

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).

Prep kit: 10x Chromium Single Cell 3' v3 (Alicia Hanley) ▼

Map feature barcodes ⓘ ☐

Barcode location ⓘ

- ☒ Bases 1 - 15
- ☐ Bases 11 - 25
- ☐ Other

Sequences ⓘ

- ☒ Partek Flow Server
- ☐ My Computer
- ☐ URL

No files selected

Keep bam files ⓘ ☐

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	CTCATTGTAACCTCCT
CD4	TGTTCCCGCTCAACT
CD8a	GCTGCGCTTTCCATT
CD11b	GACAAGTGATCTGCA
CD14	TCTCAGACCTCCGTA
CD15	TCACCAGTACCTAGT
CD16	AAGTTCACTCTTTGC
CD19	CTGGGCAATTACTCG
CD20	TTCTGGGTCCCTAGA
CD25	TTTGTCTGTACGCC
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.



Your Rating:  Results:  12 rates