Deduplicate UMIs

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The Deduplicate UMIs task identifies and removes reads mapped to the same chromosomal location with duplicate unique molecular identifiers (UMIs). The details of our UMI deduplication methods are outlined in the UMI Deduplication in Partek Flow white paper.

To invoke Deduplicate UMIs:

- Click an Aligned reads data node
- Click Post-alignment tools in the toolbox
- Click Deduplicate UMIs

The task configuration dialog content depends on whether you imported FASTQ files or BAM files into Partek Flow.

Configuring Deduplicate UMIs

Imported FASTQ

UMIs and barcodes are detected and recorded by the Trim tags task in Partek Flow. You can choose whether to retain only one alignment per UMI or not (Figure 1). The default will depend on which prep kit was used in the Trim tags task.

Retain only one alignment per UMI	i	✓	
Select Annotation file			
Assembly	Homo sapiens (human) - hg38		
Gene/feature annotation	0	Ensembl Transcripts release 93	
Back Finish			

Figure 5. UMI deduplication dialog if UMIs and barcodes were processed by Trim tags

If you select *Retain only one alignment per UMI*, you will be asked to choose an assembly and gene/feature annotation file. The annotation file is used to check whether a read overlaps an exonic region. Only reads that have 50% overlap with an exon will be retained.

If you do not select *Retain only one alignment per UMI*, UMI deduplication will proceed without filtering to exonic reads. Other differences between the two options are outlined in the UMI Deduplication in Partek Flow white paper.

Imported BAM

Imported BAMs generated by other tools can be imported into Partek Flow and deduplicated by the software. Additional options are available in the task configuration dialog to allow you to specify the location of the UMI and cell barcode information typically stored in the BAM header. Specify the BAM header tags in the text fields. For example, when processing a BAM file produced by CellRanger 3.0.1, the BAM identifier tag for the UMI sequence is *UR* and the BAM identifier for the barcode sequence is *CR* (Figure 2).

BAM UMI tag	0	UR				
BAM barcode tag	0	CR				
Retain only one alignment per UMI	0	*				
Select Annotation file						
Assembly Gene/feature annotation		Mus musculus (mouse) - mm10 🔹				
		Ensembl Transcripts release 94 •				
Back Finish						

Figure 6. Specify the location of the BAM UMI and barcode tags

The option to Retain only one alignment per UMI is also available when starting from a BAM file.

Deduplicate UMIs task report

The Deduplicate UMIs task report includes a knee plot showing the number of deduplicated reads per barcode. This plot is used to filter the barcodes to include only barcodes corresponding to cells. For more information about using the knee plot to filter barcodes, please see the Cell Barcode QA/QC page. One difference between the Deduplication report and the Cell Barcode QA/QC report is that the Deduplication report gives the number of initial alignments and the number of deduplicated alignments for each sample (Figure 3). This indicates how many of your aligned reads were PCR duplicates and how many were unique molecules.

The initial number of cells is set by our automatic filter. You can set the filter manually by clicking on the plot or by typing a cutoff number in the *Cells* or *Rea ds in cells* text boxes. If there are multiple samples, each sample receives a plot and filters are set per sample.



Figure 7. The deduplication report shows the number of UMIs per barcode

The number of cells, reads in cells, median reads per cell, number of initial alignments, and number of deduplicated alignments are listed for each sample in the summary table (Figure 4).

Back to filter									
Sample	Cells	Reads in cells	Median reads per cell	Initial alignments	Deduplicated alignments				
4K PBMC	4822	87.37 %	3884	436892846	23143405				
Apply filter Reset all filters									

Figure 8. Deduplication report summary table

Clicking Apply filter at either the knee plot or the summary table will run the Filter barcodes task and generate a Filtered reads data node.

To return to the knee plot, click Back to filter.

To reset the filters for all sample to the automatic cutoff, click Reset all filters.

Additional Assistance

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

