Filter barcodes

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In droplet-based single cell isolation and library prep methods, each droplet is labeled by a unique nucleotide barcode. Because not all droplets will contain cells, it is important to filter out nucleotide barcodes that correspond to empty droplets prior to downstream analysis.

In Partek Flow, you can filter barcodes interactively using a knee plot after UMI deduplication in the UMI deduplication task report or after quantification in the Cell barcode QA/QC task report. Alternatively, you can filter using preset options in the Filter barcodes task.

To invoke Filter barcodes:

- Click a **Deduplicated reads** data node
- Click the Filtering section of the toolbox
- Click Filter barcodes

Configuring Filter barcodes

You can choose to filter barcodes using three or four options, depending on whether you have already run a Filter barcodes task for your samples in the project (Figure 1).

Filter by	 Automatic Number of cells Percent of reads in cell Previous filter 	0.0
Back Finish]	

Figure 5. Configuring Filter barcodes

Automatic

The automatic filter threshold is set for each sample individually. It picks the cutoff between cells and empty droplets by identifying where the UMI content per barcode drops precipitously when moving in descending order from the barcode with the highest number of UMIs.

Number of cells

Set the number of cells per sample to include. This is set for all samples; if set to 100, the top 100 barcodes by total UMI count for each sample will be retained.

Percent of reads in cell

Set the percent of reads in cells per sample to include. The number of barcodes included will be set to match the specified percent of reads in cells for each sample. Barcodes are included starting with the barcode with the highest number of total UMIs and proceeding in descending order of total UMIs per barcode until the specified percent of reads has been met or exceeded.

Previous filter

If you have already run a Filter barcodes task for your samples in the project, the Previous filter option will be available. This option lets you filter to the same cell barcodes that were included by the previous filter. This option is particularly useful for CITE-Seq data, where antibody barcodes and gene expression data must be processed separately, but you will want to analyze the same cell barcodes in downstream steps.

Selecting Previous filter opens a table with information about the previous barcode filters in your project (Figure 2).

Filter by	 Automatic Number of cells Percent of reads in cell 0.0 + Previous filter 					
Previous filter			Cells	Submitted time		
	٠	•	3855	21 Dec 2018, 10:46 AM CST	==	
	\bigcirc	\bigcirc	919	20 Dec 2018, 03:57 PM CST	==	
Back Finish]					

Figure 6. Previous filter options

To help you identify which previous filter you want to apply, the color of the task node on the Analyses tab, the number of cell barcodes retained (summed for all samples), and the time/date the previous filter task was submitted are included in the table. To view the number of cells and percentage of reads in cells for each sample in a previous filter, mouse over the **b** button (Figure 3).

Filter by	 Automatic Number of cells Percent of reads in cell 0.0 - Previous filter 									
Previous filter			Ce	lls		Submi	tted time			
	۲	•	38	55	21 Dec	2018, 1	0:46 AM CST	==		
	\bigcirc	0	91	S	ample	Cells	Reads in cells			
				0_	1a_S1	480	33.29%	View reads in cells breakdown chart		
Back Finish				0_	1b_\$2	439	31.68%			

Figure 7. Checking details of a previous filter

Use the radio buttons in the first column to pick which filter you want to use.

Output of Filter barcodes

After configuring the task, click Finish to run.

Filter barcodes produces a Filtered reads data node (Figure 4). Filter barcodes does not have a task report.



Figure 8. Filter barcodes produces a Filtered reads data node

Additional Assistance

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

