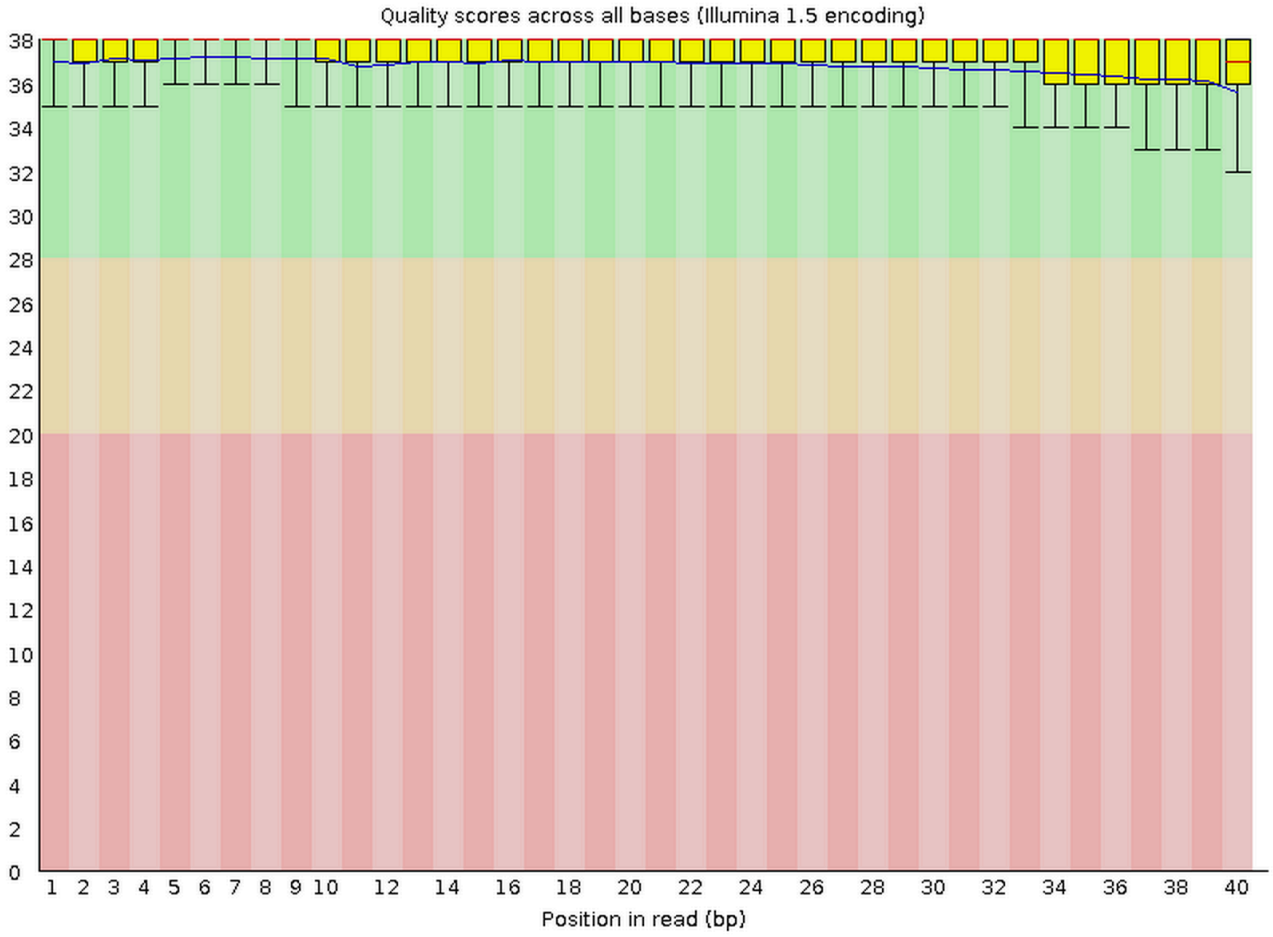
# FastQC Report

Wed 25 Mar 2015
good_sequence_short.txt

## Summary


- Basic Statistics
- Per base sequence quality
- Per tile sequence quality
- Per sequence quality scores
- Per base sequence content
- Per sequence GC content
- Per base N content
- Sequence Length Distribution
- Sequence Duplication Levels
- Overrepresented sequences
- Adapter Content
- Kmer Content


## Per base sequence quality



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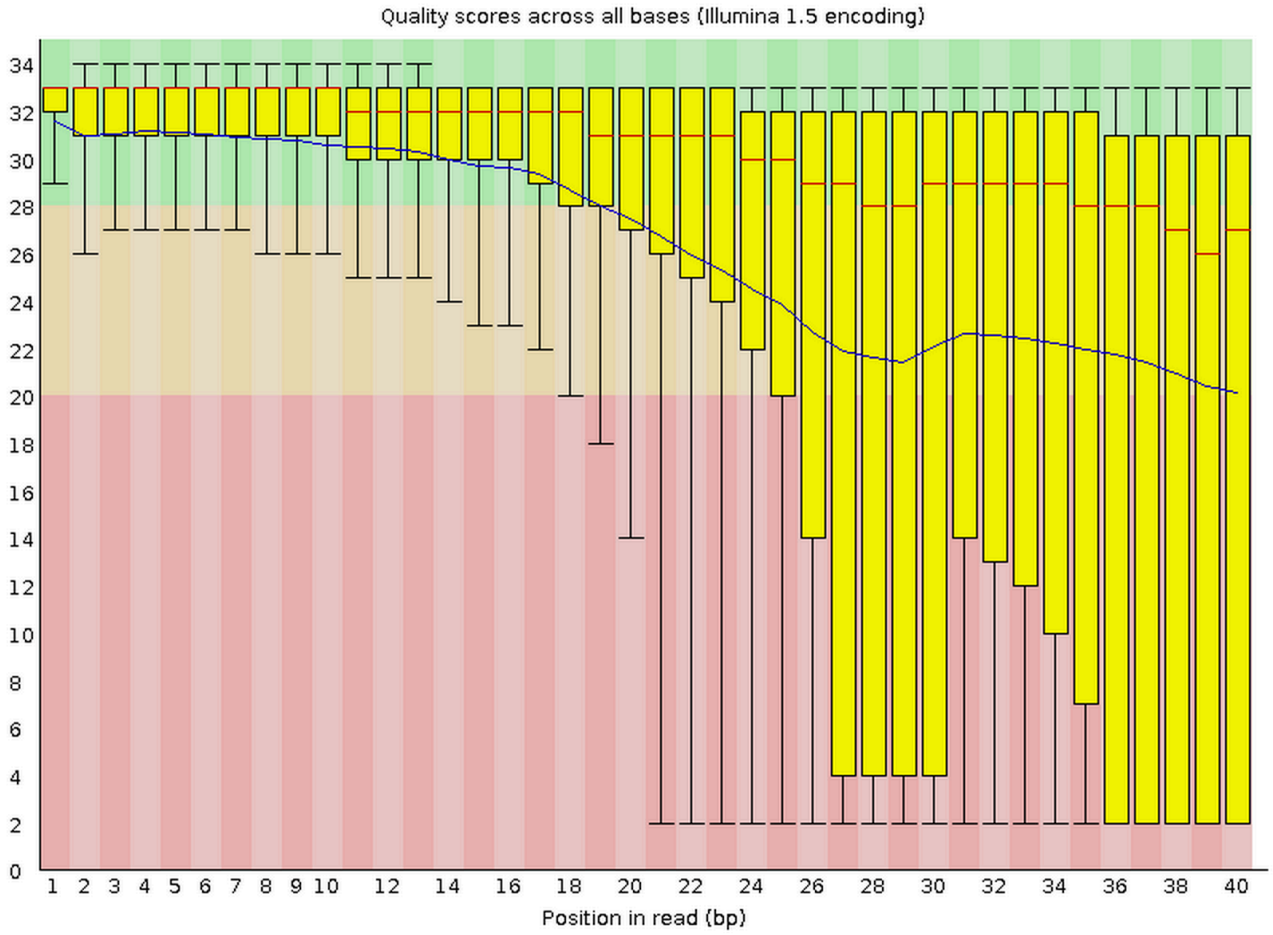
## Per base sequence quality



# What to do?

- Trim the reads?
- Start over – try sequencing it again?

frontiers in GENETICS

ORIGINAL RESEARCH ARTICLE
published: 31 January 2014
doi: 10.3389/fgene.2014.00013

# On the optimal trimming of high-throughput mRNA sequence data

**Matthew D. MacManes**[1,2]*

[1] Department of Molecular, Cellular and Biomedical Sciences, University of New Hampshire, Durham, NH, USA
[2] Hubbard Center for Genome Studies, Durham, NH, USA

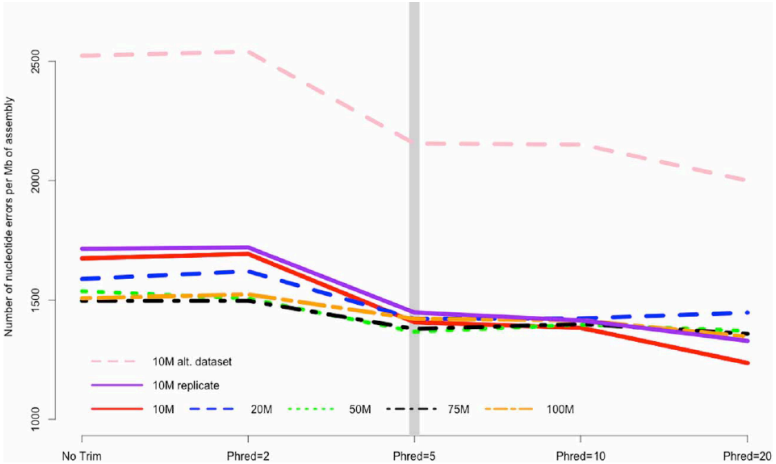
"… researchers interested in assembling transcriptomes de novo should elect for a much gentler quality trimming, or no trimming at all."

"… trimming at PHRED=2 or PHRED=5 optimizes assembly quality."

# Aggressive Trimming may be harmful, whereas light trimming could be beneficial

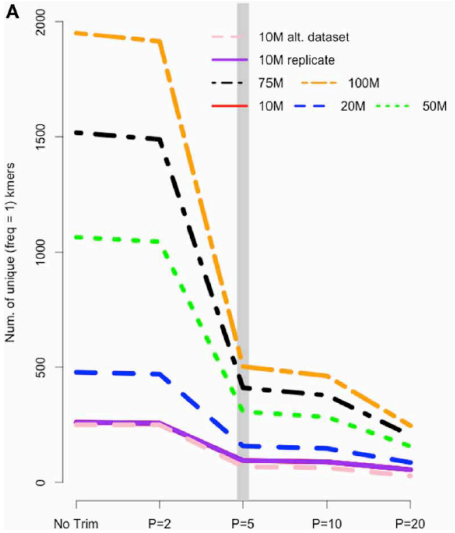
Fewer errors in the assembly

# Nucleotide errors / Mb



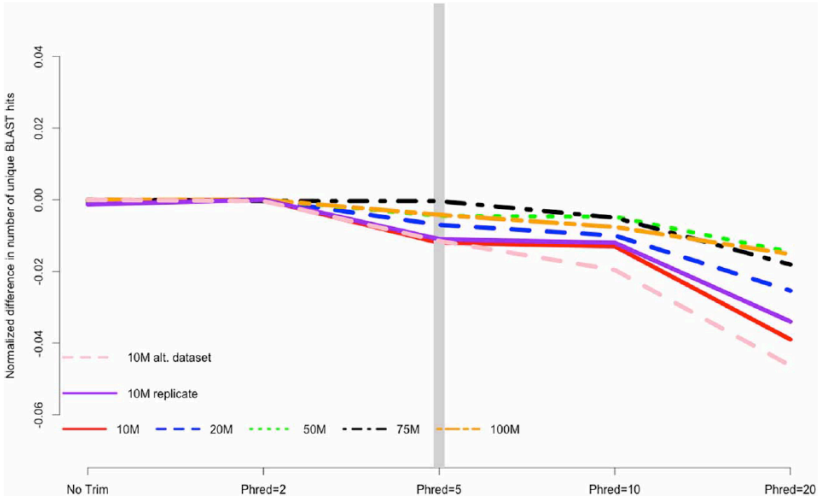

Fewer unique kmers

# unique kmers



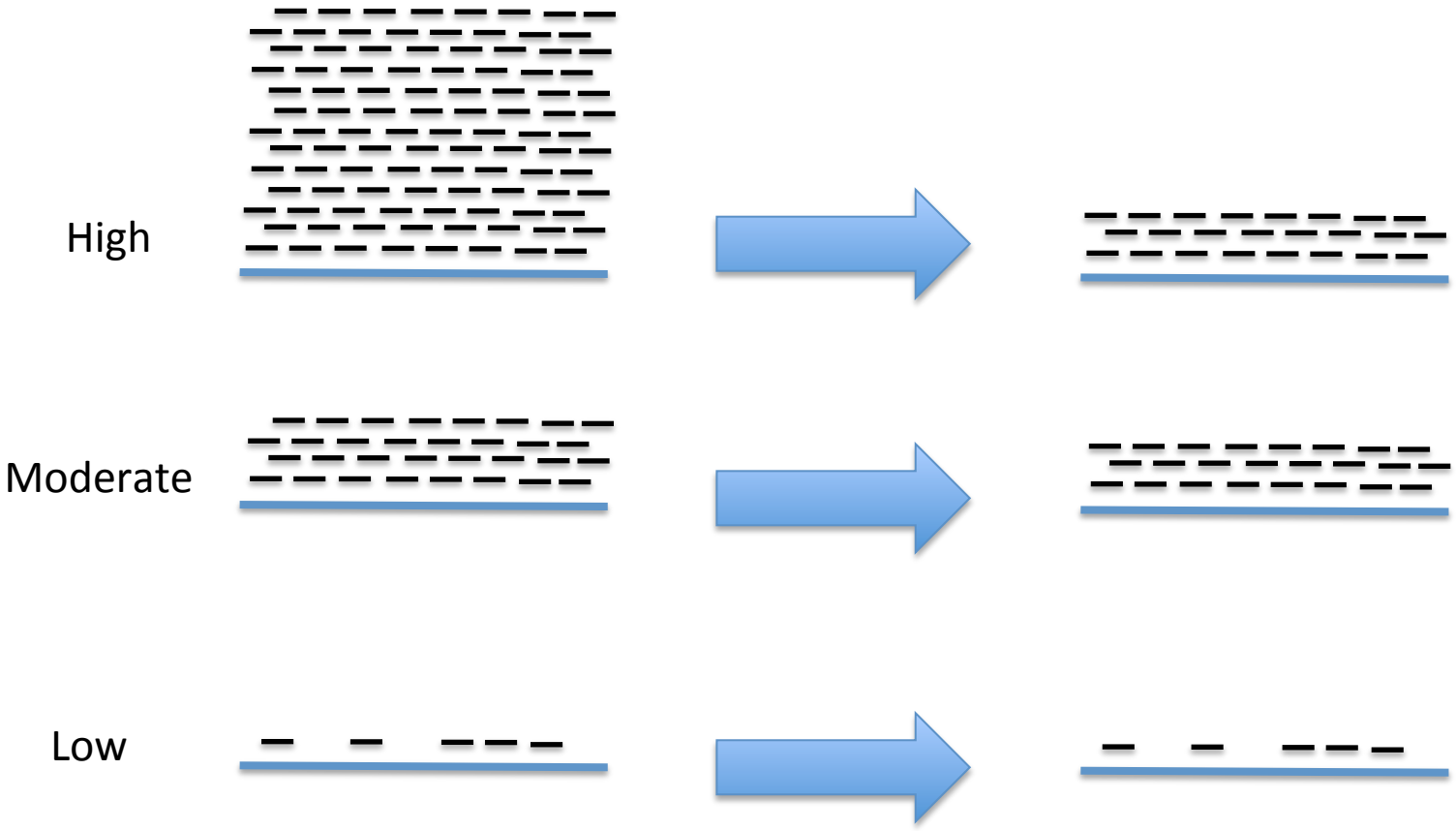

Light trimming doesn't reduce number of blast matches w/ higher sequencing depths.

Normalized # of blast matches

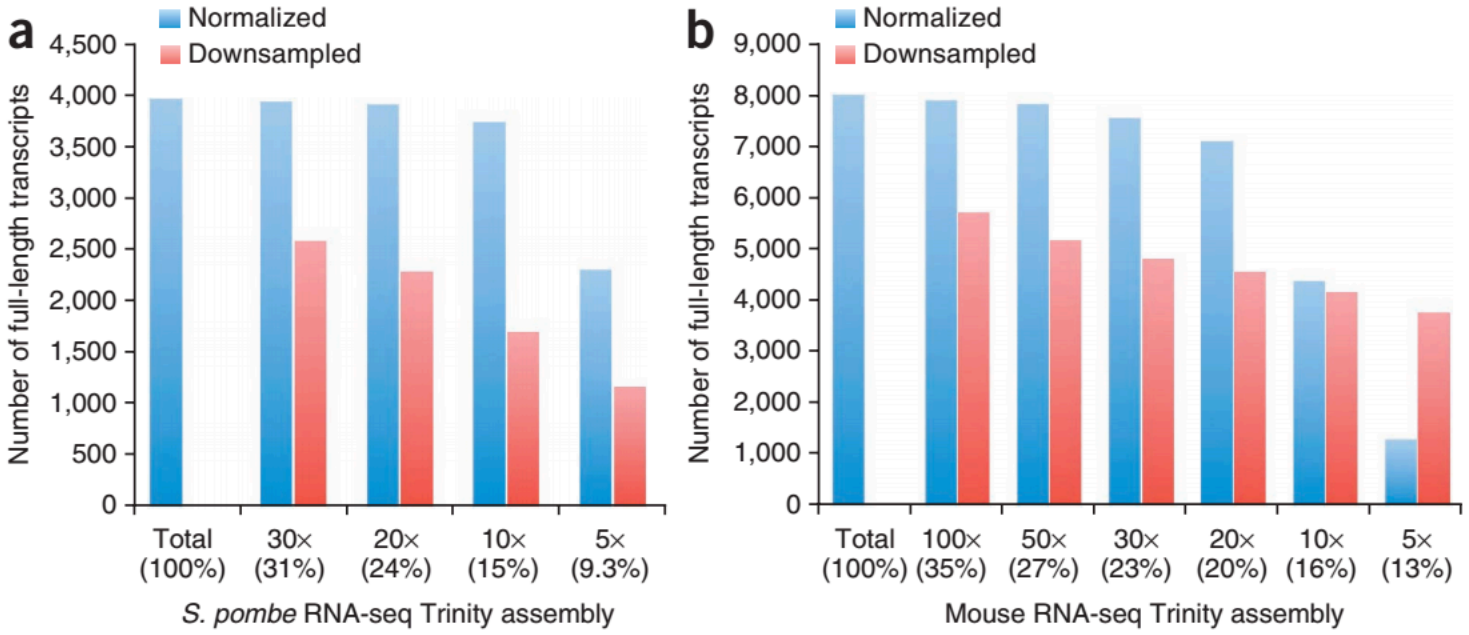



MacManes 2014

# *In silico* normalization of reads



# Impact of Normalization on *De novo* Full-length Transcript Reconstruction




Largely retain full-length reconstruction, but use less RAM and assemble much faster.

Haas et al., 2013

# Quality Trimming and Normalization via Trinity

- Quality Trimming using Trimmomatic:
  - Trinity --trimmomatic
- Normalization of reads:
  - Trinity --normalize_reads *(now on by default!)*
- You can do both in a single Trinity assembly run:
  - Trinity --trimmomatic --normalize_reads

# Fastqc, trimming, and normalization practical