

Tutorial for Cortex and Bubbleparse

Richard Leggett
richard.leggett@tgac.ac.uk

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1 Introduction

This tutorial will show you how to download the Cortex and Bubbleparse sources, how to compile the code, and how to find the SNPs between two different accessions of *Arabidopsis thaliana*.

Because of memory and processing requirements, we recommend that this tutorial is carried out on a cluster instead of a desktop computer.

2 Download and compile sources

1. Download Cortex Con sources from
https://sourceforge.net/projects/cortexassembler/files/cortex_con/.
2. Download Bubbleparse sources from
<https://github.com/richardmleggett/bubbleparse>.
3. Copy `bubbleparse.c` into the `src/util` directory of the Cortex sources.
4. To build Cortex, change into the `cortex_con` build directory (the one containing `Makefile` and the `src` directories) and type:

```
make MAXK=31 cortex_bub
```

Or, if building on Mac OS X:

```
make MAXK=31 MAC=1 cortex_bub
```

5. Assuming there are no compilation errors, a file called `cortex_con_31` will appear in the `cortex_con/bin` directory. You might like to copy this to another directory where you store tools.
6. Now to build Bubbleparse, type:

```
make MAXK=31 bubbleparse
```

Or, if building on Mac OS X:

```
make MAXK=31 MAC=1 bubbleparse
```

7. A file called `bubbleparse_31` will appear in the `cortex_con/bin` directory.

3 Download *Arabidopsis* reads

1. For this tutorial, we will use the Col-0 and Tsu-1 accessions of *Arabidopsis thaliana*.
2. Create a `data` directory somewhere to store the reads and cortex files.
3. Download two files of Col-0 reads from <http://www.ebi.ac.uk/ena/data/view/SRX000702>. You will end up with two files called `SRR013327.fastq` and `SRR013328.fastq`.
4. Download five files of Tsu-1 reads from <http://www.ebi.ac.uk/ena/data/view/SRX000704>. You will end up with files called `SRR013334.fastq`, `SRR013335.fastq`, `SRR013336.fastq`, `SRR013337.fastq`, `SRR013338.fastq`.

4 Merge reads and make CTX files

1. Within the data directory, create a file of files for the Col-0 reads. Copy the following into a text file called `col0reads.txt`:

```
SRR013327.fastq 0
SRR013328.fastq 0
```

2. Now create a file of files for the Tsu-1 reads. Copy the following into a text file called `tsu1reads.txt`:

```
SRR013334.fastq 0
SRR013335.fastq 0
SRR013336.fastq 0
SRR013337.fastq 0
SRR013338.fastq 0
```

3. We'll now create a single Cortex CTX file which includes all the Col-0 reads. Use the following command line:

```
cortex_bub_31 -k 21 -n 24 -b 55 -t fastq -c 100 -s 1 -i col0reads.txt
               -o col0.ctx -l col0.log
```

To execute this command, Cortex will require approximately 32Gb of RAM, so you may need to specify this as a parameter to your cluster's job submission system. On our cluster, this command took around 90 minutes to complete. On successful completion, Cortex will write a CTX file called `col0.ctx` and a log file called `col0.log`.

4. Now for the Tsu-1 reads. Use the following command line:

```
cortex_bub_31 -k 21 -n 24 -b 75 -t fastq -c 100 -s 1 -i tsu1reads.txt
               -o tsu1.ctx -l tsu1.log
```

To execute this command, Cortex will require approximately 32Gb of RAM. On our cluster, it took around 2 hours to complete. On successful completion, Cortex will write a CTX file called `tsu1.ctx` and a log file called `tsu1.log`.

5 Find bubbles

1. Create a new file of files called `col0tsu1files.txt` containing the following text:

```
col0.ctx 0
tsu1.ctx 1
```

2. To run Cortex bubble finding, type the following:

```
cortex_bub_31 -k 21 -n 25 -b 58 -t binary -w 1,200
               -i col0tsu1files.txt -f col0tsu1output -l col0tsu1.log
```

To execute this command, Cortex will require approximately 48Gb of RAM. On our cluster, it took around 3 hours to complete.

3. Cortex will write two files: `col0tsu1output.fasta` (containing contigs representing paths through bubbles) and `col0tsu1output.coverage` (containing the coverage of those paths).

6 Rank SNPs

1. We need to create an options file for bubbleparse. Create a new file called `boptions.txt` containing the following text:

```
EXPECTEDCOVERAGE "0,10,100,0"
EXPECTEDCOVERAGE "1,10,100,0"
MINIMUMCONTIGSIZE "100"
```

2. Now create a file of files called `allfiles.txt` containing ALL the input files, as follows:

```
SRR013327.fastq 0
SRR013328.fastq 0
SRR013334.fastq 1
SRR013335.fastq 1
SRR013336.fastq 1
SRR013337.fastq 1
SRR013338.fastq 1
```

3. Now to run bubbleparse, type:

```
bubbleparse_31 -k 21 -o boptions.txt -f col0tsu1output -i allfiles.txt
               -t table.txt -c table.csv -d bplog.txt -x
```

This should create a ranked table called `table.txt` and the same data represented in comma separated format called `table.csv`.

7 Further information

For further information, please refer to the Cortex and Bubbleparse manuals.

Please report any bugs or problems with the bubble finding options in Cortex, or with bubbleparse, to richard.leggett@tgac.ac.uk. General problems with Cortex should be referred to the appropriate authors.