

How To BatchLR

Introduction

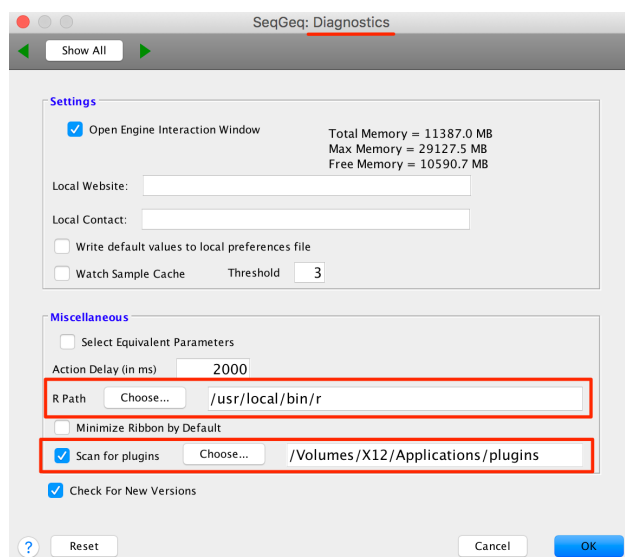
Batchelor implements a method for batch correction of single-cell (RNA sequencing) data. Batch effects refer to differences between data sets that are often an unavoidable product of differences in samples we wish to compare, including: time-points, laboratories, and even sequencing pipelines. The fastMNN method implemented in this plugin is based on detecting mutually nearest neighbors as well as simple sparsity-preserving translation of the population means. This approach does not rely on equal population compositions across batches, instead it requires that only a subset of the population be shared between batches. This allows the method to be scaled to large numbers of cells. The algorithm returns a matrix of corrected principal components that can be used for downstream analyses such as visualization and clustering, without altering the underlying data matrices. MNN batch effect correction has been implemented as an R/BioConductor library and published: Haghverdi L, Lun ATL, Morgan MD, Marioni JC (2018). “Batch effects in single-cell RNA-sequencing data are corrected by matching mutual nearest neighbors.” Nat. Biotechnol., 36(5), 421–427. <https://www.nature.com/articles/nbt.4091>.

We have developed a BatchLR plugin that integrates this functionality directly into SeqGeq. A video tutorial is available here: <https://tinyurl.com/BatchLR-v0-3-Demo>.

Please review FlowJo documentation for installing plugins <http://docs.flowjo.com/d2/plugins/installing-plugins/>.

Download and installation

1. Place the plugin .jar file in your Plugins folder, and direct SeqGeq to that folder using the Diagnostics section of the Preferences.
2. Make sure you have R installed and the R path is specified in the R Path field of the Diagnostics section of the Preferences.



3. The batchelor package needs to be installed and will run in the R environment. This plugin was tested in R version 3.6.0 & 3.6.1 and batchelor version 1.0.1. To install the required R packages, use the following commands in R:

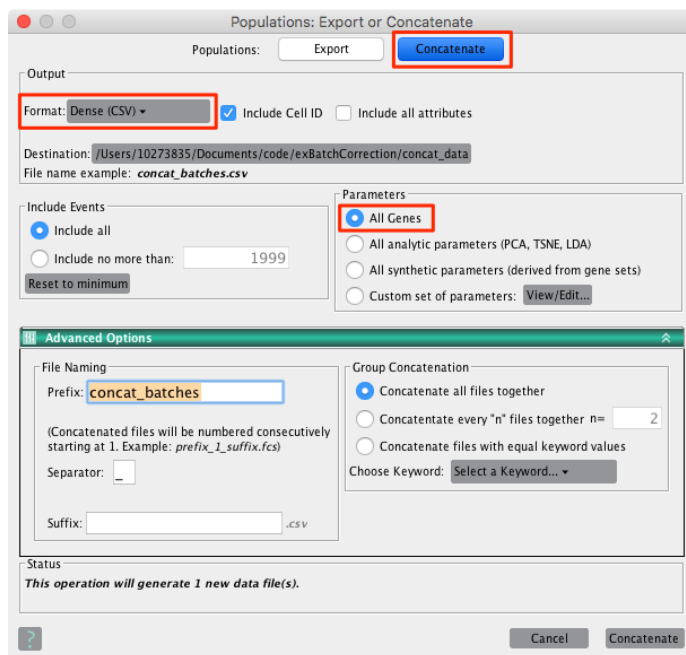
```
if (!requireNamespace("BiocManager", quietly = TRUE))  
  install.packages("BiocManager")  
BiocManager::install()
```

```
BiocManager::install(c("batchelor", "SingleCellExperiment", "scran", "scater"))
```

Note1: For older versions of R, please refer to the appropriate Bioconductor release: <https://bioconductor.org/about/release-announcements/>

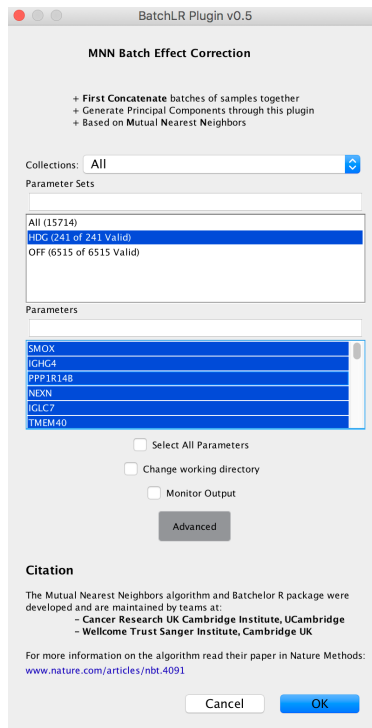
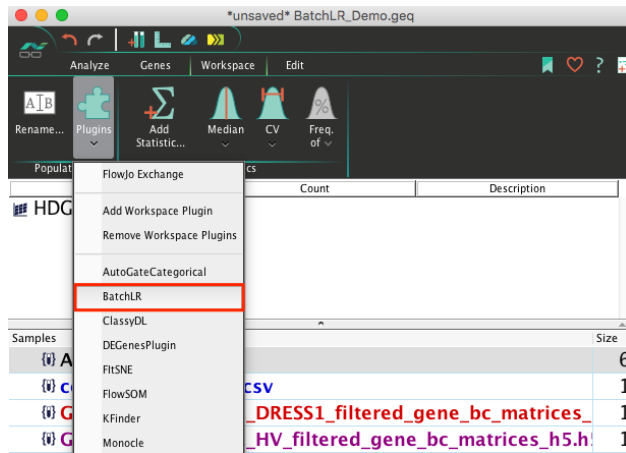
Usage

In order to run the BatchLR plugin you will first need to concatenate the different sample files together that you want to perform batch correction on. Use cmd-click(Mac) or Ctrl-click(Windows) to select multiple samples to concatenate. Right-click and choose Export/Concatenate Populations. In the resulting window, make sure to select 'All Genes', or a Custom geneset of interest:



Note: The order in which you add samples to the workspace will determine the order in which they are concatenated and assigned SampleIDs. The SampleID keywords will be used to create the batches to correct. The order in which the batches are corrected will affect the final results. The first batch is used as a reference batch against which the subsequent batches will be corrected. Corrected values of the second batch are added to the reference batch, against which the third batch is corrected, and so on.

To run the algorithm, select the concatenated data matrix, go to the Workspace tab of the SeqGeq workspace and select the **BatchLR** plugin from within the plugins drop-down menu. Note that plugins will be unavailable (greyed out) if no file is selected:



Note: We generally recommend a parameterset of the data's most high dispersed features to correct for batch effects, for best possible results downstream, based on preliminary testing.

Benchmarking

In **Fig1** batch effect correction performed between similar samples, human PBMC datasets, which were collected for entirely separate studies.

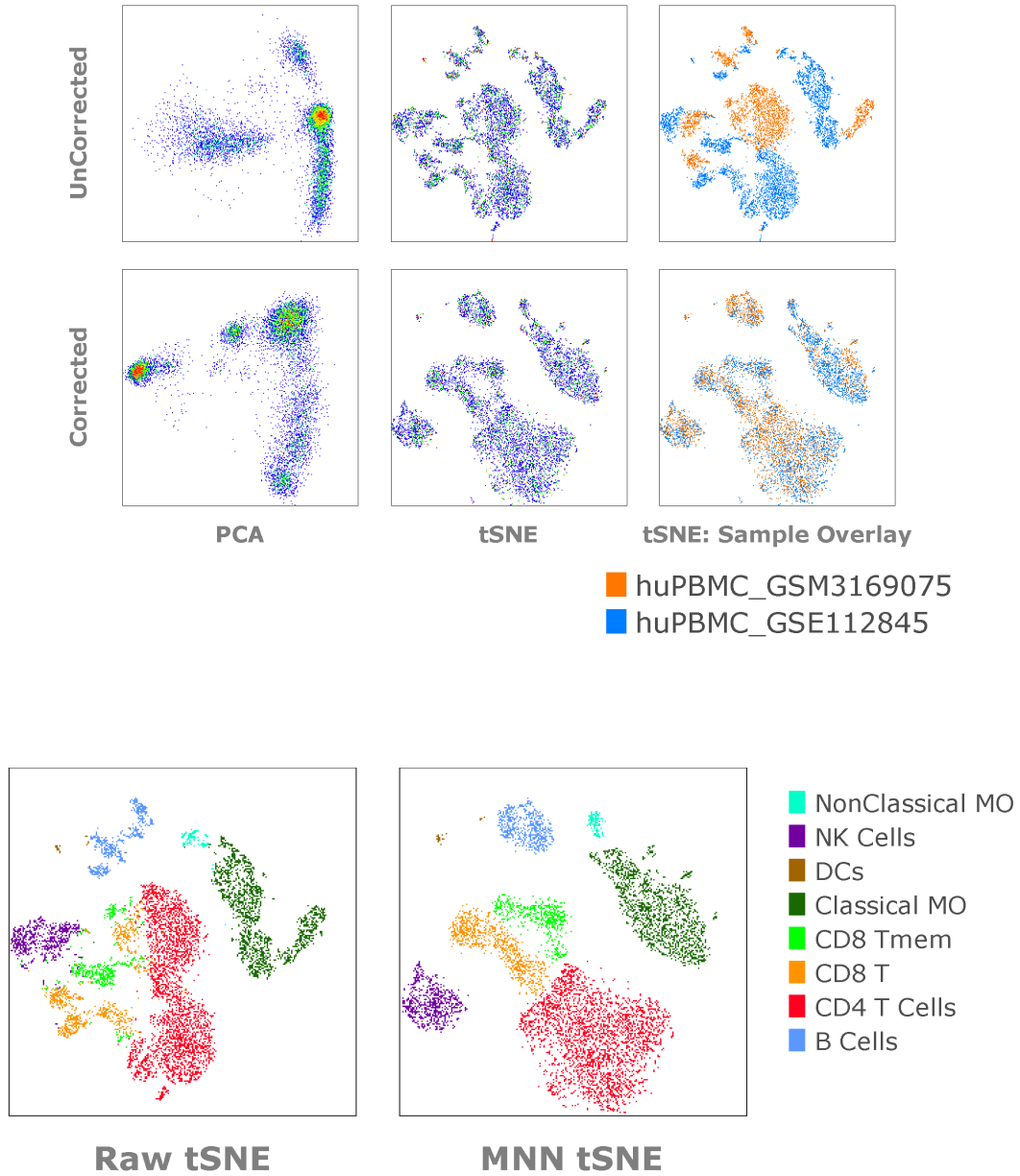


Figure 1 - Initial the combined data matrices show stark batch effects in raw PCA space, despite similar sample types, but these are nicely mitigated after MNN batch effect correction is applied.

Fig2 shows the subtle effect of batch effect correction when samples are placed in different order, by visualizing human Dendritic cells sequenced after enrichment by sorting in Flow Cytometry, combined with a dataset from total human PBMCs (PBMCs followed by DCs above, and DCs followed by PBMCs below):

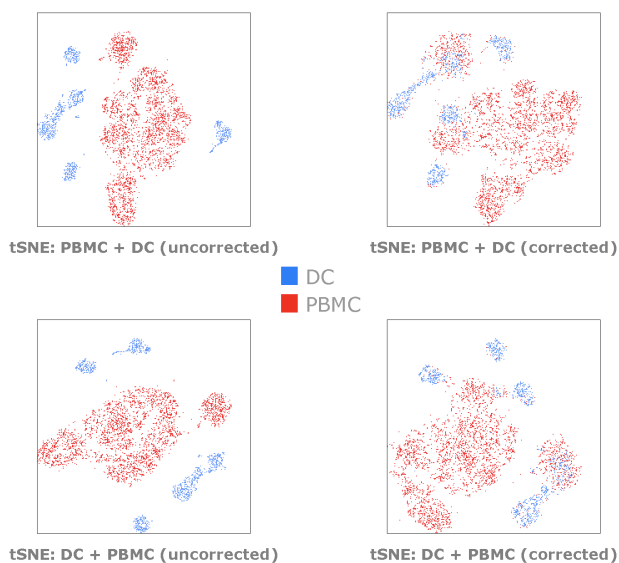


Figure 2 - The DC subsets are slightly better preserved and more easily distinguished when the PBMC batch is included first.

Fig3 illustrates the effect of batch correcting many samples (x5) from a variety of randomly selected data matrices including cutaneous T cells, monocytes, and different total PBMC files:

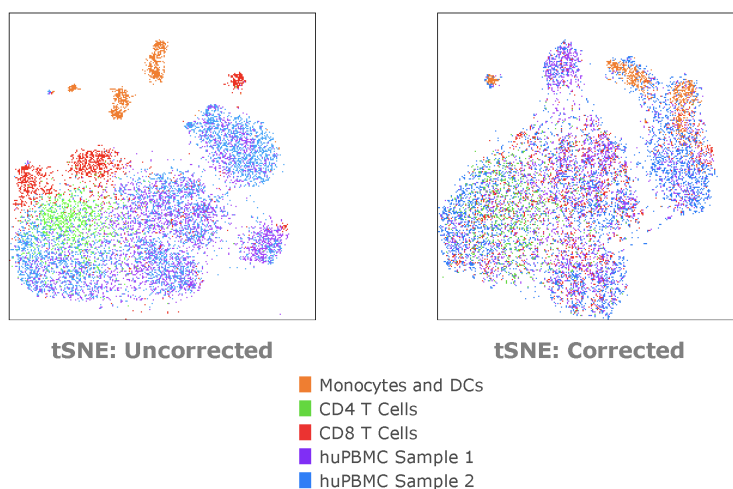


Figure 3 - Too many decidedly different sample batches, from different: Subjects, studies, and even sequencing technologies - can lead to poor quality corrections. In this case the distinction between different subsets appears to have become less clear-cut.

Statistical scores calculated per batch give some indication of the goodness of batch effect correction, **Table 1** (poor) and **Table 2** (good). By checking the **Variance Lost** as a result of the algorithm, accross batches comparatively, we can get some idea of the amount of biological signal that has been sacrificed. These scores can be viewed by accessing the terminal while BatchLR is running in SeqGeq:

```
10201397 — seqgeqJavaApplicationStub — java -cp ./Users/10201397/Docume...
OUTPUT>List of length 1
OUTPUT>names(1): corrected
OUTPUT>character-Rle of length 12104 with 5 runs
OUTPUT> Lengths: 5592 880 1482 3307 843
OUTPUT> Values : "1" "2" "3" "4" "5"
OUTPUT> Difference in variance lost between batches:
OUTPUT>      [,1]      [,2]      [,3]      [,4]      [,5]
OUTPUT>[1,] 0.018409027 0.020848848 0.023178995 0.01564450 0.010855206
OUTPUT>[2,] 0.007335017 0.009816779 0.008609919 0.00708773 0.006656182
OUTPUT>[3,] 0.010370027 0.010595828 0.017479040 0.01665410 0.005887924
OUTPUT>[4,] 0.011013407 0.011485461 0.011887923 0.01209539 0.012692322
OUTPUT> Large values, and major differences between batches indicates a loss of
true biological signal.
OUTPUT> Differences between the batch effect and biological signal are assumed t
o be orthogonal.(Haghverdi et al. 2018)
```

Table 1 - The table above is taken directly from batch effect correction shown in **Figure 3**, and is given in units: frequency of total variance.

```
10201397 — seqgeqJavaApplicationStub — java -cp ./Users/10201397/Docume...
OUTPUT> Difference in variance lost between batches:
OUTPUT>      [,1]      [,2]
OUTPUT>[1,] 0.0179505 0.006641005
OUTPUT> Large values, and major differences between batches indicates a loss of
true biological signal.
OUTPUT> Differences between the batch effect and biological signal are assumed t
o be orthogonal.(Haghverdi et al. 2018)
```

Table 2 - The table above was calculated for batch effect correction shown in **Figure 1**.

Leave us your feedback

Please write to seqgeqq@bd.com with any questions or concerns.