Cogent™ NGS Discovery Software v1.5 Quick Start Guide

The following information is provided as a high-level introduction to the software, also referred to as CogentDS. For more detailed information, please see the Cogent NGS Discovery Software v1.5 User Manual.

Before you begin

A. Supported operating systems
- Windows 7, Windows 10
- macOS Mojave (10.14) or higher
- Linux CentOS 6.9 or higher

B. Hardware requirements
- Standard laptop, desktop, or server
- Memory: 8 GB RAM or higher
- Free disk space: 2 GB or higher

C. Additional dependencies
- Internet connectivity on the computer
- R version 4.0.0 or higher
  - For Windows: RTools, version compatible with R 4.x (e.g., rtools40)
  - For MacOS: `clang-7.0.0.pkg`
- RStudio (IDE for R)
- R devtools 2.4.2 or higher

Required input files

CogentDS can take either of the following options for input (choose one):

1. `CogentDS.analysis.rda`, an rda file output from Cogent NGS Analysis Pipeline (CogentAP)—recommended input for full analysis capabilities
2. Raw gene-count matrix and stats/metadata files

Installation

1. Sign up to download the installation package from our website.
2. Run RStudio.
3. (Uninstall previous software versions): If you have an older versions of CogentDS or the Takara Bio hanta software installed, type the following command(s) at the RStudio prompt to uninstall them:

CogentDS: `remove.packages("CogentDS")`

hanta: `remove.packages("hanta")`

4. Type the following command at the RStudio prompt (all one line) to install CogentDS:

```
devtools::install_local("<PATH>/Cogent
_NGS_Discovery_Software_v1.5.zip")
```

<PATH> will be replaced by the full path where the installation ZIP file is stored on the target server.

Example:

```
devtools::install_local("C:/temp/Cogent_
NGS_Discovery_Software_v1.5.zip")
```

To run Cogent NGS Discovery Software

1. Run RStudio.
2. At the RStudio prompt, run the command:

```
CogentDS::launch()
```

The CogentDS user interface (GUI) will display in the default browser for the workstation or server.

NOTE: If prompted to download updates for R modules and additional guidance is needed, refer to the Cogent NGS Discovery Software notices page.

3. Click the [Get Started] button in the GUI.
4. *Select Input Data*: Upload the required input files by selecting one of the options from the drop-down.

NOTE: A mini dataset to test the CogentDS installation can be downloaded from the `*Example data` link on the *Select Input Data* window. The file is called `analysis_demo.rda`.

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Click [Submit] to go to the next step.

- If the "Use previous settings and calculations" option is selected, skip to Step 8, *Cluster Analysis*.

- If the "Select new settings and run all calculations" option is selected and transcript analysis was performed in CogentAP, continue to Step 5, *Select data to use for clustering*.

- If the "Select new settings and run all calculations" option is selected and transcript analysis was **not** performed in CogentAP, go to Step 6, *Quality control*.

5. *Select data to use for clustering*: If transcript analysis was performed on the data in CogentAP (e.g., full-length applications), you will be prompted to choose one of the two options:

- 'Use transcript counts' to review transcript-level expression in CogentDS

- Otherwise, select 'Use gene counts' (default behavior)

6. *Quality control:*
   NOTE: First-time users should start by using the default settings for the quality control options.

   a. QC Filter: Select how to filter non-informative cells and genes from the gene-count matrix.

   b. Click the [Next: Norm/Log] button when finished.

7. *Normalization and Transformation*: Select how you would like to normalize and/or transform the data.
   Click [Next: Clustering] to continue.

8. *Cluster Analysis*: Adjust the gene filter method, number of variable genes to cluster by, and the reduction method (either UMAP or tSNE). Click [Launch Analysis] button to run the analysis.
   This step typically takes about a minute to complete.

9. *Discover:* The main/center section of the screen typically shows the results of graph-based clustering. The User Controls and Formatting menu options in the sidebar panel to the left of the screen can be used to modify how the graph displays.

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