Assessing 454 Errors in Microbial Genomes

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Sanger/454 Hybrid Assembly

Sanger/454 Hybrid Assembly at the JGI

- 9X Sanger (3 libraries), 20X 454/Roche GS 20 sequencing depth.
- Assemble 454 reads using Newbler assembler.
- Shred 454 Newbler contigs into 1000 bp fragments with 100 bp overlapping ends.
- Assemble 454 pseudo fragments with Sanger reads.

High Quality Finishing Standard

- All consensus bases >= Q30.
- Final error rate <= 0.2 per 10 Kb (1 error in 50,000 bp).
- No single clone coverage, i.e. minimum of 2X depth everywhere.
- Manually inspect and quantify single stranded regions.
- Check all high quality discrepancies.
- Final sequence should have a base at every position (no strings of xxxx anywhere).
- Verify all repeats (perform paired ends sequencing and PCR if necessary).
- Check correctness of final assembly. Confirm questionable areas with PCR.

454 Error Rate Analysis

GOAL:

- Assess error rate and type of errors produced by 454 sequence.

- Devise a strategy to target 454 errors in Sanger/454 hybrid assembly for quality improvement.

  - detect errors by motif?
  - correlate Sanger quality with Newbler quality?
  - adjust quality based on 454 read depth?

454 Error Rate Analysis

METHOD:

- Evaluated 9 finished microbial genomes sequenced with Sanger/454.
- Masked repeats in finished sequence.
- Aligned 454 Newbler contigs with finished sequence.
- Identify base mismatch between 454 and finished sequence.
- Categorize mismatches into homopolymers and non-homopolymers, where homopolymer is defined as a stretch of 2 or more identical bases.
- Categorize errors by read depth.

Results from Analysis

RESULTS:

Number of Finished Genomes:	9
Total 454 Bases:	23,940,257
Total Sanger Bases:	24,639,388
Total Bases Aligned:	23,650,082
Total Errors	5965
Total Error Rate	1 in 3,965
Homopolymer Errors:	5,666 (95%)

- Of all homopolymers detected in finished sequence, 1 in 833 (0.12%) contain errors

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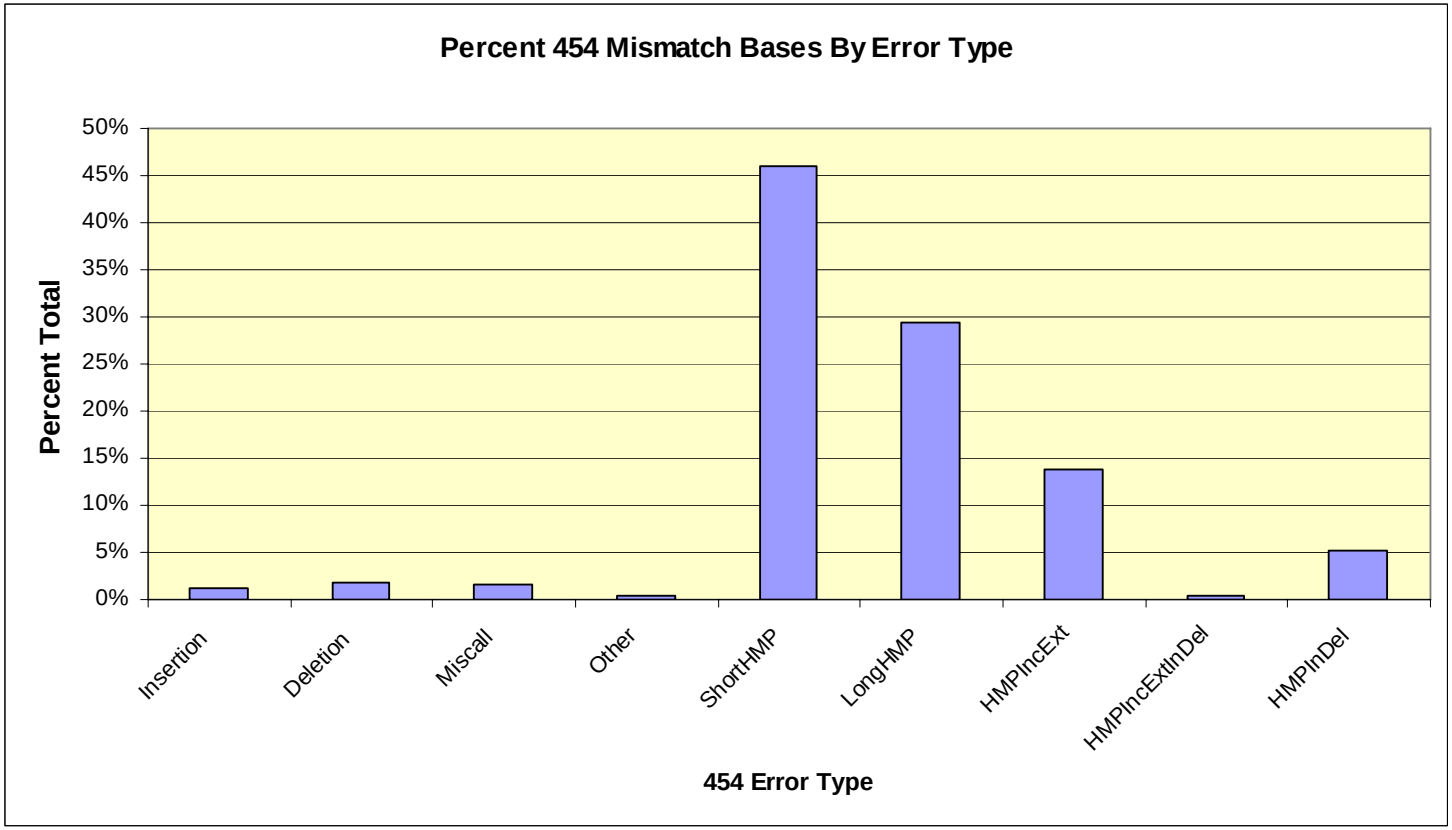
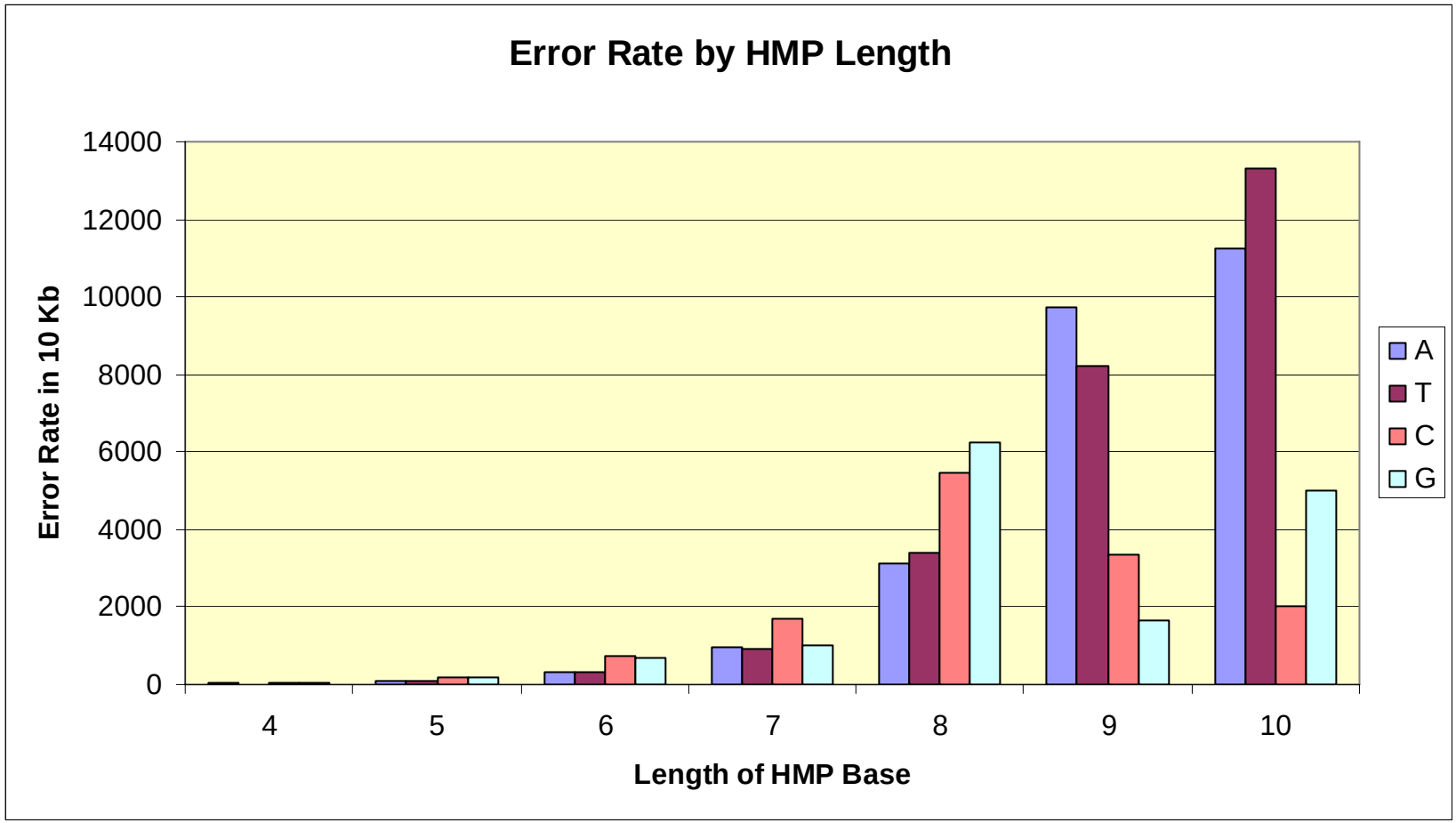
454 Mismatch Bases by Error Types



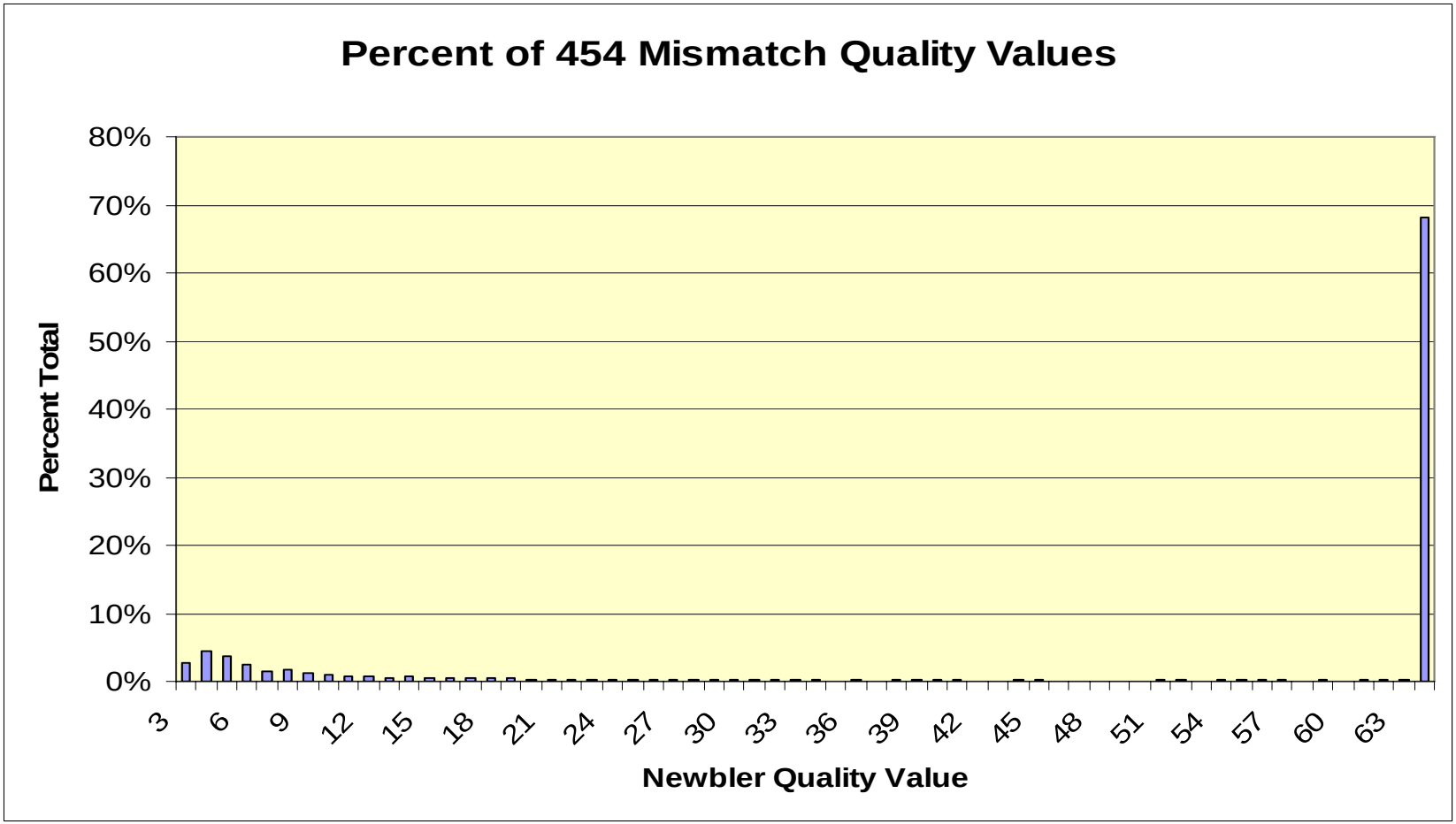
Non-homopolymer

Homopolymer

454 Error Rate by HMP Base and Length

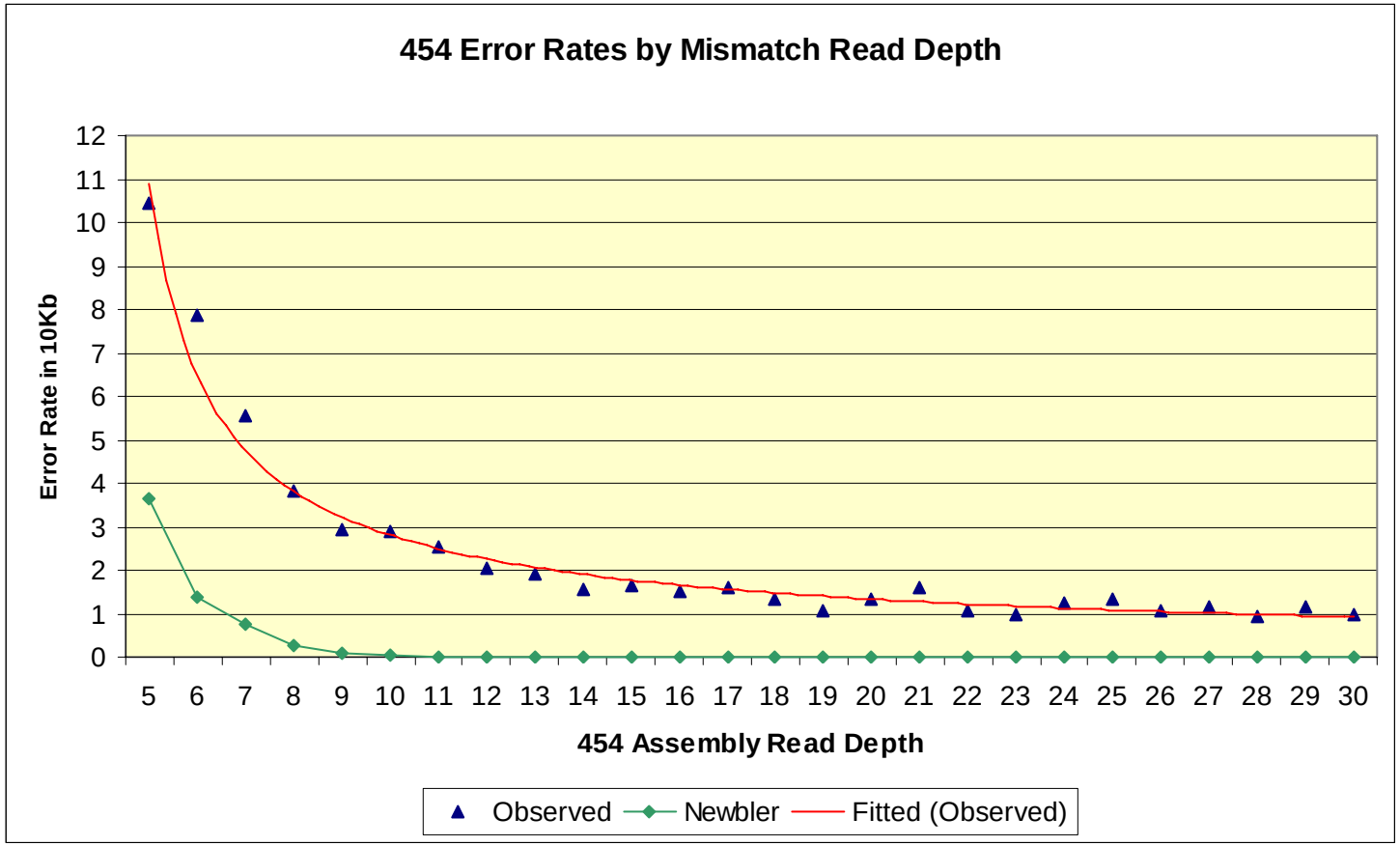


454 Mismatch Quality Values



Approximately 69% of 454 errors are assigned a Newbler consensus quality value of 64, the highest value assigned by Newbler.

Error Rate by 454 Mismatch Read Depth

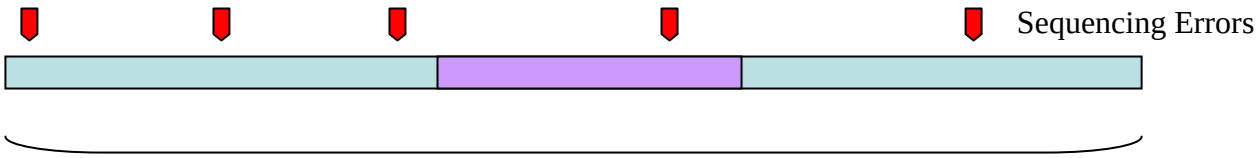


$$\text{Error Rate} = \frac{\text{Number of bases at read depth}}{\text{Number of mismatches at read depth}}$$

How Do We Use Read Depth to Target 454 Errors?

10% 454 Only Coverage

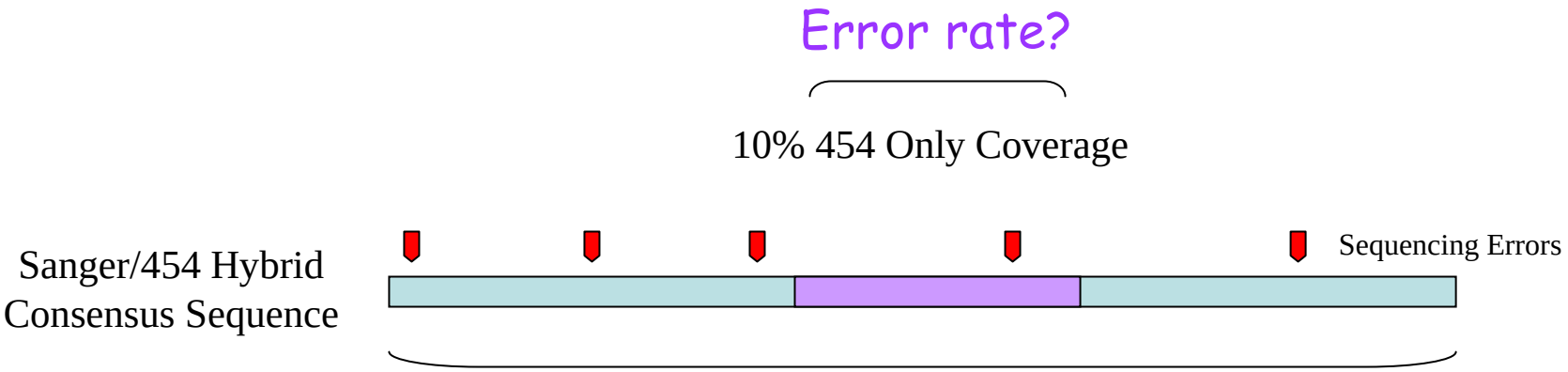
Sanger/454 Hybrid Consensus Sequence



Overall error rate <= 1 in 50 Kb

How Do We Use Read Depth to Target 454 Errors?

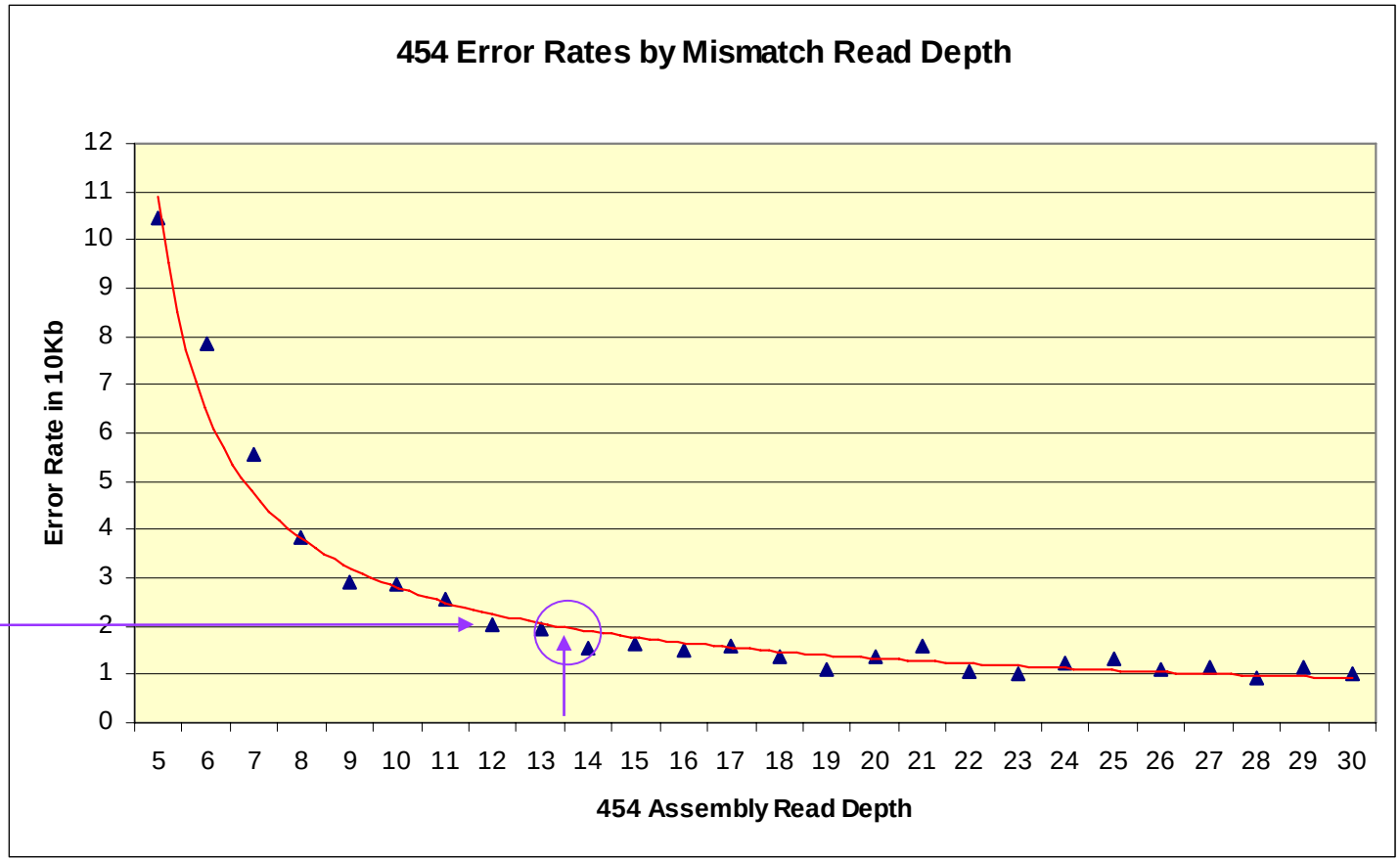
What is the error rate of the 454 only coverage such that the overall error rate is 1 in 50 Kb (assuming no errors outside 454 only coverage)?



Overall error rate <= 1 in 50 Kb

Error rate = 0.10 x 50 Kb = 5 Kb <u>OR</u> 1 in 5 Kb

Determining Minimum 454 Read Depth to Achieve Finishing Error Rate

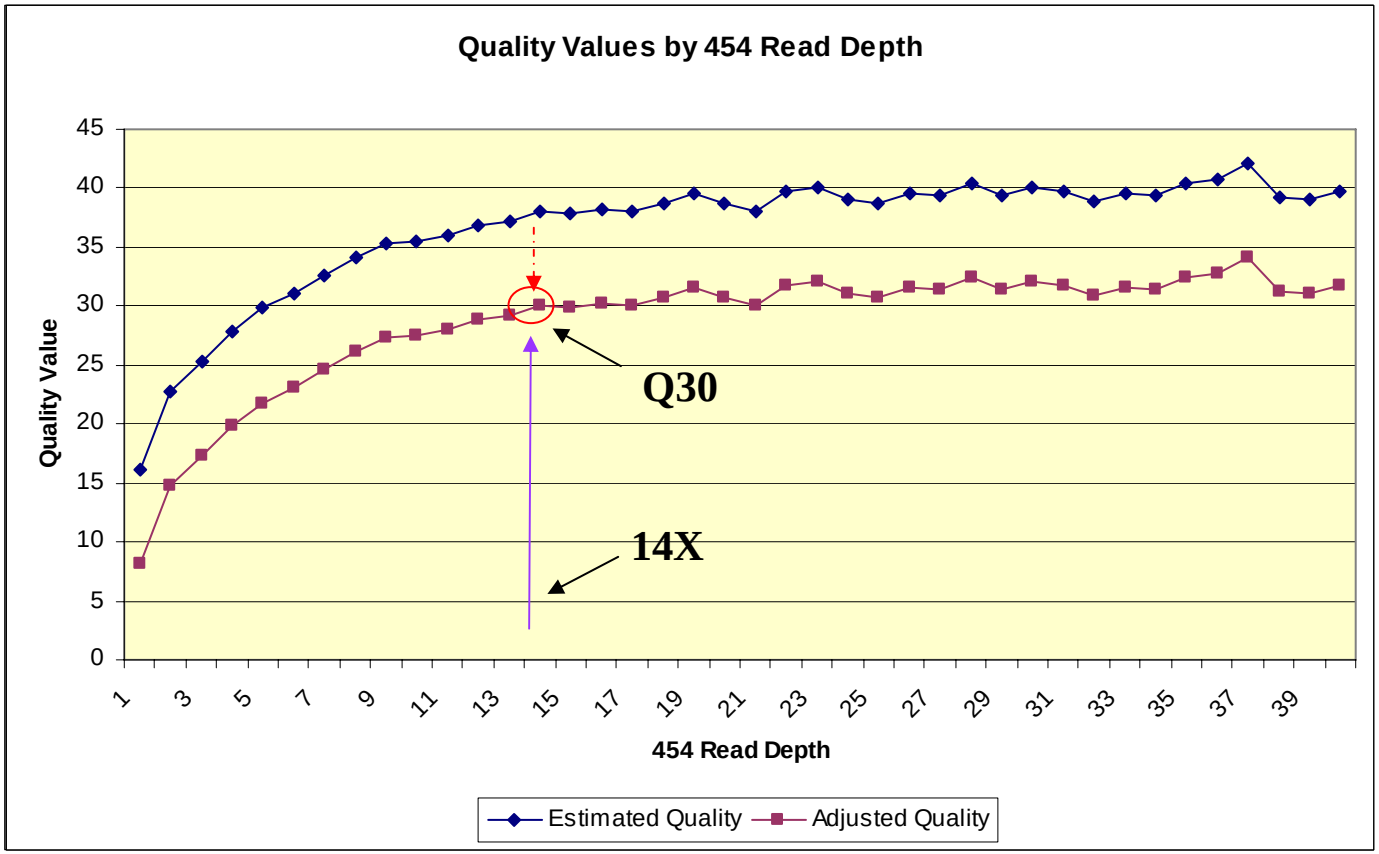


Error Rate in 5 Kb

Given Sanger/454 hybrid assembly containing 10% 454 only coverage, the minimum 454 read depth to achieve an overall error rate of 1 in 50 Kb is 14X.

Adjusted Quality Values Based on 454 Read Depth

Given Sanger/454 assembly containing 10% of areas covered only by 454 sequence



Alternative Strategies for Quality Improvement

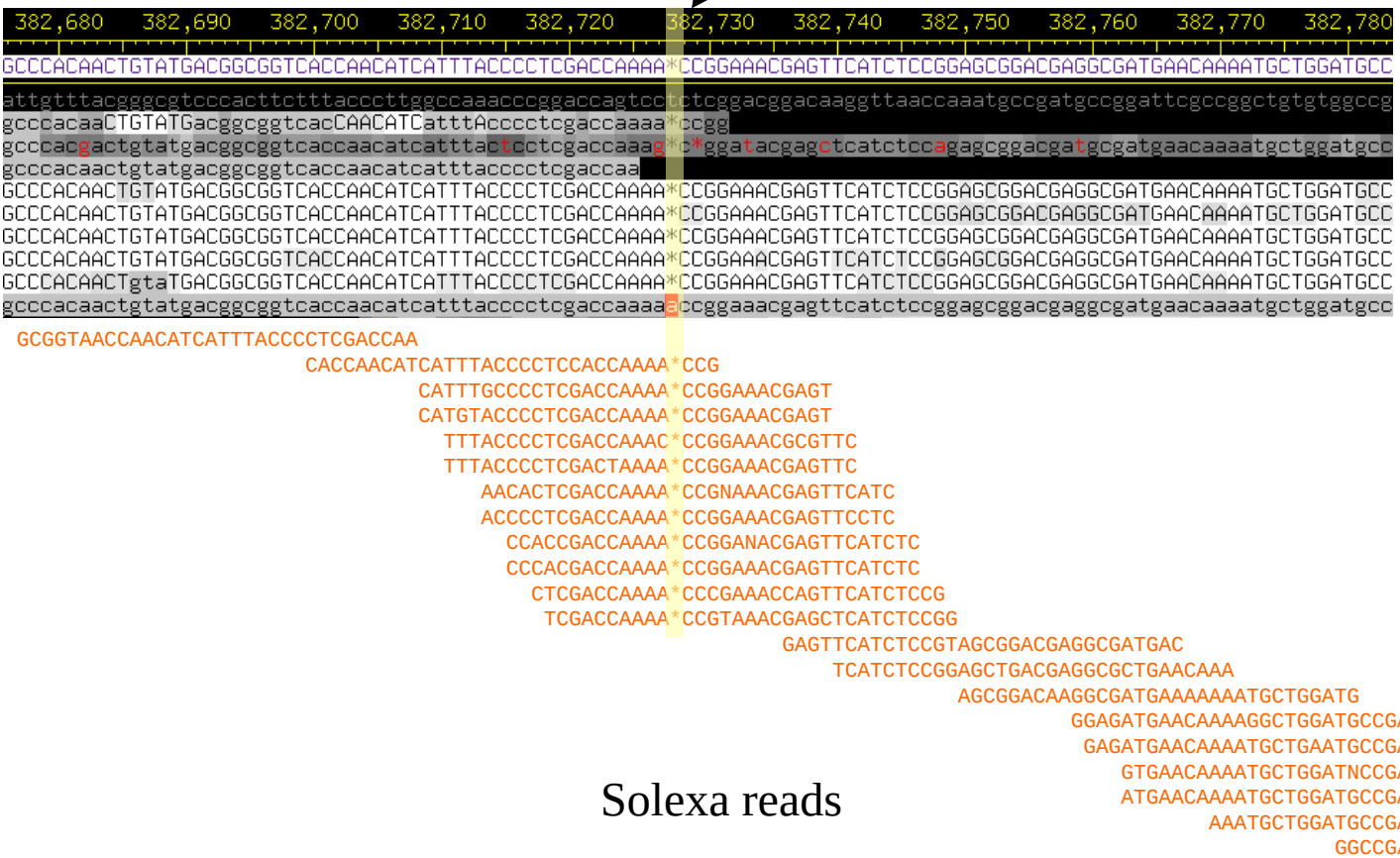
Solexa Sequencing:

- Identify and correct mismatches between Solexa and 454 only coverage

Frameshift Detection:

- HMM based frameshift detection tool (in collaboration with GeneMark developers at Georgia Institute of Technology)

Example: Solexa corrects frame shift (FS) in 454



Frame shift detected

```
 382,680   382,690   382,700   382,710   382,720    382,730   382,740   382,750   382,760   382,770   382,780
GCCCACAACTGTATGACGGCGGTCACCAACATCATTTACCCCTCGACCAAAA*CCGGAAACGAGTTCATCTCCGGAGCGGACGAGGCGATGAACAAAATGCTGGATGCC
attgtttacgggcgtcccacttctttacccttggccaaacccggaccagtcctctcggacggacaaggttaaccaaatgccgatgccggattcgccggctgtgtggccg
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gcccacgactgtatgacggcggtcaccaacatcatttactcctcgaccaaag*c*ggatacgagctcatctccagagcggacgatgcgatgaacaaaatgctggatgcc
gcccacaactgtatgacggcggtcaccaacatcatttacccctcgaccaa
GCCCACAACTGTATGACGGCGGTCACCAACATCATTTACCCCTCGACCAAAA*CCGGAAACGAGTTCATCTCCGGAGCGGACGAGGCGATGAACAAAATGCTGGATGCC
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gcccacaactgtatgacggcggtcaccaacatcatttacccctcgaccaaaaaccggaaacgagttcatctccggagcggacgaggcgatgaacaaaatgctggatgcc
 GCGGTAACCAACATCATTTACCCCTCGACCAA
                       CACCAACATCATTTACCCCTCCACCAAAA*CCG
                                CATTTGCCCCTCGACCAAAA*CCGGAAACGAGT
                                CATGTACCCCTCGACCAAAA*CCGGAAACGAGT
                                  TTTACCCCTCGACCAAAC*CCGGAAACGCGTTC
                                  TTTACCCCTCGACTAAAA*CCGGAAACGAGTTC
                                     AACACTCGACCAAAA*CCGNAAACGAGTTCATC
                                     ACCCCTCGACCAAAA*CCGGAAACGAGTTCCTC
                                       CCACCGACCAAAA*CCGGANACGAGTTCATCTC
                                       CCCACGACCAAAA*CCGGAAACGAGTTCATCTC
                                         CTCGACCAAAA*CCCGAAACCAGTTCATCTCCG
                                          TCGACCAAAA*CCGTAAACGAGCTCATCTCCGG
                                                             GAGTTCATCTCCGTAGCGGACGAGGCGATGAC
                                                                 TCATCTCCGGAGCTGACGAGGCGCTGAACAAA
                                                                           AGCGGACAAGGCGATGAAAAAAATGCTGGATG
                                                                                    GGAGATGAACAAAAGGCTGGATGCCGAGATGC
                                                                                     GAGATGAACAAAATGCTGAATGCCGAGATGCT
                                                                                        GTGAACAAAATGCTGGATNCCGAGATGCTGCC
                                                                                        ATGAACAAAATGCTGGATGCCGAGCTGCCGCC
                                                                                               AAATGCTGGATGCCGAGATGCTGCCGTACTGT
                                                                                                         GGCCGAGATGCTGCCGTTGTGTGTTGGAGCGG
```

454 contig

Solexa reads

- -Frame shift detected in 454 contig.
- -Sanger and Solexa support the finished reference.
- -Development of both GeneMark based FS detector (in collaboration with GeneMark developers) and corrective tool are in progress.

100% of positive frame shifts detected in 454 contigs were confirmed by Solexa reads

454 Error Rate Analysis

SUMMARY:

Sanger/454 sequencing:

- detect 454 errors by read depth and percent 454 only coverage.

Sanger/454/Solexa sequencing:

- detect mismatches between Solexa and 454 to correct 454 errors.
- use frame shift detection to identify discrepancies between Sanger and Solexa sequence.

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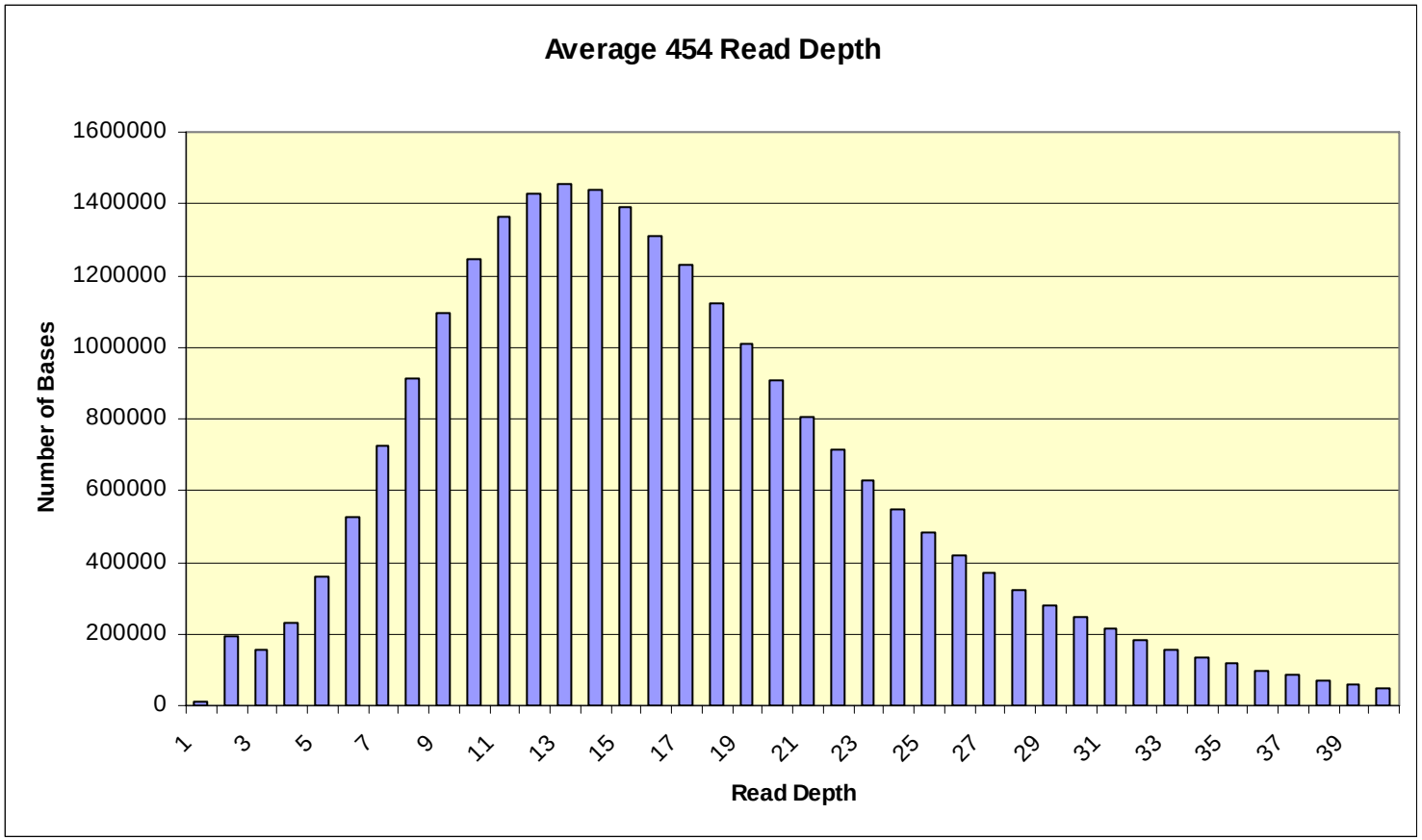
Average 454 Read Depth



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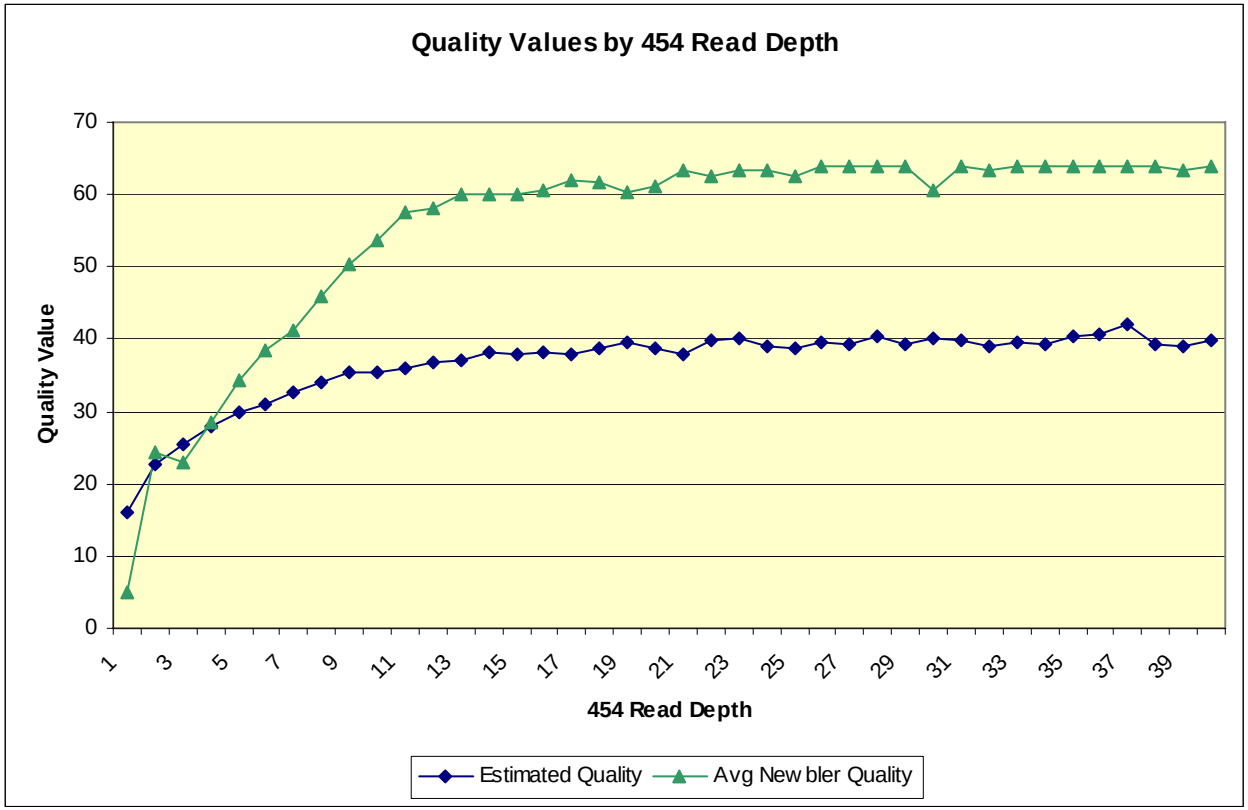
Quality Values Based on 454 Read Depth



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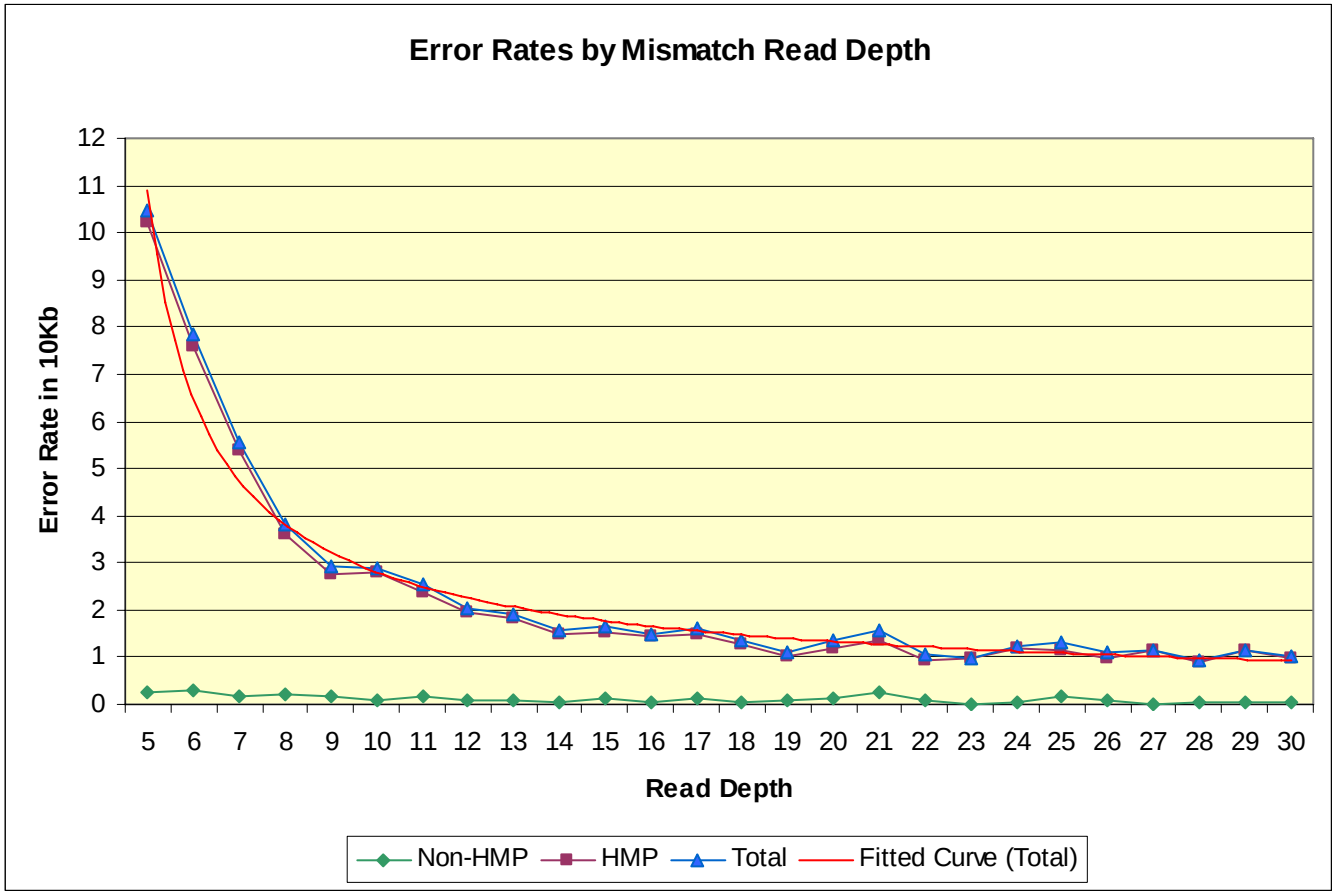
Error Rates by Mismatch Read Depth



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Distribution of 454 Only Coverage