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Based on the estimated empirical quality for a particular quality score, we were able to interpolate the expected mean quality for each base. To estimate the error probability for a base for a given quality, we used the binomial distribution (Methods). The estimated effective error rate of base for a given quality score is shown in Figure 2. Overall, the estimated base quality is poor, with only 0.08 (0.14), 0.19 (0.29), 0.26 (0.44) and 0.50 (0.81) of the expected quality for bases of 10, 11, 12 and 13 bases long, respectively, in the data generated by Roche 454 pyrosequencing. Most of these bases are very poor quality (P5), however, only a small proportion of these bases have been identified as unreliable by the Roche 454 platform, and thus, these poor-quality bases are likely to be identified as HIF using the Roche 454 sequencing platform. Indeed, the homopolymer base error estimate (Figure 2) compares favourably with that estimated using Roche 454 pyrosequencing, with the exception of >13 base homopolymers. By contrast, the estimated effective error rate in the HIF calls of the Ion Torrent PGM sequences is approximately three times higher than that estimated using Roche 454 pyrosequencing at the most homozygous bases. This is particularly marked at lower average homopolymer lengths, however, it also appears that PGM sequences can be accurately sequenced over longer homopolymers than the Roche 454 pyrosequencing. Note that we can only directly compare the effective error rates of the two sequencing platforms given their different algorithms.

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JGI genomes that were sequenced on the Ion Torrent (except for three genomes that were sequenced on an ABI SOLiD v4 instrument), NCBI genomes that were sequenced on the Ion Torrent, and Roche 454 genomes that were sequenced on the Illumina HiSeq (except for the frozen cells data which was sequenced on the Illumina MiSeq). Datasets were excluded if they had been previously used for a study of the platform or if raw sequences were publicly available as of March 2013. Datasets from organisms other than bacteria, archaea, viral or fungi were excluded due to the expected bias towards sequencing bacterial strains. Any dataset generated using both platforms was retained, as were any organisms for which a dataset had been generated previously with a different platform. An additional dataset containing sequencing reads from frozen cells was downloaded from an open access repository [20] and used as a supplementary reference dataset in this study. The high levels of indel errors detected through the various combinations of alignment algorithm and platform were much more extreme than in the sequencing data from Ilumina. Only 25% (100 bp OneTouch) to 56% (150 bp OneTouch) of the reads produced by the 100 bp flow-cell were mapped with low mismatches. We attributed this to the platform-specific sub-sampling effect of low-quality bases near the ends of the reads [10]. Ultimately, the combined biological and analytical insights from these datasets will allow us to better understand the errors inherent to each platform. We have taken the insights obtained from the analysis of technical replicates (discussed above) to design a joint method to detect error-free reads (Methods). Simulations suggest that this can result in a more than 99.99% reduction of over-call/under-call errors in amplicon sequencing as well as the ability to identify large areas of noise that would otherwise confound genotype calls (Additional file 21 : Figure S11). The power of this joint method is demonstrated in Figure 6 - bottom panel, where this approach reduces the number of ambiguous calls and detects polymorphic sites at very low frequency. 5ec8ef588b

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