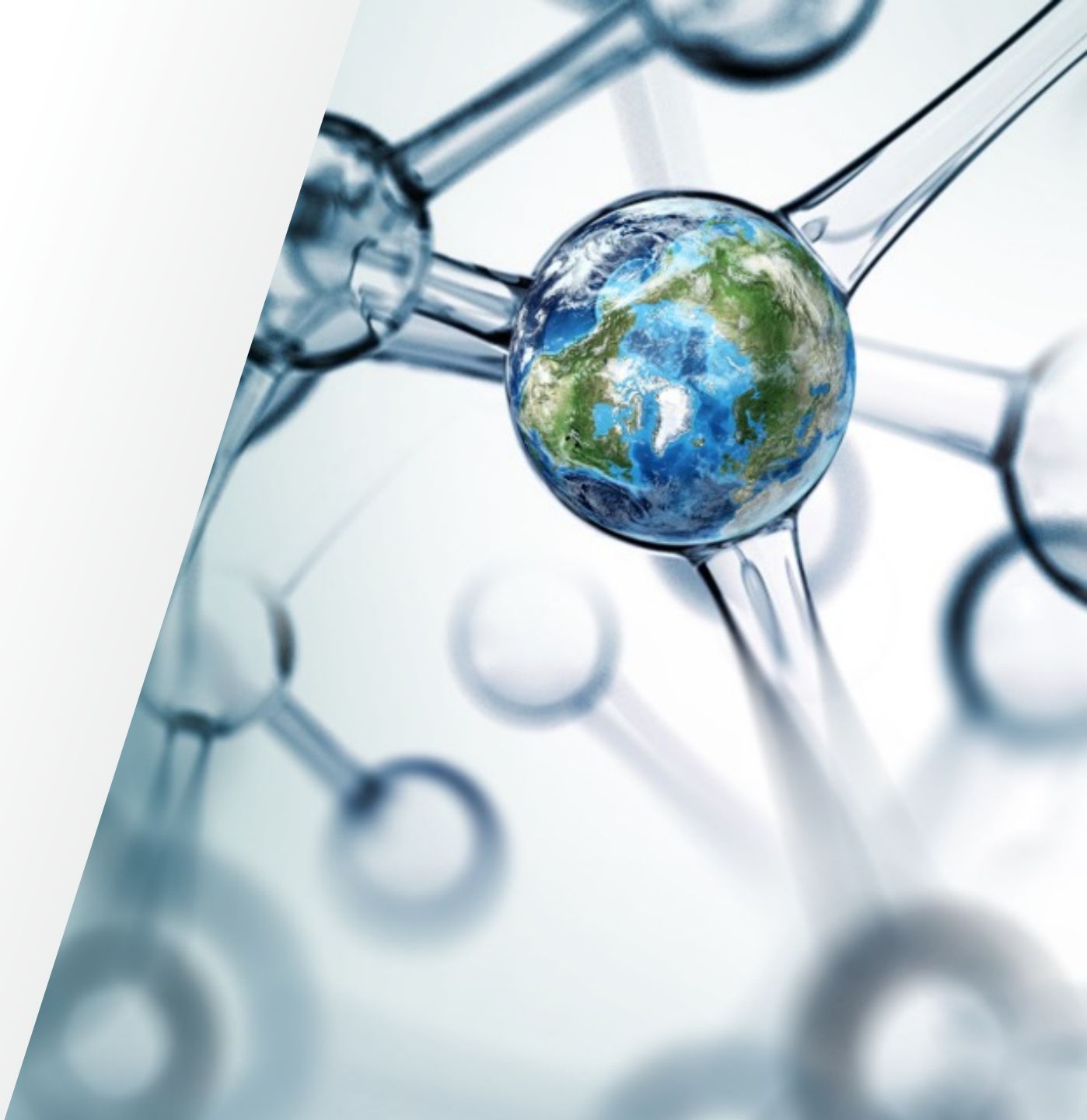
ThermoFisher SCIENTIFIC



KaryoStat+ ™ Assay Report

Client Name: **Stanford**
Quote No: **D4874281**
Date: **23-Jan-2023**
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Assay background

- Traditionally, genetic stability of pluripotent stem cells has been confirmed via g-banded karyotyping, a method performed by professional cytogeneticists. This method requires provision of log-phase cells by the researcher. Chromosomal spreads from 20 individual cells are stained and visually inspected for aberrations, and images captured under a microscope for subsequent reporting.

- Recently, researchers have adopted alternative methods employing molecular, array-based approaches to help reduce subjectivity and streamline sample submission. Conveniently, frozen cell pellets are collected instead of live cells. Resolution is often slightly higher (1Mb or less) with these assays than with traditional karyotyping (5-10 Mb). The limit of detection for percent of cells containing aberrations is lower, however, since it is a population-based assay rather than evaluating one cell at a time.

- KaryoStat+ Assay services represents one such alternative to g-banded karyotyping for **all non-transformed human cells**. This includes primary cells, stem cells, and *in vitro*-differentiated cells. The assay offers accurate genotyping (cell ID) plus whole-genome coverage for accurate detection of chromosomal abnormalities with a typical turnaround time of 3-4 weeks. This assay is **not recommended for transformed or immortalized cell lines** due to the large number of chromosomal rearrangements observed. These cell lines are unlikely to pass internal QC requirements and therefore provide inconclusive results.

- We welcome you to find out more about the services at the following site:

https://www.thermofisher.com/us/en/home/life-science/stem-cell-research/stem-cell-services/karyostat-karyotyping-service.html

Summary of Services

Project Summary:

- Stanford is interested in services provided by the Life Technologies Corporation in the analysis of three (3) client-provided samples using the KaryoStat+ assay.

Service Description:

- The KaryoStat™ assay allows for digital visualization of chromosome aberrations with a resolution similar to g-banding karyotyping. The size of structural aberration that can be detected is > 2 Mb for chromosomal gains and > 1 Mb for chromosomal losses (the resolution depends on the location of the aberration in the chromosome. Due to a lower probe density on the telomere ends and centromeres, the resolution in those locations may be closer to 5Mb). The KaryoStat array is optimized for balanced whole-genome coverage with a low-resolution DNA copy number analysis, the assay covers all 36,000 RefSeq genes, including 14,000 OMIM® targets. The assay enables the detection of aneuploidies, submicroscopic aberrations, and mosaic events.

Methods:

1. Genomic DNA purification:
    - Cells were prepared according to the PureLink™ Genomic DNA Purification Kit (Catalog #: K1820-02) and quantified using the NanoDrop ONE^{C} (Catalog # : 701-058108).

2. GeneArray® Preparation:
    - 100 ng total gDNA was used to prepare the GeneArray® for KaryoStat according to the manual, and is an array that looks for SNPs, copy number variants and single nucleotide polymorphisms across the genome.

KaryoStat+ Results: KS-13986 (VS-4)

Chromosome	Type	Cytoband Start	CN State	Size (kbp)
12	GainMosaic	p13.33	2.28	133,236
15	Loss	q11.1	1.00	3,548
20	Gain	q11.21	3.00	1,111

Table 3. KaryoStat+ analysis. Chromosomal aberrations are indicated in the table shown**.** See supplemental data for more details.

1. For iPSCs, the presence of two X chromosomes indicates a female genotype whereas presence of an X and a Y chromosome denotes a male genotype.
2. If a deletion in Chr. Y is found, it is possible that this sample is derived from the WTC cell lines, which are known to have this genotype, and originates from the donor from which the cell line was derived.

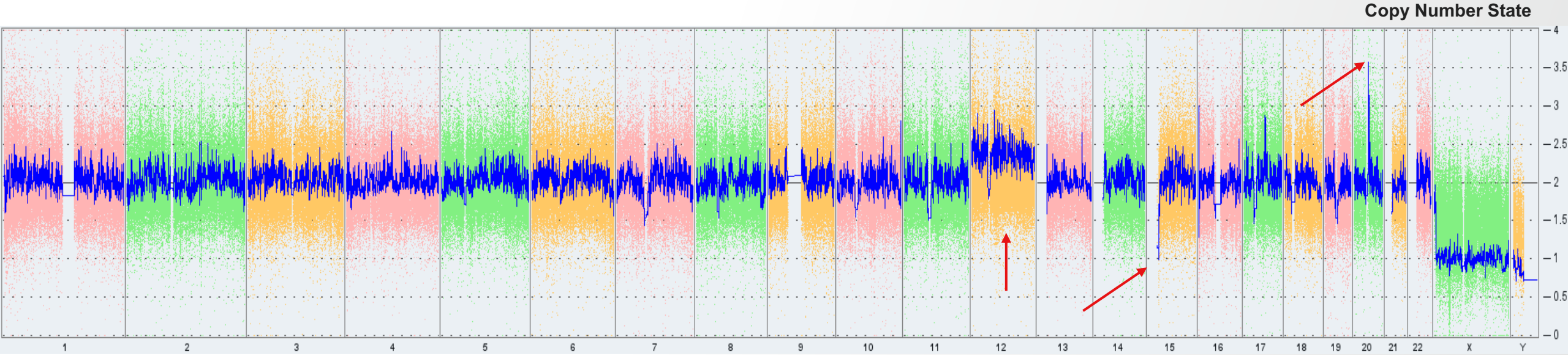

Figure 2: Whole genome view. The whole genome view displays all somatic and sex chromosomes in one frame with high level copy number. The smooth signal plot (right y-axis) is the smoothing of the log2 ratios which depict the signal intensities of probes on the microarray. A value of 2 represents a normal copy number state (CN = 2). A value of 3 represents chromosomal gain (CN = 3). A value of 1 represents a chromosomal loss (CN = 1). The pink, green and yellow colors indicate the raw signal for each individual chromosome probe, while the blue signal represents the normalized probe signal which is used to identify copy number and aberrations (if any). Aberrations when present are indicated by red arrows.