CopyNumberVariantsSequenceAnalysis

A Step-by-Step Guide

[DRAFT]

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Created: July, 2022
Last updated: 14 July 2022

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========== CNVseq Analysis Banana and Sorghum ==========

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Date 07/14/2022

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

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

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.

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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

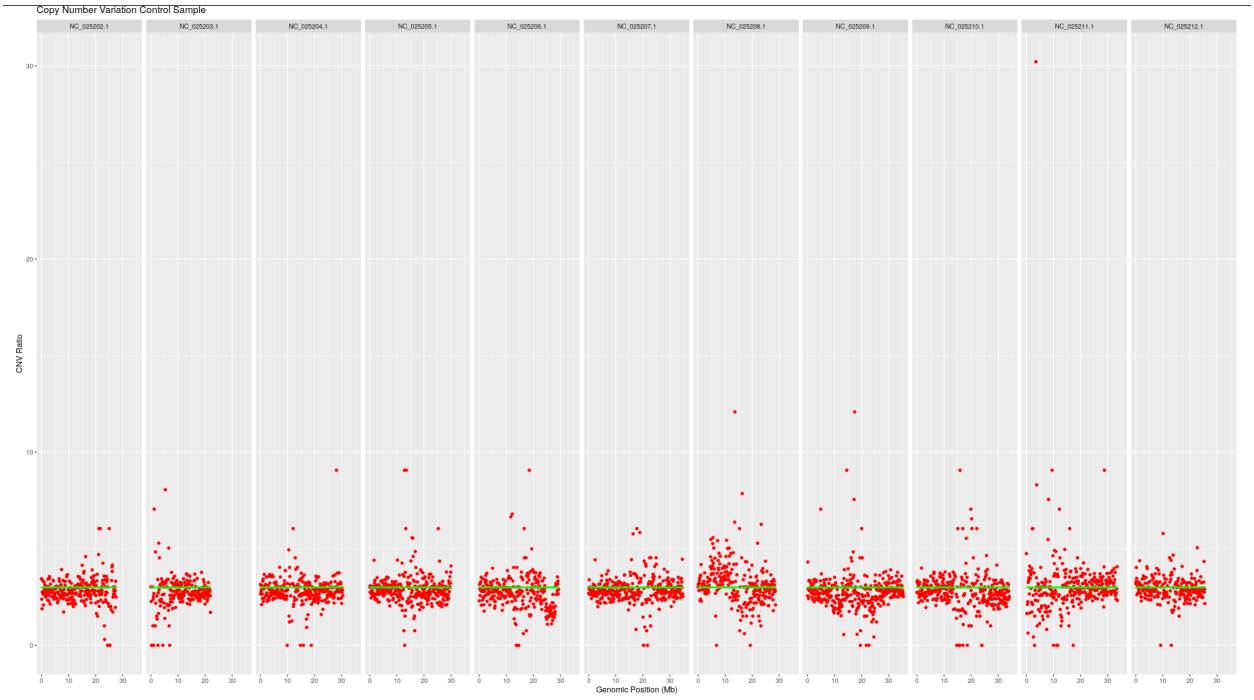
samtools view -b Novaria.dedup.sam > Novaria.bam samtools view -b Naine.dedup.sam > Naine.bam

samtools index Novaria.bam samtools index Naine.bam

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

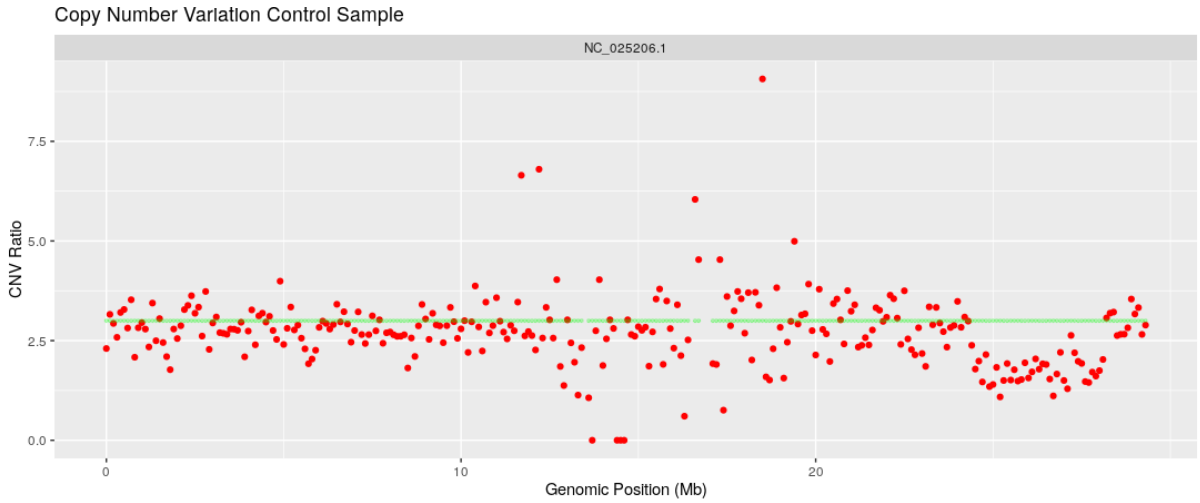
5 Download the r package rom PBGLMichael/CNVseq repository

devtools::install_github(“PBGLMichaelHall/CNVseq”) # 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 = “Novaria.Naine”,controlname = “Naine.Naine”,size = .75,alpha = .25,color=”green”)



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”)



You have two BAM files one is a “mutant” and the other is a “control”

First convert BAM to SAM The sam file must have an ending _aln.sam to work properly in python script

CONTROL

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

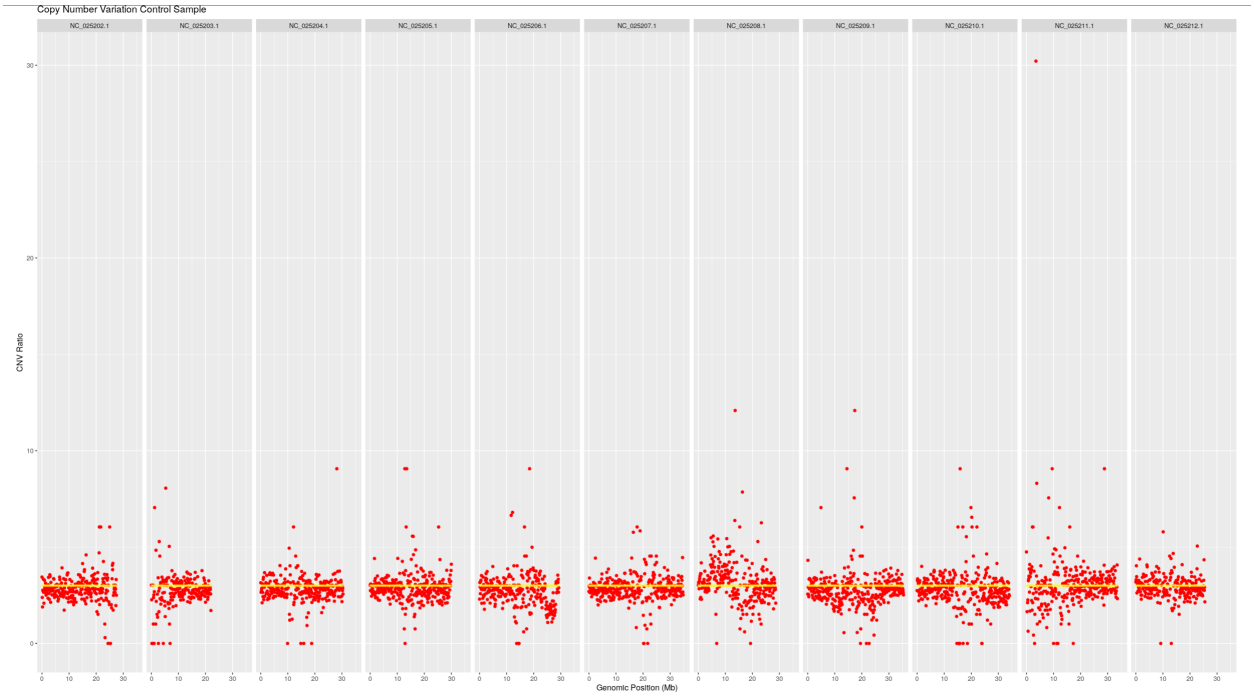
7 bin-by-sam_2.0.py python script

$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

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”)

8 PLOT


Copy Number Variation Control Sample
NC_025202.1
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
CNV Ratio
Genomic Position (Mb)

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)