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: <u>RTools</u>, version compatible with R 4.x (e.g., rtools40)
 - o For MacOS: clang-7.0.0.pkg
 - <u>RStudio</u> (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):

- 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

- 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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This document has been reviewed and approved by the Quality Department.

05.23 US

