

## 1 Using Batch Effects Interface Corrections: EB

This document focuses on explaining the components of the Batch Effects Interface (BEI) involved with creating a job, loading data, and running assessments. This document will not address statistical issues or "how to spot" batch effects.

The URL for your install should be provided to you, but will likely be something like:

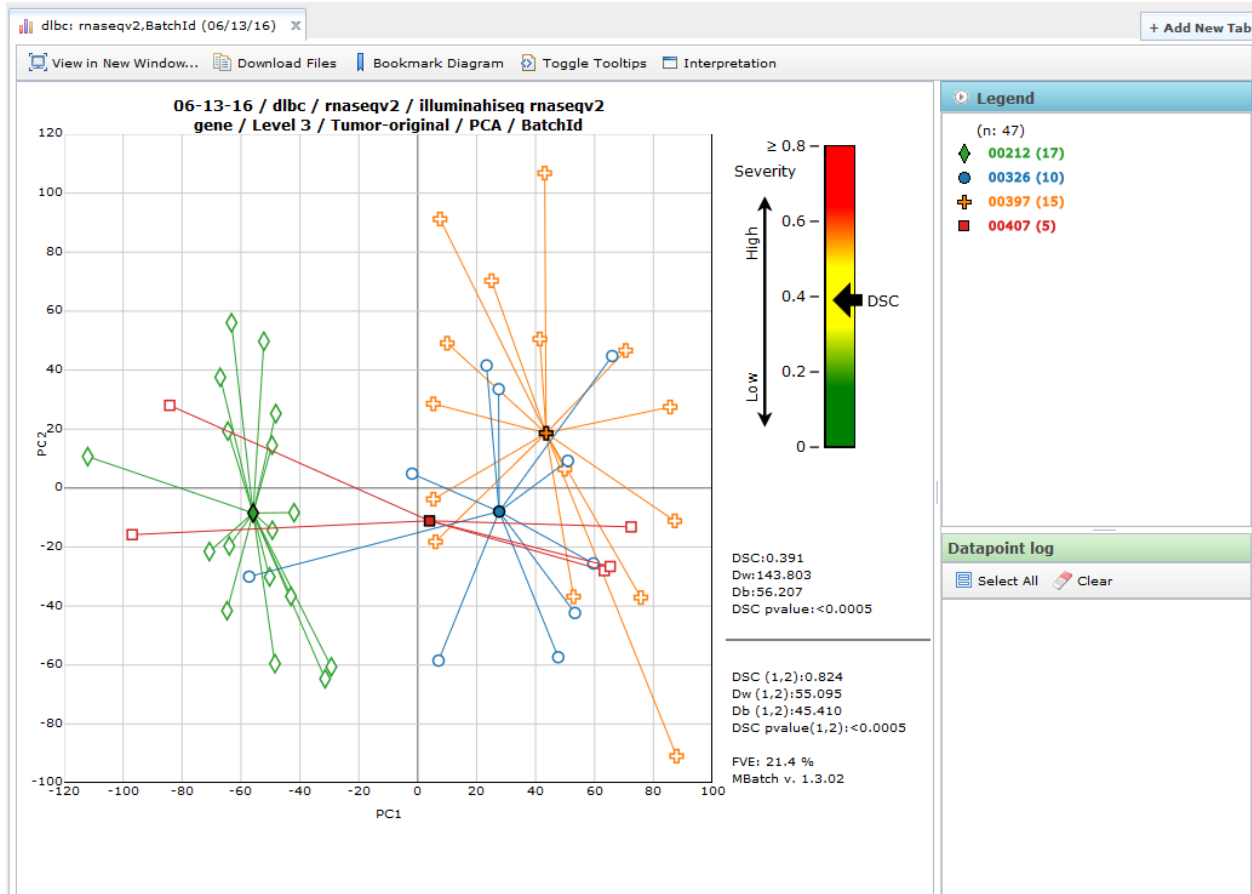
<http://your-server.your-company.com:9999/BatchEffectsInterface/>

These instructions are aimed at people familiar with R and familiar with TCGA/GDC platforms and data types. They are intended to introduce the reader to producing the given assessment. These instructions will only rarely, if ever, touch on the appropriateness of the assessment algorithm or interpretation of output. See MBatch\_01\_InstallLinux at <https://github.com/MD-Anderson-Bioinformatics/MBatch/tree/master/pdf> for instructions on downloading test data.

RBN corrections uses replicates between the two datasets to combine the two data sets based on replicates between sets.

For demonstrating Empirical Bayes (EB) corrections, we will use the DLBC RNASeq2 dataset found at: <http://bioinformatics.mdanderson.org/TCGA/databrowser> You can see the Batch Effects Assessment here: [http://bioinformatics.mdanderson.org/BatchEffects/index.jsp?path=%252F2016\\_06\\_13\\_0834-2016\\_08\\_16\\_1052%252Fdlbc%252Fmaseqv2%252Filluminahiseq\\_rnaseqv2\\_gene%252FLevel\\_3%252FTumor-original%252FPCA%252FShipDate%252FManyToMany&xaxis=PC1&yaxis=PC2](http://bioinformatics.mdanderson.org/BatchEffects/index.jsp?path=%252F2016_06_13_0834-2016_08_16_1052%252Fdlbc%252Fmaseqv2%252Filluminahiseq_rnaseqv2_gene%252FLevel_3%252FTumor-original%252FPCA%252FShipDate%252FManyToMany&xaxis=PC1&yaxis=PC2)

For this exercise, we have no idea if this is a real batch effect or biology, we are just using this as an example of removing a batch effects. You can see batch 00212 way to the left in green.



## 2 Starting a Job

See BEstack\_02A\_BEIUsingAssessments for more details about starting a job.

Use the "Start New Job" button and select "User Uploaded Data" for Step 1.a. Use the data matrix and batch file from the Standardized Data Browser website given above.

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MDAnderson Cancer Center Batch Effects Interface External 2018-04-25-1433  
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[Home](#) Job Id:1524771804717 Job State:NEWJOB\_PRIMARY\_DONE Job Message:Primary Data Available. Waiting for Secondary Data. [Edit Details](#)

✓ Step 1.a.: Specify Primary Dataset Complete

**Step 1.b.: Specify Secondary Dataset**  
Some correction algorithms use two datasets. Use the Proceed button if you do not need a second dataset.

User Uploaded Data [Proceed without Secondary Dataset](#)

**Use Data Uploaded**

Matrix File Upload: [Browse...](#) No file selected. [Upload Secondary Matrix](#)

Then for Step 1.b. select Proceed without Secondary Dataset.

### 3 Configuring Assessments

See BEstack\_02A\_BEIUsingAssessmentsExternal for more details about Configuring Assessments.

Below, we have selected Sample as the Sample Identifier, and selected BatchId as well as ShipDate as the assessment batch types. For Step 3, we have kept the defaults.

Note that the batch type you wish to correct must be one of the batch types checked.

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HomeJob Id:1524771804717Job State:MBATCHCONFIG\_STARTJob Message:MBatch Configuration in ProcessEdit Details

**Step 2: Select Batches**

Reset Defaults

Select Sample Identifier (Select Sample Id Batch Type): 

Sample

Selected Types for Batch Analysis: BatchId,ShipDate

Batch Type to Check	Batch Type Name	Batch Summary (Batch Name and Count)
<input checked="" type="checkbox"/>	BatchId	00212:17 00248:1 00326:10 00397:15 00407:5
<input type="checkbox"/>	PlateId	2213:17 2404:1 A31O:10 A38C:15 A39D:5
<input checked="" type="checkbox"/>	ShipDate	2012-03-14:17 2012-09-10:1 2013-07-24:10 2014-03-26:15 2014-04-30:5
<input type="checkbox"/>	TSS	FA - Asterand:9 FF - SingHealth:11 FM - International Genomics Consortium:1 G8 - Roswell Park:7 GR - University of Nebraska Medical Center (UNMC):6 GS - Fundacio Clinic per a la Recerca Biomedica:10 RQ - St Josephs Hospital AZ:3 VB - Global BioClinical - Georgia:1
<input type="checkbox"/>	Type	01:48

**Step 3: Select Filtering Options**

Reset Defaults

Auto-Filter to Maximum Number of Values:

Max Number of Values: 

4000000

Exclude User Specified Batches:

Batch Type for Exclusion: 

Select Optional Batch Type for Exclusion

#### 3.1 Step 4 Empirical Bayes

In Step 4, we begin by selecting Empirical Bayes-withNonParametricPriors as the optional Correction Type. (The setup for Empirical Bayes-withParametricPriors is the exact same.)

**Step 4: Select a Correction (optional)**

Reset Defaults

Correction Type: 

EmpiricalBayes-withNonParametricPriors

Correlation Density Plot (CDP) for Original versus Corrected Data:

☒ Select to generate CDP

Batch Type to Correct: 

BatchId

Minimum Valid Batch Size for Corrections: 

1

We accept the defaults for the rest of the data.

### 3.2 Do MBatch Run

See BEstack\_02A\_BEIUsingAssessments for more details about running and monitoring a run. We press Do MBatch Run from the configuration page.

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[Home](#) Job Id:1524771804717 Job State:MBATCHRUN\_START\_WAIT Job Message:MBatch Run Queued [Edit Details](#)

The MBatch run is queued and waiting for assignment to a processing node.

Here you can see the nonparametric adjustments being performed, which can take 30+ minutes.

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[Home](#) Job Id:1524771804717 Job State:MBATCHRUN\_RUNNING\_WAIT Job Message:MBatch Run in Progress [Edit Details](#)

The MBatch run is underway on a processing node.

Log File Tail (last 100 lines):

```
2018 04 26 19:50:59.439 DEBUG da7671a17f31 Check for genes with whole batch missing or no variation
2018 04 26 19:51:05.159 DEBUG da7671a17f31 Standardizing Data across genes
2018 04 26 19:51:05.172 DEBUG da7671a17f31 Standardization Model
2018 04 26 19:51:05.385 DEBUG da7671a17f31 stand.mean
2018 04 26 19:51:05.567 DEBUG da7671a17f31 Fitting L/S model and finding priors
2018 04 26 19:51:05.568 DEBUG da7671a17f31 no NAs
2018 04 26 19:51:07.712 DEBUG da7671a17f31 Find priors
2018 04 26 19:51:07.750 DEBUG da7671a17f31 Plot empirical and parametric priors
2018 04 26 19:51:07.752 DEBUG da7671a17f31 Find EB batch adjustments
2018 04 26 19:51:07.754 DEBUG da7671a17f31 Finding nonparametric adjustments
```

The DSC Permutations step will take some time, which can take 30+ minutes.

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Batch Effects Interface External 2018-04-24-1417

[Home](#) Job Id:1524669586504 Job State:MBATCHRUN\_RUNNING\_WAIT Job Message:MBatch Run in Progress [Edit Details](#)

The MBatch run is underway on a processing node.

Log File Tail (last 100 lines):

```
2018 04 25 15:44:37.058 DEBUG 1549a9e9f75d pvalueDSCwithExcerpt start
2018 04 25 15:44:37.059 DEBUG 1549a9e9f75d nrow(thePcaDataExcerpt)= 1815
2018 04 25 15:44:37.060 DEBUG 1549a9e9f75d ncol(thePcaDataExcerpt)= 1815
2018 04 25 15:44:37.061 DEBUG 1549a9e9f75d length(theBatchIdsForSamples)= 1815
2018 04 25 15:44:37.064 DEBUG 1549a9e9f75d getDSCwithExcerpt before java
2018 04 25 15:44:38.491 DEBUG 1549a9e9f75d getDSCwithExcerpt after java
2018 04 25 15:44:38.494 DEBUG 1549a9e9f75d pvalueDSCwithExcerpt after getDSCwithExcerpt
2018 04 25 15:44:38.496 DEBUG 1549a9e9f75d pvalueDSCwithExcerpt length(unique(theBatchIdsForSamples))= 38
2018 04 25 15:44:38.499 DEBUG 1549a9e9f75d pvalueDSCwithExcerpt call doDscPerms
2018 04 25 15:44:38.502 DEBUG 1549a9e9f75d doDscPerms before java
```

### 3.3 Finished Job

After the job has finished, use the Download option to get the corrected data.

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Home Job Id:1524771804717 Job State:MBATCHRUN\_END\_SUCCESS Job Message:MBatch Run Finished Successfully Edit Details

✓ **MBatch succeeded.**  
Download MBatch Results view on [the visualization website](#).

Log File Tail (last 100 lines):

```
2018 04 26 20:45:05.764 INFO da7671a17f31 CDP_Plot theData1UnmatchedReplicates= 0
2018 04 26 20:45:05.765 INFO da7671a17f31 CDP_Plot theData2UnmatchedReplicates= 0
2018 04 26 20:45:06.017 INFO da7671a17f31 CDP_Plot pairedCorr= 48
2018 04 26 20:45:06.018 INFO da7671a17f31 CDP_Plot unmatchedCorr= 0
2018 04 26 20:45:06.021 INFO da7671a17f31 CDP_Plot pairedDensity$x= 512
2018 04 26 20:45:06.022 INFO da7671a17f31 CDP_Plot pairedDensity$y= 512
2018 04 26 20:45:06.022 INFO da7671a17f31 CDP_Plot pairedDensity$bw= 0.000354899895588602
2018 04 26 20:45:06.023 INFO da7671a17f31 CDP_Plot unmatchedDensity$x= 0
2018 04 26 20:45:06.023 INFO da7671a17f31 CDP_Plot unmatchedDensity$y= 0
2018 04 26 20:45:06.024 INFO da7671a17f31 CDP_Plot unmatchedDensity$bw=
```

Click the Download MBatch Results button. Open or unzip the archive and enter the MBatch directory.

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Home Job Id:1524771804717 Job State:MBATCHRUN\_END\_SUCCESS Job Message:MBatch Run Finished Successfully Edit Details

✓ **MBatch succeeded.**  
Download MBatch Results view on [the visualization website](#).

Log File Tail (last 100 lines):

```
2018 04 26 20:45:05.764 INFO da7671a17f31 CDP_Plot theData1UnmatchedReplicates= 0
2018 04 26 20:45:05.765 INFO da7671a17f31 CDP_Plot theData2UnmatchedReplicates= 0
2018 04 26 20:45:06.017 INFO da7671a17f31 CDP_Plot pairedCorr= 48
2018 04 26 20:45:06.018 INFO da7671a17f31 CDP_Plot unmatchedCorr= 0
2018 04 26 20:45:06.021 INFO da7671a17f31 CDP_Plot pairedDensity$x= 512
2018 04 26 20:45:06.022 INFO da7671a17f31 CDP_Plot pairedDensity$y= 512
2018 04 26 20:45:06.022 INFO da7671a17f31 CDP_Plot pairedDensity$bw= 0.000354899895588602
2018 04 26 20:45:06.023 INFO da7671a17f31 CDP_Plot unmatchedDensity$x= 0
2018 04 26 20:45:06.023 INFO da7671a17f31 CDP_Plot unmatchedDensity$y= 0
2018 04 26 20:45:06.024 INFO da7671a17f31 CDP_Plot unmatchedDensity$bw=
```

Opening 1524771804717\_Results.zip

You have chosen to open:  
1524771804717\_Results.zip  
which is: Zip archive  
from: http://localhost:9999

What should Firefox do with this file?

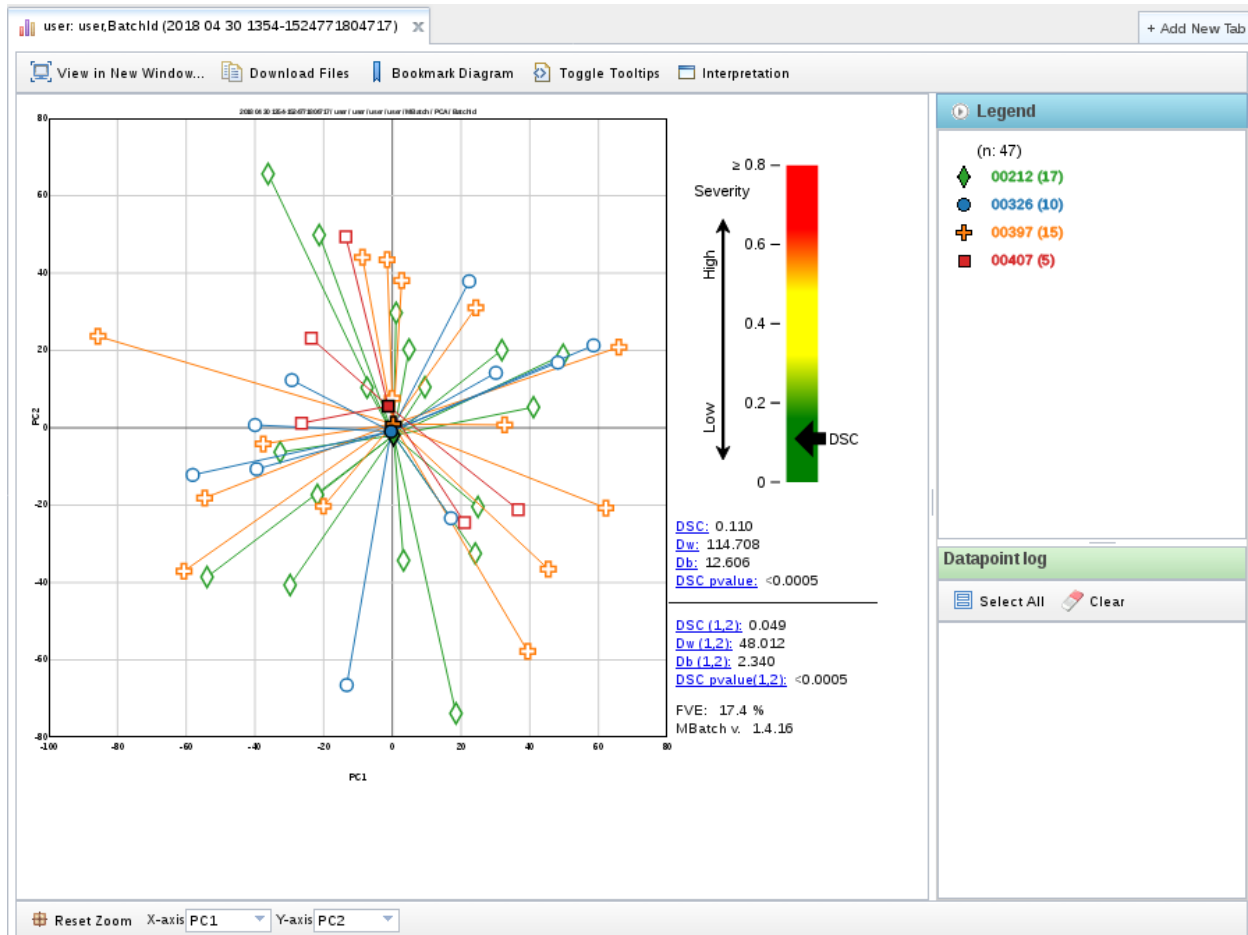
☐ Open with Xarchiver (default)

☒ Save File

☐ Do this automatically for files like this from now on.

Cancel OK

When we examine the PCA plot, we see the correction has been performed. (We will not comment on whether or not this has removed biological effects.)



The ANY\_Corrections-EBwithNonParametricPriors.tsv file contains the corrected data. Looking at an excerpt from that file below, you see the group ids have been added to the end of the sample ids (with a period to separate them).

	TCGA-FA-8693-01A-11R-2404-07	TCGA-FA-A4BB-01A-11R-A310-07	TCGA-FA-A4XK-01A-11R-A310-07	TCGA-FA-A6HN-01A-11R-A310-07
A1BG 1	8.408407463	7.833472256	7.944558757	9.107147164
A1CF 29974	-1.428007951	-1.390367717	-1.390367717	-1.390367717
A2BP1 54715	-0.954393532	-1.312007102	-1.312007102	-1.312007102
A2LD1 87769	7.282673978	7.749438326	8.210468997	7.323894016
A2ML1 144568	-0.044995866	-1.41702626	-1.41702626	-0.204374047
A2M 2	12.2499439	11.93860208	12.31336814	11.35818912
A4GALT 53947	9.414619035	7.713706942	8.516100679	7.895134388
A4GNT 51146	0.691938264	-1.900678582	-1.900678582	-1.900678582



The BatchData.tsv contains the combined batch files. Looking at an excerpt from that file below, you see the group ids have been added to the end of the sample ids (with a period to separate them).

Sample	BatchId	ShipDate
TCGA-FA-8693-01A-11R-2404-07	00248	2012-09-10
TCGA-FA-A4BB-01A-11R-A31O-07	00326	2013-07-24
TCGA-FA-A4XK-01A-11R-A31O-07	00326	2013-07-24
TCGA-FA-A6HN-01A-11R-A31O-07	00326	2013-07-24
TCGA-FA-A6HO-01A-11R-A31O-07	00326	2013-07-24
TCGA-FA-A7DS-01A-11R-A38C-07	00397	2014-03-26
TCGA-FA-A7Q1-01A-11R-A38C-07	00397	2014-03-26
TCGA-FA-A82F-01A-11R-A38C-07	00397	2014-03-26