## Instructions on How to Run This Code

This notebook is designed for the **Al Jord** team at the **Centre for Genomic Regulation**. All components in this repository operate within a *Virtual Machine* (either Docker or Singularity), ensuring consistent functionality regardless of when or where it is downloaded, as long as the working directory is the root of this repository.

## Accessing the HPC Cluster

To access the HPC Cluster, you may require assistance from **Emyr James**, the head of Scientific IT as of 2024-2025. COntact him first to grant you access to the cluster and then follow these steps to set you up:

- 1. (Optional for external access) Install Forticlient VPN:
  - Select Add New Connection and choose SSL-VPN.
  - Set https://vpn.crg.es:10000/sslvpn/ as the Remote Gateway.
  - Use your CRG credentials for the *Username* and *Password*.
- 2. SSH Client Setup:
  - For *MacOS* or *Linux*, open Terminal and run: ssh login1.hpc.crg.es -1 <your-CRG-username> Enter your CRG password when prompted.
  - For Windows, open Powershell or Terminal as Administrator and run:

```
wsl --install
wsl --set-default-version 2
wsl --install Ubuntu-20.04
Follow the installation steps for Ubuntu. Then, run:
ssh login1.hpc.crg.es -1 <your-CRG-username>
Enter your CRG password when prompted.
```

You will now be in your personal folder within the lab team directory.

## Download the Repository

Clone the repository to your HPC folder:

```
git clone https://github.com/andresgordoortiz/24CRG_ADEL_MANU_00CYTE_SPLICING.git cd 24CRG_ADEL_MANU_00CYTE_SPLICING
```

Explore the repository's folders:

ls

**Note**: These folders correspond to those in *Isilon*. Files can be uploaded or downloaded through Isilon as needed.

## Running the Analysis

Run the following pipelines to execute the complete analysis, from downloading samples to generating the final report and Excel tables:

# Important: you must pass a suitable VASTDB database as absolute path to run the pipelines sbatch workflows/full\_processing\_pipeline\_fmndko.sh /users/mirimia/projects/vast-tools/VASTDB sbatch workflows/full\_processing\_pipeline\_pladb.sh /users/mirimia/projects/vast-tools/VASTDB sbatch workflows/full\_processing\_pipeline\_spire.sh /users/mirimia/projects/wast-tools/VASTDB sbatch workflows/full\_processing\_pipeline\_spire.sh /users/mirimia/projects/wast-tools/VASTDB sbatch workflows/full\_processing\_pipeline\_spire.sh /users/mirimia/projects/wast-tools/VASTDB sbatch workflows/full\_processing\_pipeline\_spire.sh /users/mirimia/projects/wast-tools/VASTDB sbatch workflows/full\_processing\_pipeline\_spire.sh /users/mirimia/projects/wast-tools/wast-tools/wast-tools/wast-tools/wast-tools/wast-tools/wast-tools/wast-tools/wast-tools/wast-tools/w

**Important**: Ensure the workflow is provided with a valid path to the VASTDB database. The code above should run smoothly since Manu keeps a copy of it in his folder but, if not available, download the Mm2 database as follows:

```
# This wll download the VASTDB for the mouse assembly.
mkdir VASTDB
wget https://vastdb.crg.eu/libs/vastdb.mm2.23.06.20.tar.gz
tar -xzvf vastdb.mm2.23.06.20.tar.gz -C VASTDB
```

And then run the pipelines with the new VASTDB:

sbatch workflows/full\_processing\_pipeline\_fmndko.sh \$(pwd)/VASTDB
sbatch workflows/full\_processing\_pipeline\_pladb.sh \$(pwd)/VASTDB
sbatch workflows/full\_processing\_pipeline\_spire.sh \$(pwd)/VASTDB

After a few hours the analysis should finish, but you can check the estatus of your query using:

```
squeue -u <your-CRG-user>
```

After it has finished, run the R Report (it will take an hour or so)

sbatch scripts/R/run\_notebook.sh

Signed: Andrés Gordo Ortiz