Material Suplementario: Corroboración de picos y descubrimiento de motivos en *P. aeruginosa*

Anastasia Hernández Koutoucheva y Alán Fernando Muñoz González

El trabajo se basa en los datos de Chip-seq obtenidos en el artículo ChIP-seq reveals the global regulator AlgR mediating cyclic di-GMP synthesis in Pseudomonas aeruginosa.

Detección de motivos

Se obtienen las secuencias en formato .txt de GEO Accesion GSE65356 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE65356), se convierten al formato .bed requerido para el análisis, mediante:

En bash: more secuencia_DirectaDeGeo.txt | grep -v "#" | cut -f 2,3,10 > seqid.bed

Usando R:

table <- read.table("../Downloads/seqid.bed") Chrname <- rep("Chromosome",length(table[,1])) ntable <- data.frame(Chrname,table) write.table(ntable,"../Downloads/seqid_clean.bed", sep = " \hat{i} ", row.names = F, col.names = F, quote = F)

En RSat, se cambia a la base de datos de procariotes; en sequence tools -> sequences from bed/gff/vcf y se elige el organismo Pseudomonas aeruginosa pao1.ASM676v1.30; la opción Mask repeats se deja en su forma predeterminada. Tras obtener los archivos en formato .fasta, se procede a usar la herramienta Peak motifs; con los parámetros default excepto en Compare discovered motifs with databases, en la que únicamente se elige a los procariotes en RegulonDB.

Análisis de Peak Calling

Las secuencias tienen alrededor de 230 pdb lo cual es un buen indicio para los picos de ChIP-seq ya que son números menores a los que se obtendrían con picos de histonas. La composición en las secuencias es mayor para nucleótidos G/C y menor para la de A/T; pero en cada una de las parejas se observa el mismo patrón (Figura 1). En la composición de dinucleótidos se observa el mismo patrón de aumento en G/C sobre A/T. Para la secuencia de control, se observan los mismos patrones de composición de nucleótidos.

Tabla 1: Resultados de peak calling con los datos de picos obtenidos directamente del artículo original.

Peak name	Mean Length	Composition	Discovered Motifs	Bold motif (s) sequence (asmb)	Major significance
2588	230 pdb	>G/C <a t<br="">=2	10	gfoggoosgog g	14
algr	192 pdb	>G/C <a t<br="">=2	10	acogacgaso ggfog	28
exsa	309 pdb	>G/C <a t<br="">=2	5	-	2
gaca	208 pdb	>G/C <a t<br="">=2	10 10	agogooaggo ogaaoag	20 77
mext	350 pdb	>G/C <a t<="" td=""><td>10</td><td>-</td><td>3</td>	10	-	3
mvfr	227 pdb	>G/C <a t<br="">=2	10	-	10
rpon	208 pdb	>G/C <a t<br="">=2	10	atogoogagga	19
SOXT	266 pdb	>G/C <a t<br="">=2	10	-	5
vfr	218 pdb	>G/C <a t<br="">=2	10	-	8
wspr	1000 pdb	=G/C A/T	6	•	1

Tabla 2: Resultados de peak calling con los picos obtenidos con MACS2.

42	306 pdb	>G/C <a t<br="">=2	10	ttootoggogat	22
43	323 pdb	>G/C <a t<br="">=2	10	ttootoggogat	76
44	334 pdb	>G/C <a t<br="">=2	10	otoggogat	89
45	342 pdb	>G/C <a t<br="">=2	10	ttootoggogat	77
46	343 pdb	>G/C <a t<br="">=2	10	ttootoggogat	72
47	337 pdb	>G/C <a t<br="">=2	10	ttootoggogat	83
48	332 pdb	>G/C <a t<br="">=2	10	ttootoggogat	63
49	331 pdb	>G/C <a t<br="">=2	10	atogoogagga a	72
60	340 pdb	>G/C <a t<br="">=2	10	ogaogaao	70
51	330 pdb	>G/C <a t<br="">=2	10	ogaogaaoag o	67

Como nota, la secuencia usada para el análisis con los datos procesados manualmente es la número 50; debido a que toda la secuencia es completamente igual a la obtenida en el artículo. Cabe resaltar la similitud con otras como la 51, en la que obtenemos la secuencia misma y además, más información. Después las matrices con los resultados generados son guardadas en un formato .transfac

Uso de herramientas de clustering para corroborar los resultados

Para realizar este paso, se cambia al servidor Fungi para aumentar la velocidad del proceso. Matrix tools -> Matrix-clustering, todos los parámetros se quedan en su forma predeterminada, con excepción de Motif

comparison with compare-matrices-quick (100 times faster). Only for Ncor and Cor, el cual se activa.

Tabla 3: Resultados de matrix-clustering para ambos casos.

Peak Name	Input motifs	Clusters found	Thresholds
2588	10	2 (8,2)	Noor = 0.4
42	10	4(2,5,2,1)	oor = 0.6
algr	10	5(6,1,1,1,1)	Noor = 0.4
43	10	6(4,2,1,1,1,1)	oor = 0.6
exsa	5	2(4,1)	Noor = 0.4
44	10	8(2,2,1,1,1,1,1,1)	oor = 0.6
gaca	10	3(4,4,2)	Noor = 0.4
45	10	5(2,2,3,2,1)	oor = 0.6
mext	10	6(2,3,1,1,2,1)	Noor = 0.4
46	10	8(2,2,1,1,1,1,1,1)	oor = 0.6
mvfr	10	4(6,2,1,1)	Noor = 0.4
47	10	7(2,2,2,1,1,1,1)	oor = 0.6
rpon	10	4(4,3,2,1)	Noor = 0.4
48	10	4(4,3,1,1,1)	oor = 0.6
50xr	10	5(2,2,4,1,1)	Noor = 0.4
49	10	6(2,2,2,2,1,1)	oor = 0.6
vfr	10	5(4,2,2,1,1)	Noor = 0.4
50	10	5(4,2,2,1,1)	oor = 0.6
wspr	6	5(2,1,1,1,1)	Noor = 0.4
51	10	5(3,3,2,1,1)	oor = 0.6

Control Negativo

Regresas al servidor RSat de Procariotes. Se usa NGS-ChIP-seq-> random genome fragments para buscar falsos positivos en los resultados. Para esto, se utiliza el mismo organismo que en el análisis de peak calling (Pseudomonas aeruginosa pao1.ASM676v1.30) y dejas todas las opciones de forma predeterminada, con excepción de Mask repeats. Después, se usa la opción Peak-motifs en RSat de Fungi; con los mismos parámetros que en el análisis de Peak calling.

Tabla 4: Resultados de control negativo para ambos casos

Peak name	Mean Length	Composition	Discovered Motifs	Major significance	Clusters found
2588 42	230 pdb 307 pdb	>G/C <a t<br="">=2	10 10	5 21	3 (7,2,1) 6(4,2,1,1,1,1)
algr 43	192 pdb 323 pdb	>G/C <a t<br="">=2	10 10	7 40	2(6,4) 8(2,2,1,1,1,1, 1,1)
exsa 44	309 pdb 334 pdb	>G/C <a t<br="">=2	10 10	6 45	3(3,6,1) 8(2,2,1,1,1,1, 1,1)
gaca 45	208 pdb 342 pdb	>G/C <a t<br="">=2	10 10	7 48	6(2,2,3,1,1,1) 7(2,2,2,1,1,1, 1)
mext 46	350 pdb 343 pdb	>G/C <a t<br="">=2	10 10	2 72	7(2,3,1,1,1,1, 1) 7(2,1,1,3,1,1, 1)
mvfr 47	227 pdb 337 pdb	>G/C <a t<br="">=2	10 10	4 67	5(2,3,2,2,1) 6(3,2,2,1,1,1)
rpon 48 49	208 pdb 332 pdb 331 pdb	>G/C <a t<br="">=2	10 10 10	22 62 58	3(2,4,4) 6(3,3,1,1,1,1) 5(3,2,3,1,1)
SOXY	266 pdb	>G/C <a t<br="">=2	10	2	6(2,2,3,1,1,1)
vitr 50	218 pdb 340 pdb	>G/C <a t<br="">=2	10 10	10 57	6(2,2,2,2,1,1) 6(2,3,2,1,1,1)
wspr 51	1000 pdb 330 pdb	= G/C A/T >G/C <a t<br="">=2	10	1 64	5(2,1,1,1) 7(2,2,2,1,1,1, 1)

Como nota, todos los análisis de peak-motifs se realizaron con oligos, esto fue porque se intentaron usar otros enfoques (el análisis de diadas no fue usado en este punto) como el de tomar únicamente el parámetro de posición, el cual (generalmente) regresó menos motivos (con menor significancia) con las secuencias prueba. Y, en el control negativo disminuyeron aún más los motivos encontrados (pero, la significancia se mantuvo). Por ejemplo, para el caso de la secuencia 2588 la secuencia regresó 4 motivos con significancia menor a 1 y el control negativo en este experimento retornó 1 motivo con significancia de 1. Además, únicamente con este enfoque, podría usarse la secuencia completa del organismo como control.

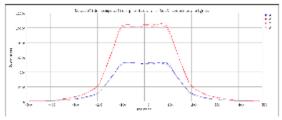


Figura 1: Porcentaje de bases obtenido en los motivos descubiertos.

Motivos de Algr

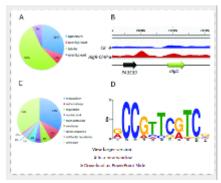


Figura 2: Descubrimiento de motivos de AlgR con MEME [1].

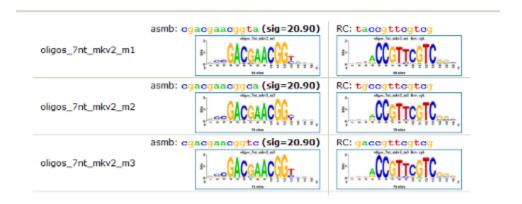


Figura 3: Descubrimiento de motivos con RSat con picos obtenidos del artículo original.

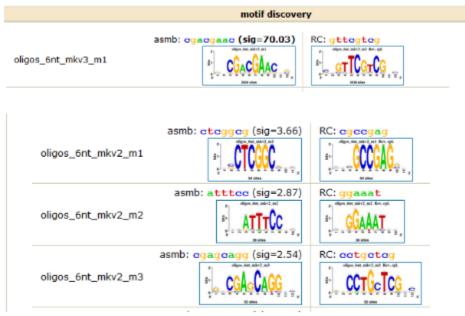


Figura 4: Descubrimiento de motivos con RSat con picos obtenidos manualmente con MACS2.

Análisis de diadas

El análisis de diadas se realizó con la herramienta dyad-analysis de RSat, en el servidor de procariotes. Para esto, se usó el archivo en formato fasta del pico de AlgR y P. aeruginosa como organismo para modelo de Background, el resto de los parámetros se quedaron en su forma predeterminada.

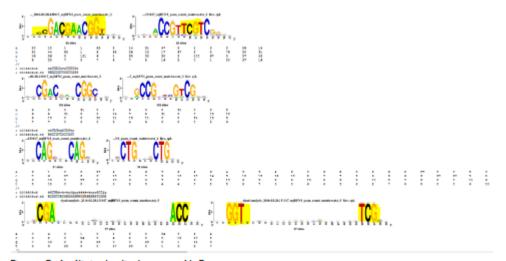


Figura 5: Análisis de diadas para AlgR

Workflow para nuestros peaks

Descargar Secuencias de lecturas cortas http://www.ebi.ac.uk/ena/data/view/SRP052880

Mapear con genoma de referencia en bowtie2 Index de genoma de Pseudomonas aeruginosa a bowtie2 http://support.illumina.com/sequencing_software/igenome.html bowtie2 build [Genome_fasta] PAO1

Mapear Lecturas de Illumina con genoma de referencia bowtie2 PAO1 seq_ name.fastq -S seq_name.sam -N 1 bowtie2 PAO1 seq_ name.fastq -S seq_name.sam -very-sensitive

El cambio daba $\sim 1\%$ de más secuencias, por lo que decidimos usar en RSAT los mapeos de la línea inicial Pseudomonas aeruginosa strain PAO1 NCBI 2000-09-13

Pseudomones	aeruginosa strain PAO1 N	ICBI 2000-09-18
SRR1776542	bowtie2 PAO1 Downloads/Fastq/SRR1776542.fast q -S SAM/SRR1776542.sam	4575282 reads; of these: 4575282 (100.00%) were unpaired; of these: 3165712 (69.19%) aligned 0 times 1380101 (30.16%) aligned exactly 1 time 29469 (0.64%) aligned >1 times 30.81% overall alignment rate
SRR1776543	bowrie2 PAO1 Downloads/fastq/SRR1776543.fast q -S SAM/SRR1776543.sam	\$308832 reads; of these: \$308832 (100.00%) were unpaired; of these: \$3284383 (61.87%) aligned 0 times \$1983911 (37.37%) aligned exactly 1 time 40538 (0.76%) aligned >1 times 38.13% overall alignment rate
SRR1776544	bowtie2 PAD1 Downloads/fastq/SRR1776544.fast q -S SAM/SRR1776544.sam	\$420068 reads; of these: \$420068 (100.00%) were unpaired; of these: 2913278 (53.75%) aligned 0 times 2459292 (45.37%) aligned exactly 1 time 47498 (0.88%) aligned >1 times 46.25% overall alignment rate
SRR1776545	bowrie2 PAD1 Downloads/fastq/SRR1776545.fast q -S SAM/SRR1776545.sam	6036968 reads; of these: 6036968 (100.00%) were unpaired; of these: 3514824 (58.22%) aligned 0 times 2474726 (40.99%) aligned exactly 1 time 47418 (0.79%) aligned >1 times 41.78% overall alignment rate
SRR1776546	bowtie2 PAO1 Downloads/fastq/SRR1776546.fast q -S SAM/SRR1776546.sam -O 20 -R 3 -N 0 -L 20 -i S,1,0.50	\$161454 reads; of these: \$161454 (100.00%) were unpaired; of these: 2239403 (43.39%) aligned 0 times 2871495 (55.63%) aligned exactly 1 time \$05556 (0.98%) aligned >1 times \$6.61% overall alignment rate
SRR1776547	bowtie Z PAO 1 Downloads/Fastq/SRR1776547.fast q -S SAM/SRR1776547.sam -D 20 -R 3 -N 0 -L 20 -i S,1,0.50	4620331 reads; of these: 4620331 (100.00%) were unpaired; of these: 1898380 (41.09%) aligned 0 times

		2678292 (57.97%) aligned exactly 1 time 43659 (0.94%) aligned >1 times 58.91% overall alignment rate
SRR1776548	bowrtie2 PAD1 Downloads/fastq/SRR1776548.fast q -S SAM/SRR1776548.sam -D 20 -R 3 -N 0 -L 20 -i S,1,0.50	5019633 reads; of these: 5019633 (100.00%) were unpaired; of these! 1142783 (22.77%) aligned 0 times 3808400 (75.87%) aligned exactly 1 time 68450 (1.36%) aligned >1 times 77.23% overall alignment rate
SRR1776549	bowtie2 PAD1 Downloads/fastq/SRR1776545,fast q -S SAM/SRR1776549,sam -D 20 -R 3 -N 0 -L 20 -i S,1,0.50	4838667 reads; of these: 4838667 (100.00%) were unpaired; of these: 823979 (17.03%) aligned 0 times 3944953 (81.53%) aligned exactly 1 time 69735 (1.44%) aligned >1 times 82.97% overall alignment rate
SRR1776550	bowtie2 PAD1 Downloads/fastq/SRR1776550.fast q -S SAM/SRR1776550.sam -D 20 -R 3 -N 0 -L 20 -i S,1,0.50	7908293 reads; of these: 7908293 (100.00%) were unpaired; of these: 2560348 (32.38%) aligned 0 times 5241027 (66.27%) aligned exactly 1 time 106918 (1.35%) aligned >1 times 67.62% overall alignment rate
SRR1776551	bowtie2 PAD1 Downloads/fastq/SRR1776551.fast q -S SAM/SRR1776551.sam -D 20 -R 3 -N 0 -L 20 -i S,1,0.50	4824339 reads; of these: 4824339 (100.00%) were unpaired; of these: 882513 (17.67%) aligned 0 times 3892440 (80.68%) aligned exactly 1 time 79386 (1.65%) aligned >1 times 82.33% overall alignment rate

bowtie2 PAO1 "JvH\ Práctica/fastq/SRR1776542.fastq" -5 "JvH\ Práctica/SAM2/SRR1776542.sam" very-sensitive	4575282 reads; of these: 4575282 (100.00%) were unpaired; of these: 3135751 (68.54%) aligned

SRR1776542	bowtie2 PAO1 "JvH\ Práctica/fastq/SRR1776542.fastq" -5 "JvH\ Práctica/SAMZ/SRR1776542.sam"very-sensitive	4575282 reads; of these: 4575282 (100.00%) were unpaired; of these: 3135751 (68.54%) aligned 0 times 1406632 (30.74%) aligned exactly 1 time 32899 (0.72%) aligned >1 times 31.46% overall alignment rate
SRR1776543	bowtie2 PAO1 "JVH\ Práctica/fastq/SRR1776543.fastq" -5 "JvH\ Práctica/SAMZ/SRR1776543.sam"very-sensitive	\$308832 reads; of these: \$308832 (100.00%) were unpaired; of these: 3243100 (61.05%) aligned 0 times 2020361 (38.06%) aligned exactly 1 time 45371 (0.85%) aligned >1 times 38.91% overall alignment rate
SRR1776544	bowtie2 PAD1 "JVH\ Práctica/fastq/SRR1776544.fastq" -5 "JVH\ Práctica/SAM2/SRR1776544.sam"very-sensitive	5420068 reads; of these: 5420068 (100.00%) were unpaired; of these: 2862746 (52.82%) aligned 0 times 2504193 (46.20%) aligned exactly 1 time 53129 (0.98%) aligned >1 times 47.18% overall alignment rate
SRR1776545	bowtie2 PAO1 "JvH\ Práctica/fastq/SRR1776545.fastq" - S "JvH\ Práctica/SAM2/SRR1776545.sam"very-sensitive	6036968 reads; of these: 6036968 (100.00%) were unpaired; of these: 3464803 (57.38%) aligned 0 times 2519498 (41.73%) aligned exactly 1 time 53167 (0.88%) aligned >1 times 42.62% overall alignment

SRR1776546	bowtie2 PAO1 "JvH\ Práctica/fastq/SRR1776546.fastq" -5 "JvH\ Práctica/SAMZ/SRR1776546.sam"very-sensitive	\$161454 reads; of these: 5161454 (100.00%) were unpaired; of these: 2179953 (42.24%) aligned 0 times 2924332 (56.66%) aligned exactly 1 time \$7169 (1.11%) aligned >1 times 57.76% overall alignment rate
SRR1776547	bowtie2 PAO1 "JvH\ Práctica/fastq/SRR1776547.fastq" -5 "JvH\ Práctica/SAMZ/SRR1776547.sam"very-sensitive	4620331 reads; of these: 4620331 (100.00%) were unpaired; of these: 1243490 (35.90%) aligned 0 times 2727522 (59.03%) aligned exactly 1 time 48319 (1.07%) aligned >1 times 60.10% overall alignment rate
SRR1776548	bowtie2 PAO1 "JvH\ Práctica/fastq/SRR1776548.fastq" -5 "JvH\ Práctica/SAMZ/SRR1776548.sam"very-sensitive	5019633 reads; of these: 5019633 (100.00%) were unpaired; of these: 1066940 (21.26%) aligned 0 times 3875232 (77.20%) aligned exactly 1 time 77461 (1.54%) aligned >1 times 78.74% overall alignment rate
SRR1776549	bowtie2 PAD1 "JvH\ Práctica/fastq/SRR1776549.fastq" -5 "JvH\ Práctica/SAM2/SRR1776549.sam"very-sensitive	4838667 reads; of these: 4838667 (100.00%) were unpaired; of these: 744936 (15.40%) aligned 0 times 4014732 (82.97%) aligned exactly 1 time 78999 (1.63%) aligned >1 times 84.60% overall alignment rate
SRR1776550	bowtie2 PAO1 "JvH\ Práctica/fastq/SRR1776550.fastq" -S "JvH\ Práctica/SAM2/SRR1776550.sam"	7908293 reads; of these: 7908293 (100.00%) were unpaired: of these:

	very-sensitive	2450204 (30.98%) aligned 0 times 5338526 (67.51%) aligned exactly 1 time 119563 (1.51%) aligned >1 times 69.02% overall alignment rate
SRR1776551	bowtie2 PAO1 "JvH\ Práctica/fastq/SRR1776551.fastq" -S "JvH\ Práctica/SAM2/SRR1776551.sam"very-sensitive	4824339 (100.00%) were 4824339 (100.00%) were unpaired; of these: 770139 (15.96%) aligned 0 times 3965453 (82.20%) aligned exactly 1 time 88747 (1.84%) aligned >1 times 84.04% overall alignment rate

MACS2 Peak Call

 $macs2\ callpeak\ -t\ SAM/\$\ bname.sam\ -f\ SAM\ -n\ peaks/\$bname\ -g\ 6.3e6\ -q\ 0.01\ -nomodel\ -shiftsize\ 100$

Comandos en el servidor:

Peak Calling: $RSAT/perl-scripts/peak-motifs - v 1 - title '2688vsmacs2' - i RSAT/public_html/tmp/apache/2016/02/20/peak-motifs.2016-02-20.195401_2016-02-20.195401_PP1XpT/peak-motifspeak_seq -max_seq_len 1000 -markov auto - disco oligos, positions - nmotifs 5 - minol 6 - maxol 7 - no_merge_lengths - 2str - origin center - motif_db regulonDB tf RSAT/public_html/motif_databases/REGULONDB/regulonDB_2015-08-07.tf - scan_markov 1 - source getfasta - task purge, seqlen, composition, disco, merge_motifs, split_motifs, motifs_vs_motifs, timelog, archive, synthesis, sma-prefix peak-motifs - noov - img_format png - outdir RSAT/public_html/tmp/apache/2016/02/20/peak-motifs.2016-02-20.195401_2016-02-20.195401_PP1XpT)$

 $\label{lem:matrix-clustering} $$RSAT/perl-scripts/matrix-clustering -v 1 -max_matrices 300 -matrix_format transfac -i $$RSAT/public_html/tmp/www-data/2016/02/22/matrix-clustering_2016-02-22.014846_eMBEdG/matrix-clustering_query_matrices.transfac -hclust_method average -title 'testfun2588' -metric_build_tree 'Ncor' -lth w 5 -lth cor 0.6 -lth Ncor 0.4 -quick -label_in_tree name -return json,heatmap -o $$RSAT/public_html/tmp/www-data/2016/02/22/matrix-clustering_2016-02-22.014846_eMBEdG/matrix-clustering 2> $$RSAT/public_html/tmp/www-data/2016/02/22/matrix-clustering_2016-02-22.014846_eMBEdG/matrix-clustering err.txt$

 $\label{lem:control_control_control} Control negativo: $RSAT/perl-scripts/random-genome-fragments-template_format len -i $RSAT/public_html/tmp/apache/20 genome-fragments_2016-02-21.202329_U9mlWz.lengths -org Pseudomonas_aeruginosa_pao1.ASM676v1.30 -return seq -rm -o $RSAT/public_html/tmp/apache/2016/02/21/random-genome-fragments_2016-02-21.202329_U9mlWz_fragments.fasta 2> $RSAT/public_html/tmp/apache/2016/02/21/random-genome-fragments_2016-02-21.202329_U9mlWz_error_log.txt$

 $$RSAT/perl-scripts/peak-motifs -v 1 -title '2588_negativectrol' -i $RSAT/public_html/tmp/www-data/2016/02/22/peak-motifs.2016-02-22.032359_2016-02-22.032359_flPJWK/peak-motifspeak_seq -ctrl $RSAT/public_html/tmp/www-data/2016/02/22/peak-motifs.2016-02-22.032359_2016-02-22.032359_flPJWK/peak-motifscontrol_seq -max_seq_len 1000 -markov auto -disco oligos,positions -nmotifs 5 -minol 6 -maxol 7 -no_merge_lengths -2str -origin center -motif_db regulonDB tf $RSAT/public_html/motif_databases/REGULONDB/regulonI 08-07.tf -scan_markov 1 -task purge, seqlen, composition, disco, merge_motifs, split_motifs, motifs_vs_motifs, timelog, archive, synt-prefix peak-motifs -noov -img_format png -outdir $RSAT/public_html/tmp/www-data/2016/02/22/peak-motifs.2016-02-22.032359 2016-02-22.032359 flPJWK$

\$RSAT/perl-scripts/retrieve-seq -org Pseudomonas_aeruginosa_PAO1_uid57945 -feattype gene -type upstream -format fasta -label name -from -1000 -to 200 -noorf -rm -all (?)

Peak Calling Statistics

Llamamos datos:

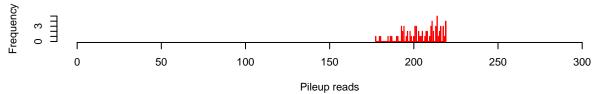
```
chip_art <- read.table("GSE65356_algrmacs2e15_peaks.txt", header = T)
chip_ours <-read.table("SRR1776550_peaks.xls", header=T)</pre>
```

Ploteamos la distribución de reads de acuerdo al pileup:

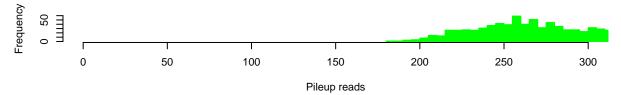
```
par(mfrow=c(3,1))
hist(chip_art$pileup,
    main="Reads Distribution (Article)",
    ylab="Frequency",
    xlab="Pileup reads",
    xlim = c(0,300),
    col="red",
    border = "red",
    breaks = 500)
```

```
hist(chip_ours$pileup,
     main="Reads Distribution (Ours)",
     ylab="Frequency",
     xlab="Pileup reads",
     xlim = c(0,300),
     col="green",
     border = "green",
     breaks = 500)
hist(chip_ours$pileup,
     main="Reads Distribution (Ours) *Expanded",
     ylab="Frequency",
     xlab="Pileup reads",
     xlim = c(0,700),
     col="green",
     border = "green",
     breaks = 500)
```

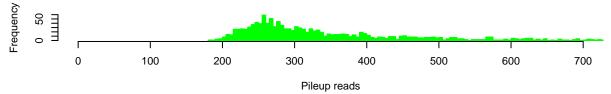
Reads Distribution (Article)



Reads Distribution (Ours)



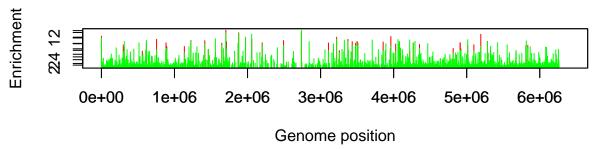
Reads Distribution (Ours) *Expanded



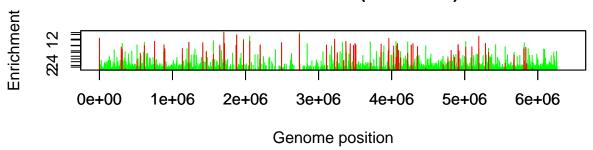
Ploteamos el enriquecimiento de picos uno sobre otro y viceversa, para ver dónde coinciden y con cuánta precisión.

```
xlim = c(0,6400000),
     col="red", type = "h")
par(new=T)
plot(chip_ours$abs_summit,
     chip_ours$fold_enrichment,
    xlim = c(0,6400000),
    ylab = "",
    xlab = "",
     col="green", type = "h")
plot(chip_ours $abs_summit,chip_ours$fold_enrichment,
     main="Fold Enrichment (Ours over)",
     ylab="Enrichment",
    xlab="Genome position",
    xlim = c(0,6400000),
     col="green", type = "h")
par(new=T)
plot(chip_art$abs_summit,
     chip_art$fold_enrichment,
    xlim = c(0,6400000),
    ylab = "",
    xlab = "",
     col="red", type = "h")
```

Fold Enrichment (Article over ours)



Fold Enrichment (Ours over)



 $summit <- chip_ artabs_summit peaks <- apply(summit, 1, subset(chip_ours, start < chip_artabs_ summit \& end> chip_ art\$abs_ summit)))$