Illumina Read Preprocessing

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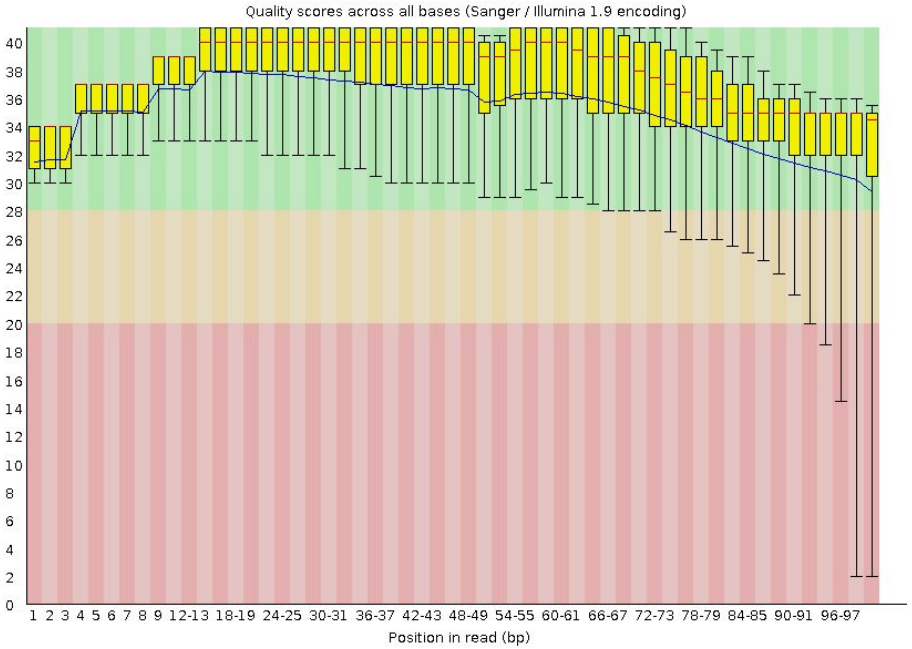
Tuesday 22 August 2017

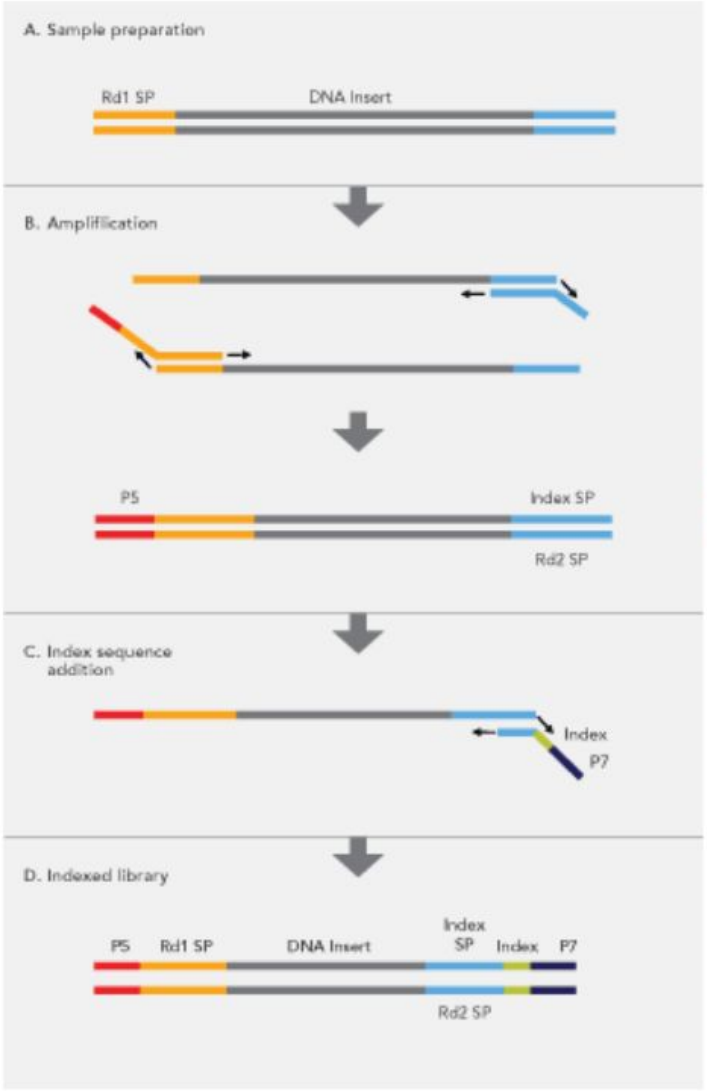
Read Preprocessing

Steps taken to improve the overall data quality.

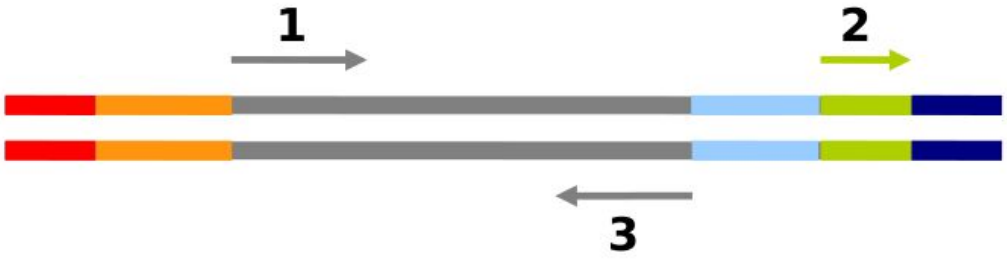
Typically includes removal of adapter sequences and low quality bases.



Adapter Contamination



Reads 1 and 3 are "forward" and "reverse" reads from your DNA-of-interest, and they are on opposite strands.

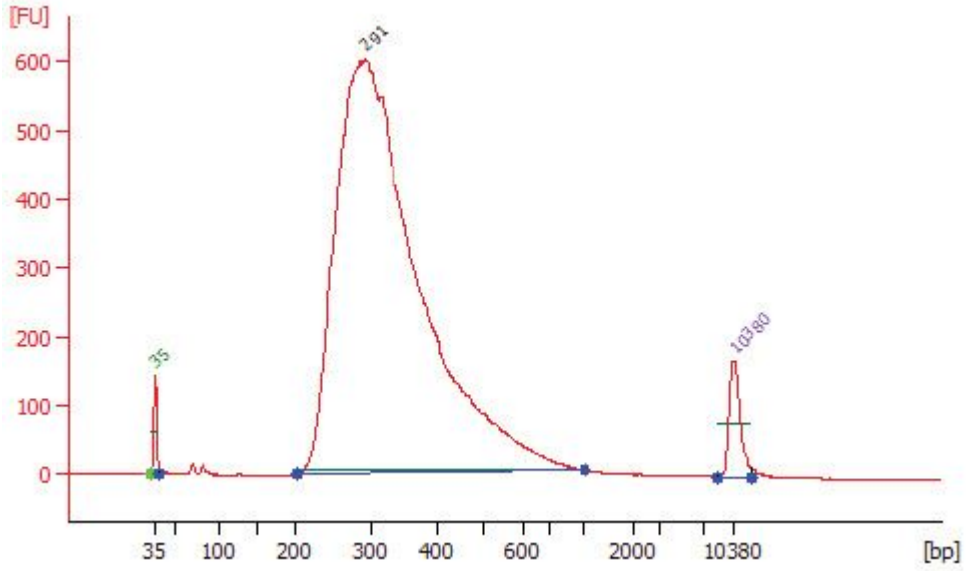


Read (2) is the "barcode," which identifies particular reads as belonging to a particular sample.

Adapter Contamination

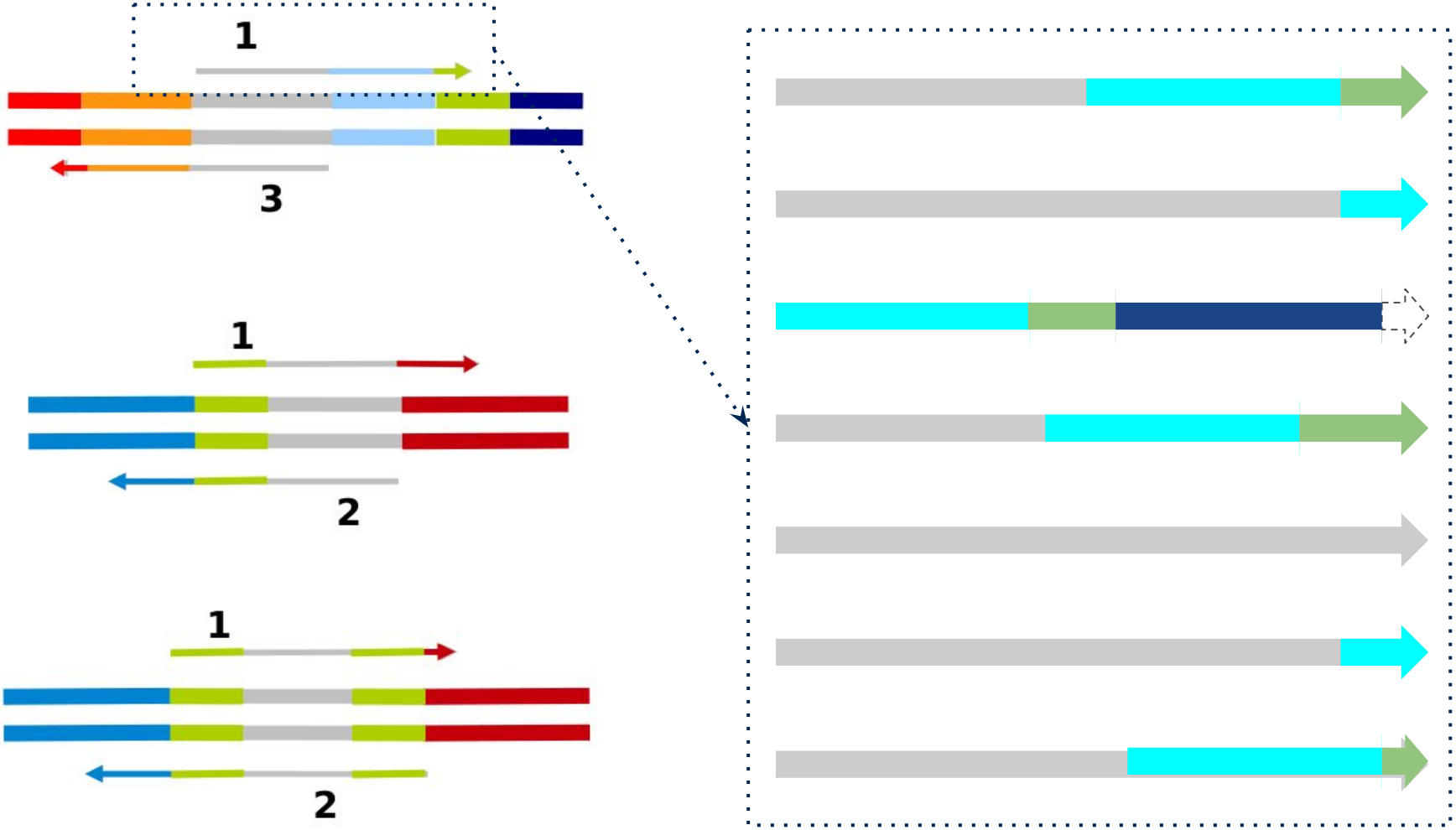
All sequencing libraries contain a distribution of molecule sizes. The smaller molecules (such as adapter dimers) will more easily find a place on the flowcell.

Any DNA insert that is shorter than the read length will generate some adapter contamination.



Adapter Contamination

Contamination is the result of the sequencer *reading through* a short read, into adapter sequence that *didn't come from your sample*!



Adapter Contamination

Where can you find adapter sequences?

- Illumina Adapter Sequences Document
- *Find them in your data*
- Contact the library kit manufacturer
- Google "github ucdavis-bioinformatics", look for Scythe, look for "*_adapters.fa"
- Check Seqanswers.com

Adapter Contamination

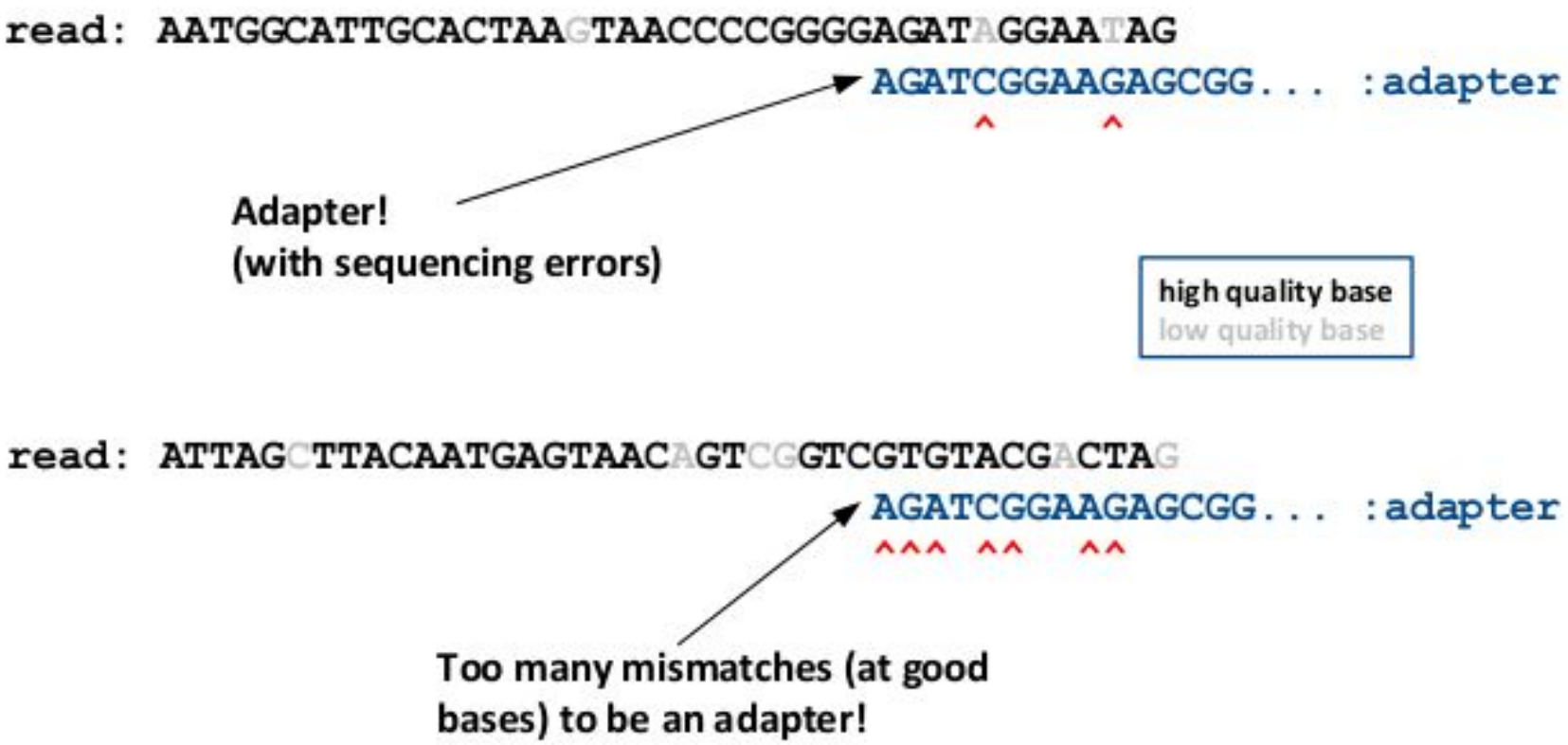
```
>TruSeq_forward_contam
AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC[8bp index]ATCTCGTATGCCGTCTTCTGCTTGAAAAA
>TruSeq_reverse_contam
AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT[8bp index]GTGGTCGCCGTATCATTAAAAA
```

```
>Nextera_forward_contam
CTGTCTCTTATACACATCTCCGAGCCCACGAGAC[8bp index]ATCTCGTATGCCGTCTTCTGCTTG
>Nextera_reverse_contam
CTGTCTCTTATACACATCTGACGCTGCCGACGA[8bp index]GTGTAGATCTCGGTGGTCGCCGTATCATT
```

```
>TruSeq_SmallRNA_forward_contam
TGGAATTCTCGGGTGCCAAGGAACTCCAGTCAC[6bp adapter]ATCTCGTATGCCGTCTTCTGCTTG
>TruSeq_SmallRNA_reverse_contam
GATCGTCGGACTGTAGAACTCTGAACCTGTCG
```

Adapter Removal: Scythe

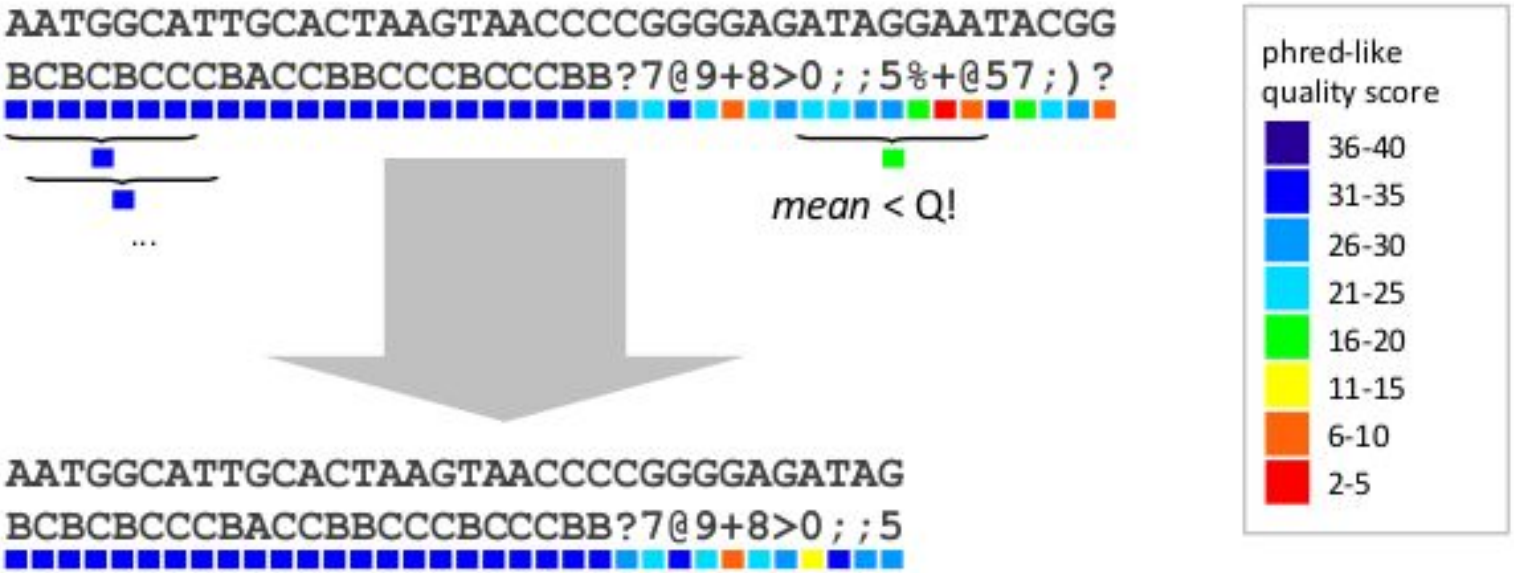
Scythe (*Vince Buffalo, Joe Fass*) is an adapter trimmer for Illumina reads that employs a Bayesian model that considers base qualities.



Low Quality Trimming: Sickle

Sickle (*Nik Joshi, Joe Fass*) is a sliding window trimmer for Illumina reads that tries to keep the longest high quality 5'-justified sequence from each read.

Moving from 5' to 3', windows of N bases are tested for average quality > Q. In the first window that fails, bases are trimmed starting with the first base with quality < Q.



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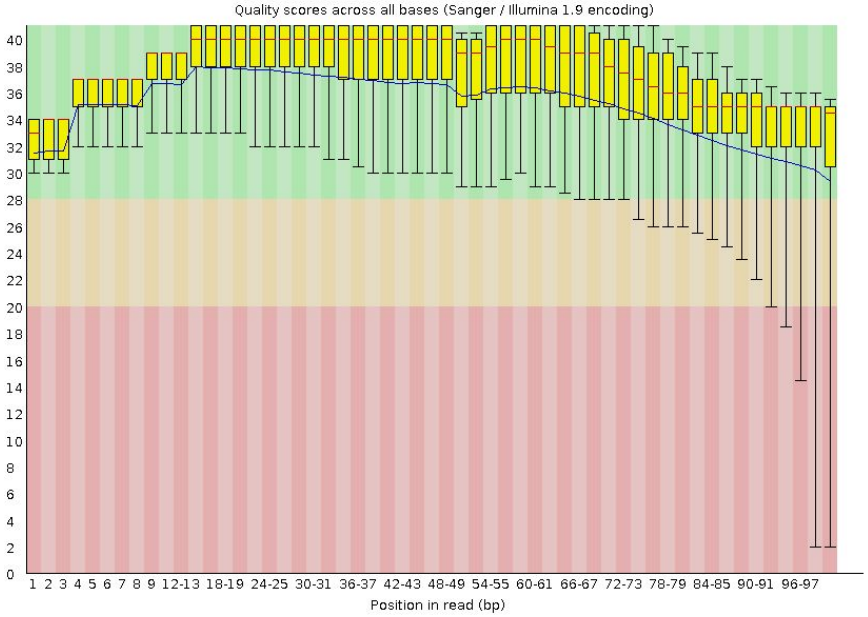
Before



After