MuG - CHi-C Pipeline Documentation

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CHAPTER 1

Requirements and Installation

1.1 Requirements

1.1.1 Software

- Python 2.7.12+
- R >=3.1.2
- bedtools
- perl
- HiCUP
- bwa
- GEM
- TADbit
- samtools>1.3

1.1.2 Python Modules

- mg-tool-api
- pylint
- pytest
- pandas
- rtree

1.1.3 R Modules

- argparser
- devtools
- Chicago

To Run runChicago.py and process_runChicago.py, the R script runChicago.R from https://bitbucket.org/chicagoTeam/chicago/src/ceffddda8ea392a1e84e4db9593f8fc35ac88048/chicagoTools/?at=master should be downloded and added to PATH.

1.2 Installation

Directly from GitHub:

```
git clone https://github.com/pabloacera/C-HiC.git
```

Using pip:

```
pip install git+https://github.com/pabloacera/C-HiC.git
```

Install R modules, use the following R code:

install.packages("argparser") install.packages("devtools") library(devtools) install_bitbucket("chicagoTeam/Chicago", subdir="Chicago")

CHAPTER 2

Full Installation

The following document is for the full installation of all software required by the C-HiC module and all programmes that it uses. The document has been written with Ubuntu Linux, although many of the commands are cross platform (*nix) complient.

If you already have certain packages installed feel free to skip over certain steps. Likewise the bin, lib and code directories are relative to the home dir; if this is not the case for your system then make the required changes when running these commands.

2.1 Setup the System Environment

```
sudo apt-get install -y make build-essential libssl-dev zlib1g-dev       \\
libbz2-dev libreadline-dev libsqlite3-dev wget curl llvm libncurses5-dev \\
libncursesw5-dev xz-utils tk-dev unzip mcl libgtk2.0-dev r-base-core     \\
libcurl4-gnutls-dev python-rpy2 git libtbb2 pigz liblzma-dev libhdf5-dev \\
texlive-latex-base

cd ${HOME}
mkdir bin lib code
echo 'export PATH="${HOME}/bin:$PATH"' >> ~/.bash_profile
```

2.2 Setup pyenv and pyenv-virtualenv

This is required for managing the version of Python and the installation environment for the Python modules so that they can be installed in the user space.

```
git clone https://github.com/pyenv/pyenv.git ~/.pyenv
echo 'export PYENV_ROOT="$HOME/.pyenv"' >> ~/.bash_profile
echo 'export PATH="$PYENV_ROOT/bin:$PATH"' >> ~/.bash_profile
echo 'eval "$(pyenv init -)"' >> ~/.bash_profile
```

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```

# Add the .bash_profile to your .bashrc file
echo 'source ~/.bash_profile"' >> ~/.bashrc

git clone https://github.com/pyenv/pyenv-virtualenv.git ${PYENV_ROOT}/plugins/pyenv-virtualenv

pyenv install 2.7.12
pyenv virtualenv 2.7.12 C-HiC

# Python 3 environment required by iNPS
pyenv install 3.5.3
ln -s ${HOME}/.pyenv/versions/3.5.3/bin/python ${HOME}/bin/py3
```

2.3 Installation Process

2.3.1 bedtools and libspatialindex-dev

```
sudo apt-get install bedtools
sudo apt-get install libspatialindex-dev
```

2.3.2 Bowtie2 Aligner

```
cd ${HOME}/lib
wget --max-redirect 1 https://downloads.sourceforge.net/project/bowtie-bio/bowtie2/2.3.4/bowtie2-2.3.4-linux-x86_64.zip
unzip bowtie2-2.3.4-linux-x86_64.zip
```

2.3.3 HiCUP

```
 cd ${HOME}/lib
 wget https://www.bioinformatics.babraham.ac.uk/projects/hicup/hicup_v0.6.1.tar.gz
 tar -xzf hicup_v0.6.1.tar.gz
 cd hicup_v0.6.1
 chmod a+x *
```

2.3.4 BWA Sequence Aligner

```
cd ${HOME}/lib
git clone https://github.com/lh3/bwa.git
cd bwa
make
```

2.3.5 SAMtools

```
cd ${HOME}/lib
git clone https://github.com/samtools/htslib.git
cd htslib
autoheader
autoconf
./configure --prefix=${HOME}/lib/htslib
make
make install

cd ${HOME}/lib
git clone https://github.com/samtools/samtools.git
cd samtools
autoheader
autoconf -Wno-syntax
./configure --prefix=${HOME}/lib/samtools
make
make install
```

2.3.6 Install CHiCAGO

```
sudo apt-key adv --keyserver keyserver.ubuntu.com --recv-keys␣
↪E298A3A825C0D65DFD57CBB651716619E084DAB9
sudo add-apt-repository 'deb [arch=amd64,i386] https://cran.rstudio.com/bin/linux/
↪ubuntu xenial/'
sudo apt-get update -qq
sudo apt-get install r-base-core
sudo apt-get install python-rpy2


cd ${HOME}/lib
sudo apt-get install libtbb-dev
sudo apt-get install libssl-dev
cd ${HOME}/C-HiC/
echo "R_LIB=${HOME}/R" > ${HOME}/.Renviron
echo "options(repos = c(CRAN = 'http://mirrors.ebi.ac.uk/CRAN/'))" > ${HOME}/.Rprofile
echo ".libPaths('~/R')" >> ${HOME}/.Rprofile
echo 'message("Using library:", .libPaths()[1])' >> ${HOME}/.Rprofile
sudo Rscript sudo Rscript CHiC/tool/scripts/install_packages.R

cd ${HOME}/C-HiC/CHiC/tool/scripts/
wget https://bitbucket.org/chicagoTeam/chicago/raw/
↪e288015f75d36c5367d1595e0ac8099f2ce82aa1/chicagoTools/runChicago.R
wget https://bitbucket.org/chicagoTeam/chicago/raw/
↪e288015f75d36c5367d1595e0ac8099f2ce82aa1/chicagoTools/bam2chicago.sh
wget https://bitbucket.org/chicagoTeam/chicago/raw/
↪e288015f75d36c5367d1595e0ac8099f2ce82aa1/chicagoTools/makeDesignFiles.py
chmod +x bam2chicago.sh
```

2.4 Setup the symlinks

```
cd ${HOME}/bin



ln -s ${HOME}/lib/hicup_v0.6.1/* ${HOME}/bin/

ln -s ${HOME}/lib/bwa/bwa bwa

ln -s ${HOME}/lib/bowtie2-2.3.4-linux-x86_64/bowtie2 bowtie2
ln -s ${HOME}/lib/bowtie2-2.3.4-linux-x86_64/bowtie2-align-s bowtie2-align-s
ln -s ${HOME}/lib/bowtie2-2.3.4-linux-x86_64/bowtie2-align-l bowtie2-align-l
ln -s ${HOME}/lib/bowtie2-2.3.4-linux-x86_64/bowtie2-build bowtie2-build
ln -s ${HOME}/lib/bowtie2-2.3.4-linux-x86_64/bowtie2-build-s bowtie2-build-s
ln -s ${HOME}/lib/bowtie2-2.3.4-linux-x86_64/bowtie2-build-l bowtie2-build-l
ln -s ${HOME}/lib/bowtie2-2.3.4-linux-x86_64/bowtie2-inspect bowtie2-inspect
ln -s ${HOME}/lib/bowtie2-2.3.4-linux-x86_64/bowtie2-inspect-s bowtie2-inspect-s
ln -s ${HOME}/lib/bowtie2-2.3.4-linux-x86_64/bowtie2-inspect-l bowtie2-inspect-l

ln -s ${HOME}/lib/htslib/bin/bgzip bgzip
ln -s ${HOME}/lib/htslib/bin/htsfile htsfile
ln -s ${HOME}/lib/htslib/bin/tabix tabix


ln -s ${HOME}/lib/samtools/bin/ace2sam ace2sam
ln -s ${HOME}/lib/samtools/bin/blast2sam.pl blast2sam.pl
ln -s ${HOME}/lib/samtools/bin/bowtie2sam.pl bowtie2sam.pl
ln -s ${HOME}/lib/samtools/bin/export2sam.pl export2sam.pl
ln -s ${HOME}/lib/samtools/bin/interpolate_sam.pl interpolate_sam.pl
ln -s ${HOME}/lib/samtools/bin/maq2sam-long maq2sam-long
ln -s ${HOME}/lib/samtools/bin/maq2sam-short maq2sam-short
ln -s ${HOME}/lib/samtools/bin/md5fa md5fa
ln -s ${HOME}/lib/samtools/bin/md5sum-lite md5sum-lite
ln -s ${HOME}/lib/samtools/bin/novo2sam.pl novo2sam.pl
ln -s ${HOME}/lib/samtools/bin/plot-bamstats plot-bamstats
ln -s ${HOME}/lib/samtools/bin/psl2sam.pl psl2sam.pl
ln -s ${HOME}/lib/samtools/bin/sam2vcf.pl sam2vcf.pl
ln -s ${HOME}/lib/samtools/bin/samtools samtools
ln -s ${HOME}/lib/samtools/bin/samtools.pl samtools.pl
ln -s ${HOME}/lib/samtools/bin/seq_cache_populate.pl seq_cache_populate.pl
ln -s ${HOME}/lib/samtools/bin/soap2sam.pl soap2sam.pl
ln -s ${HOME}/lib/samtools/bin/varfilter.py varfilter.py
ln -s ${HOME}/lib/samtools/bin/wgsim wgsim
ln -s ${HOME}/lib/samtools/bin/wgsim_eval.pl wgsim_eval.pl
ln -s ${HOME}/lib/samtools/bin/zoom2sam.pl zoom2sam.pl
```

2.5 Prepare the Python Environment

2.5.1 Install APIs and Pipelines

Checkout the code for the DM API and the C-HiC pipelines:

```
cd ${HOME}/code
pyenv activate C-HiC
pip install git+https://github.com/Multiscale-Genomics/mg-dm-api.git
pip install git+https://github.com/Multiscale-Genomics/mg-tool-api.git
pip install git+https://github.com/Multiscale-Genomics/mg-process-fastq.git


git clone https://github.com/pabloacera/C-HiC.git
cd C-HiC
pip install -e .
pip install -r requirements.txt
pip install dill
```

CHAPTER 3

Pipelines

3.1 Map and parse CHi-C reads

This pipeline will take as input two fastq files, RE sites, the genome indexed with GEM and the same genome in FASTA file. This pipeline uses TADbit to map, filter and produce a bed file that will be used later on to produce bam file compatible with CHiCAGO algorithm. More information about filtering and mapping https://3dgenomes.github.io/TADbit/

3.1.1 Running from the command line

Parameters

config [str] Configuration JSON file

in_metadata [str] Location of input JSON metadata for files

out_metadata [str] Location of output JSON metadata for files

Returns

Wd [folders and files] path to the working directory where the output files are

Example

REQUIREMENT - Needs two fastq files single end, FASTA genome and bowtie2 indexed genome.

When running the pipeline on a local machine without COMPSs:

```
python process_hicup.py \
   --config tests/json/config_hicup.json \
   --in_metadata tests/json/input_hicup.json \
```

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```
   --out_metadata tests/json/output_hicup.json \
   --local
```

When using a local version of the [COMPS virtual machine](https://www.bsc.es/research-and-development/software-and-apps/software-list/comp-superscalar/):

```
runcompss                     \
   --lang=python              \
   --library_path=${HOME}/bin \
   --pythonpath=/<pyenv_virtenv_dir>/lib/python2.7/site-packages/ \
   --log_level=debug          \
   process_fastq2bed.py         \
      --config tests/json/config_hicup.json \
      --in_metadata tests/json/input_hicup.json \
      --out_metadata tests/json/output_hicup.json
```

3.1.2 Methods

class `process_hicup.`**`process_hicup`**(*configuration=None*)

This class run hicup tool which run hicup, doing the alignment and filtering of the reads and convert them into a BAM file.

run(*input_files*, *metadata*, *output_files*)

This is the main function that runs

Parameters

- **input_files** (*dict*) – fastq1 fastq2
- **metadata** (*dict*) –
- **output_files** (*dict*) –

  out_dir: str directory to write the output

Returns

- **results** (*bool*)
- **output_metadata** (*dict*)

3.2 Create CHiCAGO input RMAP

3.3 Create CHiCAGO input BAITMAP

3.4 Create CHiCAGO input Design files

This script use as input .rmap and .baitmap files and generate the Design files. NPerBin file (.npb): <baitID> <Total no. valid restriction fragments in distance bin 1> … <Total no. valid restriction fragments in distance bin N>, where the bins map within the "proximal" distance range from each bait (0; maxLBrownEst] and bin size is defined by the binsize parameter. NBaitsPerBin file (.nbpb): <otherEndID> <Total no. valid baits in distance bin 1> … <Total no. valid baits in distance bin N>, where the bins map within the "proximal" distance range from each other end (0; maxLBrownEst] and bin size is defined by the binsize parameter. Proximal Other End (ProxOE) file (.poe): <baitID> <otherEndID> <absolute distance> for all combinations of baits and other ends that map within the "proximal" distance range from

each other (0; maxLBrownEst]. Data in each file is preceded by a comment line listing the input parameters used to generate them.

3.4.1 Running from the command line

Parameters

config [str] Configuration JSON file

in_metadata [str] Location of input JSON metadata for files

out_metadata [str] Location of output JSON metadata for files

Returns

"nbpb" : .nbpb file "npb" : .npb file "poe" : .poe file

Example

REQUIREMENT - Needs RMAP and BAITMAP files

When running the pipeline on a local machine without COMPSs:

```
python process_design.py \
   --config tests/json/config_design.json \
   --in_metadata tests/json/input_design.json \
   --out_metadata tests/json/output_design.json \
   --local
```

When using a local version of the [COMPS virtual machine](https://www.bsc.es/research-and-development/software-and-apps/software-list/comp-superscalar/):

```
runcompss                      \
   --lang=python               \
   --library_path=${HOME}/bin \
   --pythonpath=/<pyenv_virtenv_dir>/lib/python2.7/site-packages/ \
   --log_level=debug           \
   process_design.py          \
      --config tests/json/config_design.json \
      --in_metadata tests/json/input_design.json \
      --out_metadata tests/json/output_design.json
```

3.4.2 Methods

class process_design.**process_design**(*configuration=None*)

This class generates the Design files and chinput files, imput for CHiCAGO. Starting from rmap and baitmap and capture HiC BAM files.

run(*input_files*, *metadata*, *output_files*)

Main function to run the tools, MakeDesignFiles_Tool.py and bam2chicago_Tool.py

Parameters

- **input_files** (*dict*) – designDir: path to the folder with .rmap and .baitmap files rmapFile: path to the .rmap file baitmapFile: path to the .baitmap file bamFile: path to the capture HiC bamfiles
- **metadata** (*dict*) – input metadata
- **output_files** (*dict*) – outPrefixDesign : Path and name of the output prefix, recommend to be the same as rmap and baitmap files. sample_name: Path and name of the .chinput file

Returns

- *bool*
- *output_metadata*

3.5 Convert BAM file into chicago input files .chinput

3.6 Data normalization and peak calling

This pipeline runs the normalization of the data and call the real chomatine interactions

3.6.1 Running from the command line

Parameters

config [str] Configuration JSON file

in_metadata [str] Location of input JSON metadata for files

out_metadata [str] Location of output JSON metadata for files

Returns

output_dir: directory with all output folders and files

Example

REQUIREMENT - Needs a reference genome

- **Needs file with the capture sequences with FASTA format**
  - settings file
  - **design dir:** .rmap .baitmap .npb .nbpb .poe

When running the pipeline on a local machine without COMPSs:

```
python process_run_chicago.py \
   --config tests/json/config_chicago.json \
   --in_metadata tests/json/input_chicago.json \
   --out_metadata tests/json/output_chicago.json \
   --local
```

When using a local version of the [COMPS virtual machine](https://www.bsc.es/research-and-development/ software-and-apps/software-list/comp-superscalar/):

```
runcompss                      \
   --lang=python               \
   --library_path=${HOME}/bin \
   --pythonpath=/<pyenv_virtenv_dir>/lib/python2.7/site-packages/ \
   --log_level=debug           \
   process_runChicago.py          \
      --config tests/json/config_chicago.json \
      --in_metadata tests/json/input_chicago.json \
      --out_metadata tests/json/output_chicago.json
```

3.6.2 Methods

class process_run_chicago.**process_run_chicago**(*configuration=None*)

Function for processing capture Hi-C fastq files. Files are aligned, filtered and analysed for Cpature Hi-C peaks

run(*input_files*, *metadata*, *output_files*)

This main function that run the chicago pipeline with runChicago.R wrapper

Parameters

- **input_files** (*dict*) – location with the .chinput files. chinput_file: str in case there is one input file chinput_file: comma separated list in case there is more than one input file.
- **metadata** (*dict*) – Input metadata, str
- **output** (*dict*) – output file locations

Returns

- **output_files** (*dict*) – Folder location with the output files
- **output_metadata** (*dict*) – Output metadata for the associated files in output_files

3.7 Run the entire CHi-C pipeline

CHAPTER 4

Tools for processing fastq C-HiC files

4.1 Map and parser reads

4.1.1 hicup_tool

class CHiC.tool.hicup_tool.**hicup**(*configuration=None*)

Tool to run hicup, from fastq to bam files

digest_genome(*genome_name*, *re_enzyme*, *genome_loc*, *re_enzyme2*)

This function takes a genome and digest it using a restriction enzyme specified

Parameters

- **genome_name** (*str*) – name of the output genome
- **re_enzyme** (*str*) – name of the enzyme used to cut the genome format example A^GATCT,BglII .
- **genome_loc** (*str*) – location of the genome in FASTA format
- **re_enzyme2** (*str*) – Restriction site 2 refers to the second, optional (other DNA shearing techniques such as sonication may be used) enzymatic digestion. This restriction site does NOT form a Hi-C ligation junction. This is the restriction enzyme that is used when the Hi-C sonication protocol is not followed. Typically the sonication protocol is followed.

static get_hicup_params(*params*)

Function to handle to extraction of commandline parameters and formatting them for use with hicup

Parameters **params** (*dict*) –

--bowtie	Specify the path to Bowtie
--bowtie2	Specify the path to Bowtie 2
--config	Specify the configuration file
--digest	Specify the digest file listing restriction fragment coordinates

--example	Produce an example configuration file
--format	Specify FASTQ format Options: Sanger, Solexa_Illumina_1.0, Illumina_1.3, Illumina_1.5
--help	Print help message and exit
--index	Path to the relevant reference genome Bowtie/Bowtie2 indices
--keep	Keep intermediate pipeline files
--longest	Maximum allowable insert size (bps)
--nofill	Hi-C protocol did NOT include a fill-in of sticky ends prior to ligation step and therefore FASTQ reads shall be truncated at the Hi-C restriction enzyme cut site (if present) sequence is encountered
--outdir	Directory to write output files
--quiet	Suppress progress reports (except warnings)
--shortest	Minimum allowable insert size (bps)
--temp	Write intermediate files (i.e. all except summaryfiles and files generated by HiCUP Deduplicator) to a specified directory
--threads	Specify the number of threads, allowing simultaneous processing of multiple files
--version	Print the program version and exit
--zip	Compress output

Returns

Return type list

hicup_alig_filt(***kwargs*)

This function aling the HiC read into a reference genome and filter them

Parameters

- **bowtie2_loc** –
- **genome_index** (*str*) – location of genome indexed with bowtie2
- **digest_genome** (*str*) – location of genome digested
- **fastq1** (*str*) – location of fastq2 file
- **fastq2** (*str*) – location of fastq2

Returns

Return type Bool

run(*input_files*, *input_metadata*, *output_files*)

Function that runs and pass the parameters for all the functions

Parameters

- **input_files** (*dict*) –
- **metadata** (*dict*) –

- **output_files** (*dict*) –

untar_index (***kwargs*)

Extracts the Bowtie2 index files from the genome index tar file. :param genome_file_name: Location string of the genome fasta file :type genome_file_name: str :param genome_idx: Location of the Bowtie2 index file :type genome_idx: str :param bt2_1_file: Location of the <genome>.1.bt2 index file :type bt2_1_file: str :param bt2_2_file: Location of the <genome>.2.bt2 index file :type bt2_2_file: str :param bt2_3_file: Location of the <genome>.3.bt2 index file :type bt2_3_file: str :param bt2_4_file: Location of the <genome>.4.bt2 index file :type bt2_4_file: str :param bt2_rev1_file: Location of the <genome>.rev.1.bt2 index file :type bt2_rev1_file: str :param bt2_rev2_file: Location of the <genome>.rev.2.bt2 index file :type bt2_rev2_file: str

Returns Boolean indicating if the task was successful

Return type bool

4.2 Create CHiCAGO input files

4.2.1 makeRmap

4.2.2 makeBaitmap

4.2.3 makeDesignFiles

class CHiC.tool.makeDesignFiles.**makeDesignFilesTool** (*configuration=None*)

Tool for makeing the design files as part of the input for Chicago capture Hi-C

static get_design_params (*params*)

This function handle chicago parameters, selecting the given ones and passing to the command line.

makeDesignFiles (***kwargs*)

make the design files and store it in the specify design folder. It is a wrapper of makeDesignFiles.py

Parameters

- **designDir** (*str,*) – Path to the folder with the output files(recommended the same folder as .map and .baitmap files).
- **parameters** (*dict,*) – list of parameter already selected by get_makeDesignFiles_params().

Returns

- *bool*
- **outFilePrefix** (*str*) – writes the output files in the defined location

run (*input_files*, *input_metadata*, *output_files*)

The main function to run makeDesignFiles.

Parameters

- **input_files** (*dict*) – designDir : path to the designDir containin .rmap and .baitmap files
- **input_metadata** (*dict*) –
- **output_files** (*dict*) –

outFilePrefix [path to the output folder and prefix name of files] example: "/folder1/folder2/prefixname". Recommended to use the path to designDir and the same prefix as .rmap and .baitmap

Returns

- **output_files** (*dict*) – List of location for the output files.
- **output_metadata** (*dict*) – List of matching metadata dict objects.

4.3 Convert bam files into chicago input

4.3.1 bam2chicago

4.4 Normalize data and call C-HiC peaks

4.4.1 run_chicago

class CHiC.tool.run_chicago.**ChicagoTool**(*configuration=None*)

tool for running the CHiCAGO algorithm

chicago(***kwargs*)

Run and annotate the Capture-HiC peaks. Chicago will create 4 folders under the outpu_prefix data : output_index.Rds –> chicago data saved on Rds format output_index_params.txt –> parameters used to run Chicago output_index.export_format –> chicago output in the chosen format diag_plots : 3 plots to assest the quality of the output (see CHicago Capture-HiC documentation for details) enrichment_data: files for the feature enrichment output (in case is used) examples: output_index_proxExamples.pdf: random chosen peaks showing interactions regions see http://regulatorygenomicsgroup.org/chicago for more information

Parameters

- **input_files** (*str ot comma separated list if there is more than one replicate*) –
- **output_prefix** (*str*) –
- **output_dir** (*str (whole path for the output)*) –
- **params** (*dict*) –

Returns writes the output files in the defined location

Return type bool

static get_chicago_params(*params*)

Function to handle to extraction of commandline parameters and formatting them for use in the aligner for BWA ALN

Parameters **params** (*dict*) –

Returns

Return type list

run(*input_files*, *input_metadata*, *output_files*)

The main function to run chicago for peak calling. The input files are .chinput and are transformed from BAM files using bam2chicago.sh input files could be just one file or a comma separated files from more than one biological replicate. Technical replicates should be pooled to one .chinput

Parameters

- **input_files** (*dict*) – list of .chinput files, or str with a single .chinput file
- **input_metadata** (*dict*) –
- **output_files** (*dict with the output path*) –

Returns

- **output_files** (*Dict*) – List of locations for the output files,
- **output_metadata** (*Dict*) – List of matching metadata dict objects

static untar_chinput (*chinput_tar*)

This function take as input the tar chinput

Parameters **chinput_tar** (*str*) – path to the tar file, the tar files should have the same prefix name as the tar file

Returns

Return type list of untar files

CHAPTER 5

Architectural Design Record

5.1 25-09-2018 handling_chr_header branch merge with master

This rmap_tool.py from this branch take the chromosome format from the used the reference genome and output a file with two columns, dictionary like with number of the chromosome and the name of the chromsome from the reference genome. example 1 chr1 2 chr2 3 chr3 ect…

This file is passed to the makeBaitmap.py script and generate the .batimap file with the corresponding chromsome name. This is necesary as the rtrees used in makeBaitmap.py needs an integer instead of "chr" or any other format.

5.2 15-10-2018 mm_mods_for_makebaitmaps branch merge with master

This branch contains some modifications from Mark to solve issues with pyCOMPSs regarding makeBaitmap.py tool

CHAPTER 6

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CHAPTER 7

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