README CONDETRI

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CONDETRI

Summary

Trim FASTQ reads from the 3'-end and extract reads (or read pairs) of good quality. If the reads are paired, the filtering is done pairwise, and if one read in a pair has low quality, the remaining read is saved as single end.

Usage

```
perl condetri.pl -fastq1=file1 [-fastq2=file2 -prefix=s
-cutfirst=i -cutlast=i -rmN -notrim -hq=i -lq=i -frac=[0,1]
-lfrac=[0,1] -minlen=i -mh=i -ml=i -sc=i -pb=s]
```

Description

The trimming is performed in two steps:

(1) Trimming low quality bases from the 3'-end

(2) Overall quality check of read/pair

Details:

(1) Bases are removed from the 3'-end if the quality score is lower than some threshold (`hq`). When a base with higher quality is reached, it is kept temporarily and the preceding bases are considered. After this point, also bases with low quality can be saved, if they are surrounded by high quality bases. Up to `ml` consecutive low quality bases are saved temporarily, but if the following base also has low quality, all temporarily saved bases are removed, and the trimming starts all over again. The trimming stops either when finding `mh` consecutive high quality bases, or when the read is trimmed down to a certain length (`minlen`), whichever comes first.

(2) After the trimming step, the quality scores of the remaining read are controlled for. A read is approved if a certain fraction (`frac`) of the bases have a quality score higher than `hq`, and there is no base in the read that has a quality score below some lower bound (`lq`) OR – if `lfrac` is used – there is not more than a fraction `lfrac` bases with quality score less than the lower bound (`lq`). If the input is paired-end reads, both reads in a pair must be approved for keeping the pair. If only one of the reads is approved, it is saved in an additional, unpaired file which can be used as single-end data.

In the latest versions a number of additional parameters have been added, most of which operates on the reads *before* the above described trimming steps. `Cutfirst` (and `cutlast`) can remove a defined number of bases from the start (end), something that can be useful for HiSeq data that sometimes have an extreme quality drop in the first few bases from the 5' end. The `rmN` (remove N) parameter is used for removing Ns from the 5' end before the trimming (not useful in combination with the `cutfirst` parameter since those bases are removed anyway). Note that cutting bases might affect the performance of the quality trimming, since the `-minlen` will check the length of the read *after* cutting. If no cutting is done, `minlen=50` means that the trimming will continue at longest until position 51, while using `minlen=50` in combination with `cutfirst=10` means that the trimming will stop already at position 61. If the quality plot suggest that trimming down to pos 50 is a good idea, consider setting the `minlen` to 50-cutfirst.

If one wants to only remove a certain number of bases (for example, trim all the reads down to 35bp), without actually performing any additional quality trimming, there is a new parameter

`-notrim` that stops ConDeTri after the cutting. There is a list of all available options below.

Input parameters

(default values in brackets `[]`)

`-fastq1=file`
 `-fastq2=file`
	FASTQ file. If a second file is given, the files are trimmed as a pair. The reads must have the same order in both files. File(s) can be zipped (.gz).

`-prefix=string`	Prefix for the output file(s). The filtered FASTQ file(s) will be named *prefix_trim1.fastq* (and *prefix_trim2.fastq* if present). For pairs, a third file will be given with unpaired reads (reads from pairs where one low quality read has been removed).
`-cutfirst=i`	Remove i bases from 5'-end before any trimming [0].
`-cutlast=i`	Remove i bases from the 3'-end before any trimming [0].
`-rmN`	Remove non-ATCG characters before any trimming [no].
`-notrim`	Stop script after removing any 5'- 3'- or non-ATCG bases (skip the actual trimming and filtering step) [no].
`-hq=i`	Hiqh quality threshold `[25]`.
`-lq=i`	Low quality threshold `[10]`.
`-frac=[0,1]`	Fraction of read that must exceed hq *after* quality trimming `[0.8]`.
`-lfrac=[0,1]`	Maximum fraction of bases with qual<lq *after* quality trimming `[0]`.
`-minlen=i`	Min allowed read length – trim only down to i bases `[50]`.
`-mh=i`	When i consecutive hq bases is reached, the trimming stops `[5]`.
`-ml=i`	Max number of lq bases allowed within a stretch of hq bases `[1]`.
`-sc=i`	Illumina scoring table, Score=ASCII-sc. Used to be 64 for Illumina/Solexa. 33 is Sanger standard, and also used for newer Illumina data (1.8+). Can be set to any other integer if wanted `[64]`.
`-pb=fa|fq`	Print the removed low quality reads to a fasta or fastq file (for investigation/evaluation purposes) [no].
`-q`	Print the Solexa/Illumina scoring table (64 offset).
`-q33`	Print the Sanger/new Illumina scoring table (33 offset).
`-h`	Print a help message.

Output files

`prefix_trim1.fastq`
 `prefix_trim2.fastq`
 `prefix_trim_unpaired.fastq`
	File(s) with the trimmed reads (one file for single-end data, three for paired-end data, where the last file includes reads from the two input files whose read pair had too poor quality).

`prefix.stats`	Includes basic statistics in columns.
`prefix_badreads.fa[fq]`	(optional) fasta or fastq file with removed low quality reads.

The columns for the .stats file are the following:
PREFIX, NUMBER OF READS IN ORIGINAL FILE(S), NUMBER OF BASES IN ORIGINAL FILE(S), NO OF PAIRED READS AFTER TRIMMING,
NO OF BASES IN PAIRS AFTER TRIMMING, NO OF UNPAIRED READS AFTER TRIMMING, NO OF UNPAIRED BASES AFTER TRIMMING.

The .stats files are suitable for concatenation to make a summary table for several FASTQ files, for example one file for each lane in a flowcell or a set of transcriptome samples. Just use:

```
$ cat *.stats
```

Versions

v2.3

Added the -cutlast and the -notrim parameters. Changed the standard output slightly to make it more clear (first reports how many bases from each read that is removed, than how many bases in total that was removed before trimming). This version uses “zcat” instead of IO:Zlib, so no extra perl library installation is required (zcat is part of gzip which is standard in most Linux distributions. For Windows users, please continue to use version 2.2).

v2.2

ConDeTri can now read gzipped fastq input files (same input flags as for normal fastq files, recognizes file ending “.gz”). Note that perl library IO::Zlib is required.

v2.1

Added the -lfraq, -pb and -q33 parameters.

v2.0

Added the -cutfirst and -rmN parameters.

v1.1

Fixed some typos and changed the standard output.

v1.0

First publicly available version of ConDeTri!

filterPCRdupl.pl

Summary

Takes a pair of FASTQ files and removes redundant copies that might have emerged in the PCR step by comparing all read pairs against each other. If there are several copies of a read pair, only one pair is kept (the one with the highest base quality).

Usage

```
perl filterPCRdupl.pl -fastq1=file1 -fastq2=file2 [-prefix=s
-cmp=N]
```

Background

When sequencing with NGS techniques, PCR is often needed for adding adaptors and/or getting a sufficient amount of fragments for sequencing. However, it also infers the risk of amplifying the same fragments over and over again, and in worst case scenario only sequence a small part of the desired material. By inspecting the data and remove these so called PCR-duplicates we can prevent biases and erroneous results in the down stream analysis.

Especially in RNA-sequencing for expression analysis it is crucial no to include these ”false” fragments as they will change the gene expression for the genes with duplicated fragments and skew the analysis. Also for *de novo* assembly the duplicates should be removed not to add extra links in the scaffolding procedure. For mapping and SNP-calling, the removal of duplicates is less of importance, since most SNP-callers can recognize and flag these as unusable. However, since the duplicates can't be used anyway, one can save time and memory by removing them before performing any further analyses.

Description

All pairs are compared against each other by examination of the first N (default 50) bases of both reads in the pairs. If there are several pairs starting with exactly the same sequence in both ends, it is assumed that it's a duplication since it is unlikely that the two DNA-molecules have been fragmented at exactly the same positions at both ends just by random (and if N is large enough, it's also unlikely that one would find exactly the same pattern somewhere else in the genome). When finding several copies of a fragment, the quality scores for the pairs are compared, and the pair with the highest total score is kept while the others are discarded.

Input parameters

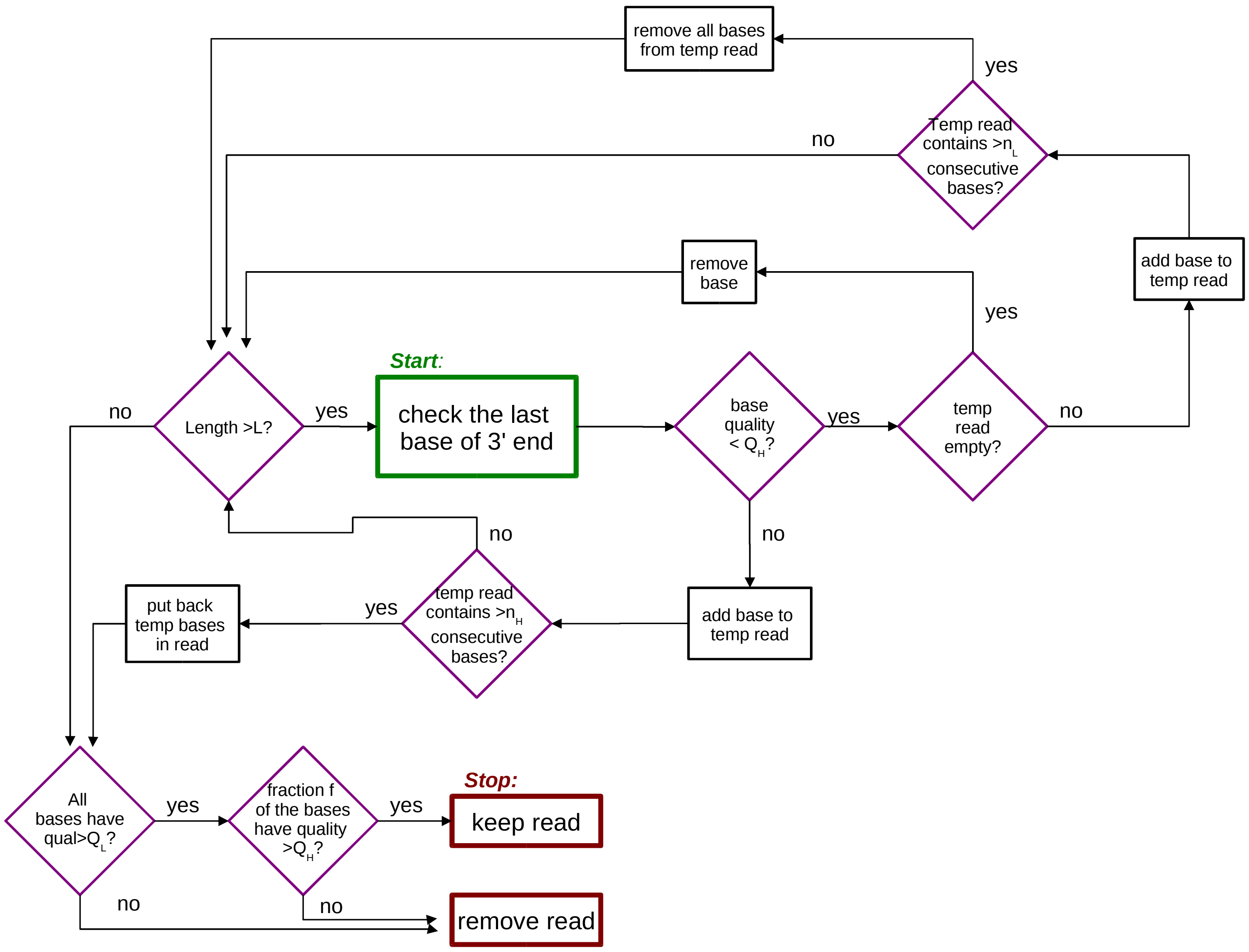
(default values in brackets `[]`)

`-fastq1=file1`	First file in a FASTQ pair.
`-fastq2=file2`	Second file in a FASTQ pair. The reads must have the same order in both files.
`-prefix=string`	Prefix for the output files. The filtered FASTQ file(s) will be named *prefix_uniq1.fastq* and *prefix_uniq2.fastq* if present [same as file1].
`-cmp=N`	Number of bases in the beginning of both reads in a pair that are used for comparison [50].
`-h`	Print a help message.

Output files

`prefix_uniq1.fastq` **`prefix_uniq1.fastq`**	Files with the filtered (non redundant) reads.
`prefix_copy.hist`	Histogram of the copy distribution.

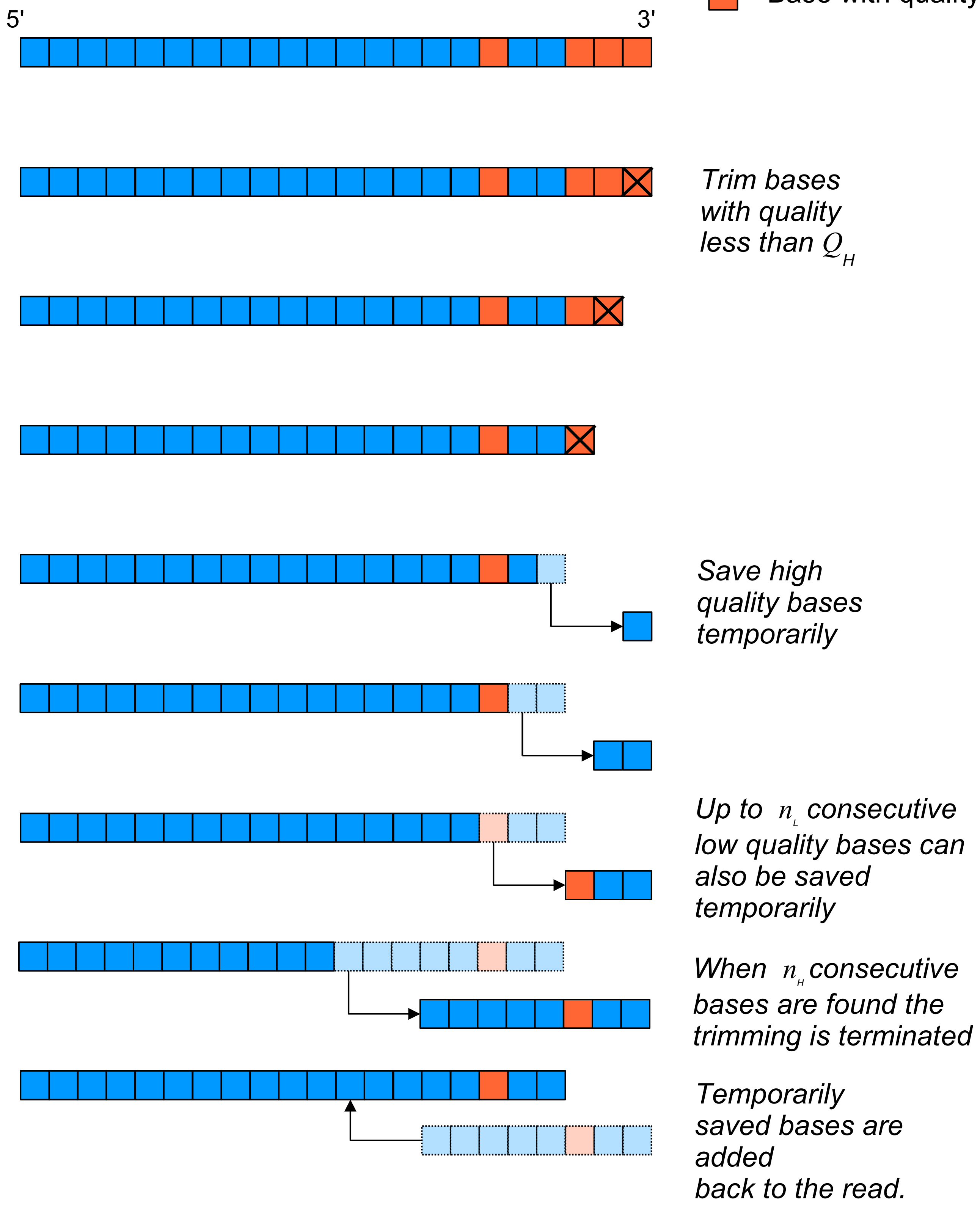
CONDETRI FLOWCHART AND TRIMMING


remove all bases from temp read
yes
Temp read contains >n_L consecutive bases?
no
remove base
add base to temp read
yes
Start:
no
Length >L?
yes
check the last base of 3' end
base quality < Q_H?
yes
temp read empty?
no
no
no
put back temp bases in read
yes
temp read contains >n_H consecutive bases?
add base to temp read
Stop:
All bases have qual>Q_L?
yes
fraction f of the bases have quality >Q_H?
yes
keep read
no
no
remove read

Example 1: Good quality read

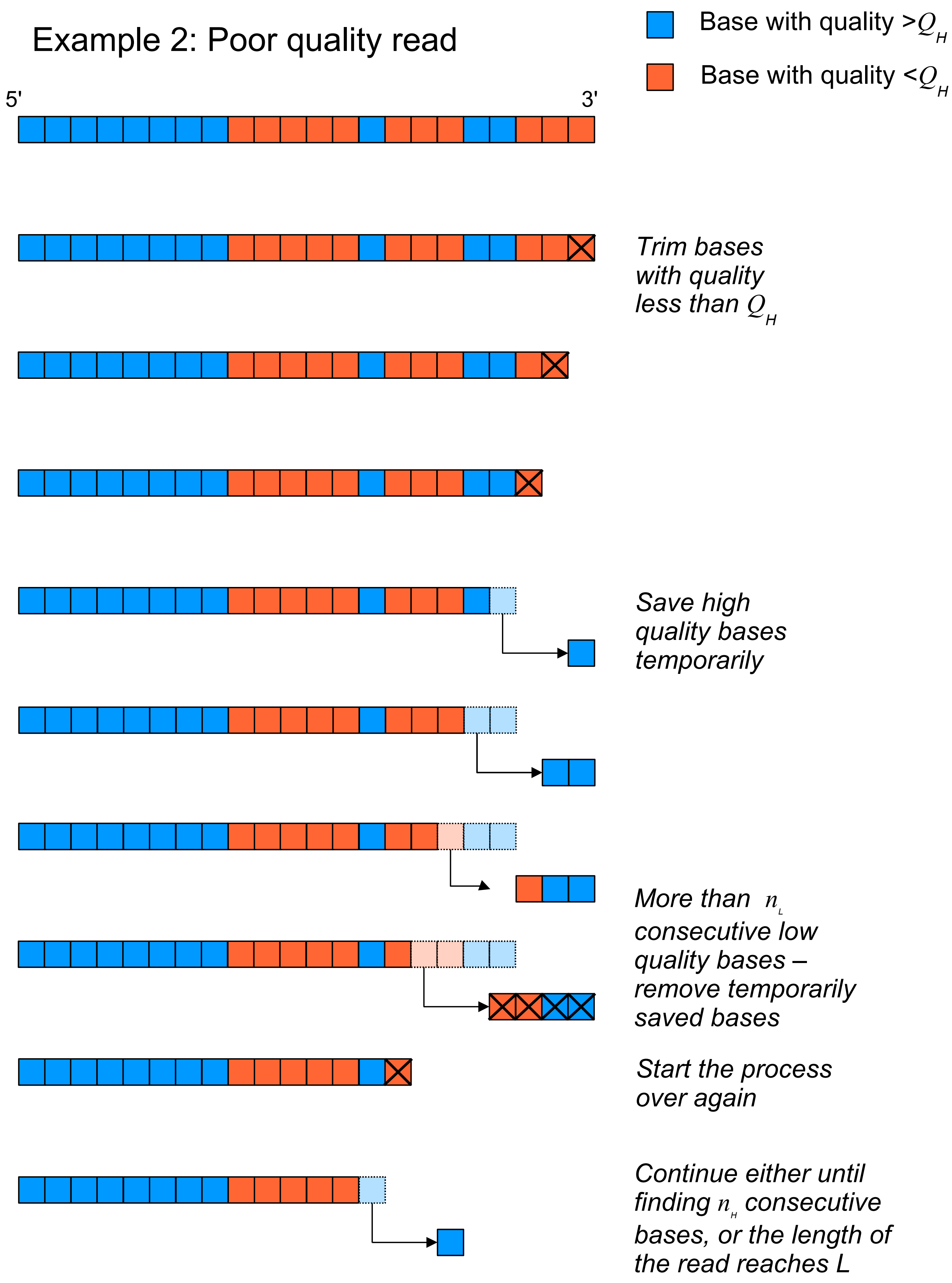
🟦 Base with quality $>Q_H$

🟧 Base with quality $<Q_H$



5'
3'
Trim bases with quality less than Q_H
Save high quality bases temporarily
Up to n_L consecutive low quality bases can also be saved temporarily
When n_H consecutive bases are found the trimming is terminated
Temporarily saved bases are added back to the read.

Example 2: Poor quality read



🟦 Base with quality $>Q_H$
🟥 Base with quality $<Q_H$
5'
3'
Trim bases with quality less than Q_H
Save high quality bases temporarily
More than n_L consecutive low quality bases – remove temporarily saved bases
Start the process over again
Continue either until finding n_H consecutive bases, or the length of the read reaches L