CopyNumberVariantsSequenceAnalysis

A Step-by-Step Guide

[DRAFT]

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Created: July, 2022
Last updated: 14 July 2022

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1 CNVseq Analysis Banana and Sorghum

1.1 Software Prerequisites

#Burrows-Wheeler-Aligner (http://bio-bwa.sourceforge.net/)(see line 126). #Download and Install BBmap https://sourceforge.net/projects/bbmap/ Bin-by-Sam-tool (see github repository) Python version 2.7(See enivornment .yaml)

Banana

Procure your raw FASTQ reads from NCBI of two Banana samples, one is a known mutant Novaria and the other is a wildtype Naine and follow the protocol. Efficient Screening Techniques to Identify Mutants with TR4 Resistance in Banana p.117 - 127 **Use clumpify script to remove duplicates**

(https://www.ncbi.nlm.nih.gov/bioproject/PRJNA627139)

#Download sratools

```
srapath SRR11579627

prefetch SRR11579627

wget https://sra-downloadb.be-md.ncbi.nlm.nih.gov/sos3/sra-pub-run-21/SRR11579627/
↪SRR11579627.1

#Convert SRA into fastq

fastq-dump --split-3 SRR11579627


srapath SRR11579628

prefetch SRR11579628

wget https://sra-downloadb.be-md.ncbi.nlm.nih.gov/sos3/sra-pub-run-21/SRR11579628/
↪SRR11579628.1

#Convert SRA into fastq

fastq-dump --split-3 SRR11579628
```

1.2 Rename FASTQ

Naine.R1.fq.gz Naine.R2.fq.gz Novaria.R1.fq.gz Novaria.R2.fq.gz

Run the clumpify python script to remove duplicates per sample.

```
./clumpify.sh in=Naine.R1.fq.gz in2=Naine.R2.fq.gz out=Naine.R1.
dedup.fastq.gz out2=Naine.R2.dedup.fastq.gz dedupe=t

./clumpify.sh in=Novaria.R1.fq.gz in2=Novaria.R2.fq.gz out=Novaria.R1.
dedup.fastq.gz out2=Novaria.R2.dedup.fastq.gz dedupe=t
```

1.3 Standard Output Clumpify python

Done! Time: 31.447 seconds. Reads Processed: 6262k 199.16k reads/sec Bases Processed: 1885m 59.94m bases/sec

Reads In: 6262958 Clumps Formed: 1730359 Duplicates Found: 3782 Reads Out: 6259176 Bases Out: 1884185686 Total time: 51.345 seconds.

NOVARIA

Done! Time: 29.438 seconds. Reads Processed: 6000k 203.82k reads/sec Bases Processed: 1837m 62.43m bases/sec

Reads In: 6000036 Clumps Formed: 1648176 Duplicates Found: 2026 Reads Out: 5998010 Bases Out: 1837286910 Total time: 50.222 seconds.

1.4 Download Reference Genome NCBI

https://www.ncbi.nlm.nih.gov/assembly/GCF_000313855.2

```
mkdir BananaGamma
mv Novaria.R1.dedup.fastq.gz Novaria.R2.dedup.fastq.gz BananaGamma/
mv Naine.R1.dedup.fastq.gz Naine.R2.dedup.fastq.gz BananaGamma/
cd BananaGamma

mkdir Genome
mv *.fna Genome/
cd Genome bwa index *.fna

cd ../

https://github.com/lh3/bwa

git clone https://github.com/lh3/bwa.git
cd bwa; make
./bwa
#Needs to be Harvard Version

./bwa mem -M -t 4 ../Genome/*.fna Novaria.R2.dedup.fq Novaria.R2.dedup.fq > Novaria.
↪dedup.sam

./bwa mem -M -t 4 Genome/*.fna Naine.R1.dedup.fastq.gz Naine.R2.dedup.fastq.gz > Naine.
↪dedup.sam

samtools sort -O sam -T sam -T Novaria.sort -o Novaria_aln.sam Novaria.dedup.sam
samtools sort -O sam -T sam -T Naine.sort -o Naine_aln.sam Naine.dedup.sam

samtools view -b Novaria.dedup.sam > Novaria.bam
samtools view -b Naine.dedup.sam > Naine.bam

samtools index Novaria.bam
samtools index Naine.bam
```

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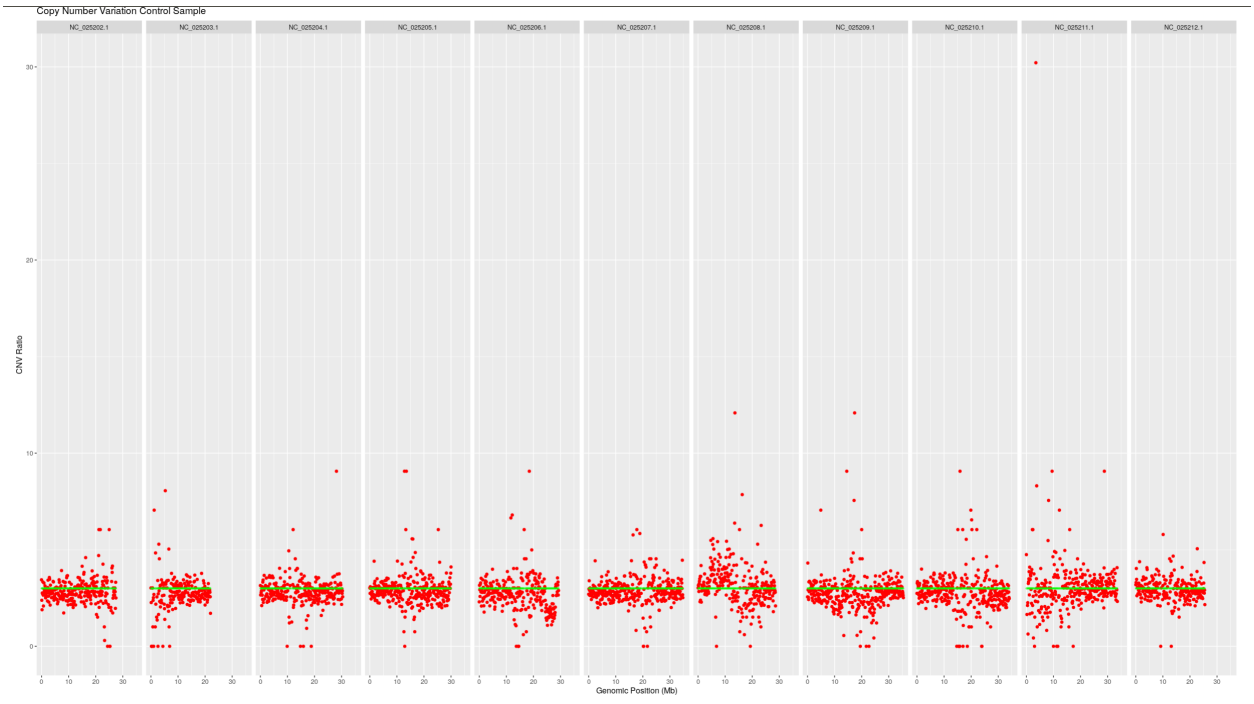
```
mv Novaria_aln.sam Naine_aln.sam Bin-by-Sam-tool/
cd Bin-by-Sam-tool
python bin-by-sam_2.0.py -o N3_100kbin.txt -s 100000 -b -p 3 -c Naine_.aln.sam
```

1.5 Download the r package rom PBGLMichael/CNVseq repository

```
# Banana CNV

setwd("/home/michael/Desktop/Banana/Banana_LC_WGS")
devtools::install_github(repo = "PBGLMichaelHall/CNVseq",force = TRUE)
library(CNV)

CNV::CNV(file = "N3_100kbin.txt",Chromosome =  c("NC_025202.1","NC_025203.1","NC_025203.1
↪","NC_025204.1","NC_025205.1",
"NC_025206.1","NC_025207.1","NC_025208.1","NC_025209.1","NC_025210.1","NC_025211.1","NC_
↪025212.1"),
mutantname = "Novaria.Naine",controlname = "Naine.Naine",size = .75,alpha = .25,color=
↪"green")
```



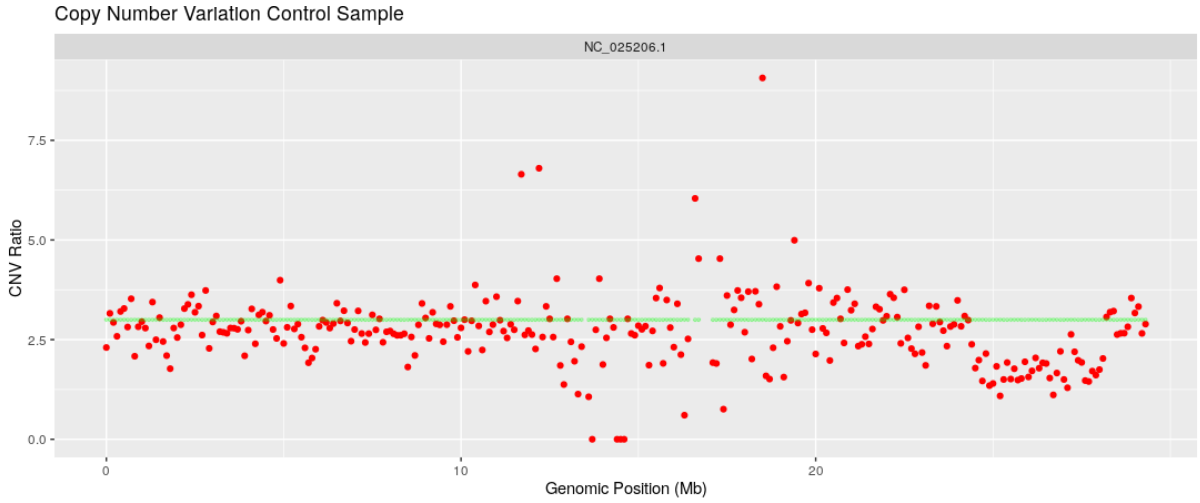
```
'data.frame':   3323 obs. of  7 variables:
 $ Chrom        : chr  "NC_025202.1" "NC_025202.1" "NC_025202.1" "NC_025202.1" ...
 $ Strt         : int  1 100001 200001 300001 400001 500001 600001 700001 800001 900001 ...
 $ End          : int  100000 200000 300000 400000 500000 600000 700000 800000 900000 1000000 ...
 $ Naine        : int  197 192 241 208 235 185 184 283 229 197 ...
 $ Novaria      : int  163 154 164 180 187 172 189 203 186 175 ...
 $ Naine.Naine  : num  3 3 3 3 3 3 3 3 3 3 ...
 $ Novaria.Naine: num  2.97 2.88 2.45 3.11 2.86 ...
```

	Chrom	Strt	End	Naine	Novaria	Naine.Naine	Novaria.Naine
1	NC_025202.1	1	100000	197	163	3	2.974
2	NC_025202.1	100001	200000	192	154	3	2.883
3	NC_025202.1	200001	300000	241	164	3	2.446
4	NC_025202.1	300001	400000	208	180	3	3.111
5	NC_025202.1	400001	500000	235	187	3	2.860
6	NC_025202.1	500001	600000	185	172	3	3.342
7	NC_025202.1	600001	700000	184	189	3	3.692
8	NC_025202.1	700001	800000	283	203	3	2.578
9	NC_025202.1	800001	900000	229	186	3	2.920
10	NC_025202.1	900001	1000000	197	175	3	3.193
11	NC_025202.1	1000001	1100000	228	173	3	2.727
12	NC_025202.1	1100001	1200000	127	124	3	3.510
13	NC_025202.1	1200001	1300000	172	145	3	3.030
14	NC_025202.1	1300001	1400000	208	164	3	2.834

table	3323 obs. of 7 variables
$ Chrom	: chr "NC_025202.1" "NC_025202.1" "NC_…
$ Strt	: int 1 100001 200001 300001 400001 50…
$ End	: int 100000 200000 300000 400000 5000…
$ Naine	: int 197 192 241 208 235 185 184 283 …
$ Novaria	: int 163 154 164 180 187 172 189 203 …
$ Naine.Naine	: num 3 3 3 3 3 3 3 3 3 3 ...
$ Novaria.Naine	: num 2.97 2.88 2.45 3.11 2.86 ...
Functions	

1.6 Chromosome 5

```
CNV::CNV(file = "N3_100kbin.txt",Chromosome =  c("NC_025206.1"),mutantname = "Novaria.
↪Naine",
controlname = "Naine.Naine",size = .75,alpha = .25,color="green")
```




```
samtools view -h con-2_S1-Chromes-04-05-09.bam > con-2_S1-Chromes-04-05-09_aln.sam

**MUTANT**
```

```
samtools view -h D2-1_S7-Chromes-04-05-09.bam > D2-1_S7-Chromes-04-05-09_aln.sam
```

1.7 bin-by-sam_2.0.py python script

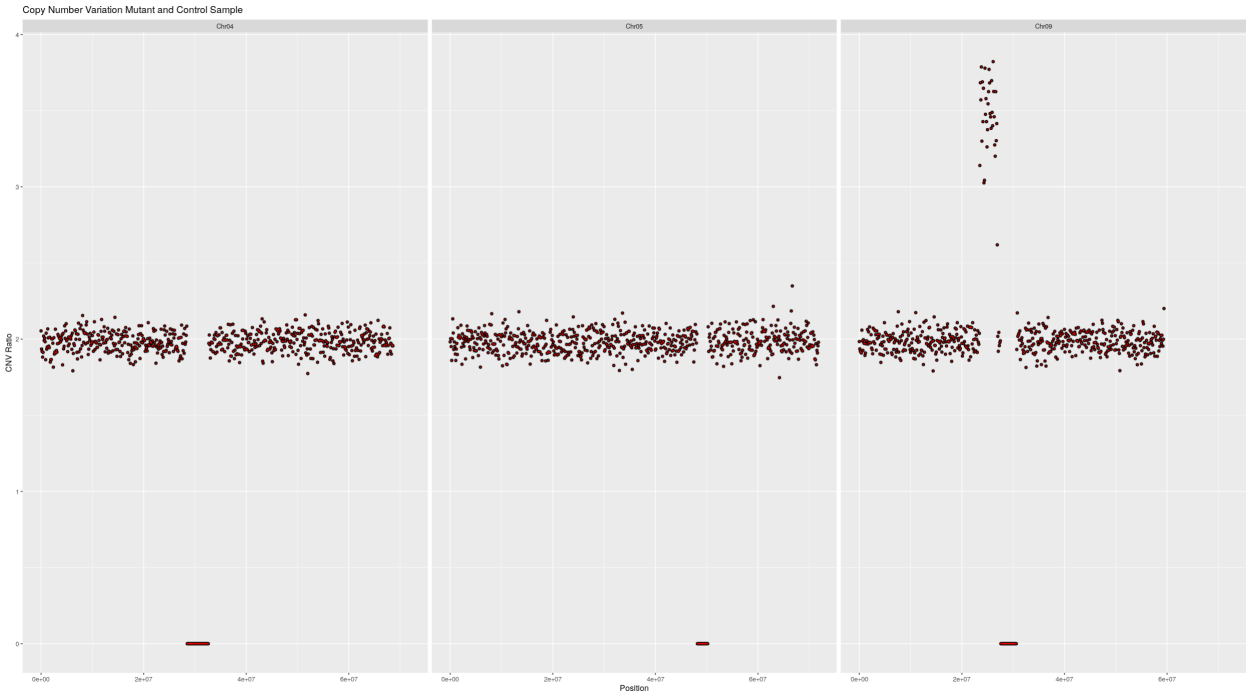
```
$python bin-by-sam_2.0.py -o N3_100kbin.txt -s 100000 -b -p 3 -c con-2_S1-Chromes-04-05-
↪09_aln.sam
```

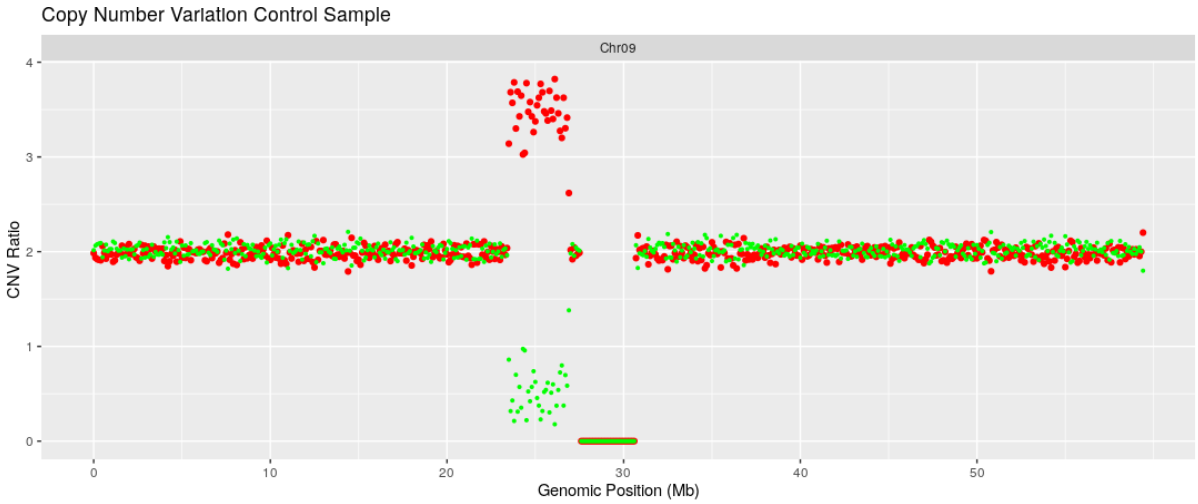
1.8 Sorghum CNV

```
CNV::CNV(file = "N3_100kbin.txt",Chromosome = c("Chr04","Chr05","Chr09"),
mutantname = "con.2.NA",controlname = "D2.2.NA",size = .75,alpha = 5.0,color="green")
```

1.9 Chromosome 9

```
CNV::CNV(file="N3_100kbin.txt",Chromosome=c("Chr09"),mutantname="con.2.NA",controlname=
↪"D2.2.NA",size=.75,alpha=5.0)
```


Copy Number Variation Mutant and Control Sample
Chr04
Chr05
Chr09
CNV Ratio
Position


Copy Number Variation Control Sample
Chr09
CNV Ratio
Genomic Position (Mb)