# Identification of Contamination in PGS Samples

Recommendations to identify contamination and avoid misdiagnosis.

## Introduction

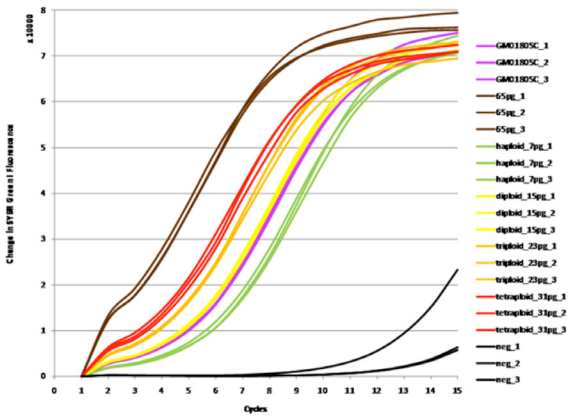
24sure™ and VeriSeq™ technologies use whole-genome amplification to increase the quantity of DNA in a single cell or a few cells. This amplification ensures sufficient amounts of DNA for microarray hybridizations or next-generation sequencing of preimplantation genetic screening (PGS) samples.

Incorporation of extraneous material containing DNA, prior to amplification, is a concern with any DNA amplification technology, and it can lead to misleading results. In the case of PGS samples, such contamination could occur during the biopsy process, from non-sterile reagents or equipment, or even air-borne DNA. At worst, DNA contamination can result in the misdiagnosis of an embryo, e.g., an aneuploid embryo appearing euploid. The laboratory protocols detail ways to avoid contamination, e.g., single-use consumables, filter tips, and ultraviolet (UV) treament of equipment. This technical note summarizes methods to assess whether a sample has DNA contamination.

## Recommendations

1. Illumina recommends amplifying both some biopsy media (e.g., one media sample per wash droplet used) and some phosphate-buffered saline (PBS) as negative controls. This will test whether these solutions are DNA-free and assess whether the process has been DNA-free up to this point. Agarose gel electrophoresis or double-stranded (ds) DNA quantification of these test reactions, along with the SurePlex sample amplifications, should be sufficient to assess contamination. Negative controls should give no bands or a barely discernible signal when compared to the positive controls, otherwise contamination is likely.
2. 24sure only: Contamination can be detected by noting suppression of X-separation for the amplified 15 pg control (Positive Control 1) vs. SureRef Male DNA. Similarly, lower X-separation for all cells in experimental samples, when compared to SureRef Female DNA and SureRef Male DNA, indicates DNA contamination.
3. VeriSeq only: Contamination with non-human DNA can be identified by a low percentage of mapped reads (< 40%) aligning to the human genome.
4. Suspect contamination when autosomal noise is very low compared to that expected for a single cell. For 24sure, if the profile is cleaner than the SureRef hybridizations or the 15 pg control (Positive Control 1), then the starting material would be closer to 10 cells' worth of DNA. When contamination occurs, it is usually in large or overwhelming amounts of DNA compared to the amount from a single cell.
5. Suspect contamination if all embryos in a cycle are the same sex, i.e., all female or all male. Overwhelming contamination with DNA may obscure the true sex chromosome shifts.
6. 24sure only: The use of SYBR Green in a real-time PCR machine can assess contamination during the amplification step (step 3) of the SurePlex protocol. Non-contaminated cells should fall into the same curve as the 15 pg diploid curve. Contamination would result in a left shift of the amplification curve, i.e., a decrease in $C_T$ value (Figure 1).
7. Some labs also run a panel of highly polymorphic short-tandem repeat (STR) PCR markers on the amplified products.

**Figure 1: Real-Time PCR Detection**




A plot of $C_T$ values from a SYBR Green real-time PCR analysis of SurePlex samples prior to use in a 24sure assay

Overall, taking some of the steps described and, in particular, paying attention to X/Y separation/autosomal noise addresses the issue of detecting sample contamination. In-house experience with running 24sure assays revealed that contamination occurred rarely. There were only 2 cases of contamination in 300 amplifications. In these cases, the contamination was detected through amplification of biopsy media and associated gel images.

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