# MuG - CHi-C Pipeline Documentation Release 0.1

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# Requirements and Installation

### 1.1 Requirements

### 1.1.1 Software

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

### 1.1.2 Python Modules

- mg-tool-api
- pylint
- pytest
- rtree
- pyenv and pyenv-virtualenv
- rpy2
- matplotlib
- pandas
- rtree

- numpy
- scipy

### 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("chicago", subdir="Chicago")

**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 zliblg-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
ceho 'export PYENV_ROOT="$HOME/.pyenv"' >> ~/.bash_profile
ceho 'export PATH="$PYENV_ROOT/bin:$PATH"' >> ~/.bash_profile
ceho 'eval "$(pyenv init -)"' >> ~/.bash_profile
```

```
# Add the .bash_profile to your .bashrc file
ccho '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
In -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 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
```

### 2.3.5 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/
12
   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 CHiC/tool/scripts/install_packages.R
   cd ${HOME}/C-HiC/CHiC/tool/scripts/
   wget https://bitbucket.org/chicagoTeam/chicago/raw/
19
   →e288015f75d36c5367d1595e0ac8099f2ce82aa1/chicagoTools/runChicago.R
   wget https://bitbucket.org/chicagoTeam/chicago/raw/
20
   \rightarrowe288015f75d36c5367d1595e0ac8099f2ce82aa1/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/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-1 bowtie2-build-1
12
   ln -s ${HOME}/lib/bowtie2-2.3.4-linux-x86_64/bowtie2-inspect bowtie2-inspect
13
   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
18
   ln -s ${HOME}/lib/htslib/bin/tabix tabix
19
20
21
   ln -s ${HOME}/lib/samtools/bin/ace2sam ace2sam
22
   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
31
   ln -s ${HOME}/lib/samtools/bin/plot-bamstats plot-bamstats
32
   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 --upgrade setuptools pip

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
```

**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 filein\_metadata [str] Location of input JSON metadata for filesout\_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 \
```

```
--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/):

### 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): <batil D> <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): <batil D> <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 filein_metadata [str] Location of input JSON metadata for filesout_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/output_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 filein_metadata [str] Location of input JSON metadata for filesout_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

### 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,BgIII.
- **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 co-

ordinates

--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 Maximum allowable insert size (bps) --longest --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 (params, genome\_digest, genome\_index, genome\_loc, fastq1, fastq2, outdir\_tar) 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

hicup\_alig\_filt\_runner(\*\*kwargs) This function runs the hicup\_alig\_filt

### **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, metadata, output files)

Function that runs and pass the parameters for all the functions

#### **Parameters**

- input\_files (dict) -
- metadata (dict) -
- output\_files (dict) -

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>.rev.1.bt2 index file :type bt2\_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

### 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 necessary 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

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