

# PRJNA297664

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## Download the sra files

```
wget ftp://ftp-trace.ncbi.nlm.nih.gov/sra/sra-instant/reads/ByStudy/sra/SRP%2FSRP064%2FSRP
wget ftp://ftp-trace.ncbi.nlm.nih.gov/sra/sra-instant/reads/ByStudy/sra/SRP%2FSRP064%2FSRP
wget ftp://ftp-trace.ncbi.nlm.nih.gov/sra/sra-instant/reads/ByStudy/sra/SRP%2FSRP064%2FSRP
wget ftp://ftp-trace.ncbi.nlm.nih.gov/sra/sra-instant/reads/ByStudy/sra/SRP%2FSRP064%2FSRP
wget ftp://ftp-trace.ncbi.nlm.nih.gov/sra/sra-instant/reads/ByStudy/sra/SRP%2FSRP064%2FSRP
wget ftp://ftp-trace.ncbi.nlm.nih.gov/sra/sra-instant/reads/ByStudy/sra/SRP%2FSRP064%2FSRP
```

## Generating the fastq files

```
fastq-dump -I --split-files SRR2549634.sra
fastq-dump -I --split-files SRR2549636.sra
fastq-dump -I --split-files SRR2549638.sra
fastq-dump -I --split-files SRR2549635.sra
fastq-dump -I --split-files SRR2549637.sra
fastq-dump -I --split-files SRR2549639.sra
```

## Download the index file for bowtie2

```
wget ftp://igenome:G3nom3s4u@uscd-ftp.illumina.com/Saccharomyces_cerevisiae/Ensembl/R64-1-1-
```

## Generating the directories with the index files

```
gzip -d Saccharomyces_cerevisiae_Ensembl_R64-1-1.tar.gz
tar xvf Saccharomyces_cerevisiae_Ensembl_R64-1-1.tar
```

## Short read alignment using bowtie2

```
bowtie2 -x Saccharomyces_cerevisiae/Ensembl/R64-1-1/Sequence/Bowtie2Index/genome -U SRR254
bowtie2 -x Saccharomyces_cerevisiae/Ensembl/R64-1-1/Sequence/Bowtie2Index/genome -U SRR254
bowtie2 -x Saccharomyces_cerevisiae/Ensembl/R64-1-1/Sequence/Bowtie2Index/genome -U SRR254
bowtie2 -x Saccharomyces_cerevisiae/Ensembl/R64-1-1/Sequence/Bowtie2Index/genome -U SRR254
bowtie2 -x Saccharomyces_cerevisiae/Ensembl/R64-1-1/Sequence/Bowtie2Index/genome -U SRR254
bowtie2 -x Saccharomyces_cerevisiae/Ensembl/R64-1-1/Sequence/Bowtie2Index/genome -U SRR254
```

## From sam to bam using samtools and sorting the reads

```
samtools view -bS SRR2549634_1.sam | samtools sort - SRR2549634_1
samtools view -bS SRR2549636_1.sam | samtools sort - SRR2549636_1
samtools view -bS SRR2549638_1.sam | samtools sort - SRR2549638_1
samtools view -bS SRR2549635_1.sam | samtools sort - SRR2549635_1
samtools view -bS SRR2549637_1.sam | samtools sort - SRR2549637_1
samtools view -bS SRR2549639_1.sam | samtools sort - SRR2549639_1
```

## Creating the file bamfiles.txt with the names of the bam files

```
SRR2549634_1.bam  
SRR2549636_1.bam  
SRR2549638_1.bam  
SRR2549635_1.bam  
SRR2549637_1.bam  
SRR2549639_1.bam
```

It can be done with

```
ls *.bam > bamfiles.txt
```

## Using Rsamtools for counting the reads aligned

```
library(Rsamtools)  
library(GenomicFeatures)  
sampleTable = read.table("bamfiles.txt")  
dirActualData = paste(getwd(), "/", sep="")  
fls = paste(dirActualData, sampleTable[,1], sep="")  
bamLst = BamFileList(fls, index=character(), yieldSize=100000, obeyQname=TRUE)  
gtfFile = "../Saccharomyces_cerevisiae/Ensembl/R64-1-1/Annotation/Genes/genes.gtf"  
txdb = makeTxDbFromGFF(gtfFile, format="gtf")  
genes = exonsBy(txdb, by="gene")  
library(GenomicAlignments)  
PRJNA297664 = summarizeOverlaps(features = genes, read=bamLst,  
  mode="Union",  
  singleEnd=TRUE, ## No son lecturas apareadas  
  ignore.strand=TRUE,  
  fragments=FALSE)  
SampleName = c("GSM1900735", "GSM1900737", "GSM1900739", "GSM1900736",  
  "GSM1900738", "GSM1900740")  
Run = c("SRR2549634", "SRR2549636", "SRR2549638", "SRR2549635",  
  "SRR2549637", "SRR2549639")  
treatment = c(0, 0, 1, 0, 1, 1)  
treatment = factor(treatment, levels=0:1, labels=c("Wild", "SEC66 deletion"))  
replication = c(1, 3, 2, 2, 1, 3)  
colData(PRJNA297664) = DataFrame(SampleName, Run, treatment, replication)
```

## Adding identifiers

```
a = AnnotationDbi::select(org.Sc.sgd.db,keys=rownames(PRJNA297664),
                           columns=c("ORF","ENTREZID","ENSEMBL"),keytype="ORF")
b = match(rownames(PRJNA297664),a[, "ORF"])
rowData(PRJNA297664) = a[b,]
PRJNA297664 = PRJNA297664[which(!is.na(rowData(PRJNA297664)[, "ORF"])),]
sel = match(unique(rowData(PRJNA297664)[, "ORF"]),rowData(PRJNA297664)[, "ORF"])
PRJNA297664 = PRJNA297664[sel,]
```

## Saving data set

```
save(PRJNA297664,file="PRJNA297664.rda")
```