

Cogent™ NGS Discovery Software Quick Start Guide

The following information is provided as a high-level introduction to the software. For more detailed information, please see the [Cogent NGS Discovery Software User Manual](#).

Before You Begin

- A. Supported operating systems
 - Windows 11
 - macOS Mojave (10.14) or higher
 - Linux CentOS 6.9 / Red Hat 7 or higher
- B. Hardware requirements
 - Laptop, desktop, or server

Standard analysis requirements

 - Memory: 8 GB RAM or higher
 - Free disk space: 20 GB or higher

Shasta Total RNA-Seq analysis requirements

 - Memory: 64 GB RAM or higher
 - Disk space: 60 GB or higher
- C. Additional software dependencies
 - Internet connectivity on the computer
 - [R](#) version 4.4 or higher, and:
 - For Windows: [RTools](#) 4.4 or higher, based on the R version
 - For MacOS: Install [Homebrew](#); after R installation, please install `xcode tools` and run `brew install libomp` and `brew install gcc`; for DNA-seq analysis, install [XQuartz](#)
 - For Linux (CentOS): use `yum install` to install `libomp-devel` and `gcc`
 - [RStudio](#) (IDE for R)

Input files

scRNA app > Analysis Mode & Bulk RNA app

Choose one of the first two required options:

- **(Recommended)** `CogentDS.analysis.rds`, an `rds` file output from [Cogent NGS Analysis Pipeline](#) (CogentAP)—for full analysis capabilities
- Raw gene-count matrix and stats/metadata files (`.csv/.csv.gz`)
- (Optional) `metadata.csv`, which contains additional identifying information associated with the barcodes or samples

scDNA app

- `CogentDS.analysis.rds`, from CogentAP

scRNA Discovery Mode

- Processed `CogentDS.analysis.rds` file, saved after the scRNA Analysis Mode workflow

scRNA Barcode Rank Plot

- `demultiplexed_fastqs_counts_all.estimated.csv` file, generated by CogentAP using the `--dry_run` argument. Refer to the [Cogent NGS Analysis Pipeline User Manual](#) (Section V.B.1.b).

Installation

1. [Sign up](#) and download the installation package from our website. Unzip the installation package on the desired laptop, desktop, or server.
2. Ensure software dependencies are installed.
3. Run RStudio.

- If a previous version of CogentDS (v1.X) or hanta™ software is installed on the same computer where CogentDS will be installed, proceed to step 4
- If this is a new install, skip to step 5


4. (Uninstall previous versions): Type the appropriate command at the RStudio prompt to uninstall:
 CogentDS: `remove.packages("CogentDS")`
 hanta: `remove.packages("hanta")`
5. Type the following commands in RStudio to install the R packages required by CogentDS:
`setwd('<PATH>')`
`source('setup_CogentDS.R')`
 where `<PATH>` is the full path of the folder created by unzipping the software zip file (Step 1).
6. Select a CogentDS installation option from the selection prompt options. '1' is the default option.

Running Cogent NGS Discovery Software


1. Run RStudio.
2. At the RStudio prompt, run the command:
`setwd('<PATH>')`
`source('launch_CogentDS.R')`
 where `<PATH>` is replaced by the full path of the folder created by unzipping the software zip file.

IMPORTANT: The CogentDS user interface (GUI) will display in the default browser for the computer.


General Usage Instructions (All Apps)

- Upload your input files on the first screen (module) of the selected application type (app).
- Fill in subsequent data fields according to your data reporting needs. Defaults are prepopulated but can be modified.
- Where action buttons exist in the module screens (blue buttons, white text), especially buttons with upload icons , click them prior to proceeding to the next module.

If the buttons are paired (e.g., an action + skip action), choose one option, not both.

NOTE: Buttons to save plots or data with a download icon , or [Reset plot] buttons are not required actions.

- The buttons with the prefix `Next`, (lower right corner of the page), should be clicked to proceed stepwise to the next module in the pipeline.

If there is no `Next` button, the page being viewed is the final step of the workflow. Click the [Home] icon ( Home) to return to the main app menu.

- Use the left-side navigation menu to step back in the pipeline. If changes are made, you must proceed sequentially from that point to continue.
- When the option is available, it is recommended at the end of each application workflow to download the analysis report and modified CogentDS processed .rds data to save the results of your work.

Single-Cell RNA-Seq (scRNA app)

- From the main CogentDS interface window, select the [Launch scRNA app]. The app will launch in a new browser tab.

scRNA Analysis Mode

1. From the scRNA web page, select [Analysis Mode].
2. On the *Upload Data* page:
 - a. (Required) Select one of the following:
 - 'Processed Data from CogentAP' and the CogentAP .rds file as the dataset
 - 'Raw count matrix', the raw count matrix file as the dataset, and the gene info file
 - 'Example data' for the mini dataset included with CogentDS for app feature exploration
 - b. (Optional) Select metadata to upload

3. Click [Prepare Data for downstream analysis] to enact the data upload.
4. Follow the **General Usage Instructions (All Apps)** for the rest of the workflow.

NOTE: The *Find Markers* module requires two action buttons be selected, [Perform DE analysis] then [Generate plots], before continuing.

scRNA Discovery Mode

1. From the scRNA web page, select [Discovery Mode].
2. On the *Upload Data* page, select the preprocessed .rds data file and click [Submit upload].
3. Follow the **General Usage Instructions (All Apps)** for the rest of the workflow.
4. From the *Expression* module, click [Home] to exit.

scRNA Barcode Rank Plot

1. From the scRNA web page, select [Barcode Rank Plots].
2. Select the `demultiplexed_fastqs_counts_all.estimated.csv` file and click [Generate Rank Plot].
3. Save output (if desired) then click [Home] to exit.

Bulk RNA Analysis (BulkRNA app)

1. From the main CogentDS interface window, select the [Launch BulkRNA app]. The app will launch in a new browser tab.
2. From the BulkRNA web page, select [Analysis Mode].
3. On the *Upload Data* page:
 - a. (Required) Select one of the following:
 - 'Processed Data from CogentAP' and the CogentAP .rds file as the dataset
 - 'Raw count matrix', the raw count matrix file as the dataset
 - 'Example data' for the mini dataset included with CogentDS
 - b. (Optional) Select a metadata to upload
4. Click [Upload Raw Data] to enact the data upload.
5. Follow the **General Usage Instructions (All Apps)** for the rest of the workflow.
6. Save output (if desired) then click [Home] to exit.

Single-Cell DNA-Seq (scDNA app)

- From the main CogentDS interface window, select the [Launch scDNA app]. The app will launch in a new browser tab.
- From the scDNA web page, select [CNV Analysis Mode].
- On the *Upload Data* page, select one of the following:
 - 'Processed Data from CogentAP' and the CogentAP .rds file as the dataset
 - 'Example data' for the mini dataset included with CogentDS
- Click [Upload Raw Data] to enact the data upload.
- Follow the **General Usage Instructions (All Apps)** for the rest of the workflow.

NOTE: For the *CN Profile* module, select between 1–3 barcodes to generate the plot.

- Save output (if desired) then click [Home] to exit.

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