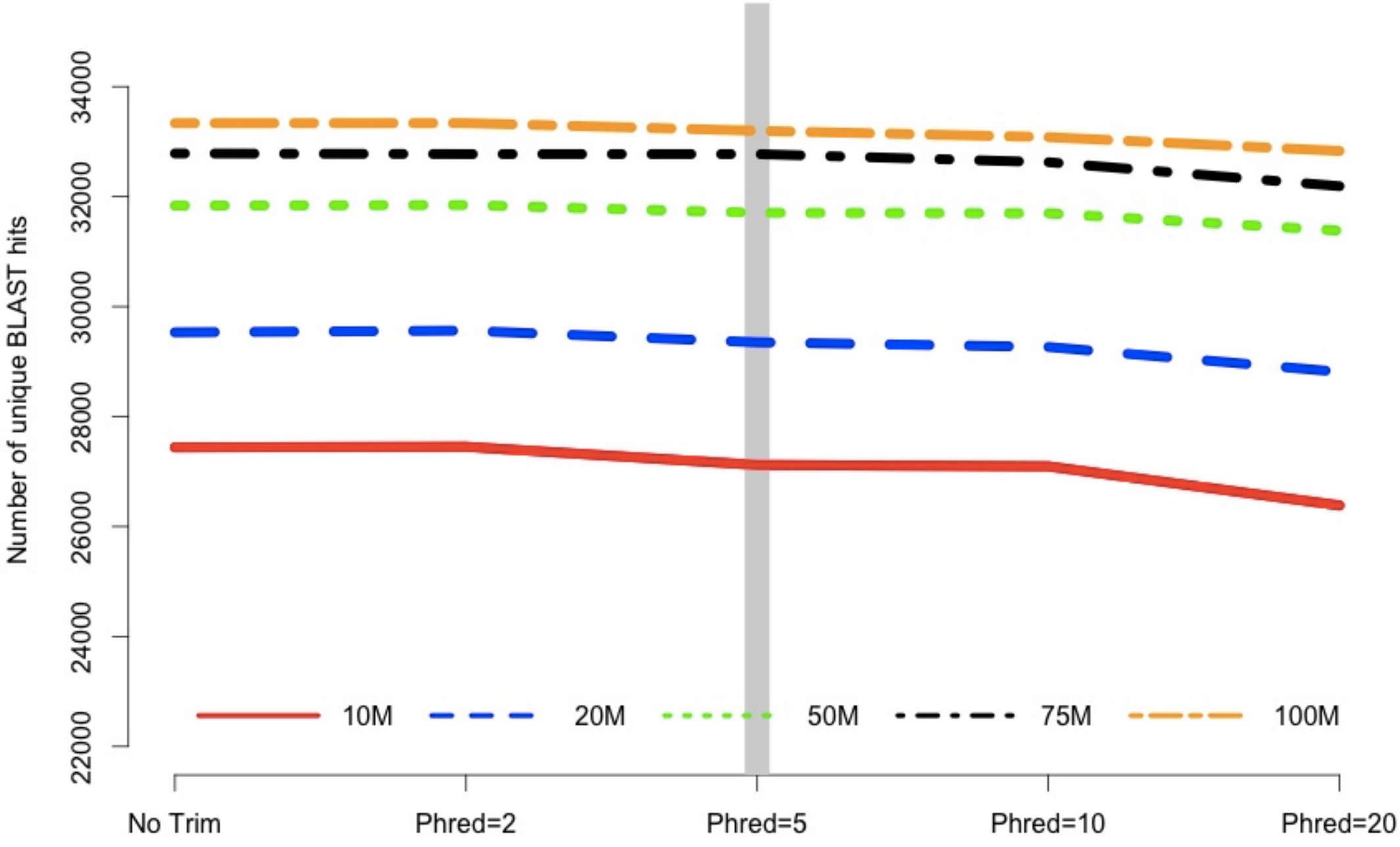
How many reads/reps/samples

- For *de novo* transcriptome assembly, diff expression
  - SNP calling
- For reference generation?? (20-30M? Francis et al 2013)

How many reads/reps/samples



How many reads/reps/samples

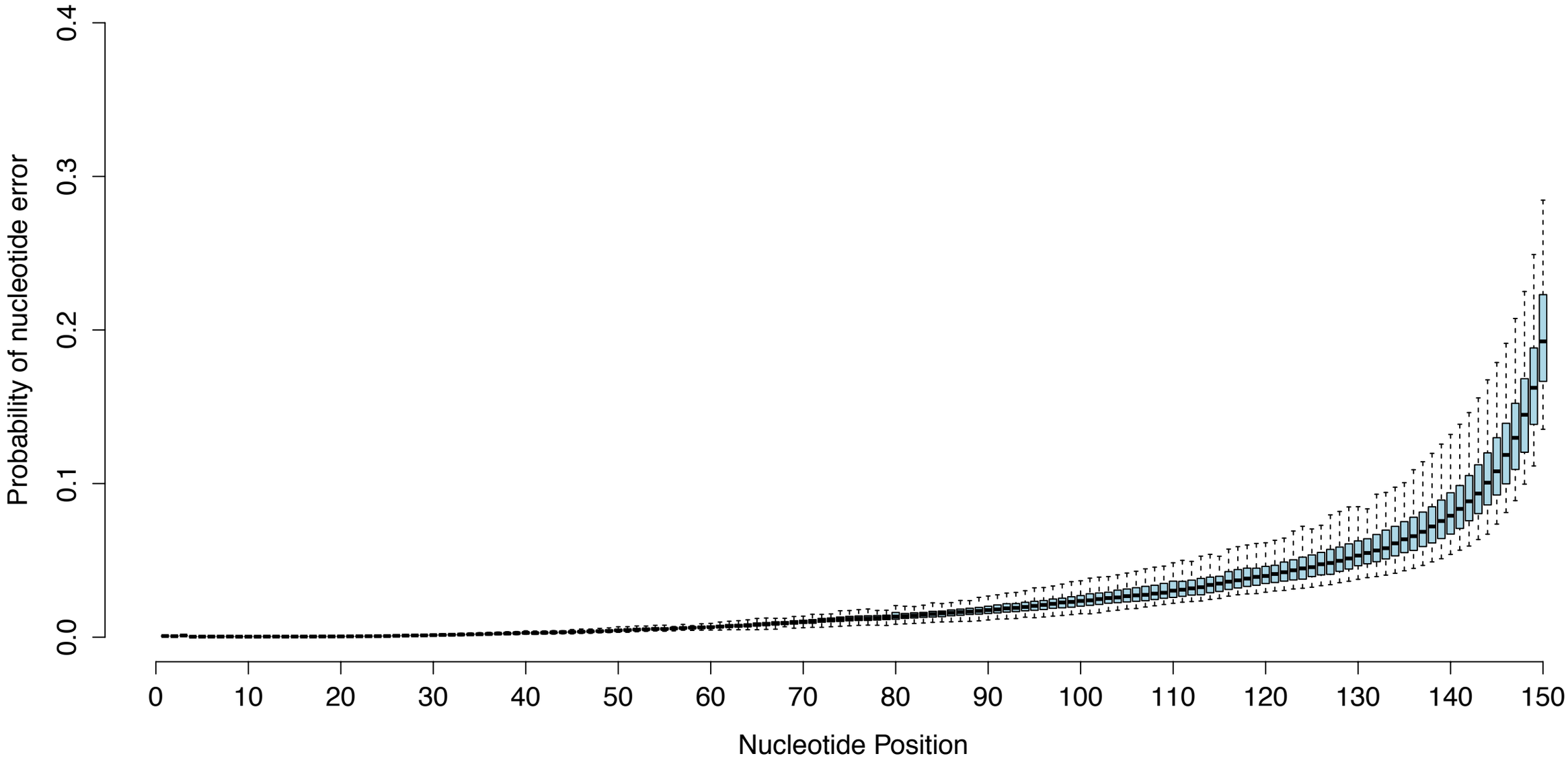
- For *de novo* transcriptome assembly, diff expression
  - SNP calling
- For reference generation??
- What is the n treatments are very different?
- Now you have a reference, how many reads for replicates?
- How to sequence these samples?

Now we have sequence reads…

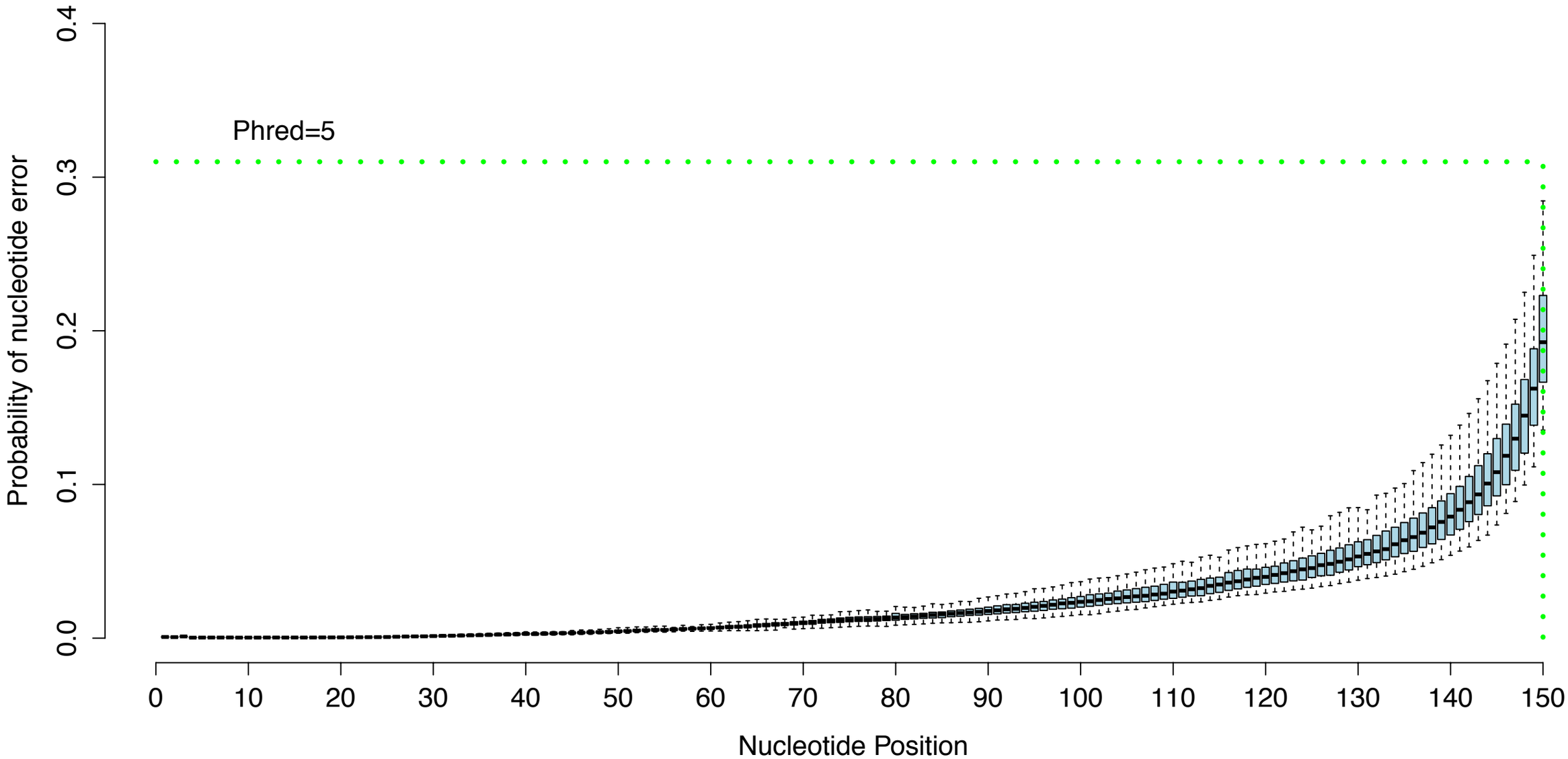
- Adapter trimming (Trimmomatic)
- Quality trimming

Quality trimming of NGS data

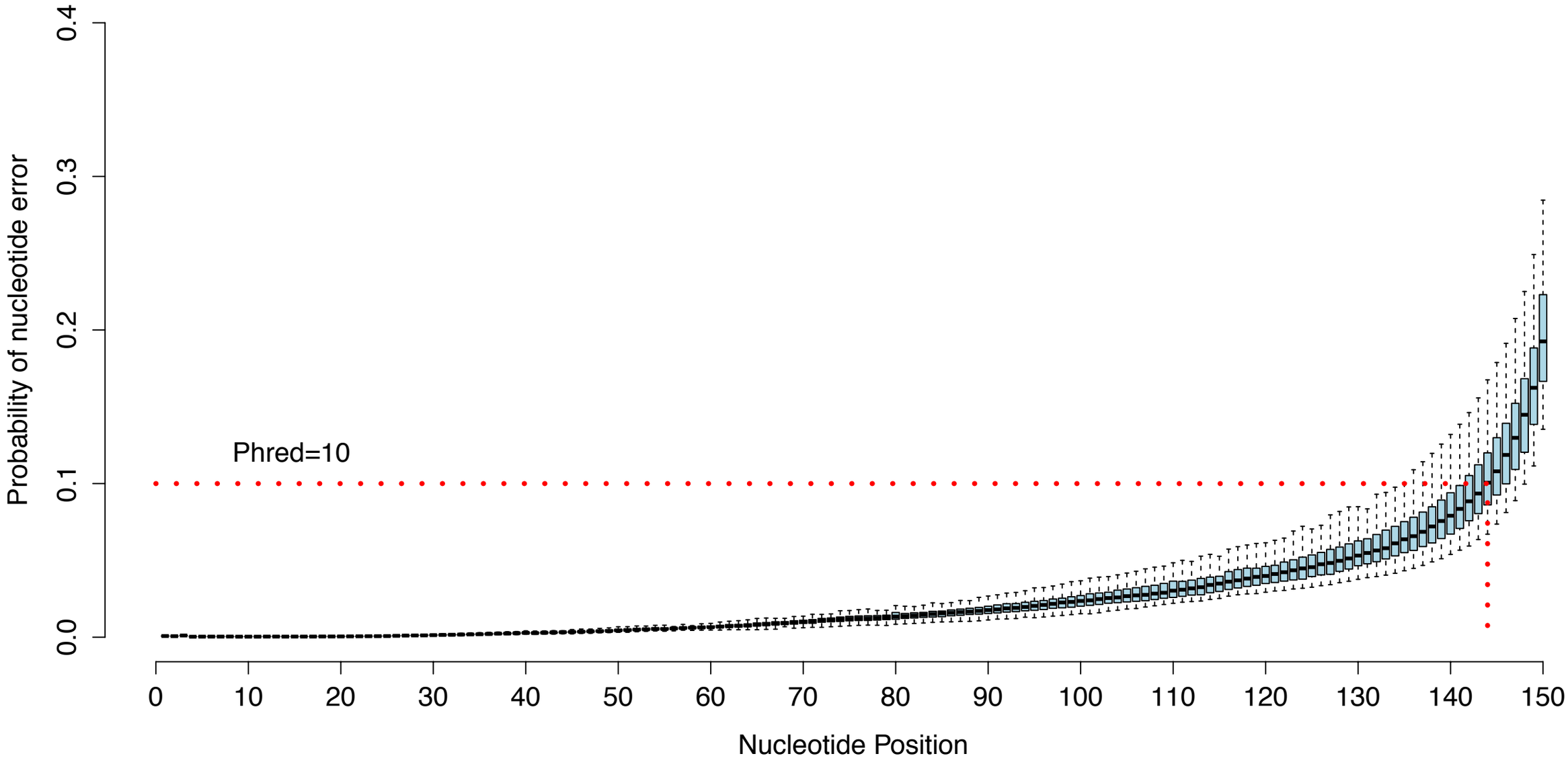
- Universal practice



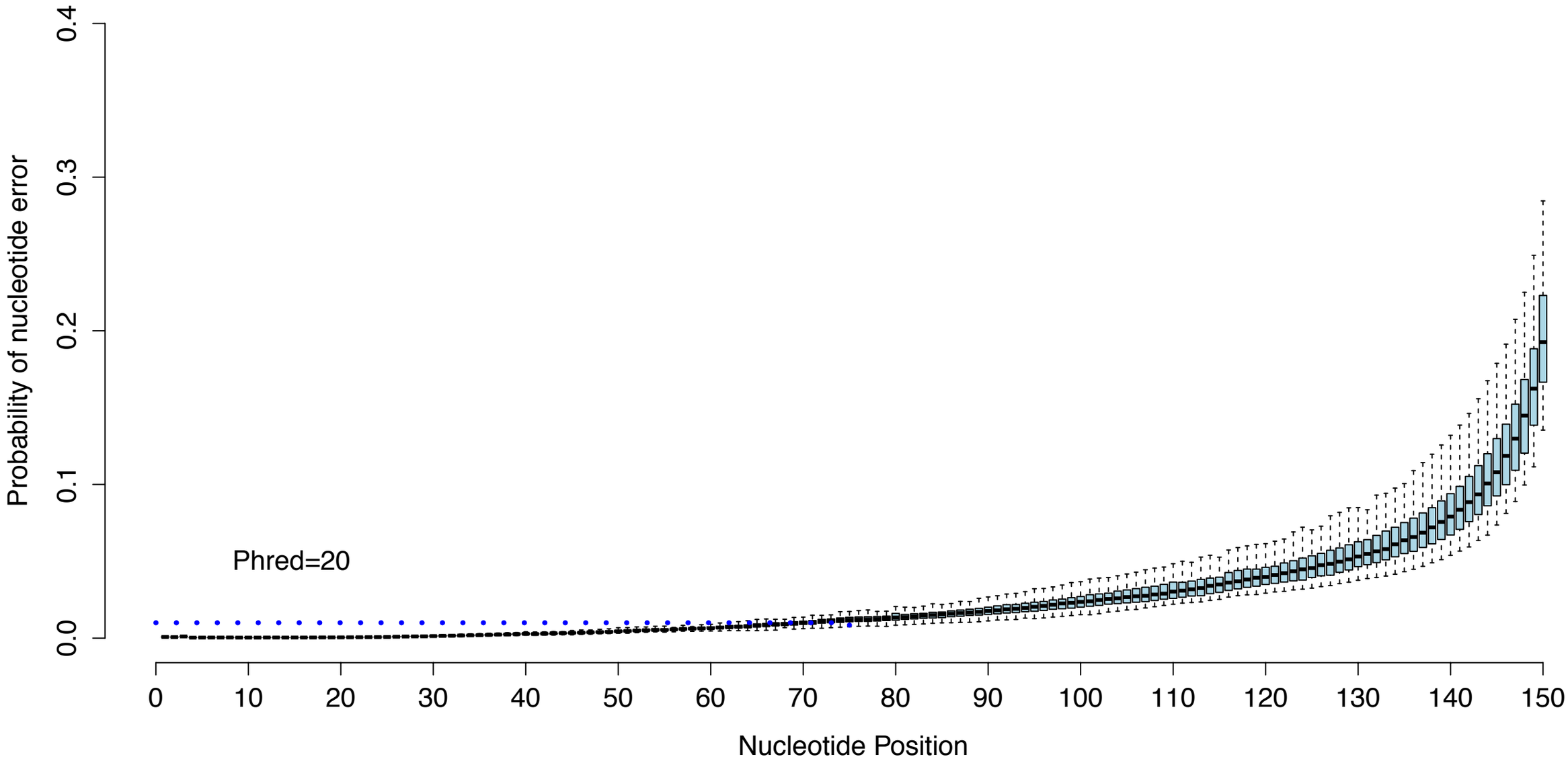
Quality trimming of NGS data



Quality trimming of NGS data



Quality trimming of NGS data



Trimming Experiment

- 2 Illumina datasets > adapter trimmed.
- Subsampled to 10M, 20M, 50M, 75M, 100M PE reads.
- Trimmed at Phred 0,2,5,10,20
- Assembled using Trinity and SOAPdenovo-Trans
- Developed metrics for evaluating transcriptome assemblies.

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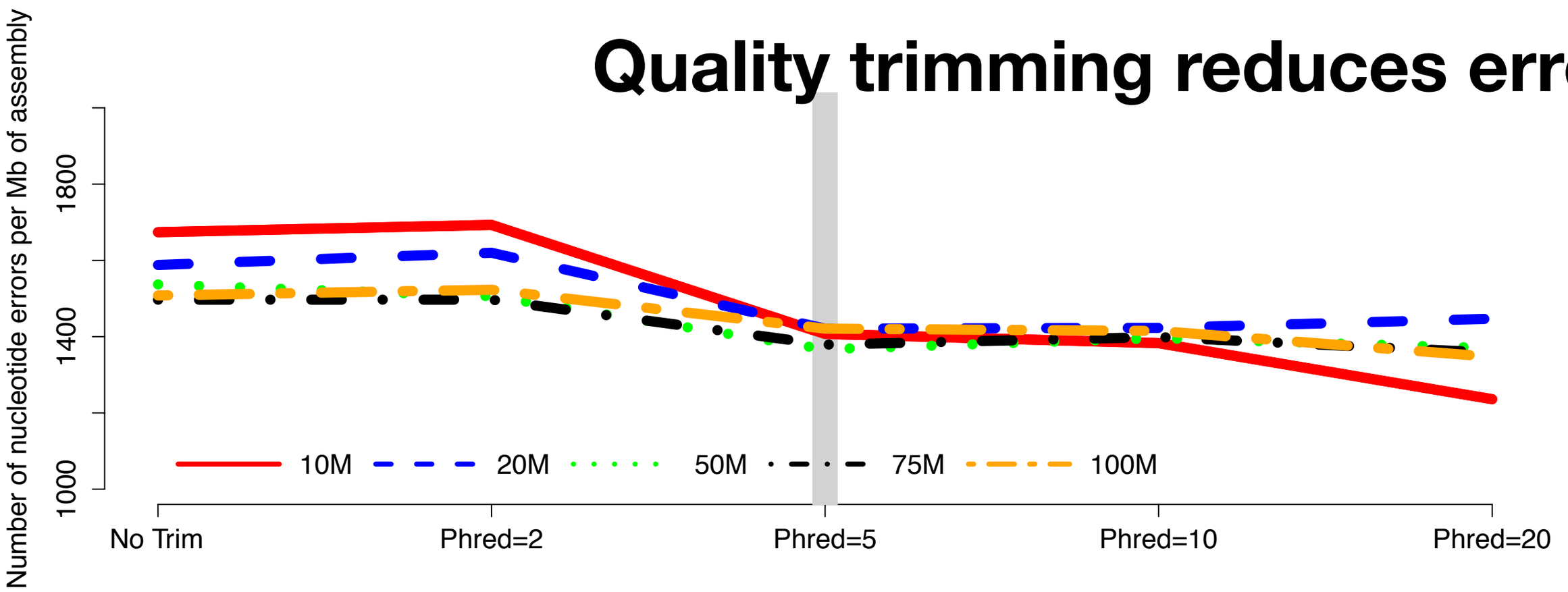
Quality trimming reduces error



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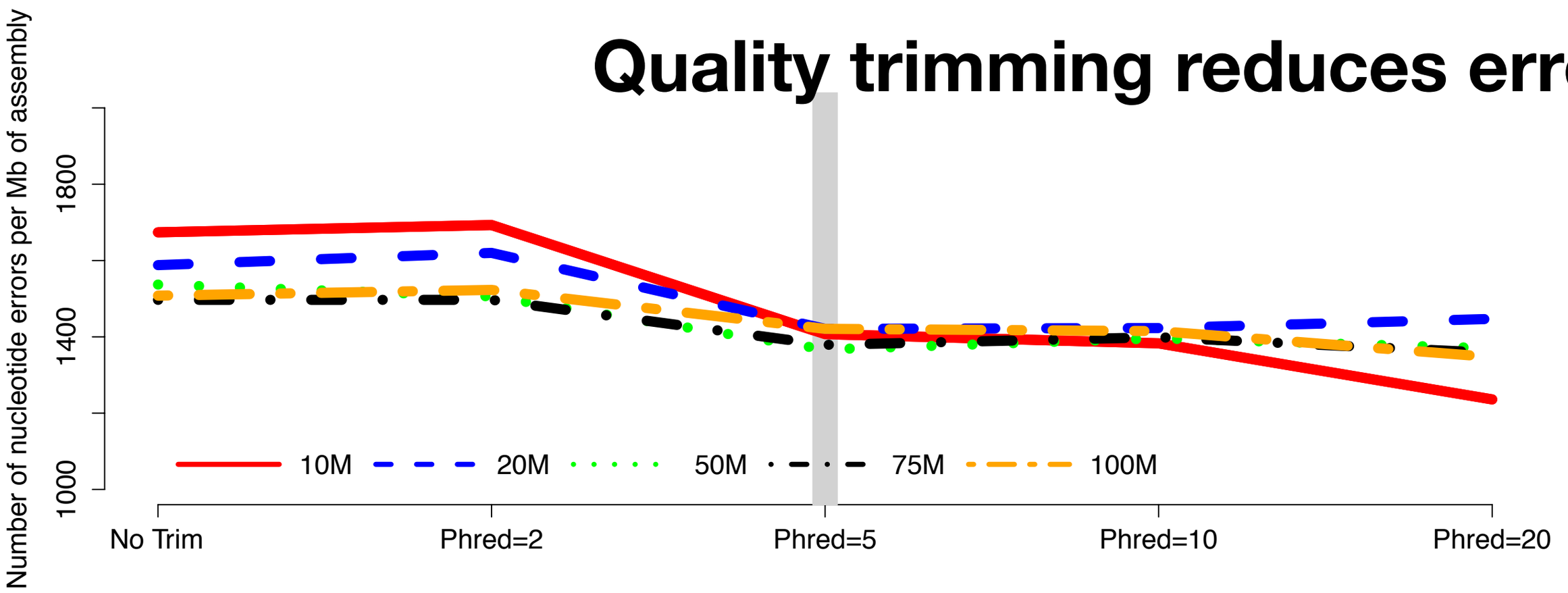
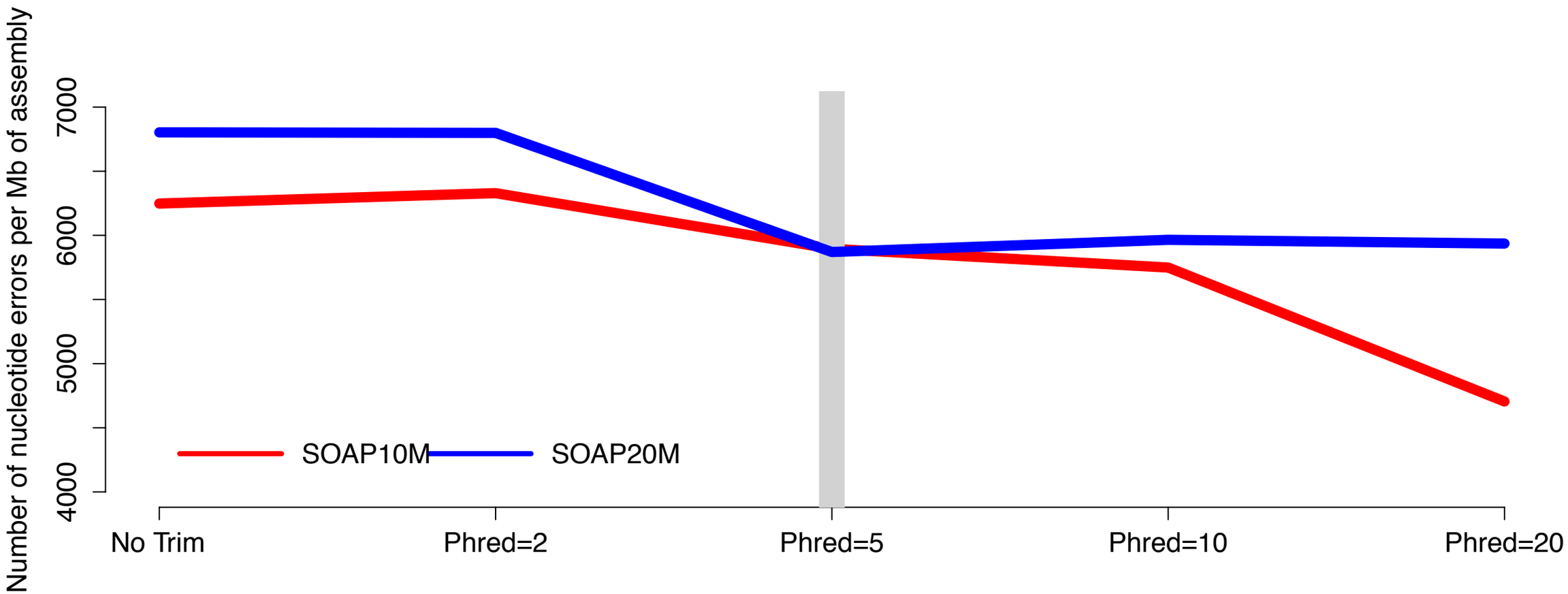
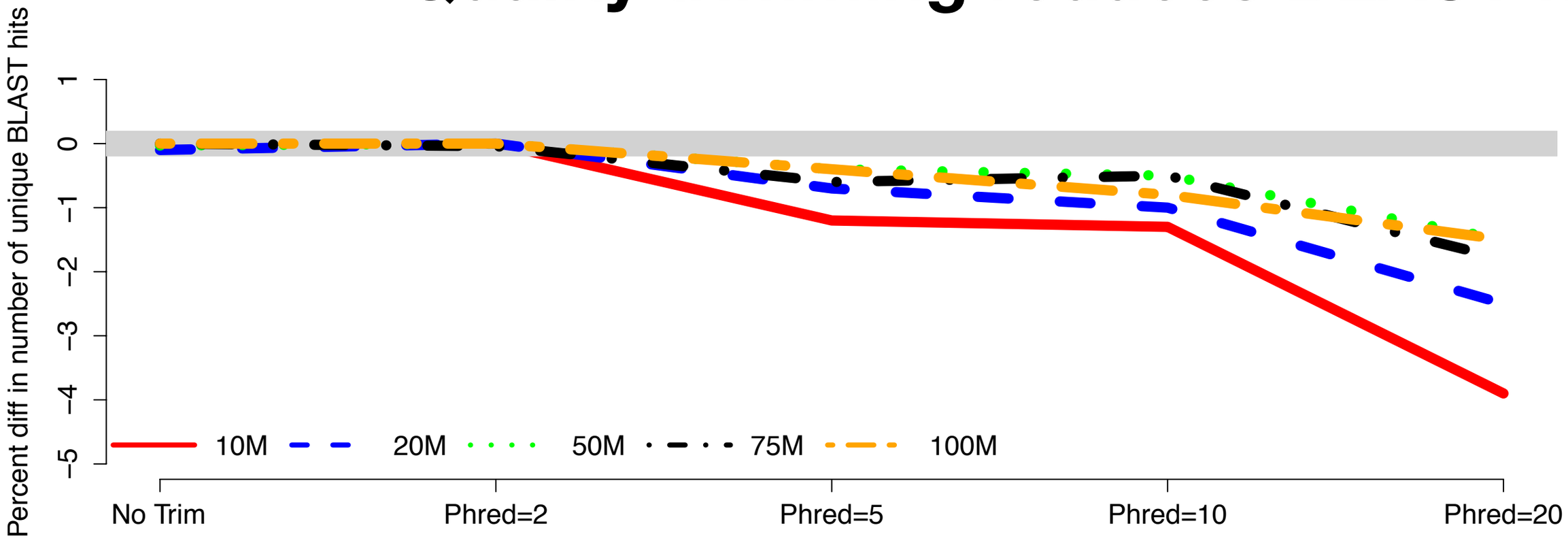
Quality trimming reduces error





Quality trimming reduces BLAST hits



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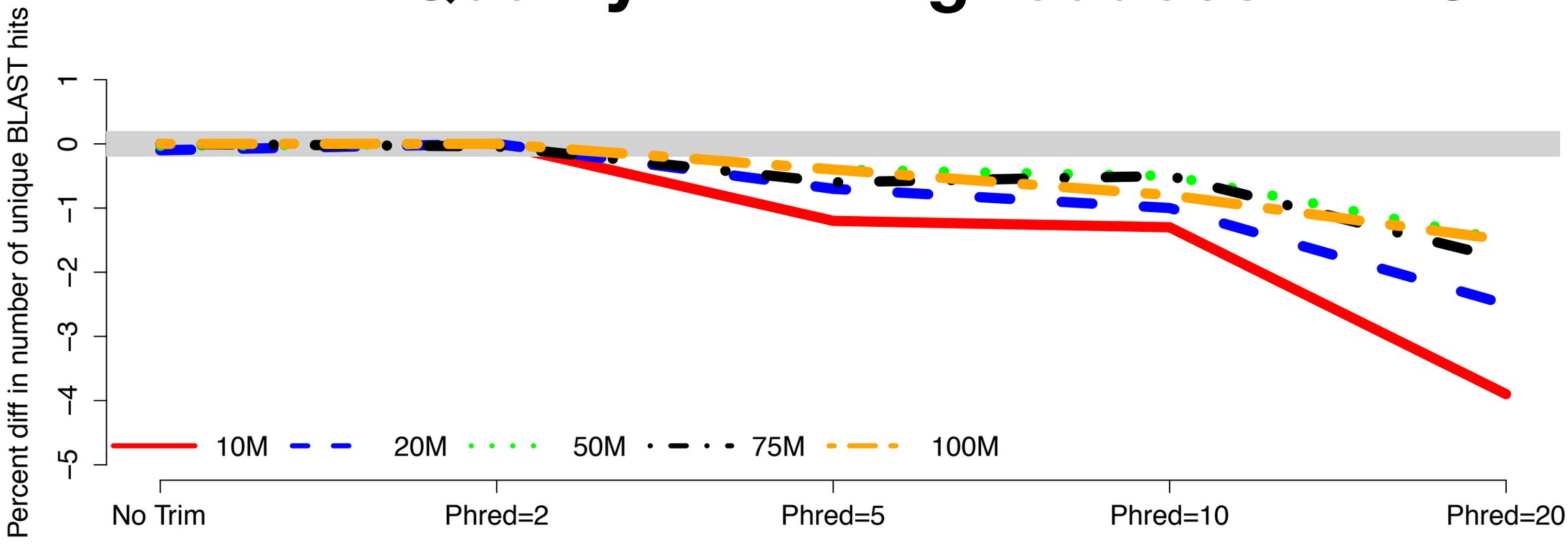
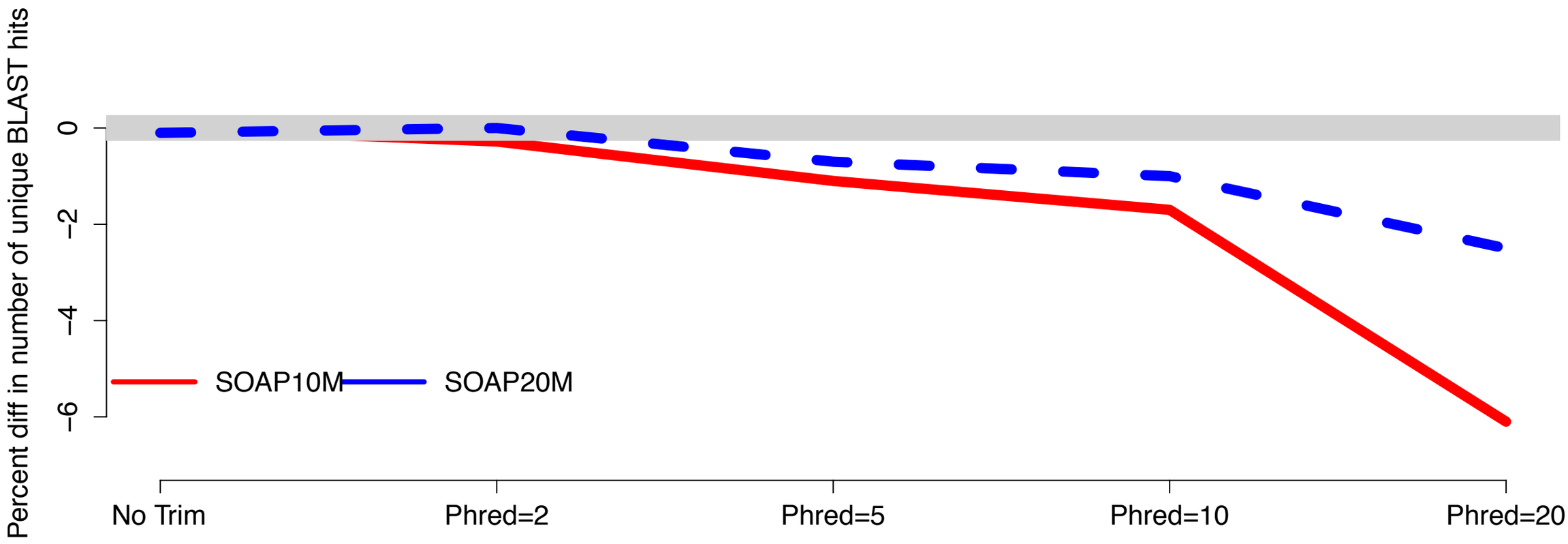
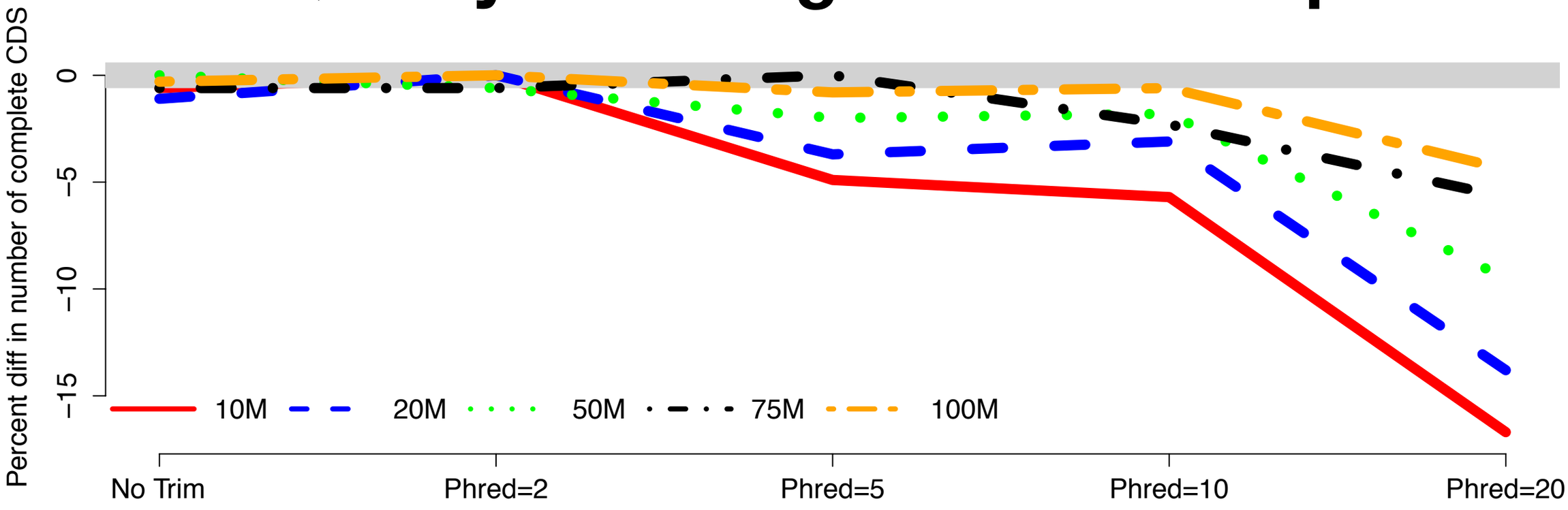
Quality trimming reduces BLAST hits





Quality trimming reduces complete CDS



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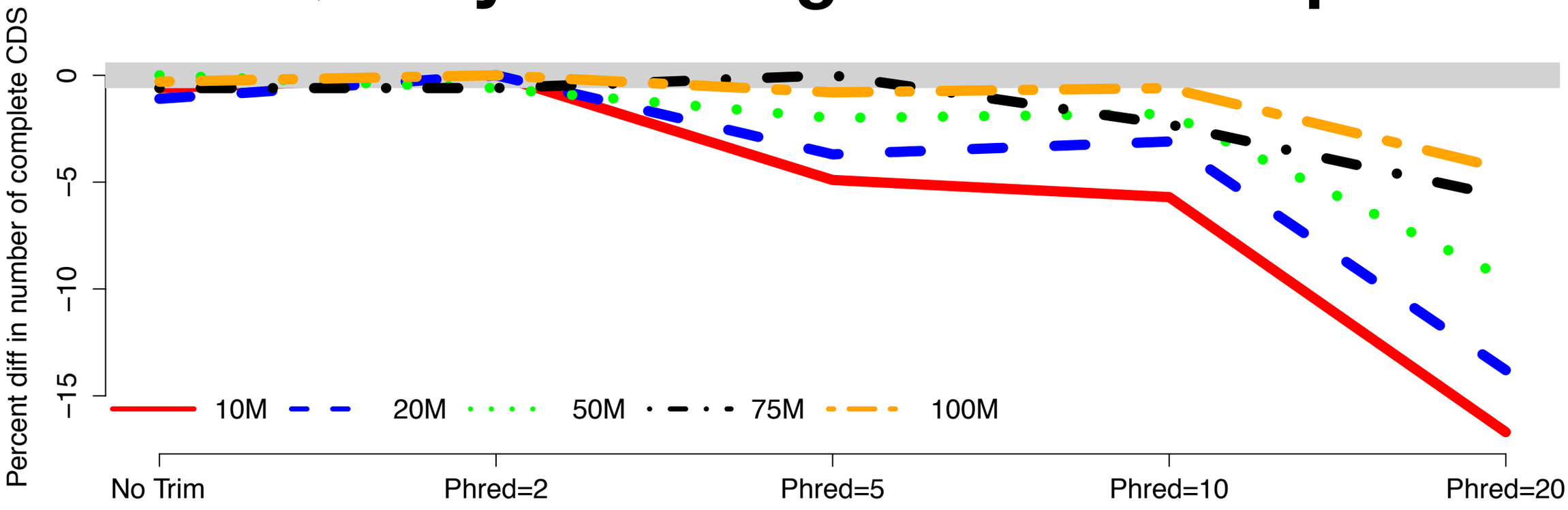
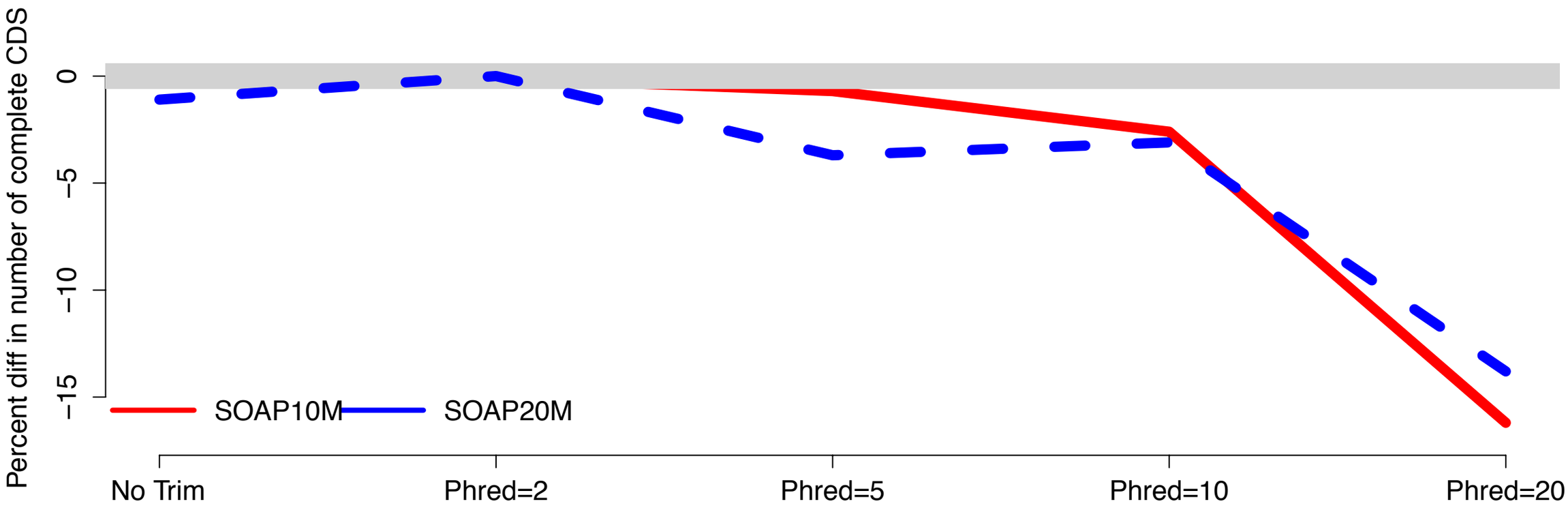
Quality trimming reduces complete CDS





Summary

- Trimming does reduce assembly error, but at the cost of content & contiguity.
- **<u>Proposed guidelines.</u>**
    1. To max assembly content and contiguity ➠ Trim at 0 or 2ish
    2. If concerned about error ➠ Trim at Phred=5
    3. Usually probably never trim at Phred ≥ 10

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Links

- https://www.youtube.com/watch?v=5NiFibnbE8o
- http://liorpachter.wordpress.com/
  - http://liorpachter.wordpress.com/2014/04/30/estimating-number-of-transcripts-from-rna-seq-measurements-and-why-i-believe-in-paywall/
  - http://liorpachter.wordpress.com/2014/03/01/using-statistical-methods-to-estimate-and-take-into-account-experimental-measurement-errors-a-case-study-using-high-throughput-proteomics-data/
  - http://liorpachter.wordpress.com/2013/10/17/non-uniform-coverage-of-transcripts-in-rna-seq-experiments/

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