

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](#) and [Cogent NGS Analysis Pipeline User Manual](#).

Before You Begin

A. Supported operating systems

- Windows 11
- macOS Mojave (10.14) or higher
- Ubuntu (24.04.2)

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 $\geq 4.4.0$ or $\leq 4.5.0$, 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/Ubuntu: use apt or apt-get to install libomp-dev, gcc, zlib1g-dev, libcurl-dev, libssl-dev, libxml2-dev, libpng-dev, libicu-dev, libcairo2-dev, perl, libfontconfig-dev, libfribidi-dev, libharfbuzz-dev, libblas-dev, liblapack-dev, gfortran, cmake, gsfonts, libglpk-dev, libmagick++-dev, libnode-dev, pandoc, libcurl4-openssl-dev, libX11-dev, and r-base ($\geq v4.4.0$)

NOTE: To identify Linux OS-level dependencies of R packages required by CogentDS, use the [pak R package](#). Some OS-level dependencies may not be listed here; they may need to be installed for CogentDS installation on a Linux OS.

- [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`, a rds file output from [Cogent NGS Analysis Pipeline](#) (CogentAP)—for full analysis capabilities
- Raw gene-count matrix and stats/metadata files (CSV/CSV.GZ). Please note that the gene info file will be required for using the raw gene-counts matrix input

(Optional) `metadata.csv`, which contains additional identifying information associated with the barcodes or samples

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)

scDNA CNV Analysis Mode

`CogentDS_scDNA_analysis.rds`, saved after the DNA-seq CNV analysis (Cogent NGS Analysis Pipeline User Manual, Section V.C.1)

scDNA SNV Analysis Mode

`CogentDS_SNV_analysis.rds`, from DNA-seq SNV analysis (Cogent NGS Analysis Pipeline User Manual, Section V.C.2)

Installation

1. [Sign up](#) to 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 (v1.5) or hanta™ software is installed on the same computer where CogentDS v2.2 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).

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  and [Reset plot] button 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 () 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 [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—[Perform DE analysis] then [Generate plots]—to be selected 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 [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 data 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 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 [Launch scDNA app]. The app will launch in a new browser tab.

scDNA CNV Analysis Mode

1. From the scDNA web page, select [CNV Analysis Mode].
2. On the *Upload Data* page, select one of the following:
 - 'Processed Data from CogentAP' and the CogentAP RDS data file generated by CNV analysis (Cogent NGS Analysis Pipeline User Manual, Section V.C.1) as the dataset
 - 'Example data' for the mini dataset included with CogentDS

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

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

5. Save output (if desired), then click [Go to Main Page] or [Home] to exit.

scDNA SNV Analysis Mode

1. From the scDNA web page, select [SNV Analysis Mode].
2. On the *Upload Data* page, select one of the following:
 - 'Processed Data from CogentAP' and the CogentAP RDS data file generated by SNV calling analysis (Cogent NGS Analysis Pipeline User Manual, Section V.C.2) as the dataset
 - 'Example data' for the mini dataset included with CogentDS
3. Click [Upload Data] to enact the data upload.
4. Follow the **General Usage Instructions (All Apps)** for the rest of the workflow.
5. Save output (if desired), then click [Home] to exit.

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tel: 800.662.2566, option 1 (toll-free)	tel: 800.662.2566, option 2 (toll-free)
fax: 800.424.1350 (toll-free)	fax: 800.424.1350 (toll-free)
web: takarabio.com/service	web: takarabio.com/support
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Takara Bio USA, Inc.

2560 Orchard Parkway, San Jose, CA 95131, USA

United States/Canada: +1.800.662.2566 • Asia Pacific: +1.650.919.7300 • Europe: +33.(0)1.3904.6880 • Japan: +81.(0)77.565.6999