PBGL CNV-seq Analysis v1.0

A Laboratory Manual

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Contents

1 Background

Copy number variation (CNV) analysis using CNVseq, R, Jupyter Notebooks, Miniconda3, Mamba, and Git.

All the commands run in a Linux terminal are preceded by the *$* prompt sign. To run a command, copy/past the command without the *$* sign. Those commands run in a Jupyter Notebook are preceded by the *In [ ]:*

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2 Installations - Virtual Environments and Software Packages

Before installing any necessary software, it is recommended to check if the computer is running 32-bit or 64-bit for downloading Miniconda3. Run the following to verify the system:

```
$ uname -m
```

2.1 Miniconda3 (conda) and Mamba

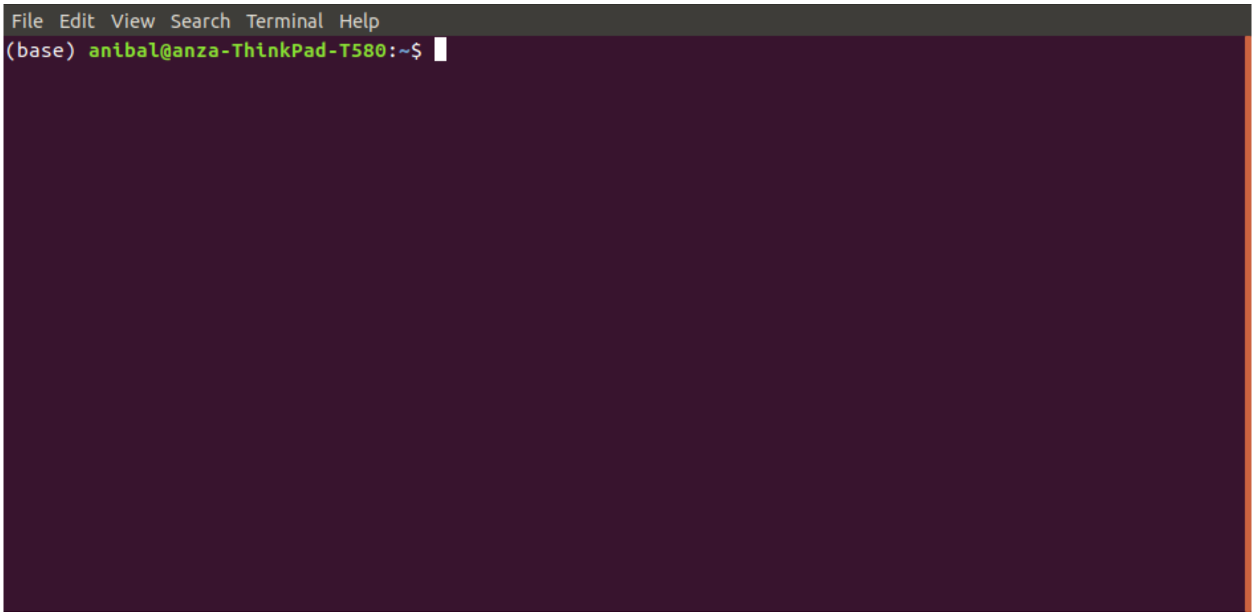
Download the Miniconda3, or simply "conda", installer:

- Miniconda3 installer for Linux

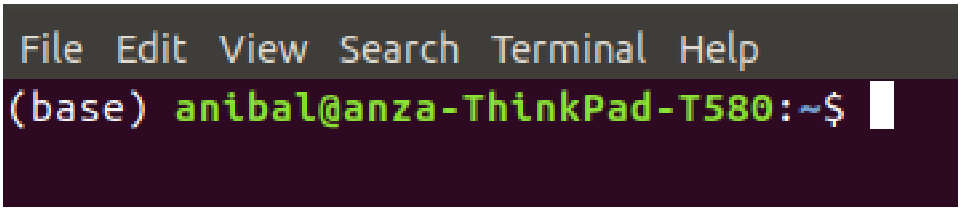
Run the downloaded installer (for a 64-bit system):

```
$ bash Miniconda3-latest-Linux-x86_64.sh
```

Open a new terminal window for conda to take effect. The word *(base)* should appear in front of the computer name in the terminal window, like so:



```
(base) anibal@anza-ThinkPad-T580:~$
```



```
(base) anibal@anza-ThinkPad-T580:~$
```

Verify the installation and update conda in new terminal window with:

```
$ conda env list
$ conda update --all
$ conda upgrade --all
```

Install mamba library/package manager that will be used for installing software dependencies of the tool:

```
$ conda install mamba --yes
```

2.2 Git Installation and Repo Cloning

Git is required for cloning locally (downloading a copy to your local computer) the PBGL CNVseq Github repository. Git and Github are used for version control of software. It keeps track of development, releases, and issues of a software project.

Install **git** for cloning the **pbgl-cnvseq** software repository from Github, where the latetest version of the tool resides:

```
$ mamba install git --yes
```

After the instalation, clone PBGL's CNVseq repository, **pbgl-cnvseq**, to the local computer in any desired directory.

```
$ git clone https://github.com/pbgl/pbgl-cnvseq.git
```

The cloning process will depict the following:

```
Cloning into 'pbgl-cnvseq'...
remote: Enumerating objects: 628, done.
remote: Counting objects: 100% (336/336), done.
remote: Compressing objects: 100% (239/239), done.
remote: Total 628 (delta 109), reused 292 (delta 78), pack-reused 292
Receiving objects: 100% (628/628), 11.79 MiB | 7.03 MiB/s, done.
Resolving deltas: 100% (190/190), done.
```

The **pbgl-cnvseq** repository should have been clones successfully. Verify that the download is complete by listing the folders/files in the directory.

```
$ ls -l
```

The folder called **pbgl-cnvseq** should be listed in the directory.

2.3 Required Libraries with Mamba

CNVseq has multiple dependencies, listed below:

- Samtools
- R
  - configr
  - ggplot2
  - BiocManager
  - Bioconductor-GenomicAlignments
  - Bioconductor-GenomeInfoDb
- Jupyter Notebook
  - IRkernel

The necessary R packages are installed through the Jupyter Notebook. It proves as a faster and error-free way to install **configr**, **ggplot2**, **Biocmanager**, **Bioconductor-GenomicAlignments**, and **Bioconductor-GenomeInfoDb** packages.

There are two ways to install the rest of the necessary libraries to run CNV-seq: automatically or manually.

2.3.1 Automatically (faster)

One YAML file, **environment.yml**, is provided to automatically create a virtual environment and install the dependent libraries through mamba. The file creates the **cnvseq** virtual environment, along R, Jupyter Notebook, and the R-kernel in Jupyter. It also installs the dependent R libraries. Run **environment.yml**:

```
$ mamba env create --file envs/environment.yml
```

Once done, a list of the virtual environments available can be seen by running:

```
$ conda env list
```

Activate (enter) the recently-created virtual environment **cnvseq**:

```
$ conda activate cnvseq
```

Once done, the virtual environment should be activated and all the necessary packages should be installed. This can be verified with:

```
$ conda list
```

2.3.2 Manually (slower)

To manually create and activate an environment, run:

```
$ conda create --name cnvseq
$ conda activate cnvseq
```

Start running the installations of the necessary libraries:

```
$ mamba install --channel conda-forge notebook r-irkernel r-biocmanager --yes
$ mamba install --channel bioconda samtools bioconductor-genomicalignments␣
↪bioconductor-genomeinfodb --yes
$ mamba install --channel r r-ggplot2 --yes
$ mamba install --channel pcgr r-configr --yes
```

Once done, all the necessary packages should be installed. This can be verified with:

```
$ conda list
```

3 Running Jupyter

To activate Jupyter, run the following in the terminal:

```
$ jupyter notebook
```

This command will start a Jupyter session inside the directory the command is run. The user can navigate between directories, visualize files, and edit files in a web browser by clicking on directories or files, respectively.

Look for the directory **pbgl-cnvseq** and click on it. Click on **tool** directory, which contains three directories and two Jupyter Notebooks. Here is a breakdown of each:

- *config*:
  - directory containing configuration files specifying file paths, parameter definitions, and comparison lists
- *helper-functions*:
  - directory containing R scripts with functions to calculate and plot CNVs
- *output*:
  - directory that will contain both tab-files and images output after running a CNVseq analysis
- two Jupyter Notebooks:
  - RCNV-seq-PBGL-sorghum-analysis-example.ipynb
    - example analysis of a comparison between a control and mutant of sorghum
  - RCNV-seq-template.ipynb
    - template for the user

Note: Jupyter lets the user duplicate, rename, move, download, view, or edit files in a web browser. This can be done by clicking the box next to a file and choosing accordingly.

3.1 Editing the Configuration File

In order to run the CNVseq Jupyter Notebook, the user needs to feed it with a configuration file (**config-CNVseq.yml**) that specifies the paths to the bam files, comparisons to be done, chromosomes to analyze, and parameter definitions for calculating and plotting CNVs.

The configuration file **config-CNVseq.yml** can be found in the **pbgl-cnvseq/tool/config** directory. The configuration file contains the following:

```
# parameters for CNV/window size calculations defaults from HLiang:
# https://github.com/hliang/cnv-seq
parameters:
  annotate: TRUE
  bed_file_present: FALSE
  bigger: 1.5
  log2: 0.6
  pvalue: 0.001
  window_size: 10000

# list of chromosomes to be analyzed
chromosomes:
```

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```
  - Chromosome1
  - Chromosome2
  - Chromosome3
  - AnotherChromosome

# folder to store outputs; should be inside "output" directory, example:
# output_path: output/run-name or output/organism
output_path: output/organism-being-analyzed

# path to bed file for varying window sizes (optional)
bed_path: /path/to/bed/file.bed

# paths to bam files
paths:
  control-1: &control-1 /path/to/bam/file/control-1.bam
  mutant-1: &mutant-1 /path/to/bam/file/mutant-1.bam
  mutant-2: &mutant-2 /path/to/bam/file/mutant-2.bam

# comparisons to be analyzed
comparisons:
  control-1-vs-mutant-1:
    control: *control-1
    mutant: *mutant-1
  control-1-vs-mutant-2:
    control: *control-1
    mutant: *mutant-2
```

Note: The user needs to edit **config-CNVseq.yml** to point towards bam/bed files; specify comparisons and chromosomes to analyze; and define the parameters to calculate/plot CNVs.

One example configuration files is provided (**config-CNVseq-PBGL-sorghum-analysis-example.yml**). The configuration file **config-CNVseq.yml** contains multiple fields to be defined by the user.

- *parameters*:
  - parameters used to create window sizes, thresholds, plots, etc
  - the parameter defaults are provided accroding to HLiang's original values
- *chromosomes*:
  - list of chromosome names to analyze
  - chromosome names can be found in a bam file's header using the following samtools command:

```
$ samtools view -h my_bam_file.bam | less -S
```

- *output_path*:
  - directory that will contain both tab-files and images output after running a CNVseq analysis
  - the defined path will be inside the **pbgl-cnvseq/tool/output** directory
  - *images*:
    - directory that will store the CNV image outputs per comparison of:
    - all chromosomes in one plot
    - each chromosome individually in one plot

        - any zoomed-in region plot of one chromosome
    - *tab-files*:
        - directory that will contain two types of output tab-delimited files:
            - hits used to calculate CNVs, containing chromosome, start, end, width, control coverage, mutant coverage
            - CNVs per chromosome per comparison, containing CNV number, chromosome, start, end, size, log2, and p-value

- *bed_path*:
    - path pointing to bed file containing targets if using varying window sizes
    - it is recommended to run the analysis without a **.bed** file; plots will only reflect the targets in a **.bed** file
    - if a **.bed** file is provided, the *bed_file_present* parameter under the *parameters* section has to be changed to *TRUE*
- *paths*:
    - sample names and their respective paths to **.bam** files
    - samples can be named as desired but the sample name must be repeated after the colon and prefixed with a *&* sign
    - the *&* prefix sign is used to reference the sample's path in different places of the same configuration file
    - example use:

```
paths:
  mysample: &mysample /home/username/bam_files/mysample.bam
  XYZ-123: &XYZ-123 /home/username/bam_files/XYZ-123.bam
  potato95: &potato95 /home/username/bam_files/potato95.bam
```

- *comparisons*:
    - comparison names with respective control and mutant samples per comparison
    - each comparison can be named as desired
    - the sample names to be used as *control* and *mutant* need to be prefixed by a * sign
    - the * prefixed sign is used to extract the sample's path defined in the *paths* section
    - example:

```
comparisons:
  comparison-1:
    control: *mysample
    mutant: *potato95
  a-different-comparison-278asd:
    control: *mysample
    mutant: *XYZ-123
```

3.2 Running a RCNV_seq-template Jupyter Notebook

Note: It is recommended to duplicate the **RCNV-seq-template** notebook and then renaming the copy before doing any edits to the notebook.

In the **pbgl-cvnseq/tool** directory, click on **RCNV-seq-template** and a new tab in your web-browser will open the notebook.

The notebook contains cells that are populated by text or code. Instructions are provided in the notebook to guide the user. To run a cell, click on the corresponding cell and click on the *Run* button on the top of the notebook. Another way to run a cell can be done by clicking on the corresponding cell and pressing **Ctrl + Enter** or **Shift + Enter**.

The notebook consists of 6 sections:

1. Installing Required Libraries (optional)
2. Loading Required Libraries (mandatory)
3. User Input (mandatory)
4. CNV Calculations
5. Plotting
6. Plotting a Zoomed-In Region of One Chromosome

3.2.1 Installing Required Libraries (optional)

- libraries being installed:

```
In [ ]: # install necessary libraries using R functions
        if (!requireNamespace("BiocManager", quietly = TRUE))
            install.packages("BiocManager")

        BiocManager::install(c("GenomicAlignments", "GenomeInfoDb"))
        install.packages("configr")
        install.packages("ggplot2")
```

- to be run if the mamba installations were not successful or the loading of the required libraries fails under the **Loading Required Libraries** section

3.2.2 Loading Required Libraries (mandatory)

- libraries being loaded:

```
In [ ]: # load necessary libraries
        library(GenomicAlignments)
        library(ggplot2)
        library(configr)

        # specify source R script with helper functions
        source("helper-functions/RCNV_seq-helper.R")
        source("helper-functions/cnvHLiang.R")
```

- this cell will load necessary libraries and scripts containing the necessary functions to be used
- if running this cell fails, some libraries may be missing from the installation

    - to fix this issue, run the installations under the **Installing Required Libraries**

3.2.3 User Input (mandatory)

- the user needs to write the appropriate name of the configuration file being used in the following cell:

```
In [ ]: configPath <- "config/config-CNVseq.yml"
```

- the bottom cell will extract the fields defined in the configuration file:

```
In [ ]: config <- read.config(configPath)
```

3.2.4 CNV Calculations

- function to calculate all the hits and CNVs:

```
In [ ]: cnvCalculate(config)
```

- output tabulated files are stored inside **pbgl-cnvseq/tool/output/name_defined_in_output_path_of_config/tab-files** directory

3.2.5 Plotting

- function to plot two types of images:
    1. CNVs of all chromosomes in the same plot
    2. CNVs of one chromosome per plot

```
In [ ]: cnvPlot(config, imgType="", yMin= , yMax= )
```

- function parameters are:
    - *config* - configuration file defined under **User Input** section
    - *imgType* - image extention to use; available options are: *png*, *jpeg*, *svg*, and *pdf*; default to *png*
    - *yMin* - y-axis bottom limit; default to *-5*
    - *yMax* - y-axis upper limit; default to *5*
- output images are stored inside the **pbgl-cnvseq/tool/output/name_defined_in_output_path_of_config/images** directory
- it is recommended to inspect the CNV-plots with default y-limits and then modify
- *cnvPlotting* can be used in two ways: with default values or user-defined values
    - with default values, the parameters can be omitted and set to *imgType=”png”*, *yMin=-5* and *yMax=5*
    - the following two commands have the same parameter values and will output the same plots:

```
In [ ]: cnvPlot(config)
        cnvPlot(config, imgType="png", yMin=-5, yMax=5)
```

3.2.6 Plotting a Zoomed-In Region of One Chromosome

- function to plot a specific zoomed-in region of one chromosome

```
In [ ]: cnvPlotZoom(config, tabFile, chromosome="", start= , end= , yMin= , yMax= ,␣
 ↪imgType="")
```

- function parameters are:
  - *config* - configuration file defined under **User Input** section
  - *tabFile* - tabulated file containing all hits; requires user-input to define path to all-hits tabulated file
  - *chromosome* - chromosome name to focus on; default to *NA*
  - *start* - start of window in bp; both scientific notation is accepted: *100000* or *10e4*; defaults to *NA*
  - *end* - end of window in bp; the same applies as in the *start* parameter; defaults to NA
  - *yMin* - y-axis bottom limit; default to *-5*
  - *yMax* - y-axis upper limit; default to *5*
  - *imgType* - image extention to use; available options are: *png*, *jpeg*, *svg*, and *pdf*; defaults to *png*
- requires path definition of *tabFile* parameter in the cell:

```
In [ ]: tabFile <- "output/run-name/tab-files/tab-file.tab"
```

4 Example Tutorial - CNV Analysis of Sorghum

In this section of the manual, an example analysis of sorghum will be shown in a step-by-step process. The data has been subset to three chromosomes Chr04, Chr05, and Chr09. The CNVseq analysis will depict a deletion on chromosome Chr09.

The tutorial is divided between the following sections:

1. Data Download
2. General Installations
3. Github Repository Cloning
4. Virtual Environment Creation
5. Configuration File Editing
6. Jupyter Notebook Analysis

4.1 Data Download

Running this tutorial requires two binary alignment map (BAM) files of sorghum crop: one control and one mutant. Each **.bam** file is subset for chromosomes Chr04, Chr05, and Chr09; this is reflected on their names. They have been both sorted and indexed using samtools.

The following links can be used to download the necessary **.bam** and **.bam.bai** (index) files:

- con-2_S1 (sorghum control)
  - https://bss1innov1nafa1poc1.blob.core.windows.net/sample-container/2021_Training/con-2_S1-Chromes-04-05-09.bam
  - https://bss1innov1nafa1poc1.blob.core.windows.net/sample-container/2021_Training/con-2_S1-Chromes-04-05-09.bam.bai
- D2-1_S7 (sorghum mutant)
  - https://bss1innov1nafa1poc1.blob.core.windows.net/sample-container/2021_Training/D2-1_S7-Chromes-04-05-09.bam
  - https://bss1innov1nafa1poc1.blob.core.windows.net/sample-container/2021_Training/D2-1_S7-Chromes-04-05-09.bam.bai

There are two additional ways to download the **.bam** files and their respectives indices, besides clicking the links above:

1. Linux terminal
2. Web-Browser

4.1.1 Linux Terminal

Open a new terminal and navigate to a directory of choice. We recommend creating a directory to store the data and running *wget* in the respective location:

```
$ mkdir bam_files
$ cd bam_files
$ wget https://bss1innov1nafa1poc1.blob.core.windows.net/sample-container/2021_
↪Training/con-2_S1-Chromes-04-05-09.bam
$ wget https://bss1innov1nafa1poc1.blob.core.windows.net/sample-container/2021_
↪Training/con-2_S1-Chromes-04-05-09.bam.bai
$ wget https://bss1innov1nafa1poc1.blob.core.windows.net/sample-container/2021_
↪Training/D2-1_S7-Chromes-04-05-09.bam
$ wget https://bss1innov1nafa1poc1.blob.core.windows.net/sample-container/2021_
↪Training/D2-1_S7-Chromes-04-05-09.bam.bai
```

4.1.2 Web-Browser

Open a web-browser of preference. Copy/paste the links provided above in the address bar. This should automatically begin the download. Move the downloaded files to a location of personal preference.

4.2 General Installations

Open a web browser and copy/paste the following link to download Miniconda3:

- https://docs.conda.io/en/latest/miniconda.html#linux-installers

After download, open a new terminal window, navigate to the directory with the downloaded Miniconda3 installer, and run the installation.

Note: The donwloaded Miniconda3 installer file might not match the one run in this example. Please, type the corresponding name of the **.sh** file downloaded.

```
$ bash Miniconda3-latest-Linux-x86_64.sh
```

Once the Miniconda3 installation is done, close the terminal and open a new one. The word *(base)* should be present to the left of the computer name in the prompt. Update conda and install mamba.

```
$ conda update --all --yes
$ conda upgrade --all --yes
$ conda install mamba --yes
```

4.3 Github Repository Cloning

Git or Github is used for storage and version control of software projects. Git is used to manage in a local Linux terminal. First, git will need to be installed. In a new terminal window, run the installation command:

```
$ mamba install git --yes
```

After installing git, create a location to store Github repoitories, navigate into it, and clone (download a copy locally) of PBGL's **pbgl-cnvseq** repository.

```
$ mkdir Github
$ cd Github
$ git clone https://github.com/pbgl/pbgl-cnvseq
```

A successful cloning will output the following:

```
Cloning into 'pbgl-cnvseq'...
remote: Enumerating objects: 628, done.
remote: Counting objects: 100% (336/336), done.
remote: Compressing objects: 100% (239/239), done.
remote: Total 628 (delta 109), reused 292 (delta 78), pack-reused 292
Receiving objects: 100% (628/628), 11.79 MiB | 7.03 MiB/s, done.
Resolving deltas: 100% (190/190), done.
```

Navigate into the cloned repository,

```
$ cd pbgl-cnvseq
```

4.4 Virtual Environment Creation

Once inside the **pbgl-cnvseq** directory, create the **cnvseq** virtual environment, install the necessary libraries, and activate the newly created **cnvseq** virtual environment.

```
$ mamba env create --file envs/environment.yml
$ conda activate cnvseq
```

The name *(cnvseq)* environment should be reflected to the left of the computer name in the terminal command prompt.

4.5 Configuration File Editing

Open a Jupyter session by running,

```
$ jupyter notebook
```

Navigate to **pbgl-cnvseq/tool/config** and click on **config-CNVseq-PBGL-sorghum-analysis-example.yml**. This will open the configuration file in a new web-browser tab. Copy/paste the following in the configuration file.

Note: The paths to the bam files will not match. They need to be edited accordingly to point towards the location of the bam files stored locally in the user's computer.

```
# parameters for CNV/window size calculations defaults from HLiang:
# https://github.com/hliang/cnv-seq
parameters:
  annotate: TRUE
  bed_file_present: FALSE
  bigger: 1.5
  log2: 0.6
  pvalue: 0.001
  window_size: 10000

# list of chromosomes to be analyzed
chromosomes:
```

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```
  - Chr04
  - Chr05
  - Chr09

# folder to store outputs; should be inside "output" directory, example:
# output_path: output/run-name or output/organism
output_path: output/PBGL-sorghum-analysis-example

# path to bed file for varying window sizes (optional)
bed_path:

# paths to bam files
paths:
  con-2_S1: &con-2_S1 /home/anibal/bam_files/sorghum/con-2_S1-Chromes-04-05-09.bam
  D2-1_S7: &D2-1_S7 /home/anibal/bam_files/sorghum/D2-1_S7-Chromes-04-05-09.bam

# comparisons to be analyzed
comparisons:
  con-2_s1-vs-D2-1_S7:
    control: *con-2_S1
    mutant: *D2-1_S7
```

Save the file and close the tab.

4.6 Jupyter Notebook Analysis

In the open Jupyter session, navigate to the **pbgl-cnvseq/tool** directory and click on the **RCNV-seq-template.ipynb** Jupyter Notebook. Begin by running the **Loading Required Libraries** section. This can be done by clicking on the cell to be run followed by clicking the *Run* button on the top of the Jupyter Notebook.

```
In [ ]:  # load necessary libraries
         library(GenomicAlignments)
         library(ggplot2)
         library(configr)

         # specify source R script with helper functions
         source("helper-functions/RCNV_seq-helper.R")
         source("helper-functions/cnvHLiang.R")
```

If loading the libraries is unsuccessful (specified in the cell output), an attempt to install the missing libraries can be done by running the **Installing Required Libraries** section,

```
In [ ]:  # install necessary libraries using R functions
         if (!requireNamespace("BiocManager", quietly = TRUE))
             install.packages("BiocManager")

         BiocManager::install(c("GenomicAlignments", "GenomeInfoDb"))
         install.packages("configr")
         install.packages("ggplot2")
```

Run the **User Input** section after correctly editing the name/path of the configuration file:

```
In [ ]: configPath <- "config/config-CNVseq.yml"
```

Load the contents of the configuration file by running the cell,

```
In [ ]: config <- read.config(configPath)
```

Copy number variants (CNVs) are calculated under the **CNV Calculations** section by running the cell,

```
In [ ]: cnvCalculate(config)
```

The output tabulated files of all-hits and CNVs can be inspected in the **pbgl-cnvseq/tool/output/PBGL-sorghum-analysis-example/tab-files** directory. These can be used to import data to other softwares of choice for downstream data analysis. A successful run has the following output right under the input cell,

```
Comparison: con-2_s1-vs-D2-1_S7
Comparing samples:
/home/anibal/bam_files/sorghum/con-2_S1-Chromes-04-05-09.bam
vs.
 /home/anibal/bam_files/sorghum/D2-1_S7-Chromes-04-05-09.bam

[1] "chromosome:  Chr04"
[1] "chromosome:  Chr05"
[1] "chromosome:  Chr09"
[1] "cnv_id:  1  of  25"
[1] "cnv_id:  2  of  25"
[1] "cnv_id:  3  of  25"
[1] "cnv_id:  4  of  25"
[1] "cnv_id:  5  of  25"
[1] "cnv_id:  6  of  25"
[1] "cnv_id:  7  of  25"
[1] "cnv_id:  8  of  25"
[1] "cnv_id:  9  of  25"
[1] "cnv_id:  10  of  25"
[1] "cnv_id:  11  of  25"
[1] "cnv_id:  12  of  25"
[1] "cnv_id:  13  of  25"
[1] "cnv_id:  14  of  25"
[1] "cnv_id:  15  of  25"
[1] "cnv_id:  16  of  25"
[1] "cnv_id:  17  of  25"
[1] "cnv_id:  18  of  25"
[1] "cnv_id:  19  of  25"
[1] "cnv_id:  20  of  25"
[1] "cnv_id:  21  of  25"
[1] "cnv_id:  22  of  25"
[1] "cnv_id:  23  of  25"
[1] "cnv_id:  24  of  25"
[1] "cnv_id:  25  of  25"

CNV percentage in genome: 1.6%
CNV nucleotide content: 3140000
CNV count: 25
Mean size: 125600
Median size: 85000
Max Size: 750000
Min Size: 20000

cnv chromosome      start   end     size    log2    p.value
CNVR_1      Chr05   66247501        66267500        20000   1.013742        1.222196e-
↪59
CNVR_2      Chr09   23497501        23527500        30000   -2.288917       3.556107e-
↪238
```

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```
CNVR_3      Chr09   23547501        23917500        370000  -2.684822       0
CNVR_4      Chr09   23927501        24132500        205000  -2.639143       0
CNVR_5      Chr09   24142501        24302500        160000  -2.359219       0
CNVR_6      Chr09   24337501        24417500        80000   -1.907744       0
CNVR_7      Chr09   24437501        24467500        30000   -0.912225       1.86383e-
↪68
CNVR_8      Chr09   24512501        24652500        140000  -2.799081       0
CNVR_9      Chr09   24657501        24692500        35000   -2.466217       9.479478e-
↪297
CNVR_10     Chr09   24747501        24887500        140000  -2.46515        0
CNVR_11     Chr09   24917501        24942500        25000   -1.980229       9.759448e-
↪172
CNVR_12     Chr09   24947501        25007500        60000   -2.378318       0
CNVR_13     Chr09   25017501        25102500        85000   -2.464295       0
CNVR_14     Chr09   25107501        25132500        25000   -2.254819       4.908193e-
↪196
CNVR_15     Chr09   25137501        25202500        65000   -2.90878        0
CNVR_16     Chr09   25212501        25422500        210000  -3.111295       0
CNVR_17     Chr09   25427501        25512500        85000   -2.883919       0
CNVR_18     Chr09   25522501        25637500        115000  -2.617425       0
CNVR_19     Chr09   25647501        25722500        75000   -2.367854       0
CNVR_20     Chr09   25727501        26477500        750000  -2.388962       0
CNVR_21     Chr09   26482501        26507500        25000   -1.727876       9.894624e-
↪147
CNVR_22     Chr09   26512501        26552500        40000   -1.624917       3.444569e-
↪216
CNVR_23     Chr09   26562501        26787500        225000  -2.290655       0
CNVR_24     Chr09   26817501        26937500        120000  -1.704837       0
CNVR_25     Chr09   26947501        26972500        25000   -1.303558       1.960969e-
↪100
```

The output tabulated files under **pbgl-cnvseq/tool/output/PBGL-sorghum-analysis-example/tab-files** are the following:

- con-2_s1-vs-D2-1_S7-all-chroms-CNVs.tab
- con-2_s1-vs-D2-1_S7-window-10000-all-hits.tab

A depiction of the files are shown below,

After the CNV calculations are done, plot the CNVs under the **Plotting** section. Here, the parameters will be set to the following,

```
In [ ]: cnvPlot(confg, imgType="png", yMin=-5, yMax=5)
```

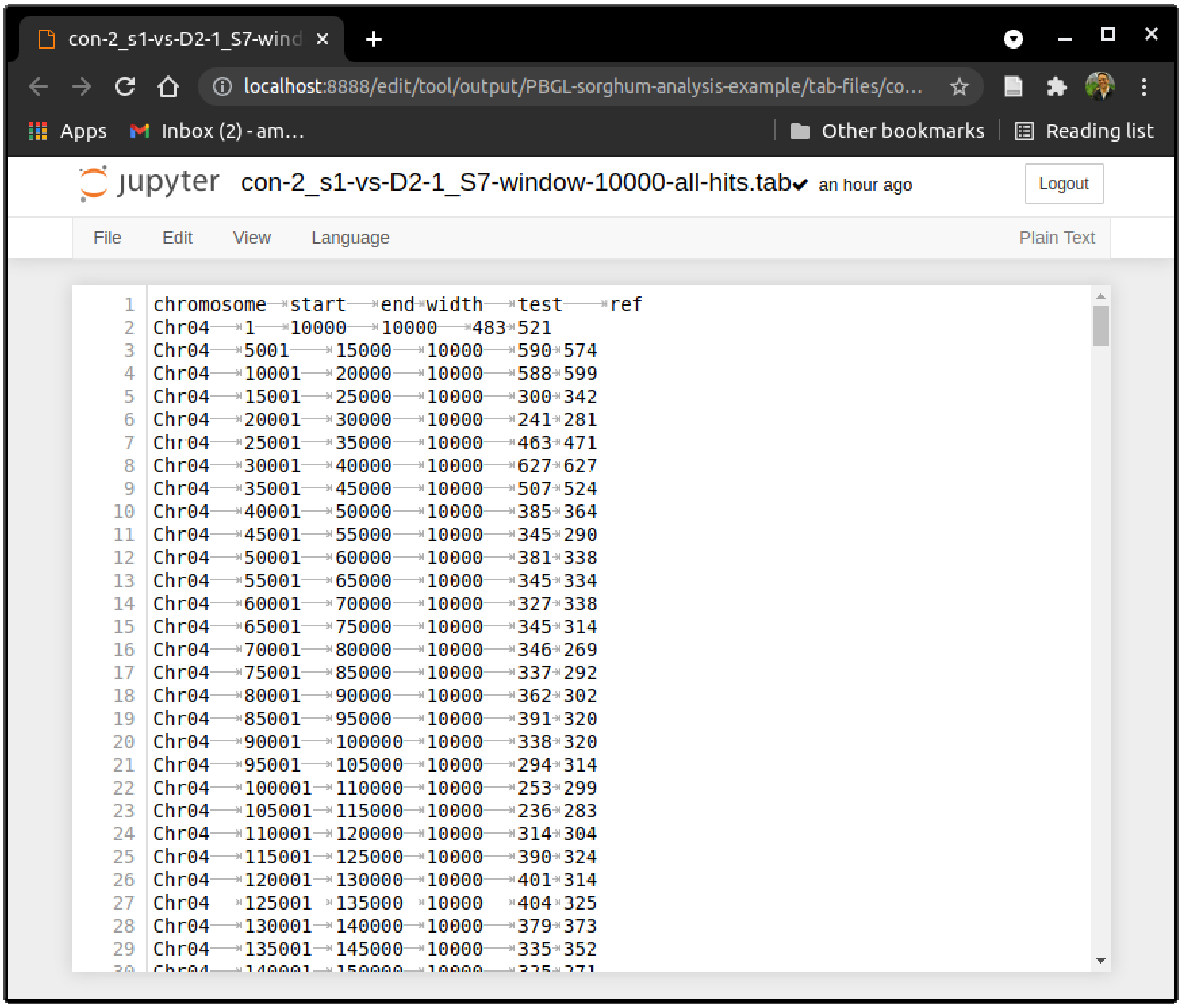
The output images under **pbgl-cnvseq/tool/output/PBGL-sorghum-analysis-example/images** are the following:

- con-2_s1-vs-D2-1_S7-chromosome-all-window-10000.png
- con-2_s1-vs-D2-1_S7-chromosome-Chr04-window-10000.png
- con-2_s1-vs-D2-1_S7-chromosome-Chr05-window-10000.png
- con-2_s1-vs-D2-1_S7-chromosome-Chr09-window-10000.png

The output plots are the following,

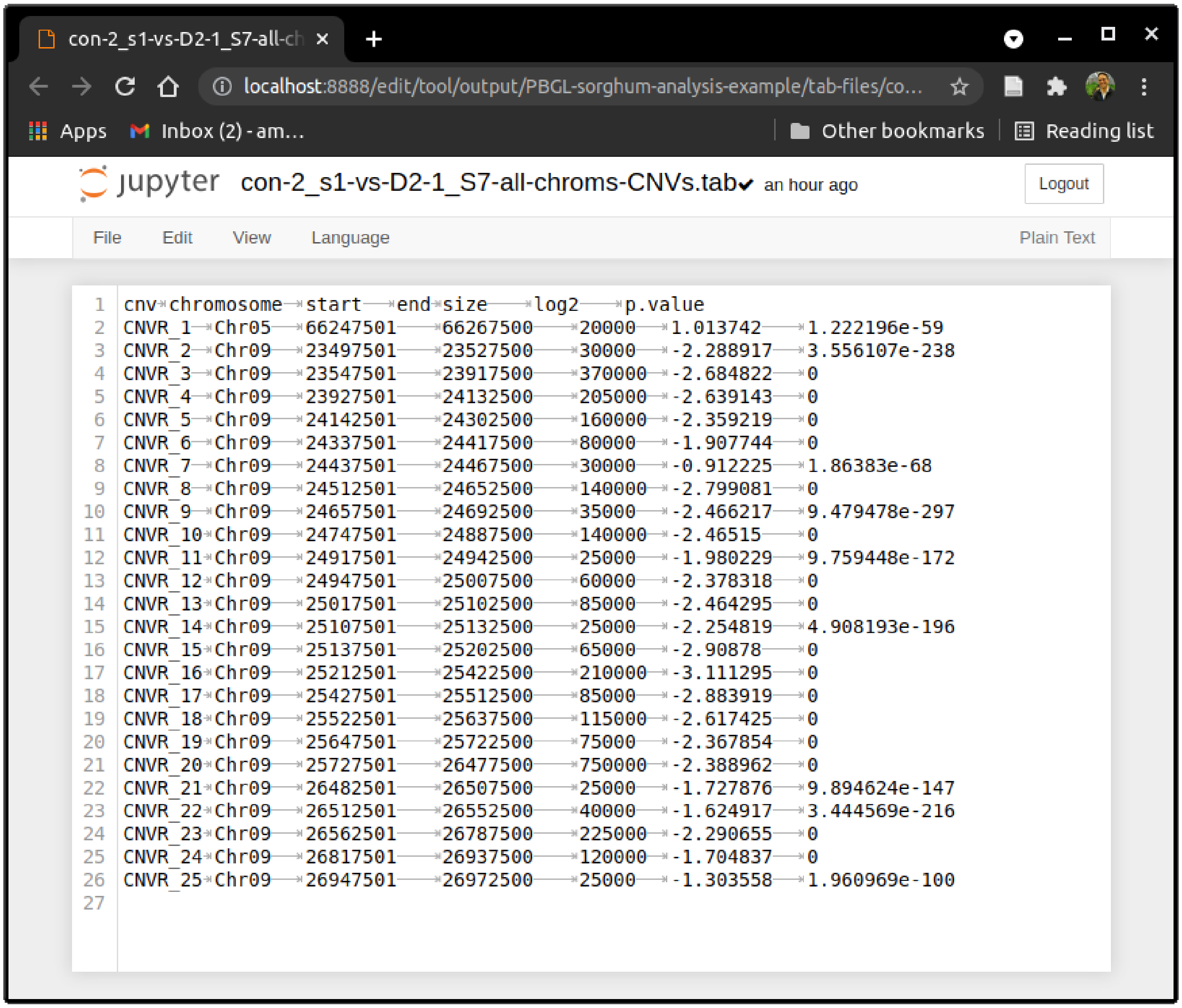
As seen above, each plot contains:

- a title of the chromosome being analyzed



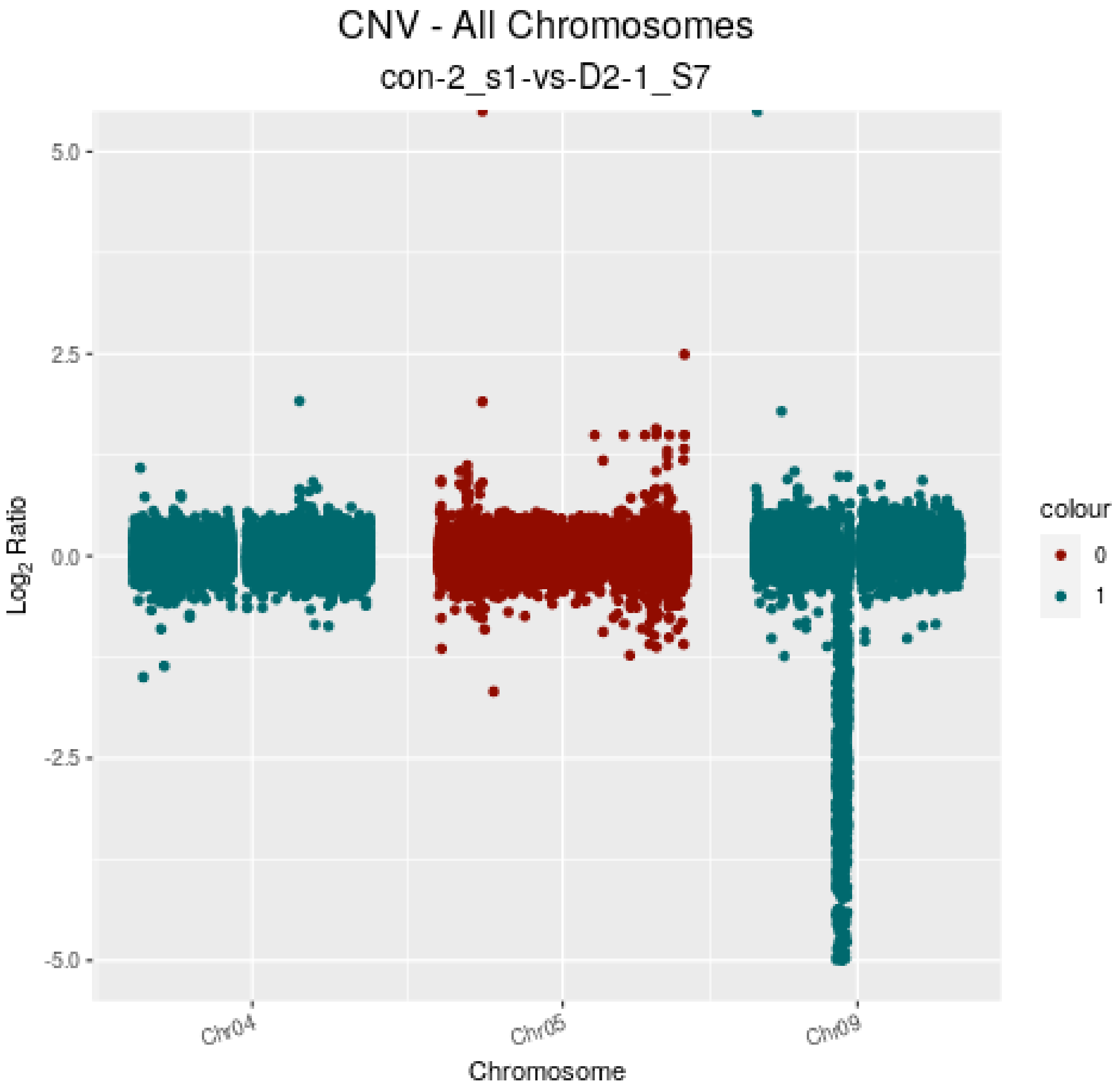
```
chromosome  start   end     width   test    ref
Chr04   1       10000   10000   483     521
Chr04   5001    15000   10000   590     574
Chr04   10001   20000   10000   588     599
Chr04   15001   25000   10000   300     342
Chr04   20001   30000   10000   241     281
Chr04   25001   35000   10000   463     471
Chr04   30001   40000   10000   627     627
Chr04   35001   45000   10000   507     524
Chr04   40001   50000   10000   385     364
Chr04   45001   55000   10000   345     290
Chr04   50001   60000   10000   381     338
Chr04   55001   65000   10000   345     334
Chr04   60001   70000   10000   327     338
Chr04   65001   75000   10000   345     314
Chr04   70001   80000   10000   346     269
Chr04   75001   85000   10000   337     292
Chr04   80001   90000   10000   362     302
Chr04   85001   95000   10000   391     320
Chr04   90001   100000  10000   338     320
Chr04   95001   105000  10000   294     314
Chr04   100001  110000  10000   253     299
Chr04   105001  115000  10000   236     283
Chr04   110001  120000  10000   314     304
Chr04   115001  125000  10000   390     324
Chr04   120001  130000  10000   401     314
Chr04   125001  135000  10000   404     325
Chr04   130001  140000  10000   379     373
Chr04   135001  145000  10000   335     352
Chr04   140001  150000  10000   325     371
```

Fig. 1: Tabulated file containing all hits of all chromosomes


con-2_s1-vs-D2-1_S7-all-ch
localhost:8888/edit/tool/output/PBGL-sorghum-analysis-example/tab-files/co...
Apps
Inbox (2) - am...
Other bookmarks
Reading list
jupyter
con-2_s1-vs-D2-1_S7-all-chroms-CNVs.tab
an hour ago
Logout
File
Edit
View
Language
Plain Text

```
cnv	chromosome	start	end	size	log2	p.value
CNVR_1	Chr05	66247501	66267500	20000	1.013742	1.222196e-59
CNVR_2	Chr09	23497501	23527500	30000	-2.288917	3.556107e-238
CNVR_3	Chr09	23547501	23917500	370000	-2.684822	0
CNVR_4	Chr09	23927501	24132500	205000	-2.639143	0
CNVR_5	Chr09	24142501	24302500	160000	-2.359219	0
CNVR_6	Chr09	24337501	24417500	80000	-1.907744	0
CNVR_7	Chr09	24437501	24467500	30000	-0.912225	1.86383e-68
CNVR_8	Chr09	24512501	24652500	140000	-2.799081	0
CNVR_9	Chr09	24657501	24692500	35000	-2.466217	9.479478e-297
CNVR_10	Chr09	24747501	24887500	140000	-2.46515	0
CNVR_11	Chr09	24917501	24942500	25000	-1.980229	9.759448e-172
CNVR_12	Chr09	24947501	25007500	60000	-2.378318	0
CNVR_13	Chr09	25017501	25102500	85000	-2.464295	0
CNVR_14	Chr09	25107501	25132500	25000	-2.254819	4.908193e-196
CNVR_15	Chr09	25137501	25202500	65000	-2.90878	0
CNVR_16	Chr09	25212501	25422500	210000	-3.111295	0
CNVR_17	Chr09	25427501	25512500	85000	-2.883919	0
CNVR_18	Chr09	25522501	25637500	115000	-2.617425	0
CNVR_19	Chr09	25647501	25722500	75000	-2.367854	0
CNVR_20	Chr09	25727501	26477500	750000	-2.388962	0
CNVR_21	Chr09	26482501	26507500	25000	-1.727876	9.894624e-147
CNVR_22	Chr09	26512501	26552500	40000	-1.624917	3.444569e-216
CNVR_23	Chr09	26562501	26787500	225000	-2.290655	0
CNVR_24	Chr09	26817501	26937500	120000	-1.704837	0
CNVR_25	Chr09	26947501	26972500	25000	-1.303558	1.960969e-100
```

Fig. 2: Tabulated file containing all CNVs of all chromsomes

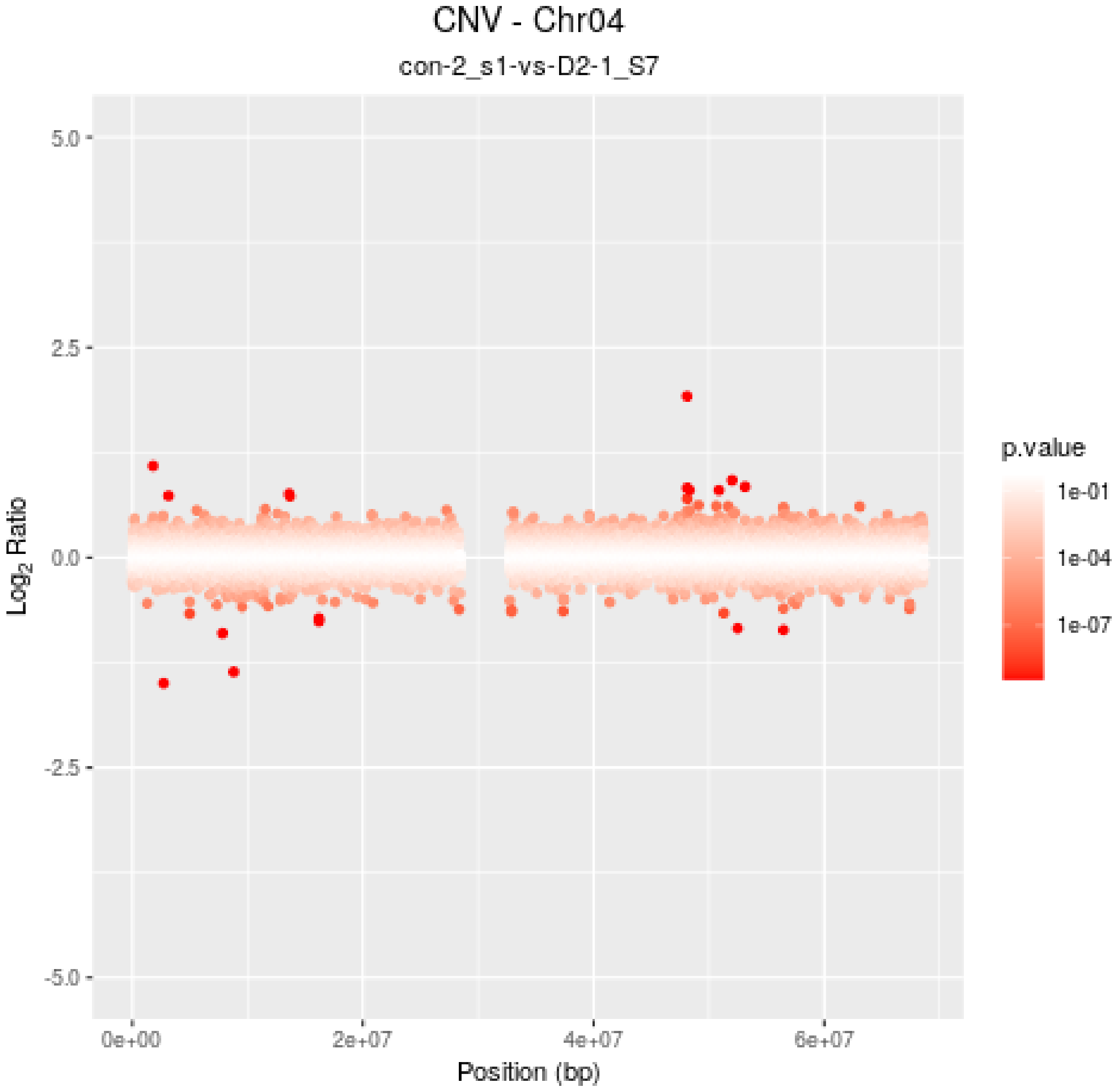


CNV - All Chromosomes

con-2_s1-vs-D2-1_S7

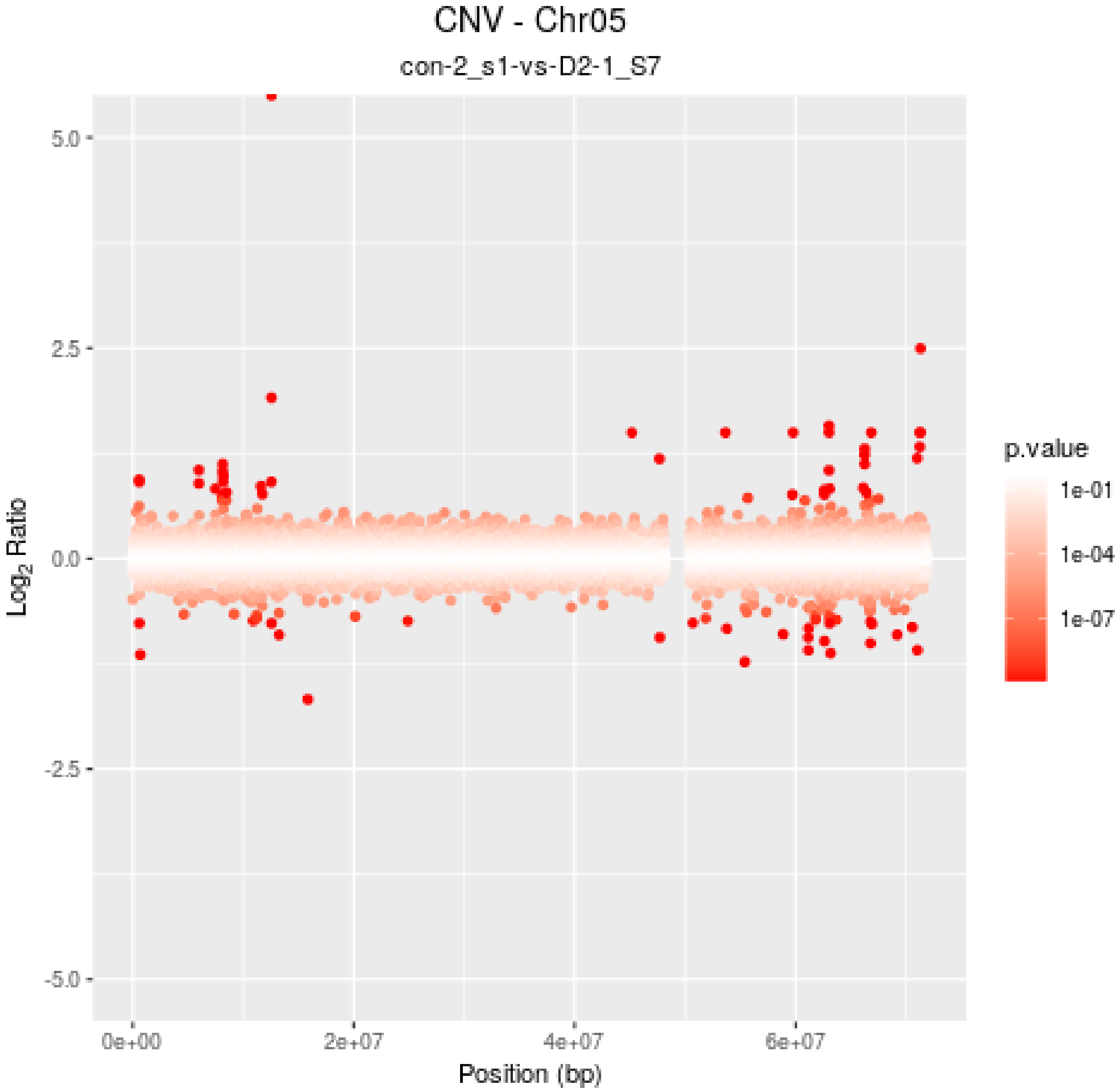
output/PBGL-sorghum-analysis-example/images/con-2_s1-vs-D2-1_S7-chromos

Fig. 3: CNV plot of all chromosomes



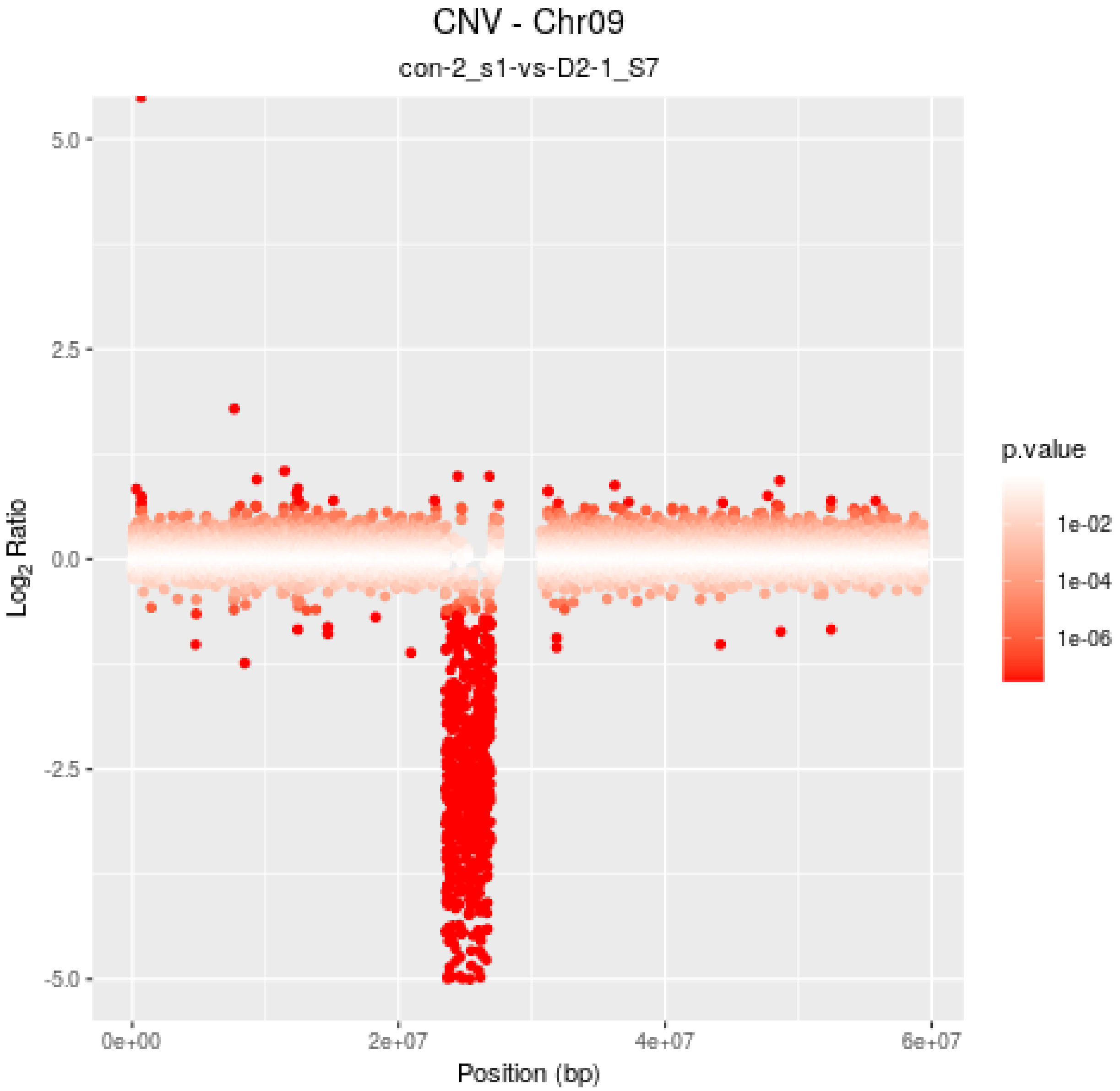
output/PBGL-sorghum-analysis-example/images/con-2_s1-vs-D2-1_S7-chromosome-Chr04-window-10000.png

Fig. 4: CNV plot of chromosome Chr04



output/PBGL-sorghum-analysis-example/images/con-2_s1-vs-D2-1_S7-chromosome-Chr05-window-10000.png

Fig. 5: CNV plot of chromosome Chr05



output/PBGL-sorghum-analysis-example/images/con-2_s1-vs-D2-1_S7-chromosome-Chr09-window-10000.png

Fig. 6: CNV plot of chromosome Chr09

- a sub-title of the comparison being done
- Log2 Ratio in the y-axis
- chromosome name on the x-axis in plot of all chromosomes
- base-pair (bp) position on the x-axis in plot of one chromosome
- a label under the x-axis specifying the location of the stored image

The CNV plot of chromosome Chr09 shows an artifact. Moreover, this artifact seems to lie between base-pairs 2e07 and 3e07. To zoom in, the **Plotting a Zoomed-In Region of One Chromosome** section is provided.

Under the **Plotting a Zoomed-In Region of One Chromosome** section, first specify the location of the all-hits tabulated file of chromosome Chr09,

```
In [ ]: tabFile <- "output/PBGL-sorghum-analysis-example/tab-files/con-2_s1-vs-D2-1_
↪S7-window-10000-all-hits.tab"
```

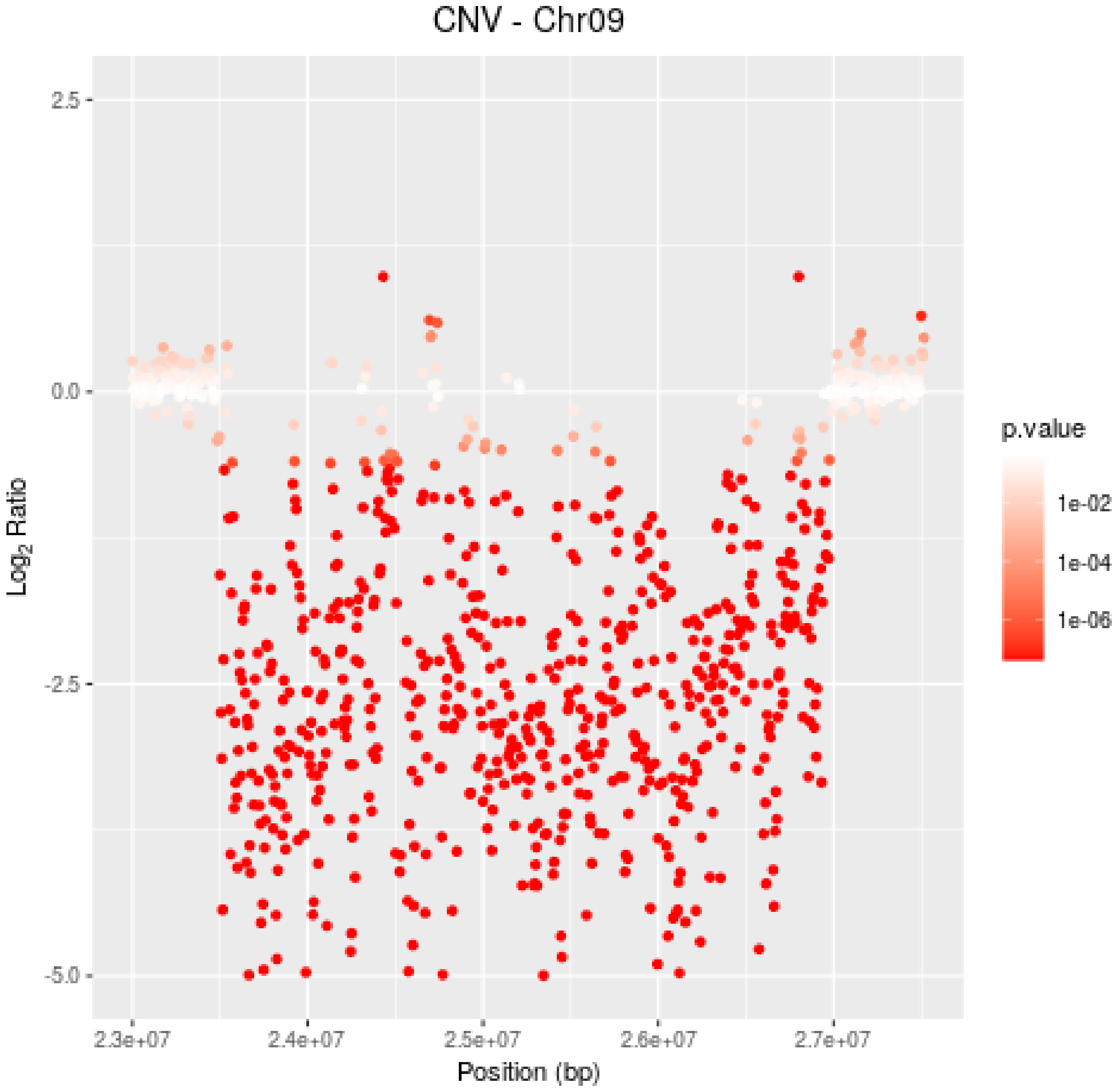
The run the zooming-in function with the following parameters,

```
In [ ]: cnvPlotZoom(config, tabFile, chromosome="Chr09", start=2.3e7 , end=2.8e7 ,␣
↪yMin=-5 , yMax=2.5, imgType="png")
```

The output plot, which is also stored in the **pbgl-cnvseq/tool/output/PBGL-sorghum-analysis-example/images**, shows a zoomed-in region of chromosome Chr09 of sorghum. The output path is also specified under the x-axis label.

Knowing where artifacts like this one is located plays a major role in identifying insertions or deletions in an organism. The user can, in turn, use different tools, like the Integrative Genomics Viewer (IGV), for further analysis.

This culminates the tutorial.



output/PBGL-sorghum-analysis-example/images/con-2_s1-vs-D2-1_S7-window-10000-chromosome-Chr09-zoom

Fig. 7: CNV plot of a zoomed-in region of chromosome Chr09

5 References

BMC Bioinformatics Publication:

- CNV-seq, a new method to detect copy number variation using high-throughput sequencing

GitHub repositories:

- hliang/cnv-seq
- Bioconductor/copy-number-analysis
- pbgl/pbgl-cnvseq
- amora197/pbgl-cnvseq