

1 Using Batch Effects Interface Corrections: MP

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:

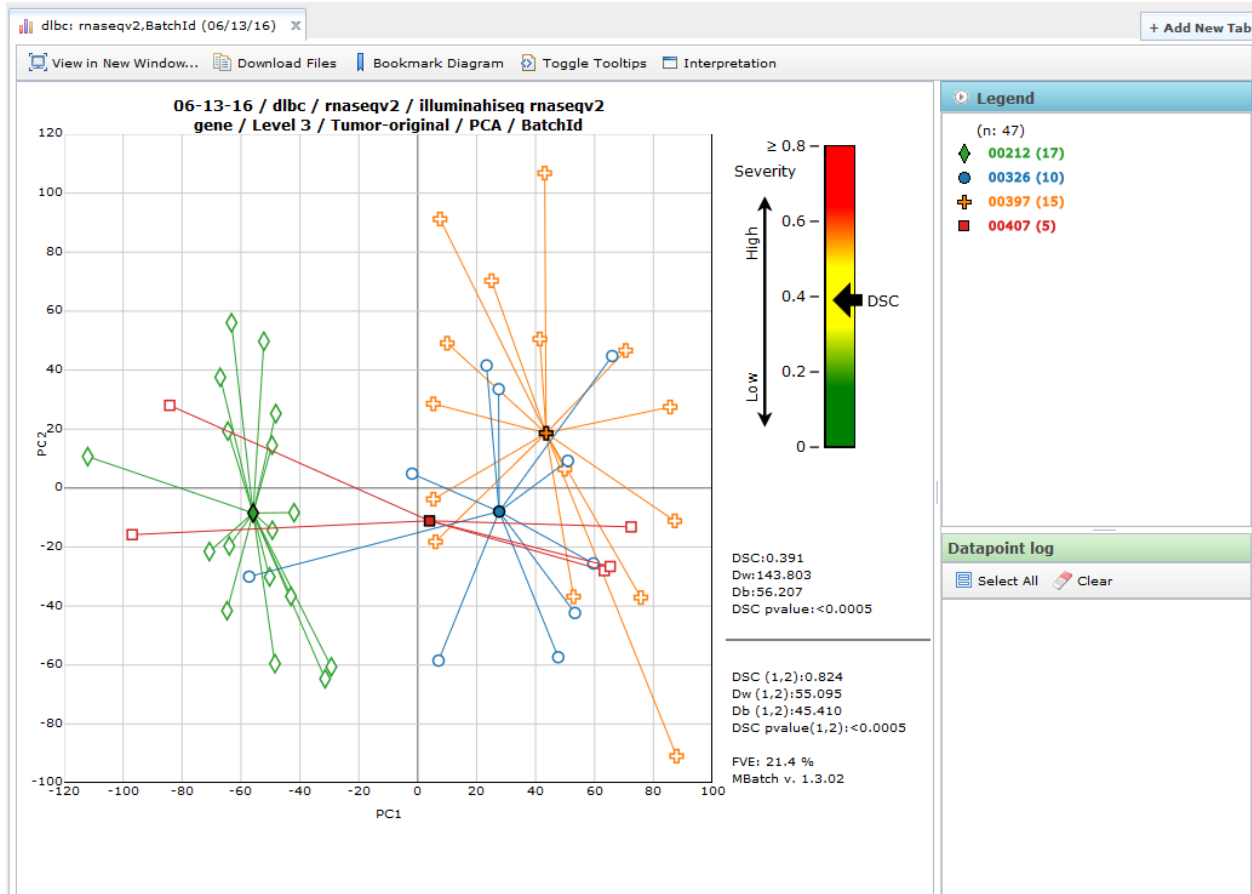
<http://your-server.your-company.com:9999/BatchEffectsInterface/>

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 Median Polish (MP) 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

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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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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Home Job Id:1524771804717 Job State:MBATCHCONFIG_START Job Message:MBatch Configuration in Process Edit Details

Step 2: Select Batches Reset Defaults
Select Sample Identifier (Select Sample Id Batch Type):
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:
Exclude User Specified Batches:
Batch Type for Exclusion:

3.1 Step 4 Median Polish Overall

In Step 4, we begin by selecting MedianPolish-Overall as the optional Correction Type. (The batch type to correct and minimum batch size are not appropriate for MP-Overall and will be removed.)

Step 4: Select a Correction (optional) Reset Defaults
Correction Type:
Correlation Density Plot (CDP) for Original versus Corrected Data:
☒ Select to generate CDP
Batch Type to Correct:
Minimum Valid Batch Size for Corrections:

We accept the defaults for the rest of the data.

3.2 Step 4 Median Polish By Batch

In Step 4, if we want “By Batch” instead, we select MedianPolish-ByBatch as the optional Correction Type. Since we want the problem within the Batch Id type corrected, we select that.

Step 4: Select a Correction (optional)

Correction Type: MedianPolish-ByBatch ?

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 ?

Reset Defaults

We accept the defaults for the rest of the data.

3.3 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.

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
```

3.4 Finished Job

After the job has finished, use the Download option to get the corrected data. Here, for reasons to be explained soon, we look at the MP-Overall Example.

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Home Job Id:1524774672723 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 20:49:09.204 INFO da7671a17f31 CDP_Plot theData1UnmatchedReplicates= 0
2018 04 26 20:49:09.204 INFO da7671a17f31 CDP_Plot theData2UnmatchedReplicates= 0
2018 04 26 20:49:09.286 INFO da7671a17f31 CDP_Plot pairedCorr= 48
2018 04 26 20:49:09.286 INFO da7671a17f31 CDP_Plot unmatchedCorr= 0
2018 04 26 20:49:09.290 INFO da7671a17f31 CDP_Plot pairedDensity$bx= 512
2018 04 26 20:49:09.291 INFO da7671a17f31 CDP_Plot pairedDensity$by= 512
2018 04 26 20:49:09.292 INFO da7671a17f31 CDP_Plot pairedDensity$bw= 0.0239307275960845
2018 04 26 20:49:09.292 INFO da7671a17f31 CDP_Plot unmatchedDensity$bx= 0
2018 04 26 20:49:09.293 INFO da7671a17f31 CDP_Plot unmatchedDensity$by= 0
2018 04 26 20:49:09.293 INFO da7671a17f31 CDP_Plot unmatchedDensity$bw=
```

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

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Home Job Id:1524774672723 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 20:49:09.204 INFO da7671a17f31 CDP_Plot theData1UnmatchedReplicates= 0
2018 04 26 20:49:09.204 INFO da7671a17f31 CDP_Plot theData2UnmatchedReplicates= 0
2018 04 26 20:49:09.286 INFO da7671a17f31 CDP_Plot pairedCorr= 48
2018 04 26 20:49:09.286 INFO da7671a17f31 CDP_Plot unmatchedCorr= 0
2018 04 26 20:49:09.290 INFO da7671a17f31 CDP_Plot pairedDensity$bx= 512
2018 04 26 20:49:09.291 INFO da7671a17f31 CDP_Plot pairedDensity$by= 512
2018 04 26 20:49:09.292 INFO da7671a17f31 CDP_Plot pairedDensity$bw= 0.0239307275960845
2018 04 26 20:49:09.292 INFO da7671a17f31 CDP_Plot unmatchedDensity$bx= 0
2018 04 26 20:49:09.293 INFO da7671a17f31 CDP_Plot unmatchedDensity$by= 0
2018 04 26 20:49:09.293 INFO da7671a17f31 CDP_Plot unmatchedDensity$bw=
```

Opening 1524774672723_Results.zip

You have chosen to open:

1524774672723_Results.zip
which is: Zip archive
from: http://localhost:9999

What should Firefox do with this file?

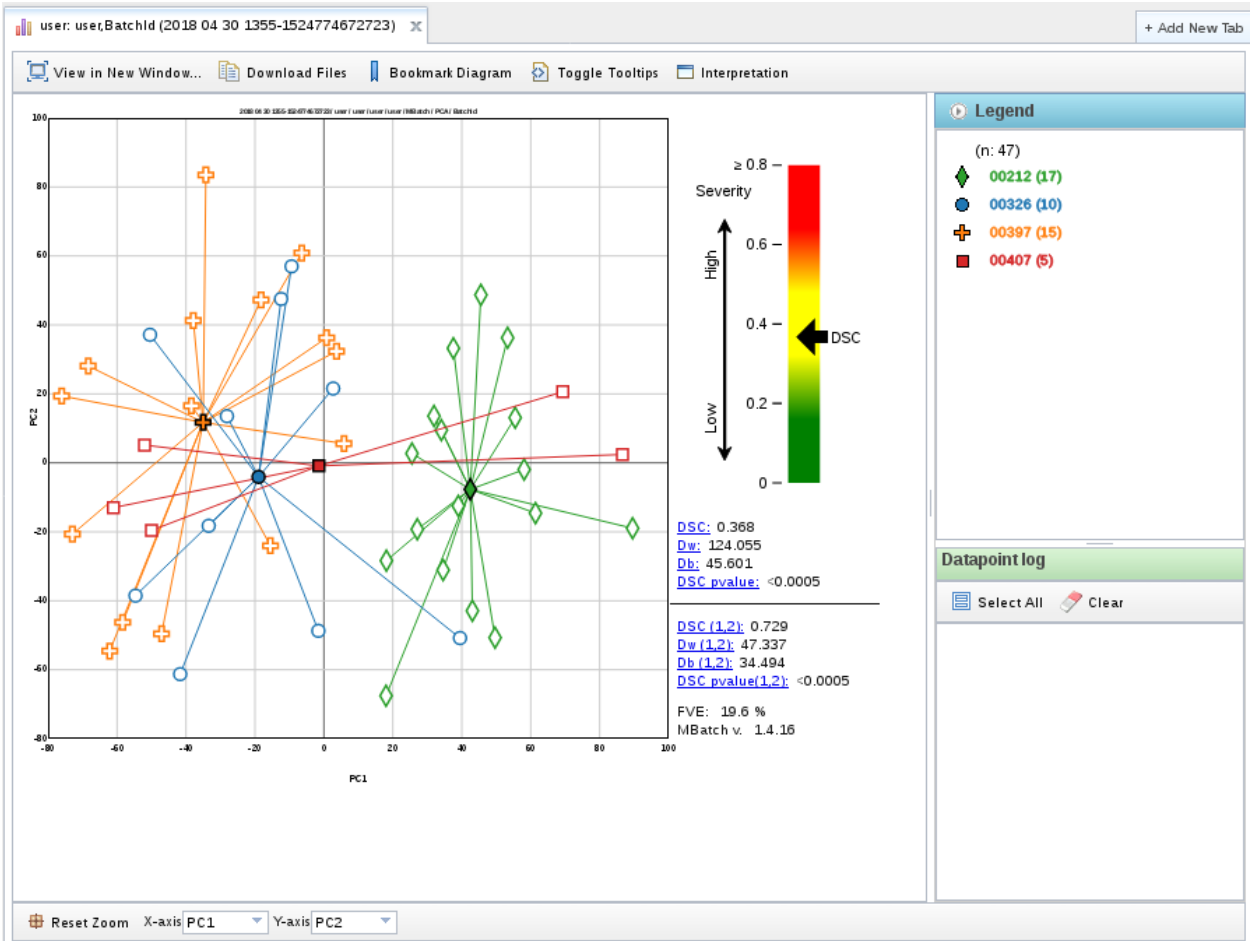
☐ Open with Xarchiver (default)

☒ 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 with minimal effectiveness. (We will not comment on whether or not this has removed biological effects.)



The ANY_Corrections-MPOverall.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 | 7.576146043 | 6.531136709 | 6.694689663 | 7.980712792 |
| A1CF 29974 | 6.98560411 | 6.945400497 | 6.98560411 | 6.980700672 |
| A2BP1 54715 | 6.98560411 | 6.945400497 | 6.98560411 | 6.980700672 |
| A2LD1 87769 | 6.371952521 | 7.069288154 | 7.663499093 | 6.593223945 |
| A2ML1 144568 | 7.703779796 | 6.386542207 | 6.42674582 | 7.705116548 |
| A2M 2 | 6.858983434 | 6.663633291 | 7.031545702 | 6.191399686 |
| A4GALT 53947 | 8.252804603 | 5.776492658 | 6.638236223 | 5.997549387 |
| A4GNT 51146 | 10.61079289 | 6.93980883 | 6.980012443 | 6.975109005 |

The BatchData.tsv contains the batch files. Looking at an excerpt from that file below, you see the batch data used.

| 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 |

3.5 Failed Job

Looking at the jobs, we notice the MB-ByBatch Example failed. Here, we will look at the log files.

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[Home](#) [Start New Job](#)

Job History:

| Job Id | Tag | Date Created | Message | Go to Job | Delete Job |
|---------------|--------------------|------------------------|----------------------------------|----------------------------|------------------------|
| 1524775346620 | ANOVA Example | 4/26/2018, 3:42:26 PM | MBatch Run Finished Successfully | Select Job | Delete |
| 1524775093543 | MB-ByBatch Example | 4/26/2018, 3:38:13 PM | MBatch Run Failed | Select Job | Delete |
| 1524774672723 | MP-Overall Example | 4/26/2018, 3:31:12 PM | MBatch Run Finished Successfully | Select Job | Delete |
| 1524771804717 | EB Example | 4/26/2018, 2:43:24 PM | MBatch Run Finished Successfully | Select Job | Delete |
| 1524687939743 | RBN Example | 4/25/2018, 3:25:39 PM | MBatch Run Finished Successfully | Select Job | Delete |
| 1524669586504 | EBNPlus Example | 4/25/2018, 10:19:46 AM | MBatch Run Finished Successfully | Select Job | Delete |
| 1524662045619 | User Data Example | 4/25/2018, 8:14:05 AM | MBatch Configuration in Process | Select Job | Delete |

The job status shows failed.

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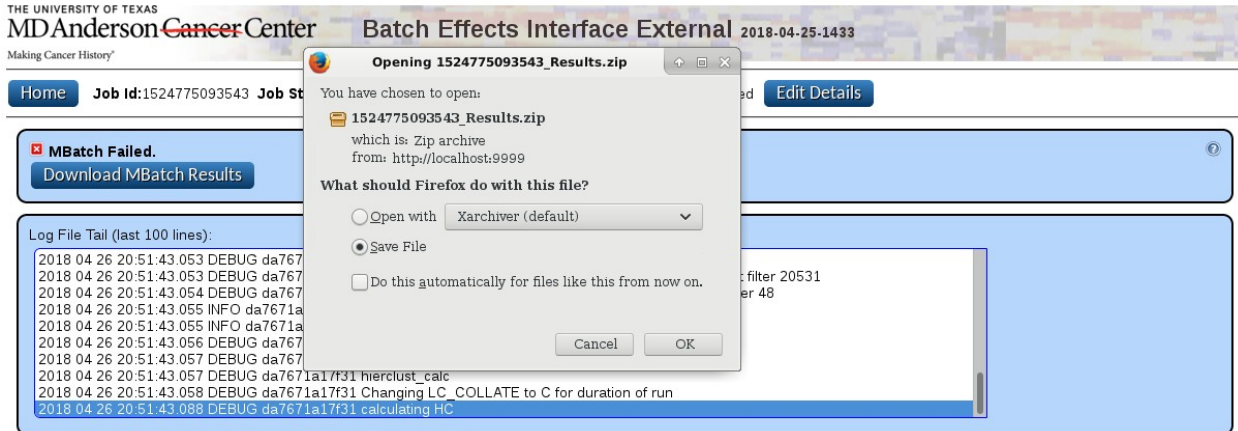
[Home](#) **Job Id:**1524775093543 **Job State:**MBATCHRUN_END_FAILURE **Job Message:**MBatch Run Failed [Edit Details](#)

✖ MBatch Failed.
[Download MBatch Results](#)

Log File Tail (last 100 lines):

```
2018 04 26 20:51:43.053 DEBUG da7671a17f31 rows post filter 20531
2018 04 26 20:51:43.053 DEBUG da7671a17f31 mbatchFilterData Prefilter, gene data had 20531 while post filter 20531
2018 04 26 20:51:43.054 DEBUG da7671a17f31 mbatchFilterData Prefilter, batch data had 48 while post filter 48
2018 04 26 20:51:43.055 INFO da7671a17f31 mbatchFilterData Finishing
2018 04 26 20:51:43.055 INFO da7671a17f31 ~~~~~
2018 04 26 20:51:43.056 DEBUG da7671a17f31 hierclust_outputGraphics
2018 04 26 20:51:43.057 DEBUG da7671a17f31 Changing LC_COLLATE to C for duration of run
2018 04 26 20:51:43.057 DEBUG da7671a17f31 hierclust_calc
2018 04 26 20:51:43.058 DEBUG da7671a17f31 Changing LC_COLLATE to C for duration of run
2018 04 26 20:51:43.088 DEBUG da7671a17f31 calculating HC
```

We download the results as before.



We open the archive and look at the mbatch.log file at the top level. At the end of that file we see these five lines:

```
2018 04 26 20:51:43.056 DEBUG da7671a17f31 hierclust_outputGraphics
2018 04 26 20:51:43.057 DEBUG da7671a17f31 Changing LC_COLLATE to C for duration of run
2018 04 26 20:51:43.057 DEBUG da7671a17f31 hierclust_calc
2018 04 26 20:51:43.058 DEBUG da7671a17f31 Changing LC_COLLATE to C for duration of run
2018 04 26 20:51:43.088 DEBUG da7671a17f31 calculating HC
```

So, we know that something happened within the hierarchical clustering calculations for this data set. This is generally a limitation of the data set itself (unsolvable solutions) or of the third party clustering algorithm. Generally, the “limitation” type errors are caught within the code and only cause a problem for the one diagram type. In this case, since there is no error message, the problem is most likely that that relatively unlimited parameters used to process the data used more than the 24GB available on the machine on which this was run and cause R to stop without notice.