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THE IMPORTANCE OF COMBINING CHROMOSOME KARYOTYPING AND DIGITAL PCR ASSAYS TO ASSESS GENOMIC INTEGRITY

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An important component of the human stem cell research and therapeutic development process is the reliable assessment of genomic integrity. Genomic instability can compromise both the safety and efficacy of derived products, yet each individual characterization method has specific weaknesses.
In alignment with the various options for genomic characterization set forth in the ISSCR guidelines[1], we evaluated the combined use of two complementary assays in the case presented: traditional G-band chromosome karyotyping, and a recently upgraded 28-probe digital PCR panel for commonly mutated sequences in human pluripotent stem cells. While karyotyping reliably identifies chromosomal rearrangements larger than 10 megabases, digital PCR (dPCR) is able to capture smaller, recurrent sequence alterations that are not always visible cytogenetically. The details of specific samples analyzed within the past year are presented to demonstrate the advantages of utilizing both techniques together in the research and development of stem cell therapy products. Considering the costs and turnaround times of other genetic characterization assays, this integrated approach provides a more rigorous and faster assessment, thus strengthening quality control practices. Together, these results support a broader adoption of dual genomic integrity testing strategies to safeguard stem cell research and accelerate progress in the cell therapy field.

MATERIAL AND METHODS

The "Duo iCS-karyo" assay, which is a combination of both G-band karyotyping and digital PCR, was performed on a total of 255 human stem cell samples received between December 1, 2024 and November 30, 2025. The majority of the samples were induced pluripotent stem cells (iPSC), 96%, with the remainder being hematopoietic stem cells (HSC), 2%, and mesenchymal stem cells (MSC), 2%. They were received from laboratories in the private industry, academic and government sectors. KaryoLogic Inc performed the G-banding by routine methods. A minimum of 20 metaphase chromosome spreads were analyzed per sample, at a band resolution ranging from 350 to 550, utilizing Leica Microsystems CytoVision karyotyping software. Stem Genomics Inc performed the digital PCR assays using a proprietary panel of 28 DNA probes. Of note is the inclusion of a probe for the chromosome 20q11.21 sequence, which has been shown to be the most common genetic abnormality in human pluripotent stem cells[2]. DNA extracted from the cell samples was set up with ddPCR Multiplex Supermix (Bio-Rad), the proprietary panel of 28 DNA probes, and a reference. PCR reaction plates were prepared using a QX200™ Droplet Generator. DNA Amplification Thermocycling conditions were run as described by the manufacturer for the ddPCR Multiplex Supermix (Bio-Rad), before samples were read on the QX600™ Droplet Reader. Copy number was assessed using Quantasoft software.

RESULTS

Of the 255 samples:
- **19 displayed an abnormal result** in either the G-banding analysis, dPCR analysis, or both. (7.45%)
- **4 samples displayed corroborating abnormal results** for both assays.

Of the remaining 15 samples, the following was observed:
- **8 samples: abnormal results for dPCR** that were undetected by G-banding
- **7 samples : abnormal results for G-banding** that were undetected by the dPCR

Concordant Abnormal Results Identified by Both Assays

Sample#	dPCR Result	G-band Karyotype Result	G-band Karyotype Explanation
1	loss Y	45,X,-Y	Loss of Y chromosome in every cell
2	gain 2q	46,XY,add(11)(p15)	Additional chromatin attached to chromosome 11 in every cell
3	gain 5q	47,XY,+5[7]/46,XY[13]	Trisomy of chromosome 5 in 35% of cells
4	trend gain 20q	46,XY,i(20)(q10)[2]/45,X,i(20)(q10)[1]/46,XY[17]	Isochromosome of a chromosome 20 q arm in 15% of cells

Abnormal Results Identified by dPCR but Undetected by G-band Karyotyping

Sample#	dPCR Result	G-band Karyotype Result	G-band Karyotype Explanation
5	gain 20q	46,XY,inv(9)(p11q13)	Pericentric inversion of chromosome 9, a benign polymorphism, in every cell
6	gain 20q, trend gain 19p	46,XY	normal
7	gain 20q	46,XY	normal
8	gain 20q	46,XY	normal
9	gain 20q	46,XY	normal
10	gain 20q	46,XY	normal
11	gain 20q	46,XY	normal
12	gain 20q	46,XY	normal

Abnormal Results Identified by G-band Karyotyping but Undetected by dPCR

Sample#	dPCR Result	G-band Karyotype Result	G-band Karyotype Explanation
13	normal	46,XY,t(9;14)(q12;p11.2)[3]/46,XY[17]	Balanced translocation between chromosomes 9 and 14 in 15% of cells
14	normal	46,XY,t(9;14)(q12;p11.2)[18]/46,XY[2]	Balanced translocation between chromosomes 9 and 14 in 90% of cells
15	normal	45,XY,der(14)t(14;15)(p11.2;q15),-15 [3]/46,XY[17]	Abnormal derivative of chromosome 14 in 15% of cells
16	normal	45,XY,der(14)t(14;15)(p11.2;q15),-15 [4]/46,XY[16]	Abnormal derivative of chromosome 14 in 20% of cells
17	normal	45,XY,der(14)t(14;15)(p11.2;q15),-15 [7]/46,XY[13]	Abnormal derivative of chromosome 14 in 35% of cells
18	normal	46,XY,del(13)(q13)[3]/46,XY,add(5)(q31)[1]/46,XY[16]	Partial deletion of chromosome 13 in 15% of cells
19	normal	46,XX,del(15)(q11.2q14)	Partial deletion of chromosome 15 in every cell

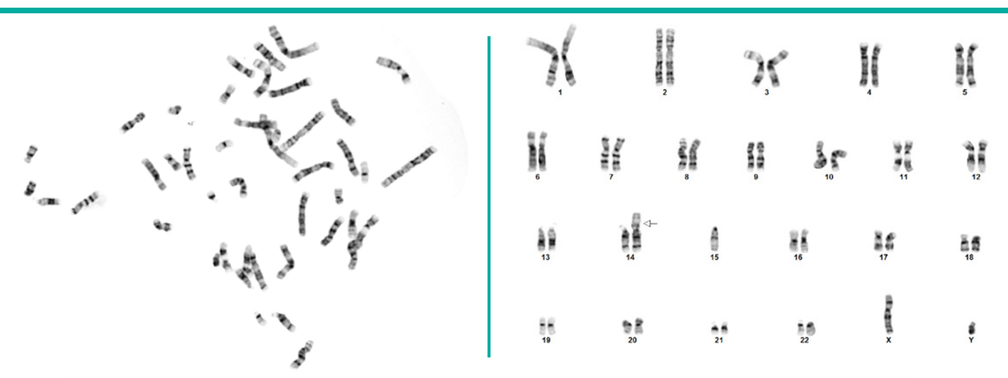

Figure 1: G-band karyotype image of Sample #15. The chromosome complement of 45,XY,der(14)t(14;15)(p11.2;q15),-15 was found in three out of twenty metaphase chromosome spreads in this sample. The dPCR did not detect any copy number variations.

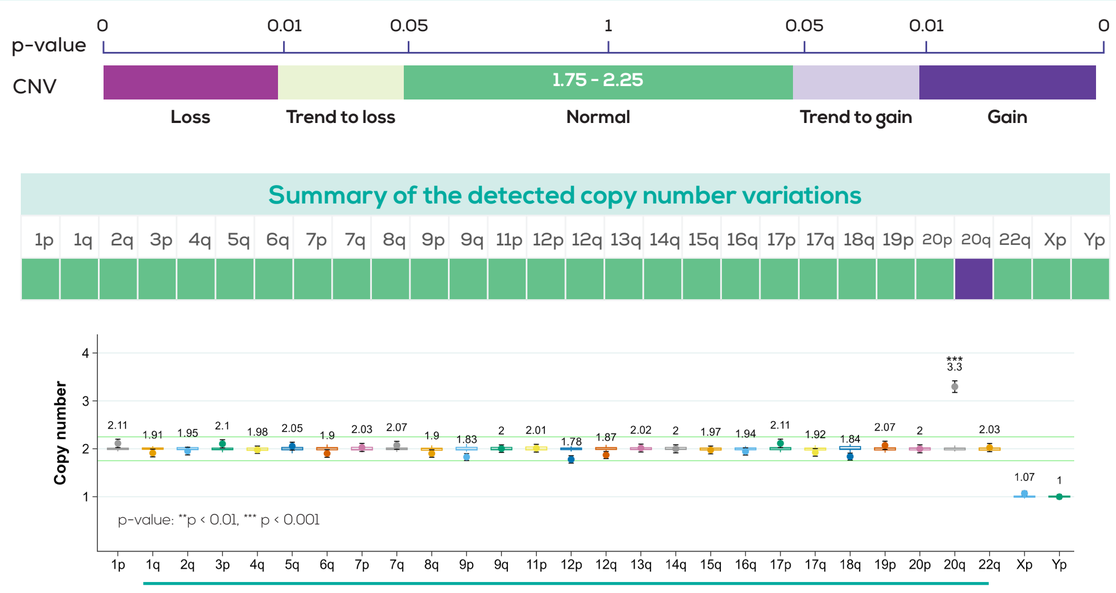

Figure 2: Sample #10 dPCR data displaying copy number variation for the various 28 chromosomal probes. The 20q sequence is elevated more than 3-fold. This increase was not detected by G-banding.

DISCUSSION

The data demonstrate that assessment of genomic integrity using both a molecular technique, dPCR, and a cytogenetic technique, G-band karyotyping, delivers a more accurate picture than using only one assay. While the majority of the samples in this study produced normal results for both assays, 7.45% of the samples produced abnormal results in either one assay, or both. The advantages of this combination Duo iCS-karyo are as follows:

1. Concordance between G-banding and dPCR strengthens interpretation, particularly when one assay yields ambiguous results (e.g., Sample #2: dPCR identified the cryptic material on chromosome 11 not recognized by G-banding; Sample #4: dPCR "trend" toward 20q11.21 gain matched a small isochromosome 20q population detected by G-banding).
2. dPCR detects submicroscopic copy-number gains below G-banding resolution, as shown in Samples #5 - #12, all exhibiting 20q amplification—a known hotspot in human pluripotent stem cells.[2]
3. Balanced rearrangements are identifiable by G-banding but invisible to dPCR (Samples #13–14). These events may disrupt gene function despite lacking copy-number change.
4. dPCR detection is probe-dependent. Aberrations outside the 28-probe panel will not be detected. G-banding revealed structural changes and partial arm deletions in Samples #13–19 that fell outside probe coverage.
5. Low-level mosaicism may escape dPCR detection, whereas G-banding (≥20 metaphases) can detect abnormalities present at ~5%. Using both assays reduces the likelihood of missing minor subclones.
6. Chromosomal inversions cannot be detected by dPCR. The inversion chromosome 9 polymorphism[3] in Sample #5 illustrates G-banding's ability to detect inversions, including potentially deleterious ones on other chromosomes.
7. Revelation of new trends in data. Trisomies are not the predominant abnormalities detected in the human stem cell samples presented here, despite their known tendency to arise during culture.
8. With its whole-genome reach, karyotyping exposes aberrations that molecular assays miss-insights that can drive smarter dPCR probe development and elevate the technology.

CONCLUSION: the Duo iCS-karyo assay combines the complementary strengths of dPCR and G-banding to give the most decisive assessment of genomic integrity in human stem cells.

References:

1. ISSCR (International Society for Stem Cell Research) Guidelines for Stem Cell Research And Clinical Translation. August 2025 Version1.2.
2. Recurrent Genetic Abnormalities in Human Pluripotent Stem Cells: Definition and Routine Detection in Culture Supernatant by Droplet Digital PCR. Assou S, Girault N, Plinet M, Bouckenheimer J, Sansac C, Combe M, Mianné J, Bourguignon C,-Fieldes M, Ahmed E, Commes T, Boureux A, Lemaître JM, De Vos J. Stem Cell Reports 2020 Jan 14;14(1):1-8.
3. Hsu LY, Benn PA, Tannenbaum HL, et al. Chromosomal polymorphisms of 1, 9, 16, and Y in 4 major ethnic groups: a large prenatal study. Am J Med Genet 1987;26:95–101.