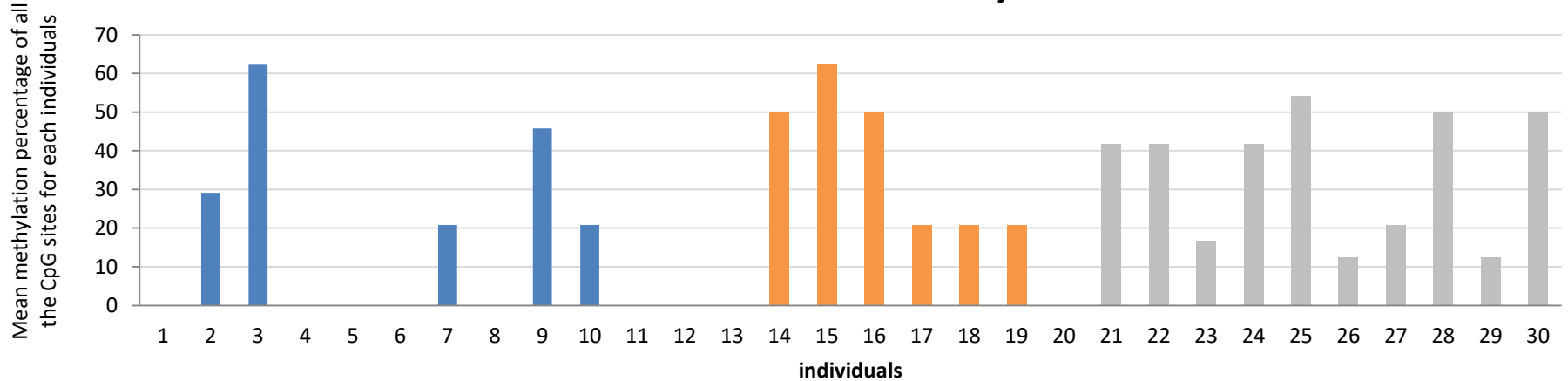


A- Comparison using the average methylation of the same 3 individuals for target analysis and BS-Seq.

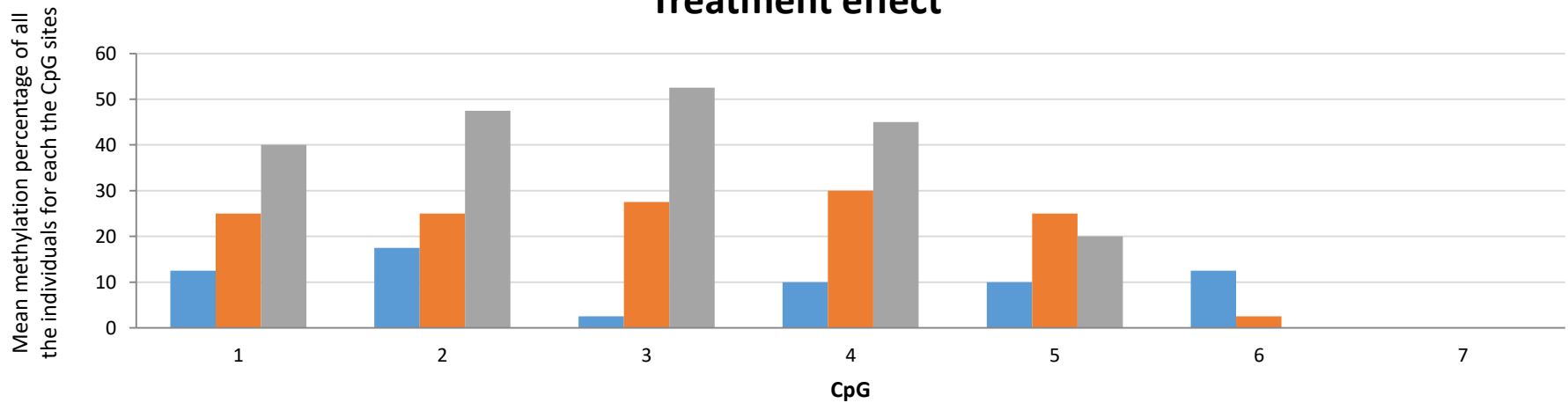
B- Comparison using the average methylation of 10 individuals for target analysis and 3 individuals for BS-Seq analysis

# Scaffold33832: 13205..13768

## Interindividual variability



## Treatment effect

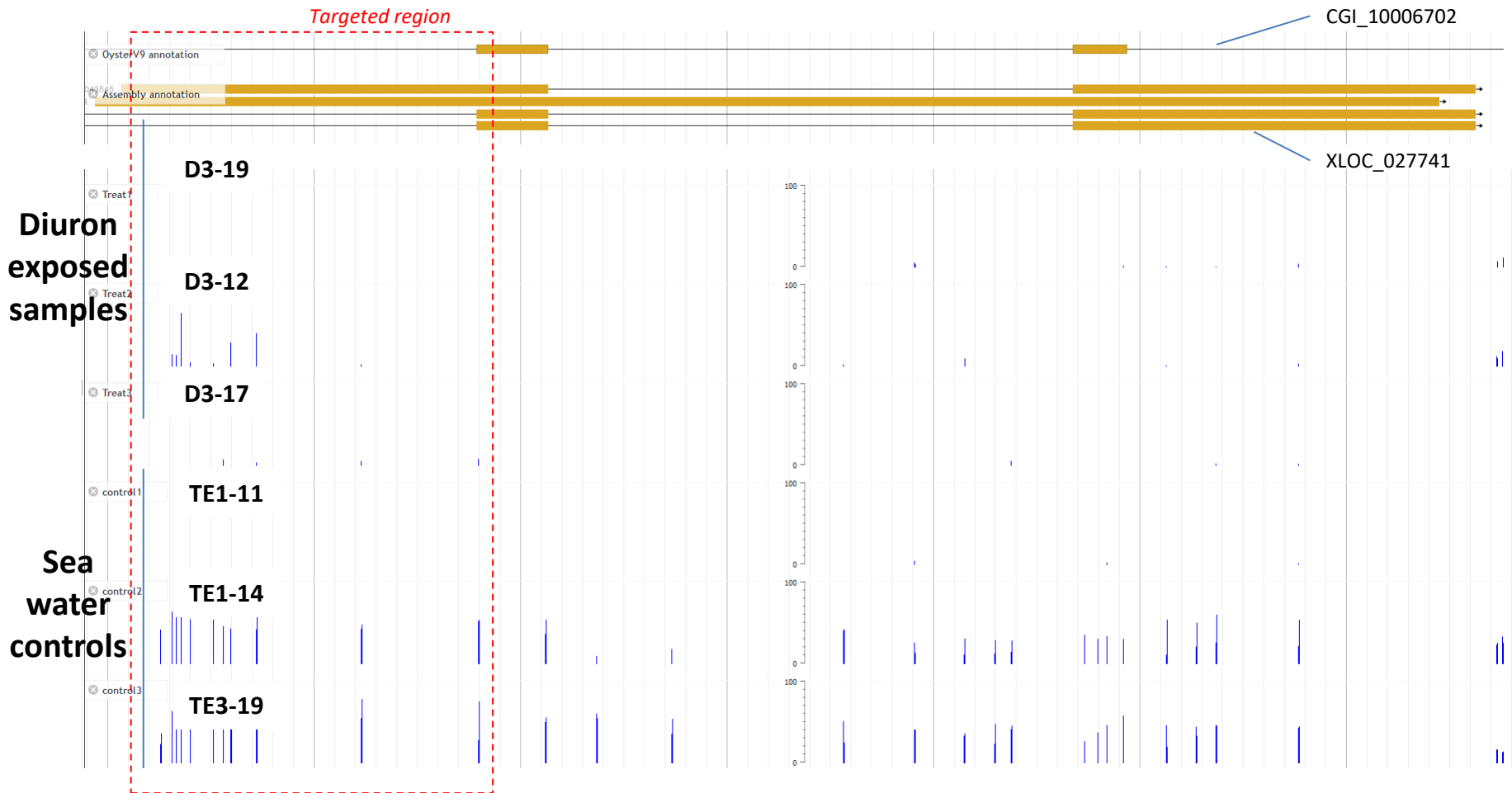




# Scaffold42366: 107142..107725

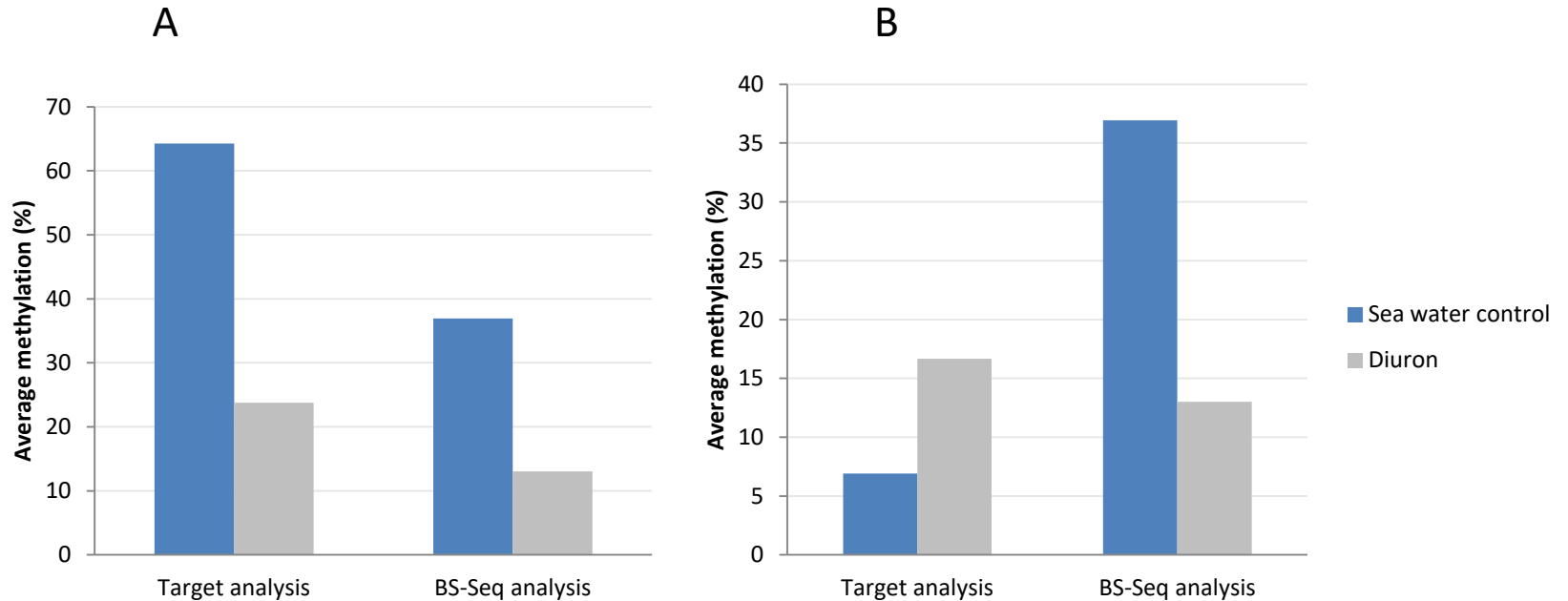
## Screen shot of BS-Seq data

*Jbrowser extract from scaffold42366:107224..108954 (1.73 Kb)*



# Scaffold42366: 107142..107725

## Comparison of Sanger/BS-Seq

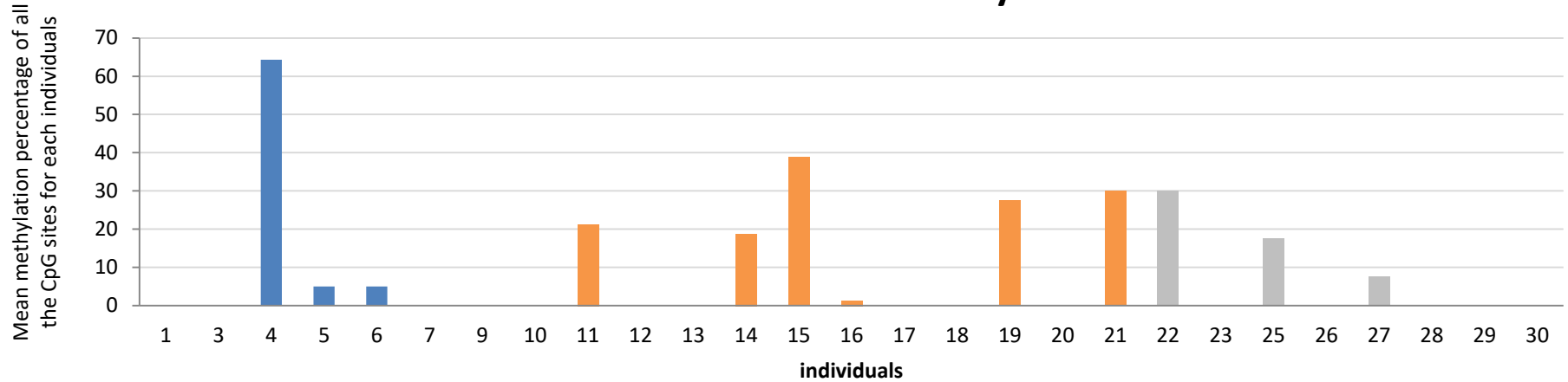


A- Comparison using the average methylation of the same 3 individuals for target analysis and BS-Seq.

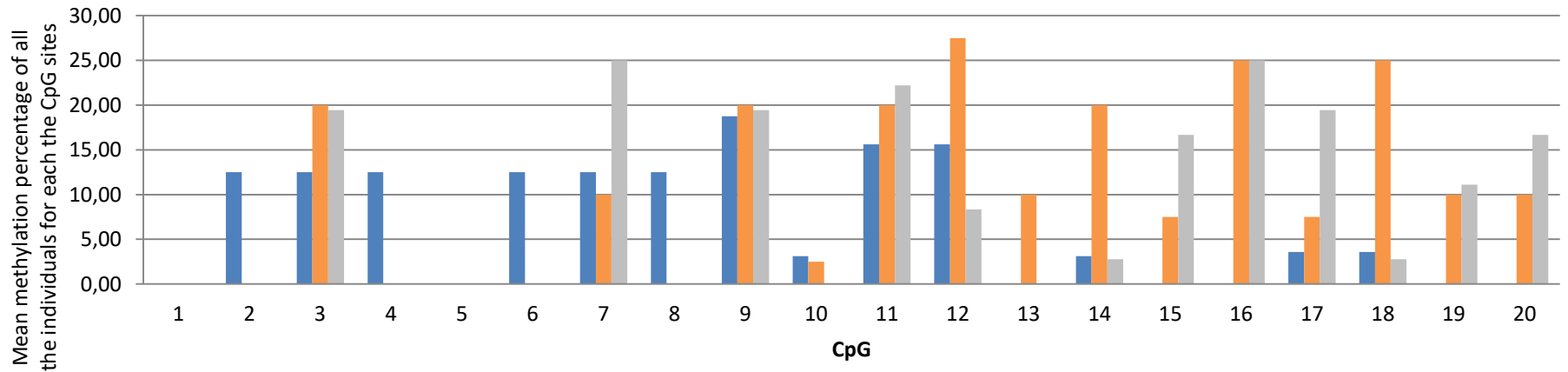
B- Comparison using the average methylation of 10 individuals for target analysis and 3 individuals for BS-Seq analysis

# Scaffold42366: 107142..107725

## Interindividual variability



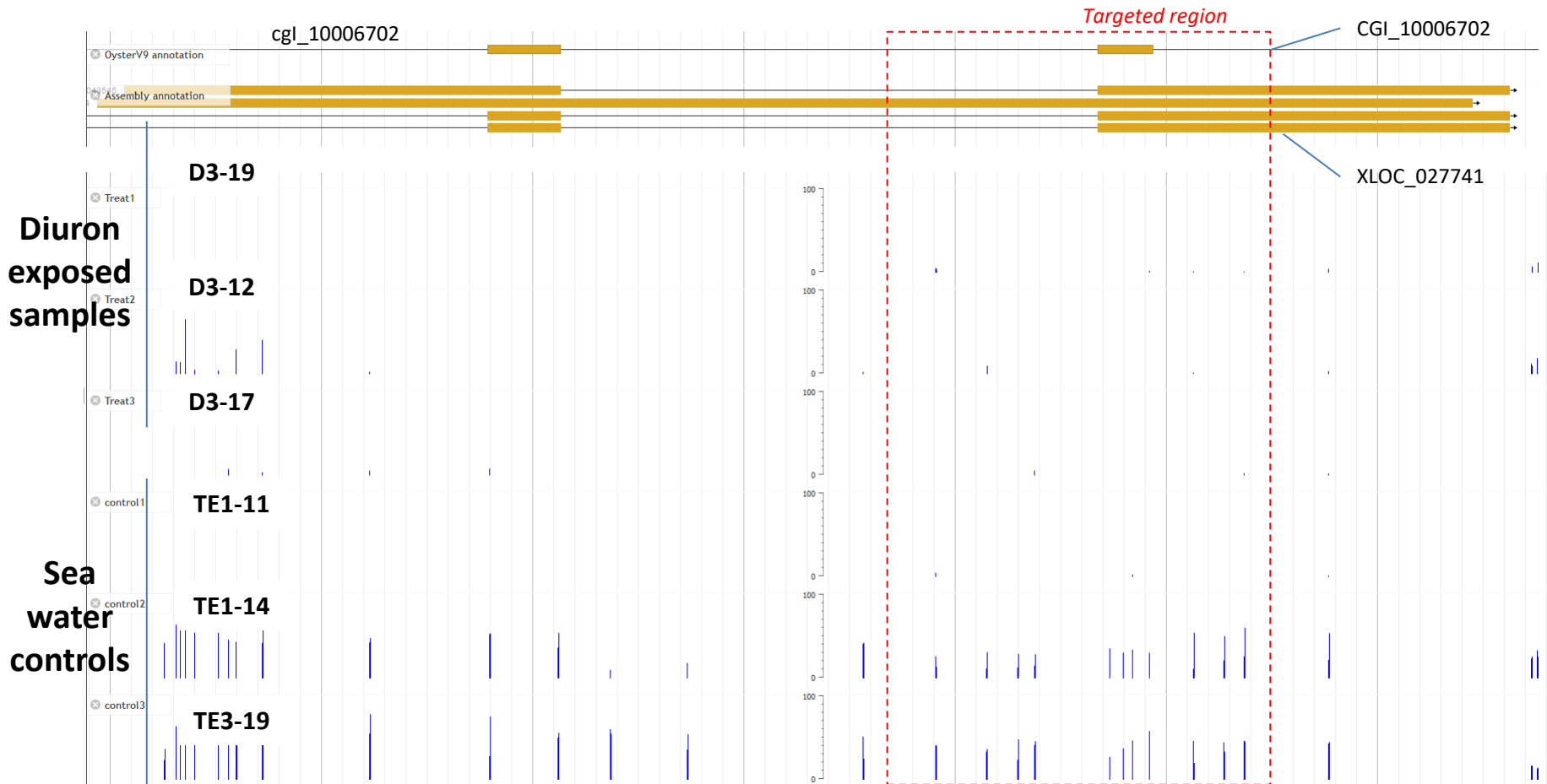
## Treatment effect



# Scaffold42366: 108172..108673

## Screen shot of BS-Seq data

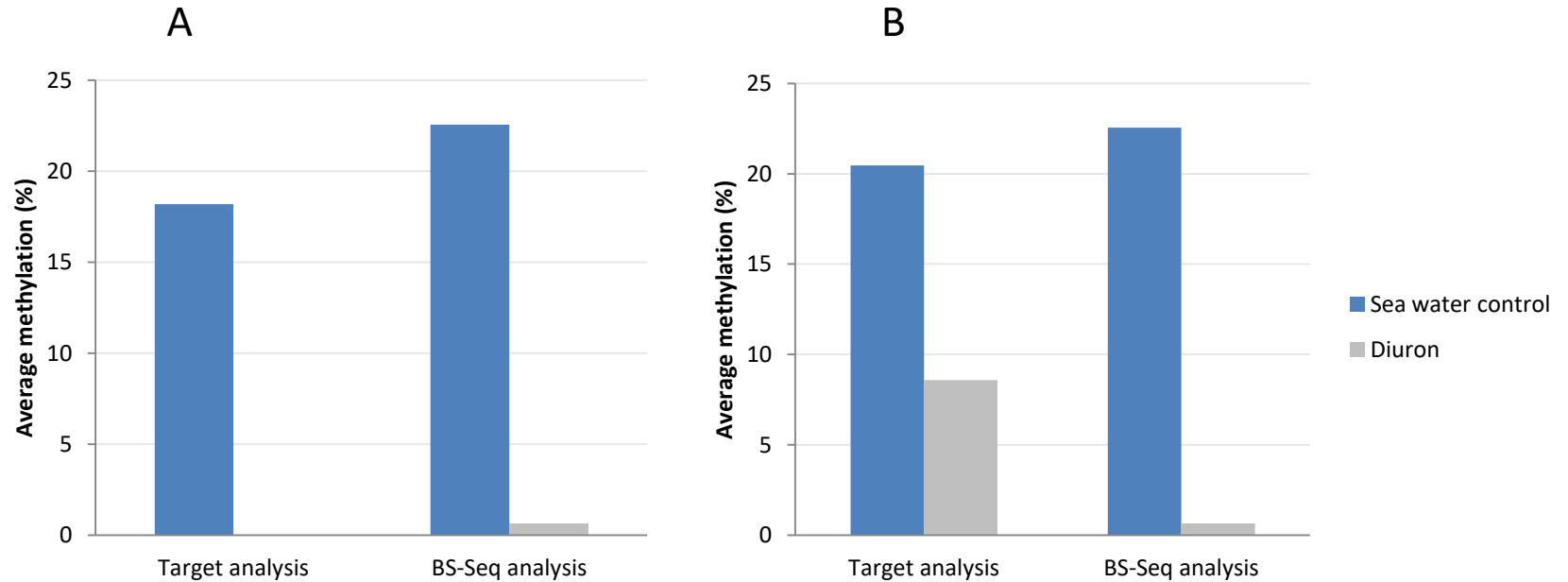
*Jbrowser extract from scaffold42366:107224..108954 (1.73 Kb)*



Blue bars represent the percentage of methylation at CpG position

# Scaffold42366: 108172..108673

## Comparison of Sanger/BS-Seq

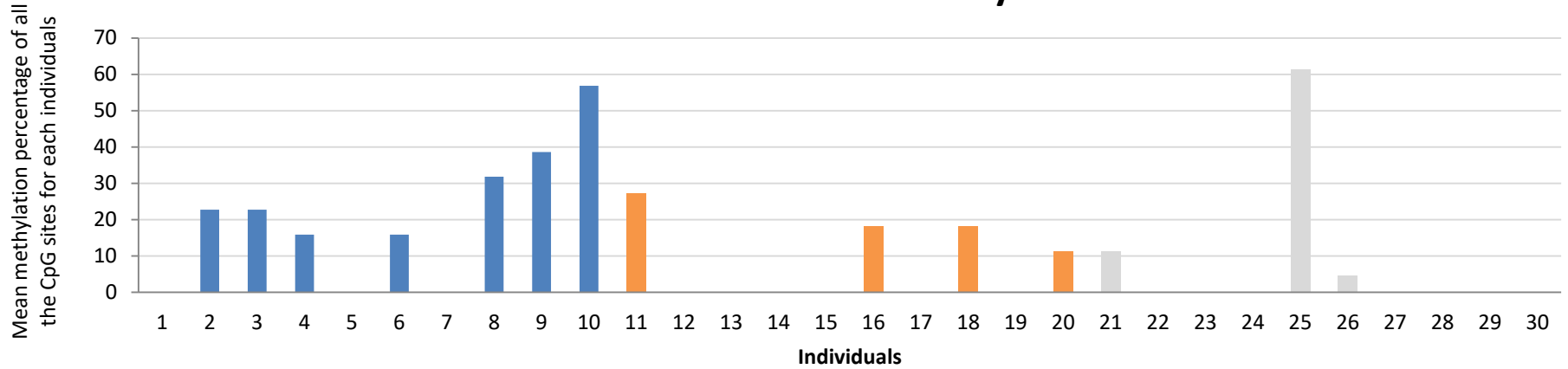


A- Comparison using the average methylation of the same 3 individuals for target analysis and BS-Seq.

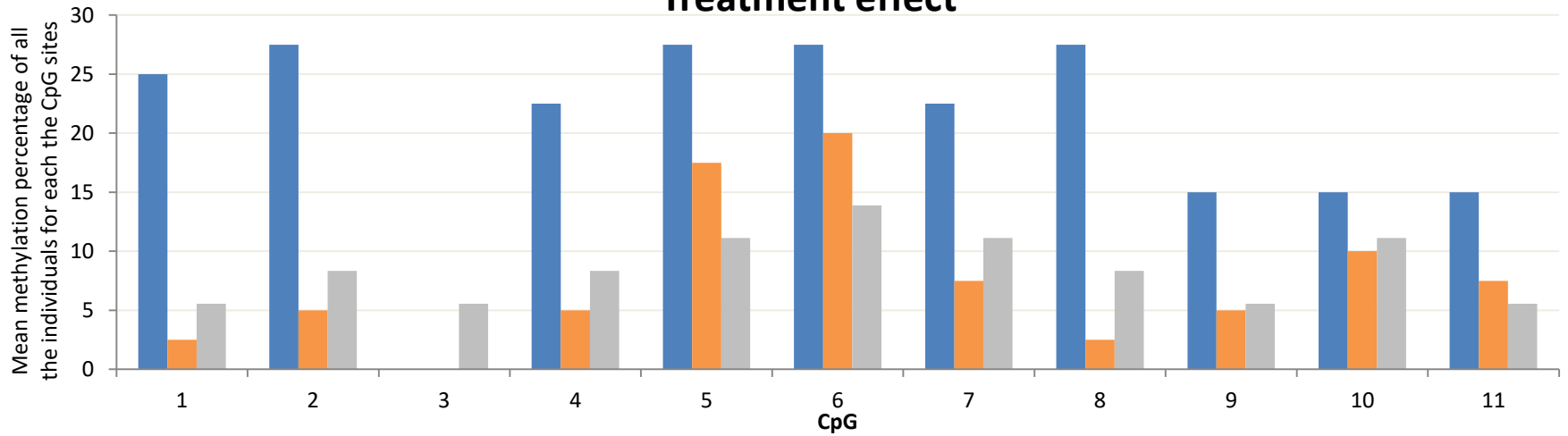
B- Comparison using the average methylation of 10 individuals for target analysis and 3 individuals for BS-Seq analysis

# Scaffold42366: 107142..107725

## Interindividual variability



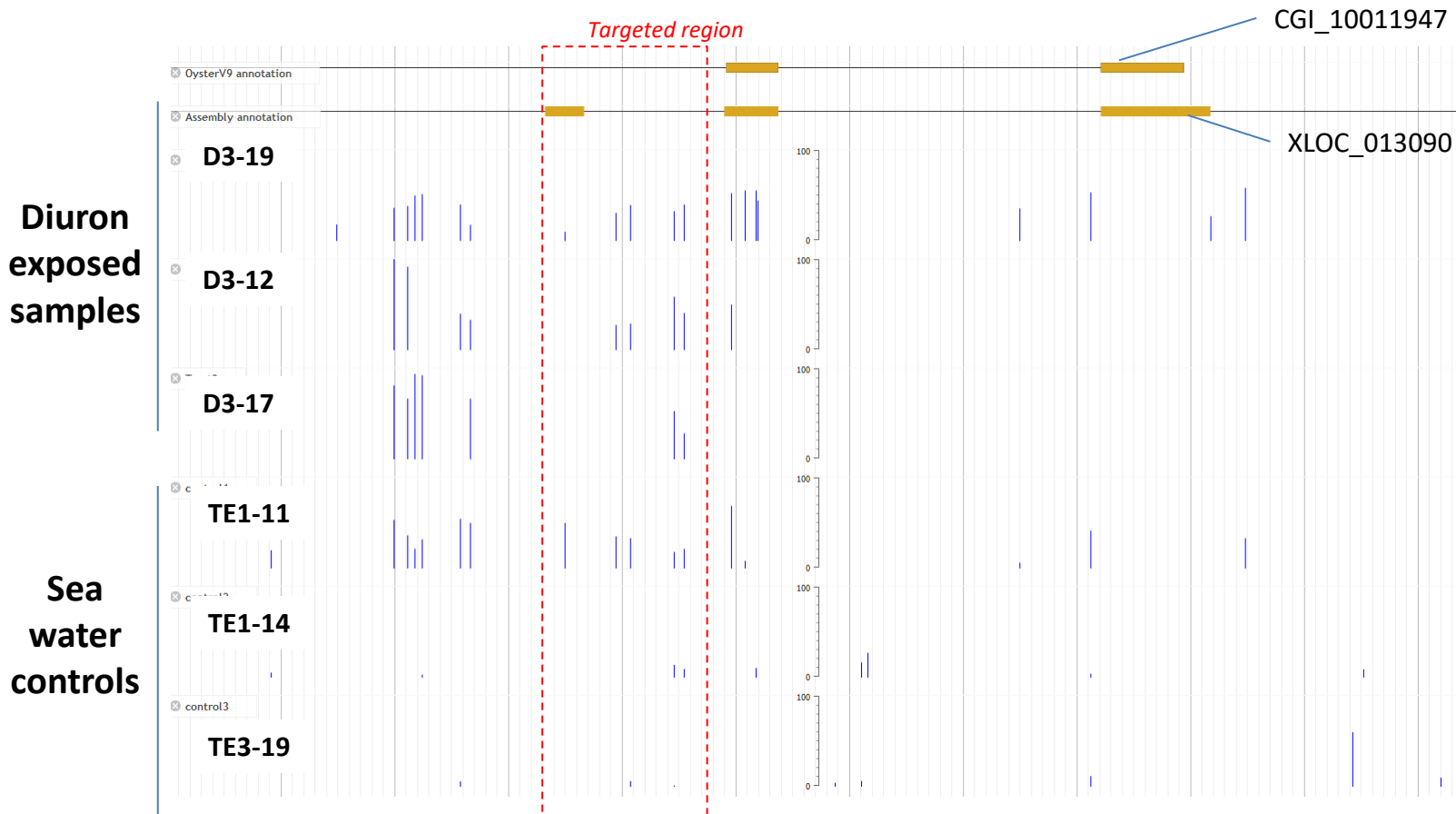
## Treatment effect



# Scaffold1720: 210182..210722

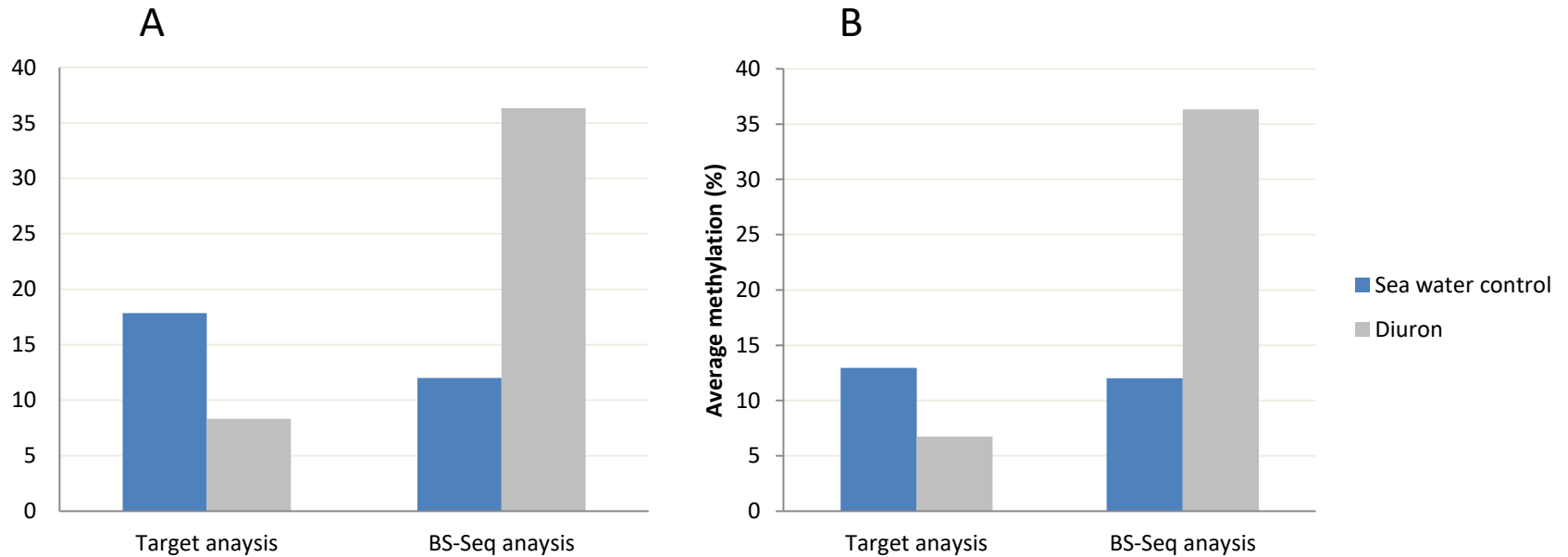
## Screen shot of BS-Seq data

*Jbrowser extract from scaffold1720:209499..212366 (2.87 Kb)*



# Scaffold1720: 210182..210722

## Comparison of Sanger/BS-Seq



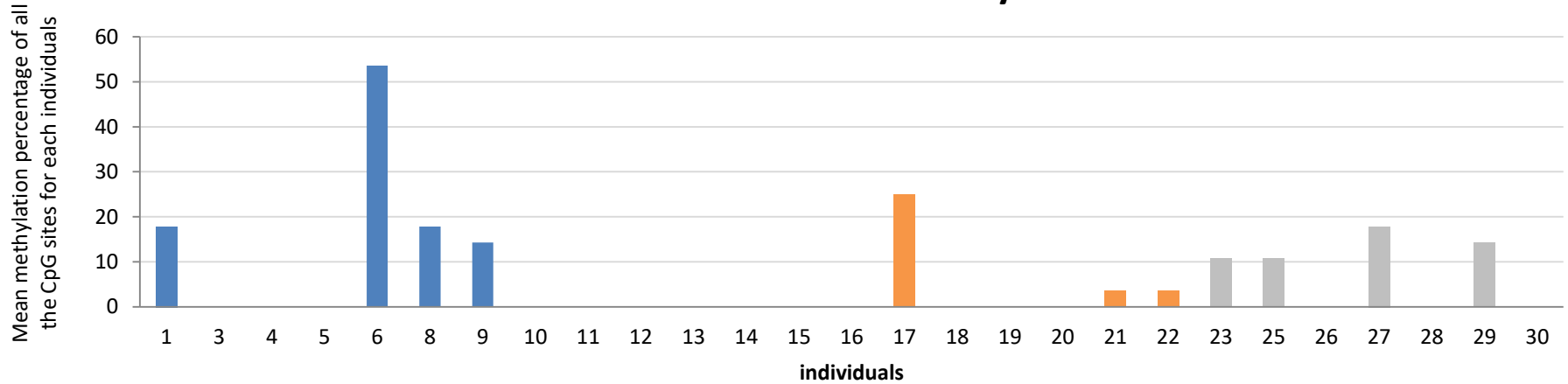
A- Comparison using the average methylation of the same 3 individuals for target analysis and BS-Seq.

B- Comparison using the average methylation of 10 individuals for target analysis and 3 individuals for BS-Seq analysis

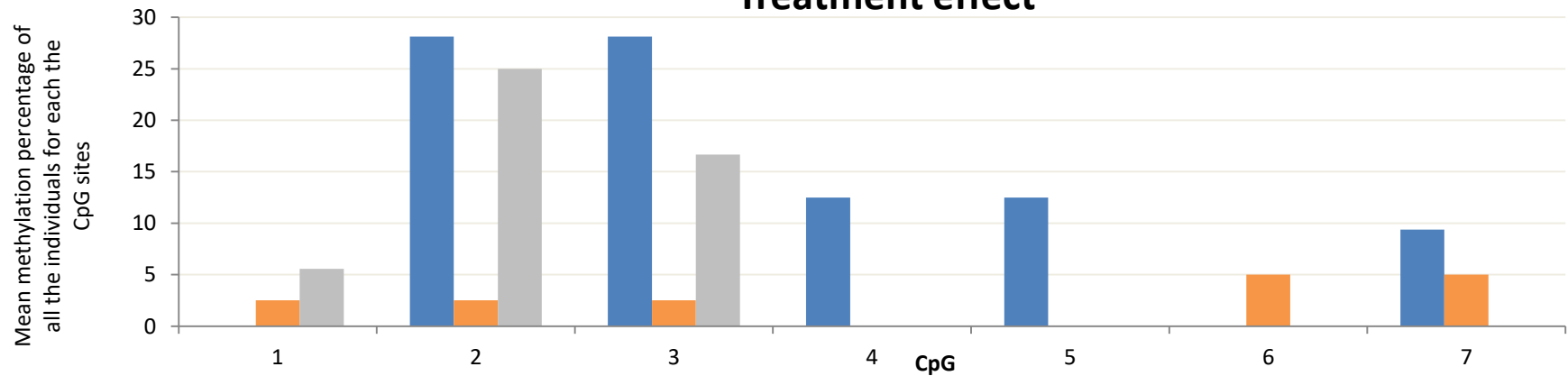


# Scaffold1720: 210182..210722

## Interindividual variability



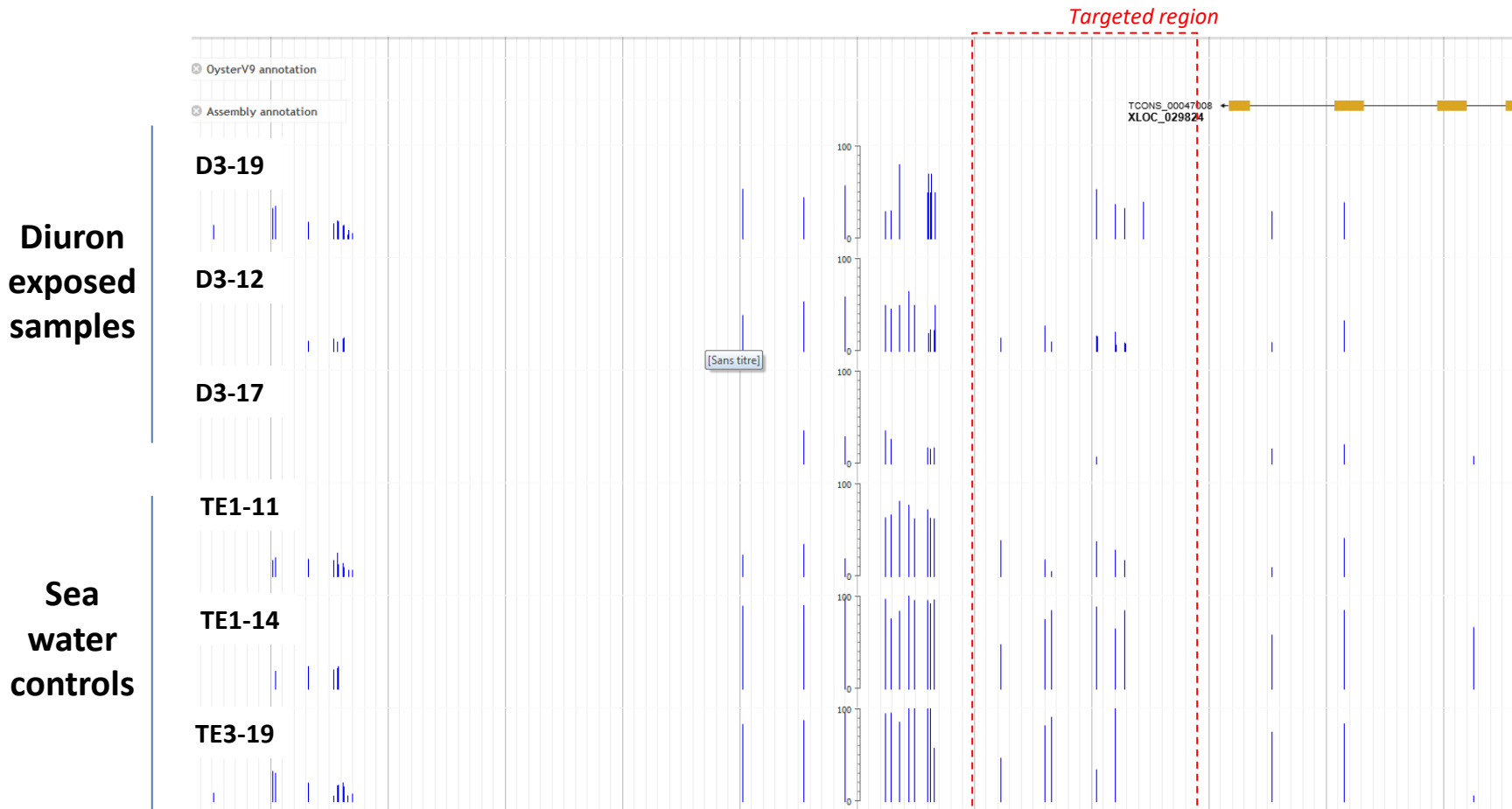
## Treatment effect



# Scaffold433: 896527..897085

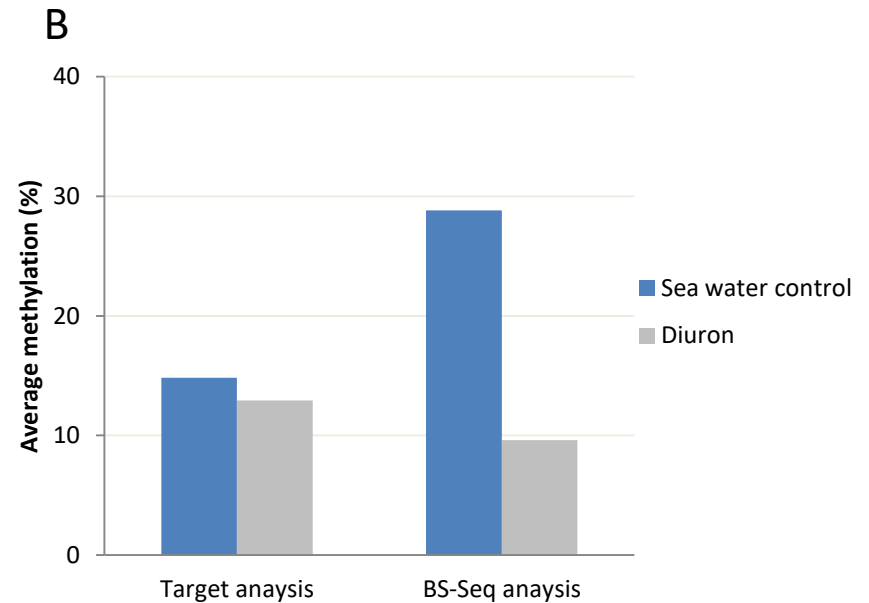
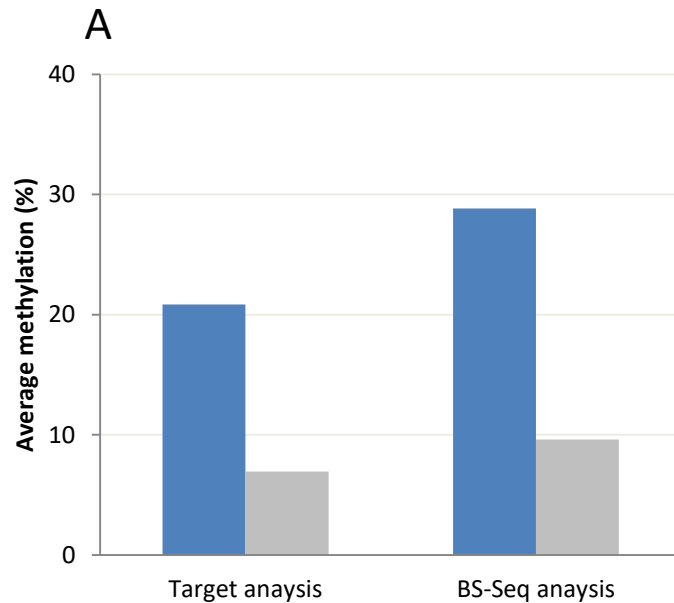
## Screen shot of BS-Seq data

*Jbrowser extract from scaffold433:894823..897668 (2.85 Kb)*



# Scaffold433: 896527..897085

## Comparison of Sanger/BS-Seq

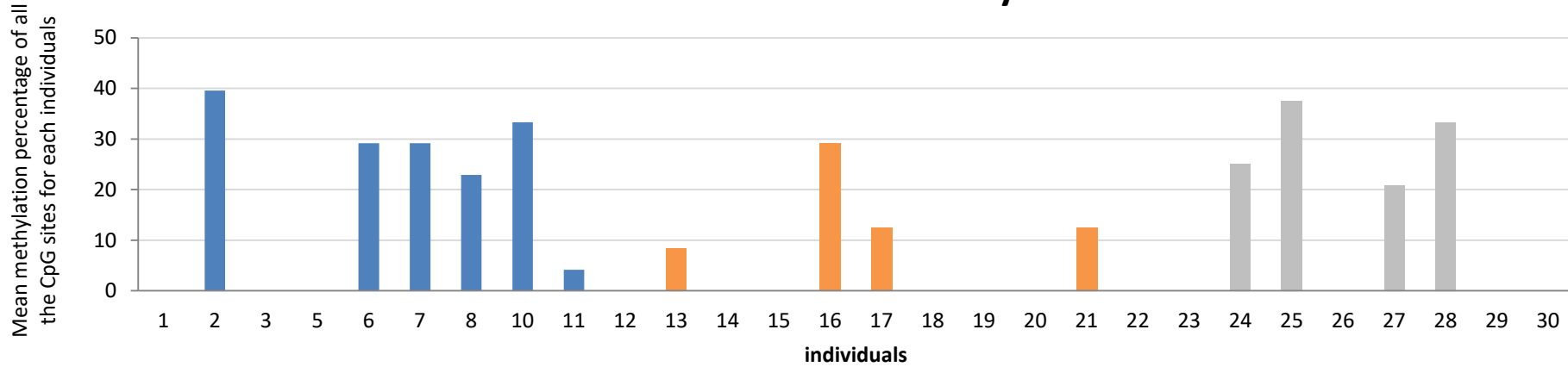


A- Comparison using the average methylation of the same 3 individuals for target analysis and BS-Seq.

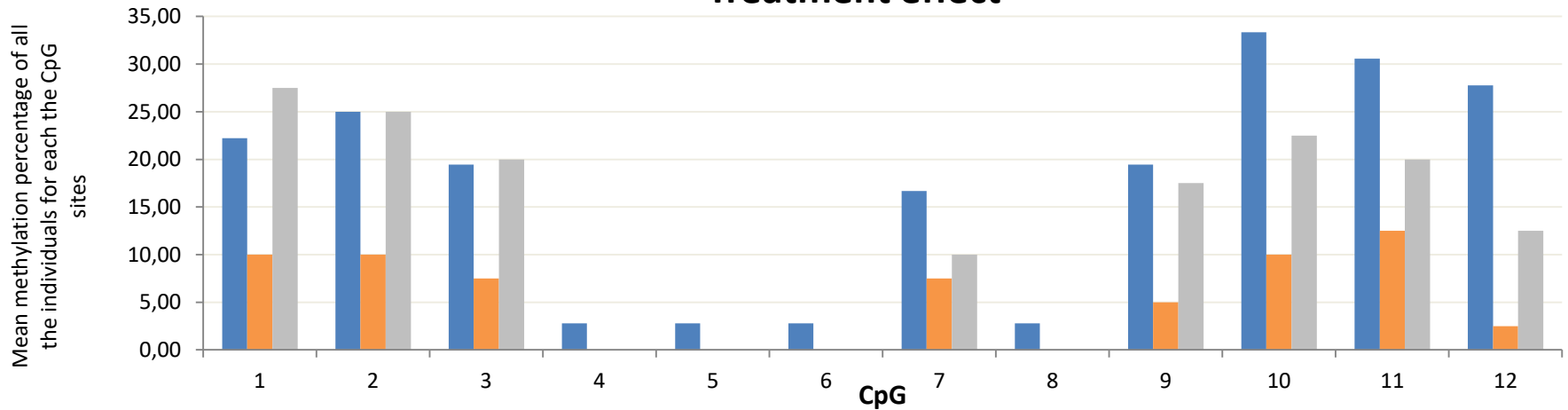
B- Comparison using the average methylation of 10 individuals for target analysis and 3 individuals for BS-Seq analysis

# Scaffold433: 896527..897085

## Interindividual variability



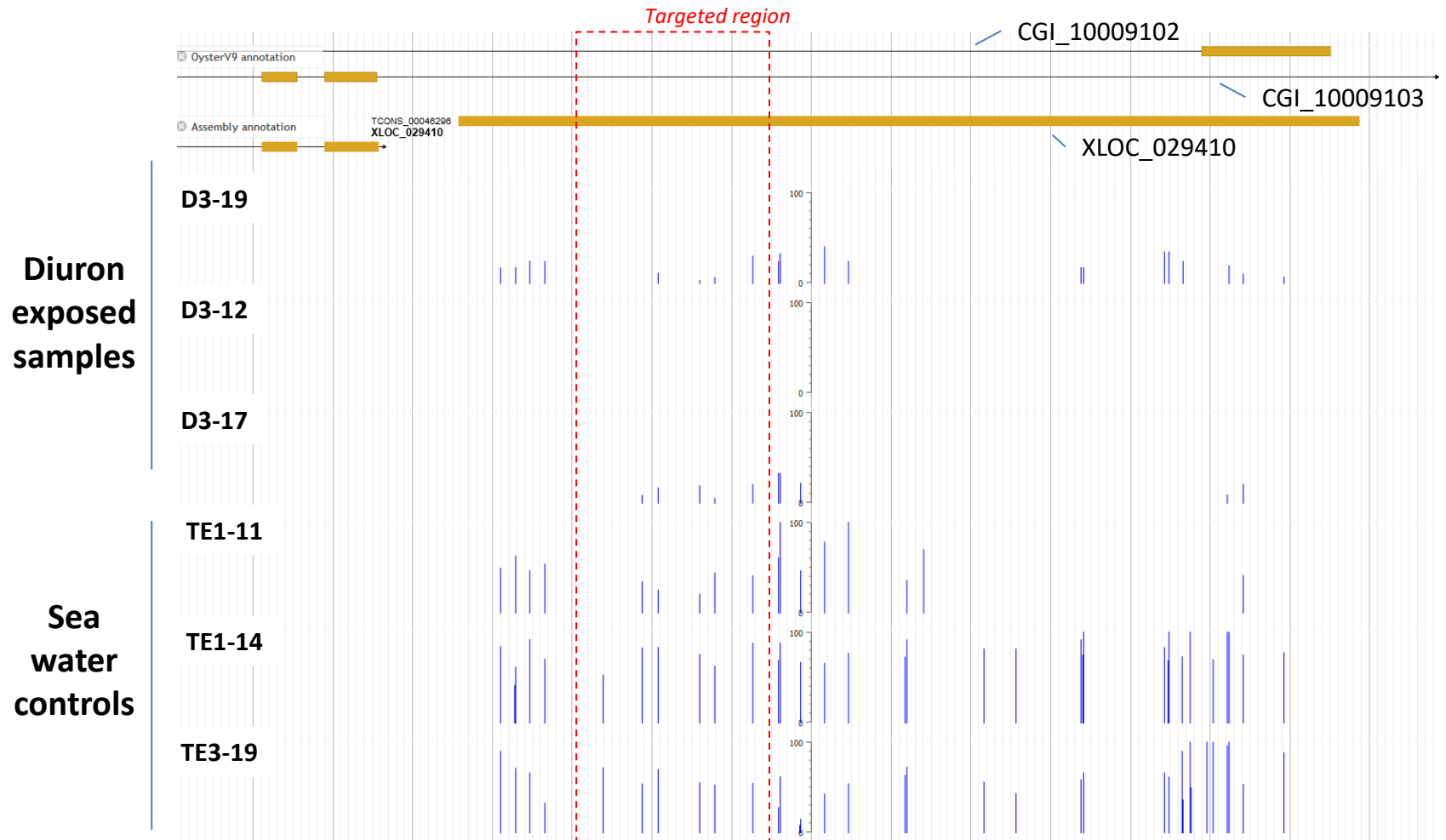
## Treatment effect



# Scaffold43170: 116309..116863

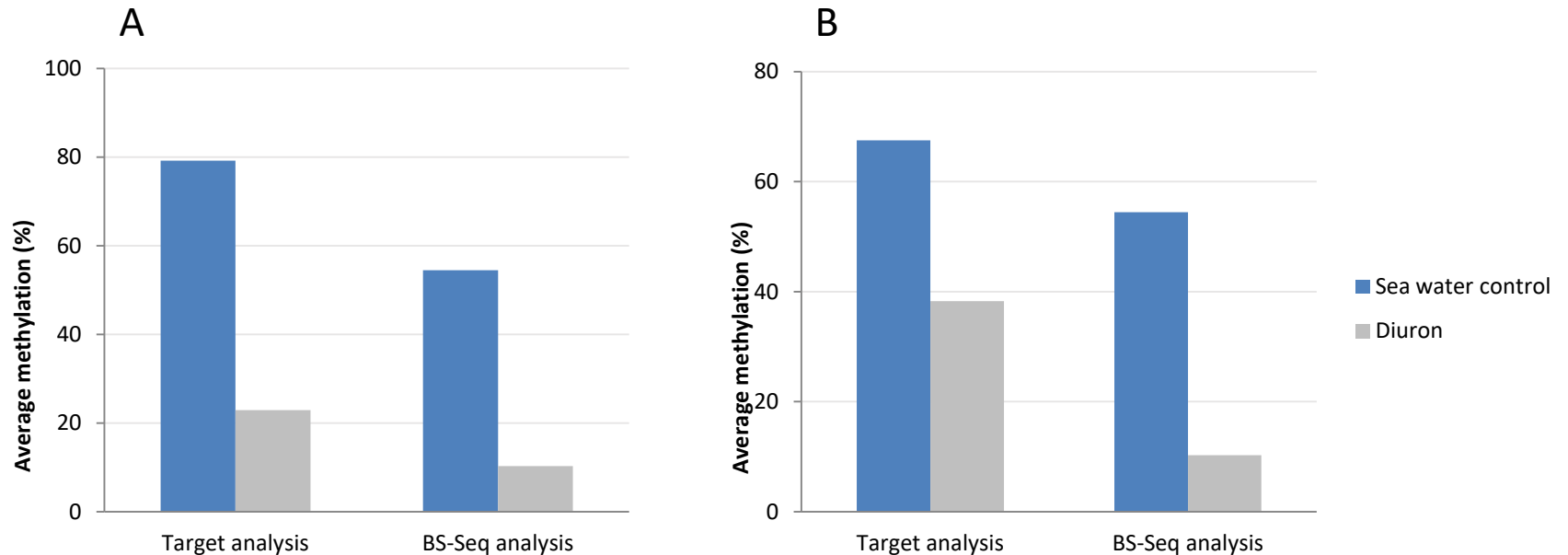
## Screen shot of BS-Seq data

*Jbrowser extract from scaffold43170:115000..118969 (3.97 Kb)*



# Scaffold43170: 116309..116863

## Comparison of Sanger/BS-Seq

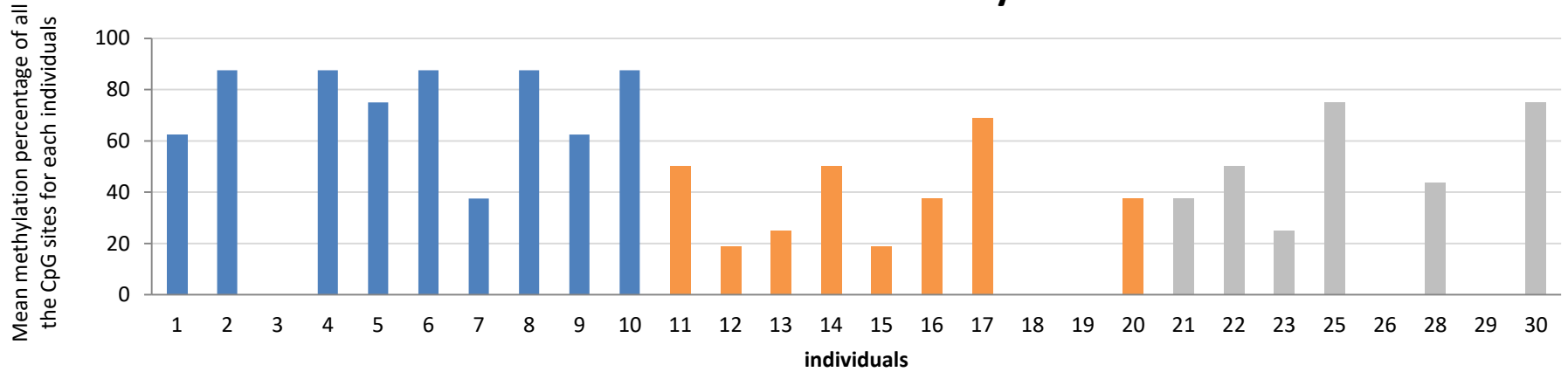


A- Comparison using the average methylation of the same 3 individuals for target analysis and BS-Seq.

B- Comparison using the average methylation of 10 individuals for target analysis and 3 individuals for BS-Seq analysis

# Scaffold43170: 116309..116863

## Interindividual variability



## Treatment effect

