Correcting reads with Blue (v1.0.1)

Blue is written in C#. This means that it will run natively on Windows, and can run under *mono* on Linux systems. Mono comes pre-installed on many Linux distributions, but can be downloaded from http://www.go-mono.com/mono-downloads/download.html and installed manually. Blue doesn't use any of the latest C# language features so it should be compatible with almost all current mono/Linux distributions. The following commands and examples are for running Blue and its related tools on Linux, and on Windows the 'mono' should be dropped (and the '.exe' is optional).

Tessel

Blue works by correcting reads using a k-mer consensus table (a set of k-mers and counts), and optionally a set of k-mer pairs. The set of kmers+counts is generated by running Tessel.

```
mono Tessel.exe -k k-merLength -g genomeLength [-t #threads]
                  [-f fasta|fastq] [-tmp tempDir] [-m minDepth]
                  cbtName readsPattern or list of reads files
```

-k	*kmerLength*	is the k-mer length to be used (normally 25 but it can be anything up to 32. Do not use k values less than 20 as Blue needs a fair degree of k-mer uniqueness).
-g	*genomeSize:*	is a guess at the size of the final (assembled) genome or genomes in the sample. This is used for the initial sizing of some arrays and any rough guess will probably be OK. *'-genome'* can also be used for this option.
-t	*noOfThreads*	is how many parallel threads to use for the tiling. This parameter is **optional** and 1 thread will be used by default. Currently around 6 is enough if you have that many processors available. *'-threads'* can also be used for this option.
-f	*reads format*	fasta or fastq. This parameter is **optional** and the file format will normally be determined by looking inside the first of the reads files. *'-format'* can also be used for this option.
-tmp	*tempDir*	is an **optional** parameter that names a directory used for the temporary k-mer singleton files. I added this parameter so I could place these files on an SSD and reduce the impact of the temp file writes on reading the sequence files. It should only be used if you have a physically separate disk drive to use for these temp files.
-m	*minDepth*	is an **optional** parameter that specifies the minimum depth needed before a k-mer (and its count) will be written to the output .cbt file. The default value for this parameter is 1, so all k-mers will be written out, regardless of how many times they appear in the reads files. '*-min*' can also be used for this option.
cbtName		is used to construct the output file names. The k-mer consensus will be written to outputcbtName_kmerLength.cbt (e.g. Cspor_25.cbt) and the k-mer repetition depth histogram will be saved as cbtName_kmerLength_histo.txt (e.g. Cspor_25_histo.txt). If this parameter is simply a name, such as 'Cspor', then the output files will be placed in the current directory. If it is a full file name or path, such as 'Healed/Cspor', then these files will be written to this directory.

reads pattern|FNs	these parameters specify the set of files to be tiled into k-mers. You either supply a list of space separated files names or a filename pattern. On Windows you would normally use a pattern and let Tessel turn it into a set of matching file names. On Linux, the same pattern will normally be turned into a list of file names by the shell, with equivalent results.

Example:

```
mono Tessel.exe -k 25 -g 6000000 -t 4 Cspor s_1_?_sequence.fastq

mono Tessel.exe -k 25 -g 6000000 -t 4 Healed/Cspor s_1_?_sequence.fastq
```

Blue can also take a 'pairs' file ('.prs'). These are pairs of short k-mers separated by a small gap, and effectively extend the view of the correction algorithm further than a single k-mer. Generating a 'pairs' file will be done by Tessel sometime soon, but before then you can use GenerateMerPairs. GenerateMerPairs will produce a pairs file with the same name as the .cbt file (except with '.cbt' replaced by '.prs')

Pairs should only be generated (and used) if there is enough depth of coverage in the sequence data. Do not use a pairs file if there is not approximately one pair for each base in the expected 'genome'. For example, for a read set corresponding to 6Mbp bacterial, you should be looking for at least 6M pairs. If there are fewer pairs than this, just delete the pairs file and Blue will do a better job of correcting the reads. The first of the numbers from the run statistics will tell you how much coverage you have: 'wrote 5475432/124412923 pairs' means that GenerateMerPairs saw 5475432 unique pairs.

```
mono GenerateMerPairs.exe [-t #threads] [-m minDepth]
                          cbtFN readsPattern or list of reads files
```

-t	*noOfThreads*	is how many parallel threads to use when generating the pairs. This parameter is **optional** and 1 thread will be used by default. Currently around 6-8 is enough if you have that many processors available. *'-threads'* can also be used for this option.
-m	*minDepth*	is an **optional** parameter that specifies the minimum depth needed before a k-mer (and its count) will be loaded into the k-mers table, and also the minimum depth needed for a k-mer within a pair. The default value for this parameter is 3. Use of this parameter can reduce the amount of memory needed by GenerateMerPairs. Setting the –min option for Tessel will have similar effects. '*-min*' can also be used for this option.
cbtFN		is the name of the .cbt file produced by Tessel from the set of reads specified by the next parameter.
reads pattern|FNs:		is a set of reads files, either a pattern or a list of file names.

Example:

```
mono GenerateMerPairs.exe -t 8 -m 10 Cspor_25.cbt s_1_?_sequence.fastq
mono GenerateMerPairs.exe -t 8 -m 10 Healed/Cspor_25.cbt s_1_?_sequence.fastq
```

Blue

Once you have a k-mer consensus file (.cbt), and optionally the set of corresponding k-mer pairs (.prs), you can go ahead and correct your reads using Blue.

```
mono Blue.exe [-r run] -m minReps [-f fasta|fastq] [-hp] [-good %]
              [-t threads] [-o outputDir]
              cbtFN readsPattern or list of reads files
```

-r	*runName*	is the name of this healing run. It is inserted into the names of each of the reads files to produce the name of the corresponding file of corrected reads. For example, the corrected form of 'ERR022075_1.fastq' will be 'ERR022075_1_runName.fastq'. This parameter is **optional**, and its default value is 'corrected_minReps'. *'-run'* can also be used for this option.
-s	*statsFN*	is the name of the file used to save the statistics from this run of Blue. This parameter is **optional** and by default Blue will generate a statistics file from the first reads file name parameter and the runName.
-m	*minReps*	is a k-mer repetition depth used (at times) to distinguish between 'good' and 'bad' k-mers. In general, this should be set to somewhere in the dip between the LHS error spike and the start of the Poisson curve derived from the good reads (more on this later). This value isn't very critical as the good/poor/bad depths are calculated dynamically for all reads if they contain any 'good' k-mers at all.
-f	*reads format*	fasta or fastq. This parameter is **optional** and the file format will normally be determined by looking inside the first of the reads files. *'-format'* can also be used for this option.
-hp		this option tells Blue to try correcting the end of every homopolymer run. These additional checks take more time, but produce better results for 454-like data.
-g	*%k-mers*	tells Blue to only save reads that look to be 'good' after correction. The '%k-mers' value specifies how many of the k-mers in a corrected read have to be above the 'good' threshold. Rejected reads are written to 'problems' files. Blue maintains the pairedness of its input files, and if one read of a pair fails the 'good' test, both the failing read and its pair will be dropped and written to the 'problems' file rather than the corrected reads files. This parameter is **optional**, and by default Blue will write all corrected reads to the output files. *'-good'* can also be used for this option.
-t	*noOfThreads*	is how many parallel threads to use for the tiling. This parameter is **optional** and 1 thread will be used by default. Currently around 6 is enough if you have that many processors available. *'-threads'* can also be used for this option.
-o	*outputDir*	specifies the output directory where Blue will write all its files, including the corrected reads. By default, these files are written to the directory where the reads were found. *'-output'* can also be used for this option.
cbtFN		is the name of the k-mer tiles file to be used (produced by Tessel). Blue will also look for a .prs file with same name and use it if it finds it.
reads pattern|FNs		these parameters specify the set of files to be tiled into k-mers. You either supply a list of space separated files names or a filename pattern. On Windows you would normally use a pattern and let Tessel turn it into a set of matching

file names. On Linux, the same pattern will normally be turned into a list of file names by the shell, with equivalent results.

Examples:

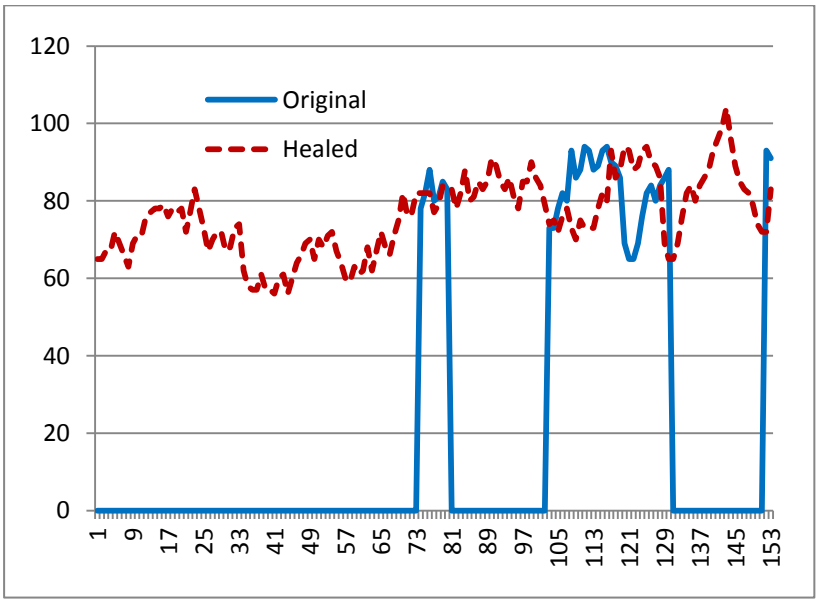
```
mono Blue.exe -m 50 -t 8 Cspor_25.cbt s_1_?_sequence.fastq

mono Blue.exe -r g80 -m 50 -t 8 -g 80 -o Healed
                 Healed/Cspor_25.cbt s_1_?_sequence.fastq
```

Setting the minReps parameter

Blue scans along each read, detecting broken k-mers by looking up their repetition depth in the k-mer consensus table produced by Tessel. Figure 1 shows the types of depths encountered along a real 454 read containing a few errors (blue line). The red line shows the depth of coverage for the read after correction. Blue examines each read and calculates two depths – an OK level for the read around 70-80 in this case; and a depth that indicates an error, around 20 here. This depth calculation can only be done if there are enough good k-mers in the read, and if there are not, Blue will use the average depth from the entire consensus to set the OK level, and the value specified for minReps for the error threshold.

The normal way to set the *minReps* parameter is to use the repetition depth histogram generated by Tessel. Figure 2 shows the histogram produced from tiling 16,000,000 Illumina reads for a 5Mbp organism. The Poisson-like curve represents the k-mers actually found in the genome of the organism being sequenced, and a *minReps* value of around 50 to 70 would be suitable for this dataset.

Figure 1: k-mer depths along a read



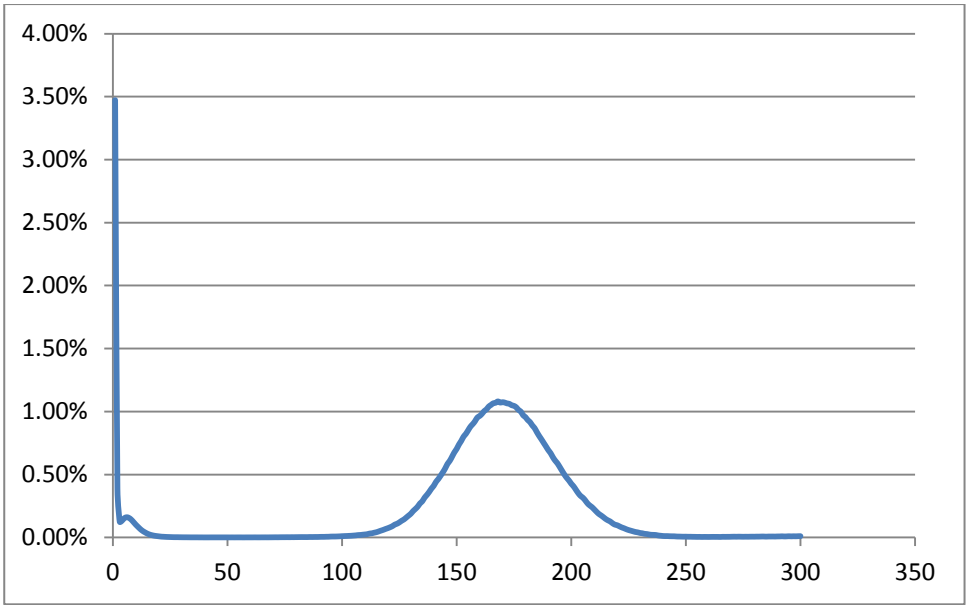


Figure 2: k-mer depth histogram

The curve shown in Figure 2 comes from a pure microbial sample – the simplest case. For diploid organisms, there will typically be two peaks, one representing homozygous k-mers, and the other heterozygous k-mers. The same procedure for choosing a *minReps* value applies though, just find somewhere in the valley between the error k-mer spike (around 1-2) and the first peak. For metagenomic datasets, there will typically be one or more peaks corresponding to the dominant organisms in the community, so just choose a minReps value somewhere on the left, and away from any peaks that may represent organisms of interest.