

Log of changes pipeline script

Блокнот: Laboratory Journal - Protein Kitchen

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Used OS :

```
> uname -a
```

```
Linux tux-meerkat 3.13.0-30-generic #55-Ubuntu SMP Fri Jul 4 21:40:53 UTC 2014 x86_64  
x86_64 x86_64 GNU/Linux
```

How to run script. Now just create a folder in your HOME directory where will locate all data. Named as "data". In this folder you could create three folders - "RUN", "OUT" and "SRC".

In RUN folder place the follow scripts: *DamID-seq_pipeline.sh*, *align_local_fq.sh*, *reads2GATC.sh*. And also .gff&config file: *parameterfile*, *DmelGATCfragments-r5_LP120507.gff*

Into *parameterfile* place this text:

```
> #by example, please change to your species
```

```
> SPECIES=fly
```

```
> FASTQ_FILES='~/data/SRC/*.fastq.gz'
```

```
> #by example, please change to your assembly
```

```
> ASSEMBLY=dm3
```

```
> OUTPUT_DIR=~/data/OUT
```

Please run the follow commands:

```
> cd ~/data/RUN
```

```
> chmod +x *.sh
```

```
> ./DamID-seq_pipeline.sh parameterfile
```

Then you need to install some program's:

```
> sudo apt-get update
```

```
> sudo apt-get install bowtie bowtie2 fastx-toolkit samtools python-htseq python-pip
```

Add this line to the end of file */etc/apt/sources.list*:

```
deb http://cran.r-project.org/bin/linux/ubuntu trusty/
```

Then:

```
> gpg --keyserver hkp://keyserver.ubuntu.com:80 --recv-keys E084DAB9
```

```
> gpg -a --export E084DAB9 | sudo apt-key add -
```

```
> sudo apt-get update
```

```
> sudo apt-get install r-base r-bioc-iranges r-bioc-biocgenerics parallel python-dev  
libevent-dev
```

```
> sudo pip install upgrade pip
```

```
> sudo pip uninstall cutadapt
```

Some changes that I made in the script *align_local_fq.sh*:

line 40-44:

```
BOWTIE2=bowtie2
CUTADAPT=cutadapt
FASTX_REVCOM=fastx_reverse_complement
BOWTIE2_INDEXES=/home/anton/data/indexes/
```

line 65-66: (remove -e option of 'echo' command)

```
ADPTR_SHORT_3=`echo ">\n${ADPTR_SHORT_5}" | ${FASTX_REVCOM} | awk 'NR > 1'`
ADPTR_LONG_3=`echo ">\n${ADPTR_LONG_5}" | ${FASTX_REVCOM} | awk 'NR > 1'`
```

line 114-120: (remove before tmp_* primary slash and point)**

```
TMP_BAM_INNER=`mktemp tmp_bam_inner.XXXXXXXXXX`
TMP_BAM_EDGE=`mktemp tmp_bam_edge.XXXXXXXXXX`
TMP_STATS_INNER=`mktemp tmp_stats_inner.XXXXXXXXXX`
TMP_STATS_EDGE=`mktemp tmp_stats_edge.XXXXXXXXXX`
TMP_FQ=`mktemp tmp_fq.XXXXXXXXXX`
TMP_FQ_EDGE=`mktemp tmp_fq_edge.XXXXXXXXXX`
TMP_FQ_INNER=`mktemp tmp_fq_inner.XXXXXXXXXX`
```

line 181: (commented line "\${CAT} \${IN_FQ}|", and changed variable)

```
${CUTADAPT} -g "${ADPTR_LONG_5}" -a "${ADPTR_LONG_3}" -O "${LEN_THRES}" --match-read-wildcards --discard-trimmed ${IN_FQ} -o "${TMP_FQ}" > ${CLIP_STATS}
```

line 193-196: (-n option. change the number of cores your processor)

```
#Get number of CPU Cores
CORE=$(lsncpu | grep 'CPU(s):' | sed -n '1p' | rev | cut -c 1)
#Run Bowtie
BOWTIE_PAR="-k 3 -p ${CORE} -t --phred33 --local -x ${BOWTIE2_INDEXES}${ASSEMBLY}"
```