User Manual for MEGANServer1.0

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1 Introduction

MEGANServer is a companion tool to MEGAN (Huson *et al.*, 2011) and allows researchers to outsource the storage of often very large metagenomic datasets to a dedicated server. For data transfer and accessibility we provide a RESTful API based on JSON. Furthermore, we extended MEGAN in that way that remote datasets can be accessed within MEGAN as simple as if they would be stored locally.

A test server has been installed with datasets extracted from a permafrost environment by (Mackelprang et al., 2011) and is available from http://megan-db.org/Permafrost/, user-Name=guest, password=guest.

2 Installation

MEGANServer is distributed as two different variants. Both version provide the same functionality but differ in how they are installed:

- **Standalone**: With the standalone version of MEGANServer we target users who would like to test the application locally. Besides the MEGANServer program this type also ships with a small application and web server (Jetty) and a script to start/stop MEGANServer out of the box.
- Web Archive: With this version we target users who would like to integrate MEGANServer in an existing web server/servlet engine environment such as Tomcat or Jetty.

2.1 Standalone

Prerequisites: MEGANServer requires an installed JAVA runtime, version 7 or 8.

Download MEGANServer_standalone.zip from http://ab.inf.uni-tuebingen.de/data/software/meganserver/download/MEGANServer_standalone.zip. The archive includes:

- **bin** folder with scripts to start and stop MEGANServer
 - start.sh: Script to start MEGANServer in Linux and OS X
 - startGUI.sh: Script to start MEGANServer with a graphical user interface in Linux and OS X
 - stop.sh: Script to stop MEGANServer in Linux and OS X
- lib folder with libraries
 - MEGANServer.war: The MEGANServer application
 - ServerStarter.jar: The graphical user interface to start MEGANServer

- jetty-runner-9.2.7.v20150116.jar: The web server which hosts the MEGANServer application
- commons-codec-1.6.jar: An additional library
- start.jar: An additional library
- TableLayout.jar: An additional library
- **properties** folder with configuration files. See Section 3
 - credentials.txt
 - log4j.properties
 - MEGANServer.properties
 - info.txt
- log folder where the application's log files are stored
- manual.pdf: This manual

The application can be started with the start.sh command. The server will then be accessible via http://localhost:8080/MEGANServer. To stop the server execute stop.sh. MEGANServer needs to know where on the file system the RMA files can be found. Please read Section 3.1 for configuration options.

Alternatively use the graphical starter by executing startGUI.sh (see Figure 1).

2.2 Web Archive

Prerequisites: MEGANServer requires an installed JAVA runtime (at least version 7). Furthermore a servlet container such as Tomcat or Jetty must be installed.

Download MEGANServer_webarchive.zip from http://ab.inf.uni-tuebingen.de/data/software/meganserver/download/MEGANServer_webarchive.zip. The archive includes:

- MEGANServer.war: The web archive containing the application and configuration files
- manual.pdf: This manual

The application has to be launched inside a servlet engine such as Tomcat (see http://tomcat.apache.org/) or Jetty (see: http://www.eclipse.org/jetty/). Configuration as described in Section 3 takes place after the first launch. Optionally unzip the war file and edit the config files in the WEB-INF/classes/ folder before the first launch.

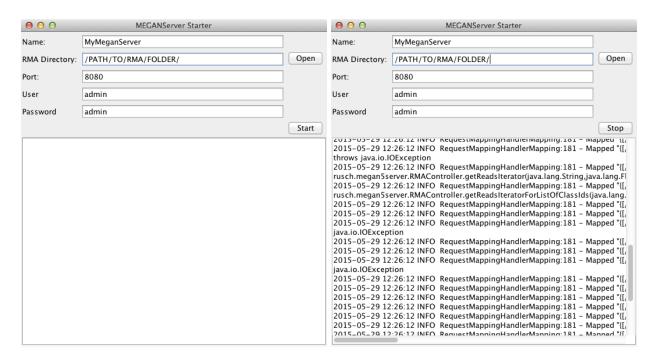


Figure 1: Graphical user interface to start MEGANServer. MEGANServer accessible under http://localhost:8080/MyMeganServer/

3 Configuration

3.1 Find metagenomic datasets

MEGAN operates on so called Read Match Archives (short RMA), a binary file format that incorporates reads, matches, metadata and classification data and is currently available in version 2 and 3. This file structure is also being used by MEGANServer in order to publish data via the web service. That is why MEGANServer must be started with a base directory which will then be recursively searched for RMA2 and RMA3 files. The base folder can be changed by modifying the entry rma.rootFolder in the MEGANServer.properties file. MEGANServer will then find RMA files in this folder and all subfolders and expose those via the web service. This property can be set before starting the server or while running. For the latter option one must then reload the properties file by calling the method described in 5.4.1.

3.2 Where to store the log files

Applicable only when using the webarchive version

MEGANServer creates by default a log file in the folder where the web application is launched. The location of this file can be changed by altering the property log4j.appender.file.File in the *log4.properties* file. This operation requires a restart of the web application.

3.3 Altering user credentials

The *credentials.txt* file contains information about all users that are allowed to access a MEGANServer instance. Its entries can be changed either by editing the file (md5 hashed) or via web service using the commands as described in Section 5.4.3.

The default user with administrative permissions has the username/password *admin*. It is advisable to change the password of this user before the first start of the server.

3.4 Information Text

The *info.txt* gives administrators the possibility to share information, such as what data is stored on the server and/or who to send an email for account requests. The *info.txt* can be found together with the other configuration files and can contain either plaintext or html.

4 MEGAN

The metagenomics analysis tool MEGAN (http://ab.inf.uni-tuebingen.de/software/megan5/) comes with a graphical user interface to connect and exchange data with MEGANServer. With this connection one can work on remote datasets just as if they would be present on a local hard drive. Please read the MEGANServer section in the MEGAN manual for further information.

5 MEGANServer REST API

5.1 Parameter

The API uses queries to retrieve data. The selection of data is performed by methods and parameters. Since some parameters are recurring in multiple methods we will introduce in this section the parameters and in the next section the methods.

5.1.1 Identifier

MEGANServer accesses datasets and data inside datasets with unique identifier. There are three types of identifier.

Dataset/File Identifier The dataset/file identifier uniquely identifies a single dataset on a MEGANServer instance. The parameter on the API is fileId and is either a number such as **217396908** or the path to the dataset on the server such as **core1_activelayer_day2.rma**

Read Identifier Each read inside a dataset has a numerical identifier. This identifier is unique inside a dataset but the identifier is not required to be unique among datasets. Two different datasets can have same read identifier. On the API we refer to the read identifier as readUid.

Match Identifier Each match inside a read has a numerical identifier. This identifier is unique inside a dataset but the identifier is not required to be unique among datasets. Two different datasets can have the same read identifier. On the API we refer to the match identifier as matchUid.

5.1.2 General Parameters

DataSelection When requesting reads or matches one can select which data fields should be returned. Currently following values are supported:

useRead, useReadName, useReadHeader, useReadSequence, useMateUId, useReadLength, useReadComplexity, useReadNumberOfMatches, useMatchText, useMatchIgnore, useMatchBitScore, useMatchLength, useMatchTaxonId, useMatchSeedId, useMatchKeggId, useMatchCogId, useMatchExpected, useMatchRefSeq

Therefore the parameter dataSelection="useReadName,useReadLength" would tell MEGANServer to return only the read name and the read length for a given query. If this parameter is skipped MEGANServer will use the default value which returns all possible data fields.

FindSelection and regEx MEGANServer supports searching for data matching regular expressions inside datasets. One has to specify where to search (FindSelection) and what to search for (regEx).

The FindSelection currently supports four data fields that can be searched:

useMatchText, useReadHeader, useReadName, useReadSequence

The regular expression can be any string or normal regular expression (See MEGAN manual for further information).

A possible combination of these both parameters which returns data on all sequences that look like "ACTG" would then be FindSelection="userReadSequence"®Ex="ACTG"

minScore and maxExpected These both parameters are meant to give users the possibility to filter matches by quality. The minScore parameter defines the minimal bitscore an alignment must have in order to appear in the results. The maxExpected value allows users to filter by E-Value. A possible parameter combination would be minScore="100"&maxExpected="0.0001".

The default values are for minScore 0 and for maxExpected 100000.

classification and classId(s) These both parameters are meant to give users the possibility to filter matches by classification and classification ids. MEGAN currently supports four classifications, namely NCBI taxonomy, SEED, COG and KEGG. Each of these classifications has identifier for e.g genes or taxa. Therefore the parameter classification="Taxonomy"&classId="562" will tell the executing method to only return reads that stem from E.coli.

5.2 General

5.2.1 help

· Name: help

• Outline: Get a list of all accessible commands of MEGANServer

Parameters

- No Parameters

• Examples:

```
- http://megan-db.org/Permafrost/
```

- http://megan-db.org/Permafrost/help

5.2.2 info

• Name: info

• Outline: Get information about the data stored on this MEGANServer instance

Parameters

- No Parameters

• Examples:

- http://megan-db.org/Permafrost/info

5.2.3 about

• Name: about

• Outline: Get a version number, contact information and build date of MEGANServer

Parameters

- No Parameters

• Examples:

- http://megan-db.org/Permafrost/About

5.3 Reading RMA files

5.3.1 listDatasets

• Name: listDatasets

• Outline: Retrieve a list of datasets that is known to the MEGANServer instance.

Parameters

- includeMetadata

* Name: includeMetadata

* Outline: include metadata to the result

* Required : false* Default Value : false

• Examples:

- http://megan-db.org/Permafrost/listDatasets
- http://megan-db.org/Permafrost/listDatasets?includeMetadata=True

5.3.2 getFileUid

• Name: getFileUid

• Outline: Get the unique identifier for a dataset

- Parameters
 - fileId

* Name: fileId (see 5.1.1)

* **Outline**: An identifier of a dataset. Either a path, a name or a number

* Required : true * Default Value : -

• Examples:

- http://megan-db.org/Permafrost/getFileUid?fileId=core1_activelayer_ day2.rma
- http://megan-db.org/Permafrost/getFileUid?fileId=217396908

5.3.3 getAuxiliary

- Name: getAuxiliary
- Outline: Get metadata and taxonomic/functional overview on a single dataset
- Parameters
 - fileId
 - * Name: fileId (see 5.1.1)
 - * Outline: An identifier of a dataset. Either a path, a name or a number
 - * Required : true* Default Value : -
- Examples:
 - http://megan-db.org/Permafrost/getAuxiliary?fileId=core1_activelayer_ day2.rma

5.3.4 getRead

- Name: getRead
- Outline: Get data on a single sequence together with all its information
- Parameters
 - fileId
 - * **Name**: fileId (see 5.1.1)
 - * Outline: An identifier of a dataset. Either a path, a name or a number
 - * Required : true
 - * Default Value : -
 - readUid
 - * Name: readUid (see 5.1.1)
 - * Outline: The numerical identifier of the read
 - * Required: true
 - * Default Value : 0
 - minScore
 - * Name: minScore (see 5.1.2)
 - * **Outline**: The minimal bitscore a match has to have in order to be present in the result
 - $* \ \textbf{Required}: false$
 - * **Default Value**: 0

- maxExpected

* Name: maxExpected (see 5.1.2)

* **Outline**: The maximal E-Value a match is allowed to have in order to be present in the result

* Required : false

* **Default Value**: 100000

- dataSelection

* Name: dataSelection (see 5.1.2)

* **Outline**: Definition of fields that should be present in the result

* Required : false

* **Default Value**: useRead,useReadName,useReadHeader,useReadSequence, useMateUId,useReadLength,useReadComplexity,useReadNumberOfMatches, useMatchText,useMatchIgnore,useMatchBitScore,useMatchLength, useMatchTaxonId,useMatchSeedId,useMatchKeggId,useMatchCogId,useMatchExpected, useMatchRefSeq

• Examples:

- http://megan-db.org/Permafrost/getRead?fileId=217396908&readUid=331
- http://megan-db.org/Permafrost/getRead?fileId=217396908&readUid=643&minScore=10
- http://megan-db.org/Permafrost/getRead?fileId=217396908&readUid=643&minScore=10&maxExpected=0.01&dataSelection="useReadName, useReadLength"

5.3.5 getAllReadsIterator

- Name: getAllReadIterator
- **Outline**: Get data of all reads of a single dataset. Returns one page of results and a link to the next page of results.

Parameters

- fileId

* Name: fileId (see 5.1.1)

* **Outline**: An identifier of a dataset. Either a path, a name or a number

* Required : true* Default Value : -

- minScore

* Name: minScore (see 5.1.2)

* **Outline**: The minimal bitscore a match has to have in order to be present in the result

* Required : false* Default Value : 0

- maxExpected

* Name: maxExpected (see 5.1.2)

* **Outline**: The maximal E-Value a match is allowed to have in order to be present in the result

* **Required** : false

* Default Value: 100000

- dataSelection

* **Name**: dataSelection (see 5.1.2)

* **Outline**: Definition of fields that should be present in the result

* Required : false

* **Default Value**: useRead,useReadName,useReadHeader,useReadSequence, useMateUId,useReadLength,useReadComplexity,useReadNumberOfMatches, useMatchText,useMatchIgnore,useMatchBitScore,useMatchLength, useMatchTaxonId,useMatchSeedId,useMatchKeggId,useMatchCogId,useMatchExpected, useMatchRefSeq

• Examples:

- http://megan-db.org/Permafrost/getAllReadsIterator?fileId=core1_ activelayer_day2.rma

5.3.6 getReadsIterator

• Name: getReadIterator

• **Outline**: Get data of all reads of a single dataset that are assigned to a node in the taxonomy or functional classification. Returns one page of results and a link to the next page of results.

Parameters

- fileId

* **Name**: fileId (see 5.1.1)

* Outline: An identifier of a dataset. Either a path, a name or a number

* Required : true* Default Value : -

- classId

* Name: classId (see 5.1.2)

* Outline: The id of a classification such as NCBI taxonomy, KEGG or SEED

* Required : true

* Default Value : -

- classification

* Name: classification (see 5.1.2)

* Outline: One of the classifications MEGAN supports

* Required : true* Default Value : -

- minScore

* Name: minScore (see 5.1.2)

* **Outline**: The minimal bitscore a match has to have in order to be present in the result

* Required : false* Default Value : 0

- maxExpected

* Name: maxExpected (see 5.1.2)

* **Outline**: The maximal E-Value a match is allowed to have in order to be present in the result

* Required : false

* Default Value: 100000

- dataSelection

* **Name**: dataSelection (see 5.1.2)

* **Outline**: Definition of fields that should be present in the result

* **Required** : false

* **Default Value**: useRead,useReadName,useReadHeader,useReadSequence, useMateUId,useReadLength,useReadComplexity,useReadNumberOfMatches, useMatchText,useMatchIgnore,useMatchBitScore,useMatchLength, useMatchTaxonId,useMatchSeedId,useMatchKeggId,useMatchCogId,useMatchExpected, useMatchRefSeq

• Examples:

- http://megan-db.org/Permafrost/getReadsIterator?fileId=core1_ activelayer_day2.rma&classification=Taxonomy&classId=14

5.3.7 getReadsForMultipleClassIds

- Name: getReadsForMultipleClassIds
- **Outline**: Get data of all reads of a single dataset that are assigned to a number of nodes in the taxonomy or functional classification. Returns one page of results and a link to the next page of results.

Parameters

- fileId

* **Name**: fileId (see 5.1.1)

* Outline: An identifier of a dataset. Either a path, a name or a number

* Required : true * Default Value : -

- classId

* Name: classId (see 5.1.2)s

* Outline: The ids of a classification such as NCBI taxonomy, KEGG or SEED

* Required : true* Default Value : -

- classification

* Name: classification (see 5.1.2)

* Outline: One of the classifications MEGAN supports

* Required : true * Default Value : -

- minScore

* Name: minScore (see 5.1.2)

* **Outline**: The minimal bitscore a match has to have in order to be present in the result

* Required : false* Default Value : 0

- maxExpected

* Name: maxExpected (see 5.1.2)

* **Outline**: The maximal E-Value a match is allowed to have in order to be present in the result

* Required : false

* Default Value: 100000

- dataSelection

* **Name**: dataSelection (see 5.1.2)

* **Outline**: Definition of fields that should be present in the result

* **Required**: false

* **Default Value**: useRead,useReadName,useReadHeader,useReadSequence, useMateUId,useReadLength,useReadComplexity,useReadNumberOfMatches, useMatchText,useMatchIgnore,useMatchBitScore,useMatchLength, useMatchTaxonId,useMatchSeedId,useMatchKeggId,useMatchCogId,useMatchExpected, useMatchRefSeq

• Examples:

- http://megan-db.org/Permafrost/getReadsForMultipleClassIds?fileId= corel_activelayer_day2.rma&classification=Taxonomy&classIds=14,-1

5.3.8 getFindAllReadsIterator

- Name: getFindAllReadIterator
- **Outline**: Get data of all reads of a single dataset and searches for text patterns. Those fulfilling the pattern are returned. Returns one page of results and a link to the next page of results.
- Parameters
 - fileId
 - * **Name**: fileId (see 5.1.1)
 - * Outline: An identifier of a dataset. Either a path, a name or a number
 - * **Required** : true
 - * Default Value : -
 - regEx
 - * **Name**: regEx (see 5.1.2)
 - * Outline: Regular expression that is being searched for
 - * Required : true
 - * Default Value : -
 - findSelection
 - * Name: findSelection (see 5.1.2)
 - * Outline: Where to search
 - * **Required** : false
 - * **Default Value**: useMatchText,useReadHeader,useReadName,useReadSequence
- Examples:
 - http://megan-db.org/Permafrost/getFindAllReadsIterator?fileId=core1_ activelayer_day2.rma®Ex=Bacteria&findSelection=useMatchText

5.3.9 loadPagedReads

- Name: loadPagedReads
- Outline: Load the next page of an readblockiterator
- Parameters

- pageId
 - * Name: pageId
 - * Outline: Next page Id. Retrieved from the previous page
 - * Required : true * Default Value : -
- **Examples**: Pages and pageIds are dynamic. This is why the link in the example will not work. Try to insert your own pageId.
 - http://megan-db.org/Permafrost/loadPagedReads?pageId=823880593

5.3.10 getNumberOfReads

- Name: getNumberOfReads
- Outline: Get number of reads for one dataset
- Parameters
 - fileId
 - * Name: fileId (see 5.1.1)
 - * Outline: An identifier of a dataset. Either a path, a name or a number
 - * **Required**: true
 - * Default Value : -
- Examples:
 - http://megan-db.org/Permafrost/getNumberOfReads?fileId=217396908

5.3.11 getNumberOfMatches

- Name: getNumberOfMatches
- Outline: Get number of alignments for one dataset
- Parameters
 - fileId
 - * Name: fileId (see 5.1.1)
 - * Outline: An identifier of a dataset. Either a path, a name or a number
 - * Required : true * Default Value : -
- Examples:
 - http://megan-db.org/Permafrost/getNumberOfMatches?fileId=217396908

5.3.12 getAllClassificationNames

- Name: getAllClassifcationNames
- Outline: Get the names of all classifications present in this dataset
- Parameters
 - fileId
 - * Name: fileId (see 5.1.1)
 - * Outline: An identifier of a dataset. Either a path, a name or a number
 - * Required : true * Default Value : -
- Examples:
 - http://megan-db.org/Permafrost/getAllClassificationNames?fileId=core1_ activelayer_day2.rma

5.3.13 getClassSize

- Name: getClassSize
- Outline: Get the number of reads assigned to one node in a classification
- Parameters
 - fileId
 - * Name: fileId (see 5.1.1)
 - * Outline: An identifier of a dataset. Either a path, a name or a number
 - * **Required**: true
 - * Default Value : -
 - classId
 - * Name: classId (see 5.1.2)
 - * Outline: The id of a classification such as NCBI taxonomy, KEGG or SEED
 - * **Required**: true
 - * Default Value : -
 - classification
 - * Name: classification (see 5.1.2)
 - * Outline: One of the classifications MEGAN supports
 - * Required : true
 - * Default Value : -
- Examples:

- http://megan-db.org/Permafrost/getClassSize?fileId=core1_activelayer_ day2.rma&classification=Taxonomy&classId=14

5.3.14 getClassificationBlock

- Name: getClassificationBlock
- Outline: Get the number of reads assigned to all nodes in a classification
- Parameters
 - fileId
 - * Name: fileId (see 5.1.1)
 - * Outline: An identifier of a dataset. Either a path, a name or a number
 - * **Required** : true
 - * Default Value : -
 - classification
 - * Name: classification (see 5.1.2)
 - * Outline: One of the classifications MEGAN supports
 - * Required : true * Default Value : -
- Examples:
 - http://megan-db.org/Permafrost/getClassificationBlock?fileId=core1_ activelayer_day2.rma&classification=Taxonomy

5.3.15 isReadOnly

- Name: isReadOnly
- **Outline**: Determines if user has write permissions. Currently all datasets are readOnly, no matter if a user is admin or not.
- Parameters
 - fileId
 - * **Name**: fileId (see 5.1.1)
 - * Outline: An identifier of a dataset. Either a path, a name or a number
 - * Required : true * Default Value : -
- Examples:
 - http://megan-db.org/Permafrost/isReadOnly?fileId=core1_activelayer_ day2.rma

5.3.16 getClassificationSize

- Name: getClassificationSize
- Outline: Get the number of nodes with reads in a classification
- Parameters
 - fileId
 - * Name: fileId (see 5.1.1)
 - * Outline: An identifier of a dataset. Either a path, a name or a number
 - * Required : true* Default Value : -
 - classification
 - * Name: classification (see 5.1.2)
 - * Outline: One of the classifications MEGAN supports
 - * Required : true* Default Value : -
- Examples:
 - http://megan-db.org/Permafrost/getClassificationSize?fileId=core1_ activelayer_day2.rma&classification=Taxonomy

5.4 Admin

Accessible with admin rights only!

5.4.1 admin/updateDatasets

- Name: admin/updateDatasets
- **Outline**: Reads the root location from the *MEGANServer.properties* file and updates the list of datasets
- Parameters
 - None
- Examples:
 - http://megan-db.org/Permafrost/admin/updateDatasets

5.4.2 admin/addUser

- Name: addUser
- Outline: Get the number of nodes with reads in a classification
- Parameters
 - userName
 - * Name: userName
 - * Outline: Name of the new user
 - * Required : true* Default Value : -
 - password
 - * Name: password
 - * Outline: password for the user
 - * Required : true* Default Value : -
 - isAdmin
 - * Name: isAdmin
 - * Outline: Determines if the new user should get admin rights
 - * Required : true* Default Value : -
- Examples:
 - http://megan-db.org/Permafrost/admin/addUser?userName=Megan&password= Server&isAdmin=false

5.4.3 admin/listUsers

- Name: listUsers
- Outline: Get the list of active users
- Parameters
 - No parameters
- Examples:
 - http://megan-db.org/Permafrost/admin/listUsers

5.4.4 admin/removeUser

• Name: admin/removeUser

• Outline: Delete user and revoke credentials

- Parameters
 - userName

* Name: userName

* Outline: Name of the new user

* Required : true* Default Value : -

- Examples:
 - http://megan-db.org/Permafrost/admin/removeUser?userName=Megan

5.4.5 admin/getLog

• Name: admin/getLog

• Outline: Returns log entries

- Parameters
 - No parameters
- Examples:
 - http://megan-db.org/Permafrost/admin/getLog

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References

Huson, D. H., Mitra, S., Weber, N., Ruscheweyh, H.-J., and Schuster, S. C. (2011). Integrative analysis of environmental sequences using MEGAN 4. *Genome Research*, **21**, 1552–1560.

Mackelprang, R., Waldrop, M., DeAngelis, K., David, M., Chavarria, K., Blazewicz, S., Rubin, E., and Jansson, J. (2011). Metagenomic analysis of a permafrost microbial community reveals a rapid response to thaw. *Nature*, **480**(7377), 368–371.