# Count feature barcodes


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## What is Count feature barcodes?

*Count feature barcodes* is a tool for quantifying the number of feature barcodes per cell from CITE-Seq, cell hashing, or other feature barcoding assays to measure protein expression. The input for *Count feature barcodes* is FASTQ files.

## Running Count feature barcodes

*Count feature barcodes* will run on any unaligned reads data node.

- Click the data node containing your unaligned reads containing feature barcodes
- Click the **Quantification** section in the toolbox
- Click **Count feature barcodes**

The task set up page allows you to configure the settings for your assay (Figure 1).

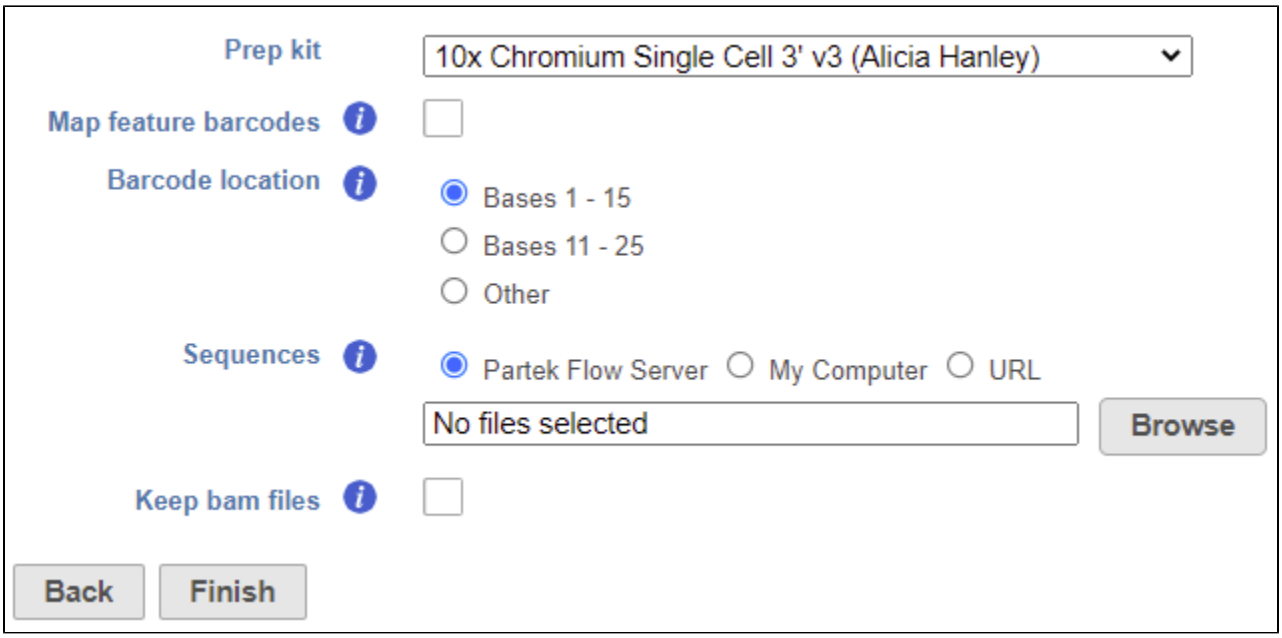


*Figure 3. Count feature barcodes task set up page*

- Choose the *Prep kit* from the drop-down menu

For more details on adding *Prep kits*, please see our documentation on Trim tags. The prep kit should include cell barcode and unique molecular identifiers (UMIs) locations.

- Check *Map feature barcodes* box (optional)

This is only necessary for processing data from 10X Genomics' Feature Barcoding assay (v3+ chemistry), which utilizes BioLegend TotalSeq-B. For all other assays, leave this box unchecked.

- Choose the *Barcode location*

For BioLegend TotalSeq-A, choose **bases 1-15**. For BioLegend TotalSeq-B/C, choose **bases 11-25**. For other locations, select **Custom** and specify the start and stop positions.

- Choose a *Sequences* text file

This tab-delimited text file should have the feature ID in the first column and the nucleotide sequence in the second column. Do not include column headers. See Figure 2 for an example.

| | |
|---|---|
| CD3 | CTCATTGTAACTCCT |
| CD4 | TGTTCCCGCTCAACT |
| CD8a | GCTGCGCTTTCCATT |
| CD11b | GACAAGTGATCTGCA |
| CD14 | TCTCAGACCTCCGTA |
| CD15 | TCACCAGTACCTAGT |
| CD16 | AAGTTCACTCTTTGC |
| CD19 | CTGGGCAATTACTCG |
| CD20 | TTCTGGGTCCCTAGA |
| CD25 | TTTGTCCTGTACGCC |
| CD27 | GCACTCCTGCATGTA |
| CD28 | TGAGAACGACCCTAA |
| CD34 | GCAGAAATCTCCCTT |
| CD45RA | TCAATCCTTCCGCTT |
| CD45RO | CTCCGAATCATGTTG |
| CD56 | TTCGCCGCATTGAGT |
| CD62L | GTCCCTGCAACTTGA |
| CD69 | GTCTCTTGGCTTAAA |
| CD80 | ACGAATCAATCTGTG |
| CD86 | GTCTTTGTCAGTGCA |
| CD127 | GTGTGTTGTCCTATG |
| CD137 | CAGTAAGTTCGGGAC |
| CD197 | AGTTCAGTCAACCGA |
| CD274 | GTTGTCCGACAATAC |
| CD278 | CGCGCACCCATTAAA |
| CD335 | ACAATTTGAACAGCG |
| PD-1 | ACAGCGCCGTATTTA |
| HLA-DR | AATAGCGAGCAAGTA |
| TIGIT | TTGCTTACCGCCAGA |
| IgG1 | GCCGGACGACATTAA |
| IgG2a | CTCCTACCTAAACTG |
| IgG2b | ATATGTATCACGCGA |

*Figure 4. Example of how the sequences tab-delimited text (.txt) file should be formatted*

- Check *Keep bam files* box (optional)

This option will retain the alignment BAM files instead of automatically deleting them when the task is complete. An extra *Aligned reads* output data node will be produced on the task graph. This option is unchecked by default to save on disk space.

- Click **Finish** to run

The output of *Count feature barcodes* is a *Single cell counts* data node.

## How does Count feature barcodes work?

*Count feature barcodes* uses a series of tasks available independently in Partek Flow to process the input FASTQ files. The output files generated by these tasks are not retained in the *Count feature barcodes* output, with the exception of BAM files if *Keep bam files* is checked.

Trim tags identifies the UMI and cell barcode sequences. The *Prep kit* is specified using the *Prep kit* setting.

Trim bases trims the insert read to include only the feature barcode sequence. *Trim bases* is set to **Both ends** for *Trim based on* with the start and stop set by the *Barcode location* preference and the *Min read length* set to **1**.

Bowtie is used to align the reads to the sequences specified in the *Sequences* text file. *Bowtie* is set to **Ignore quality limit** for the *Alignment mode*. Other settings are default.

Deduplicate UMIs consolidates duplicate reads based on UMIs. *Deduplicate UMIs* is set to **Retain only one alignment per UMI**.

Filter barcodes filters the cell barcodes to include cells and not empty droplets. *Filter barcodes* is set to **Automatic**.

Quantify barcodes counts the number of UMIs per cell for each feature in the *Sequences* file. *Quantify barcodes* uses default settings.

To perform these steps individually instead of using the *Count feature barcodes* task, you will need to generate a FASTA and GTF file containing the feature barcode IDs and sequences instead of a text file and build an index file for the Bowtie aligner.

For more information about library file management, please see Library File Management.

## Additional Assistance

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