1. Alignement, filtering, umi counting and cell calling with CellRanger v5.0.0
2. Used isOutlier() from scuttle package (v1.2.1) for cell QC on umi counts, detected genes and mithochondrial percentage as well as ratio between umi counts and detected genes. The thresholds were computed on batch 3-5, as batch 6 had outlier values.

Thresholds:

* + 14.04 % mitochondrial genes
  + detected genes 453 7087
  + the sum of umi counts: 470 to 1.8624^{4} umi counts
  + ratio detected genes/ umi counts: 0.04565117 0.72692408

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | umi\_high | genes\_high | umi\_low | genes\_low | mt\_pct |
| Cells filtered | 2294 | 74 | 0 | 3562 | 13179 |
| Threshold | 18623.92 | 7087.371 | 470.3209 | 453.2256 | 14.04298 |

1. The cell QC was performed filtering out any gene not detected in at least two cells
   * The object had 32285 genes , 63430 cells before QC and 23155 genes , 45936 cells after filtering
2. Normalisation by deconvolution with scran (v1.20.1)
3. Feature selection: used modelGeneVar() from scran (v1.20.1), with density.weights=FALSE, to model the variance of the log-expression profiles. Then selected top 15% highly variable genes (2107 HVGs)
4. PCA linear dimensional reduction, keeping 25PCs for downstream analysis
5. Batch correction by mutual nearest neighbours, with fastMNN() from Batchelor (v1.8.0)
6. Graph based clustering with clusterCells() from scran (v1.20.1) with k= 60
7. Subset for clusters with highest expression of Oligodendrocyte markers such as Plp1, Mog, Mag and Mbp; and with no expression of markers from other celltypes (such as astrocyte or OPC markers)
8. Redo all steps from 2-8 with the oligos only.

2-3b) thresholds kept the same except minum umi 5000 umi counts and mt genes (10%)

before filtering: 23155 genes 16358 cells

after filtering : 19506 genes 13583 cells

4b) same

5b) 1374 HVG

6-7b) same

8b) k=100, merging clusters with similar profile expression to obtain a total of 4 clusters

1. Differential expression between cluster 1 (KO specific) and the mean expression of all other cells with FindMarkers() from Seurat (v4.1.0).(supplementary file)