

Hashtag demultiplexing

- [Prerequisites for running Hashtag demultiplexing](#)
- [Running Hashtag demultiplexing](#)
- [References](#)

Cell Hashing enables sample multiplexing and super-loading in single cell RNA-Seq isolation by labeling each sample with a sample-specific oligo-tagged antibody against a ubiquitously expressed cell surface protein.

The *Hashtag demultiplexing* task is an implementation of the algorithm used in Stoeckius et al. 2018¹ for multiplexing cell hashing data. The task adds cell-level attributes *Sample of origin* and *Cells in droplet*.

Prerequisites for running Hashtag demultiplexing

To run *Hashtag demultiplexing*, your data must meet the following criteria:

- Data node contains number of features less than number of observations
- Data node must be output from normalization task (recommended normalization method for hashtag is CLR)

If you are processing your FASTQ files in Partek Flow, be sure to specify a different Data type for your Cell Hashing FASTQ files on import than the FASTQ files for your gene expression and any other antibody data.

If you are processing your FASTQ files using Cell Ranger, be sure to specify a different feature_type for your Cell Hashing antibodies than any other antibodies in the Feature Reference CSV File.

If you want to specify sample IDs instead of using hashtag feature IDs as the sample IDs, you will need to prepare a tab-delimited text file (.txt) with hashtag feature IDs in the first column and the corresponding sample IDs in the second column (Figure 1). A header row is required.

	A	B
1	Hashtag	Sample
2	HTO1_0251_Human	Control
3	HTO2_0252_Human	CD4 Depleted
4	HTO3_0253_Human	CD14 Depleted
5	HTO4_0254_Human	CD19 Depleted

Figure 3. Sample ID .txt file example. The first column is the hashtag feature ID and the second column is the corresponding sample ID

Running Hashtag demultiplexing

- Click the **Normalized counts** data node for your cell hashing data
- Click **Hashtag demultiplexing** in the Pre-analysis tools section of the toolbox
- Click **Browse** to select your Sample ID file (Optional)
- Click **Finish** to run

The output is a *Demultiplexed counts* data node (Figure 2).

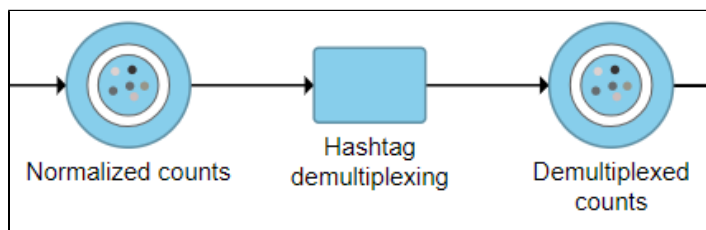


Figure 4. Hashtag demultiplexing can be run on a Normalized counts data node and outputs a Demultiplexed counts data node

Two cell-level attributes, *Cells in droplet* and *Sample of origin*, are added by this task and are available for use in downstream tasks. You can download the attribute values for each cell by clicking the *Demultiplexed counts* data node, clicking *Download*, and choosing to download *Attributes only*.

We recommend using [Annotate cells](#) to transfer the new attributes to other sections of your project after downloading the attributes text file.

It is also possible to use the *Merge matrices* task to combine your data types and attributes.

References

1. Stoeckius, M., Zheng, S., Houck-Loomis, B., Hao, S., Yeung, B.Z., Mauck, W.M., Smibert, P. and Satija, R., 2018. Cell Hashing with barcoded antibodies enables multiplexing and doublet detection for single cell genomics. *Genome biology*, 19(1), p.224.

Additional Assistance

If you need additional assistance, please visit [our support page](#) to submit a help ticket or find phone numbers for regional support.



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