The Wipe Test Revolution: Introduction of a Sequencing Based Wipe Test in a High-Throughput Genotyping Laboratory

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Motivation

Sample contaminations are a major threat for correct and reproducible HLA genotyping. They can occur if a tiny amount of DNA is transferred from one sample to another via lab surfaces, machines or staff. Ultimately this can lead to genotyping failure and at worst to the communication of wrong results with possible risks for a patient. As preventive measure, periodic wipe tests are fundamental and therefore mandatory for accredited laboratories. Even though wipe test kits can be purchased from different vendors they are generally limited to the classical HLA genes. Contaminations from other genes like KIR, MICA, MICB, ABO and Rh, which are also genotyped in our lab, would be missed. Therefore, we implemented a novel wipe test that detects DNA contaminations from both genomic DNA and amplified PCR products covering all genes analysed in our genotyping workflow.
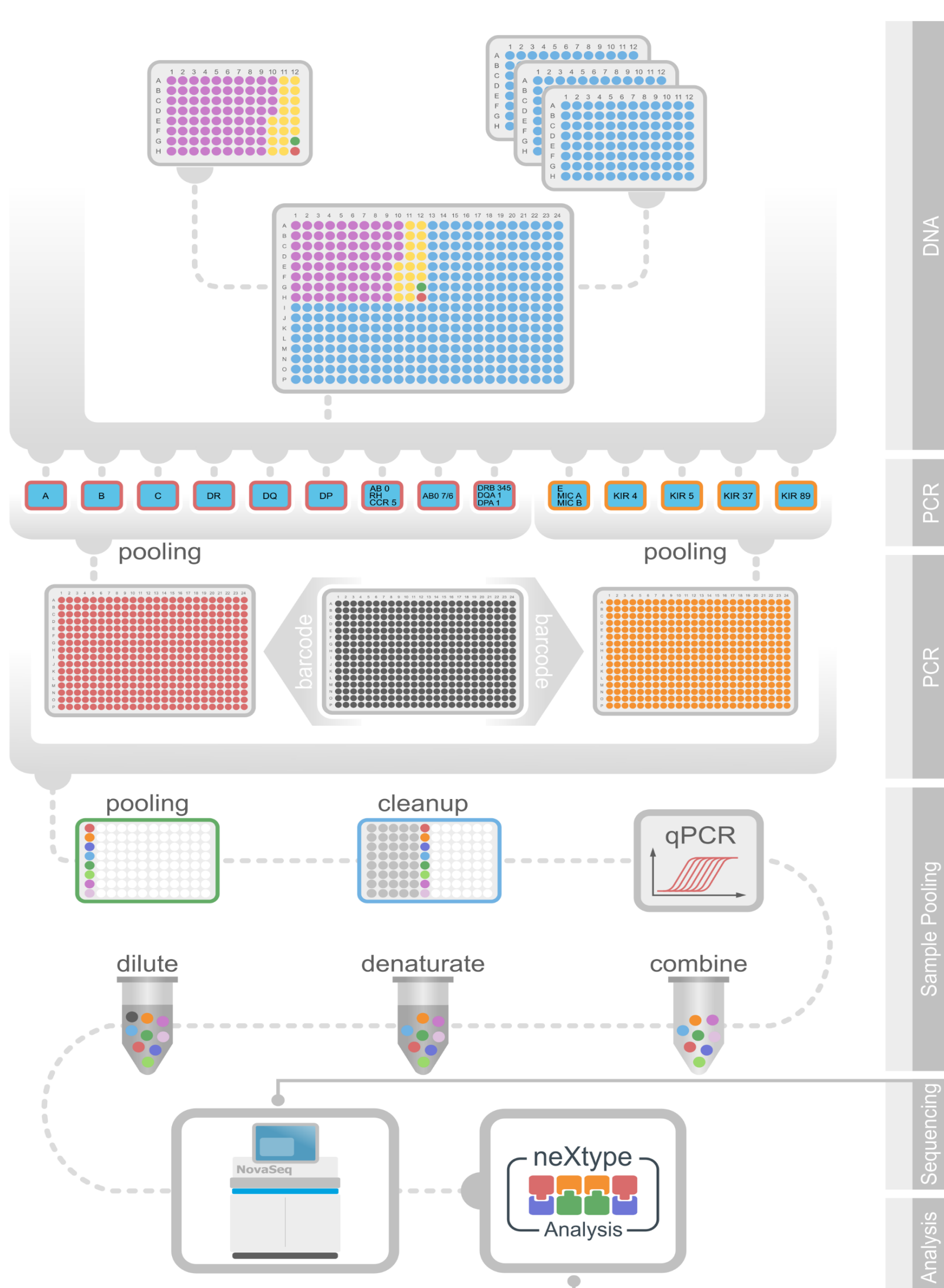
Sample Processing

Figure 1: NGS-based wipe test. A wipe test plate consists of 76 wipe test samples (violet), a dilution series of genomic DNA ranging from 15 ng/µl to 0.005 ng/µl (yellow) and controls (green/red). After DNA isolation, the wipe test plate is combined with routine genotyping samples plates and runs through our standard high-throughput HLA genotyping workflow without the need of additional standard operating procedures. After sequencing, wipe test samples are automatically visualized for analysis (Figure 2).

Analysis

Wipe test sequencing reads are counted and assigned to their corresponding PCR amplicons. Amplicons with up to 20 reads are evaluated negative (Figure 2). Samples with over 200 sequencing reads in several amplicons are evaluated positive (Figure 2, samples 216, 218, 228, 230). Wipe test samples with read counts in multiple amplicons between 20 and 200 or wipe test samples with a single amplicon over 200 reads are further analyzed in our HLA genotyping software neXtype. If a valid genotyping result can be calculated from those borderline samples, the wipe test sample is evaluated positive. Borderline sample 225 (Figure 2) was finally evaluated negative with around 100 reads assigned to HLA-B exon 2, which is not enough for the calculation of a valid result in our workflow. However, this minimal contamination might be the reason for a band in the gel-based wipe test. Interestingly, similar read counts were detected in samples 200 and 213, which did not show a band in the gel. This emphasizes the fact that the NGS-based wipe test outperforms the gel-based wipe test in samples that are close to the detection limit as it allows for a much deeper analysis.
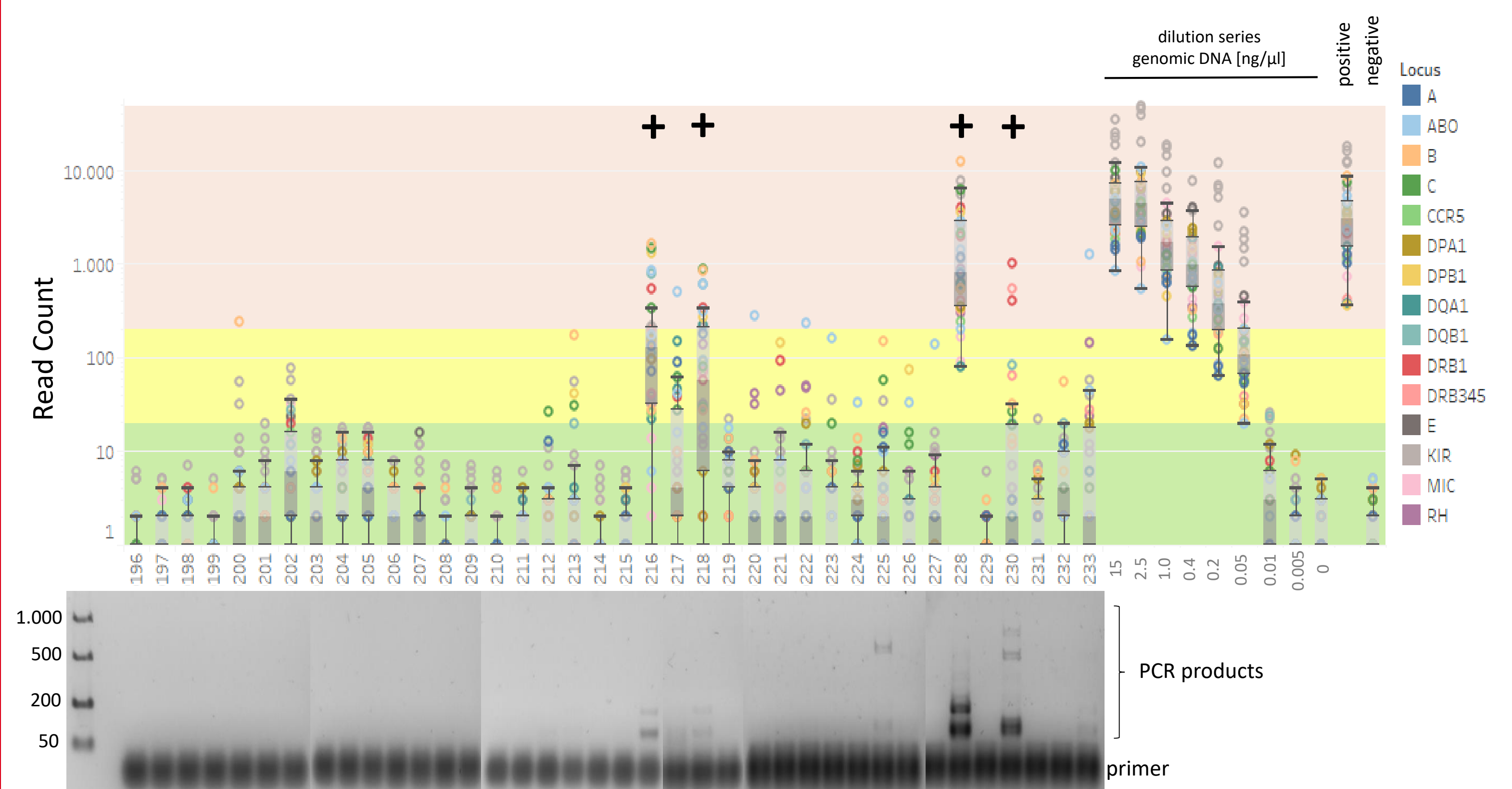

Figure 2: Comparison of the NGS-based wipe test and a commercial PCR/gel-based wipe test. The gel-based wipe test was performed using the AllSet+™GoldDNA Wipe Test kit (One Lambda, West Hills, USA). Since both wipe tests were performed on the same samples, gel lanes are shown directly beneath their corresponding NGS wipe test results for comparison. Green area: read counts between 0 and 20. Yellow area: read counts between 20 and 200. Red area: read counts above 200. The + mark is used to highlight contamination-positive samples.

Summary

As technical developments for HLA genotyping are rapidly improving, methods for quality control need to keep pace as well. A wipe test, which is processed by the same NGS-based high-throughput workflow used for genotyping, reduces costs and hands-on-time. Furthermore, the quantitative output allows standardization and continuous monitoring of all wipe locations over time and eliminates the challenge to reproducibly interpret faint bands of a gel-based wipe test kit. Most importantly, it is automatically up-to-date with the amplicons used in a lab without the need to adapt its standard operating procedures after workflow expansion.