Determining the optimal cycle number for indexing PCR using the qPCR amplification plots. Shown are the amplification plots obtained from quantifying the libraries prepared in the experiment described in 'Anticipated results'. The saturation phase of PCR starts after cycle 18 (sample libraries, red and blue) and cycle 23 (blank library, green), respectively. Assuming full amplification efficiency (i.e. a doubling of library molecules in each cycle), the optimal cycle number for indexing PCR can be determined as follows by correcting for differences in reaction volumes and the amount of template DNA: (i) qPCR was performed in 25 µl reactions, whereas indexing PCR is performed in 100 µl volume. Thus, 2 cycles should be added to allow for 4 times more end product. (ii) One microliter of a 1:20 library dilution was used for measurement, whereas 24 µl of the library are used for indexing PCR (480 times as much). This corresponds to 8.9 (rounded 9) cycles that should be deducted. Thus, 11 and 16 were estimated to be the optimal cycle numbers for indexing PCR.