ROUTINE MONITORING OF COMMON GENETIC ABNORMALITIES IN HUMAN PLURIPOTENT STEM CELLS USING THE HPSC GENETIC ANALYSIS KIT

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Introduction

Genetic aberrations in cultured human pluripotent stem cells (hPSCs) comprising numerical aneuploidies, chromosomal rearrangements and sub-microscopic changes have been widely reported (Amps *et al.*, Nat Biotechology. 2011;29:1132–44.). Genetic variants can affect hPSC growth rates, cell survival and differentiation potential. Recurrent genetic abnormalities observed in hPSCs are also observed in human cancers, an observation that raises concerns for downstream clinical applications. The hPSC Genetic Analysis Kit is a qPCR-based method designed to rapidly detect the most common genetic abnormalities observed in hPSC cultures.

Methods

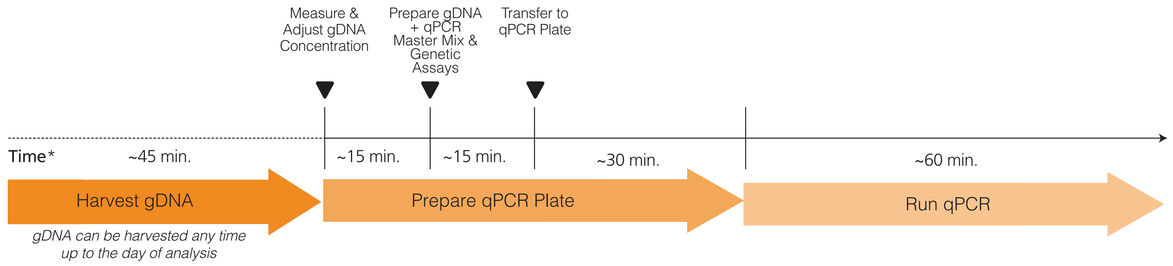
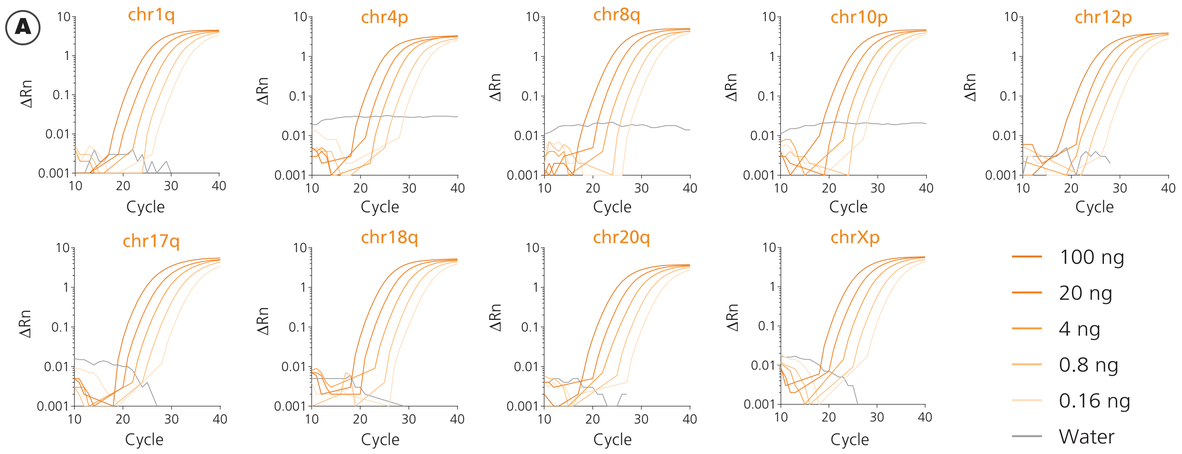


FIGURE 1. hPSC Genetic Analysis Kit Workflow

Genomic DNA (gDNA) can be harvested and analyzed the same day. This rapid analysis combined with a low cost per sample enables the screening of multiple cell lines more frequently to detect karyotypic abnormalities earlier. *Time based on analyzing 10 samples.

Results



B

	Genetic Assay								
	chr1q	chr4p	chr8q	chr10p	chr12p	chr17q	chr18q	chr20q	chrXp
Slope	-3.39	-3.34	-3.37	-3.35	-3.38	-3.46	-3.38	-3.43	-3.30
Primer Efficiency	97 ± 2%	99 ± 1%	98 ± 2%	99 ± 1%	98 ± 2%	94 ± 1%	98 ± 2%	96 ± 3%	101 ± 3%
Amplification Factor	1.97	1.99	1.98	1.99	1.98	1.94	1.98	1.96	2.01

FIGURE 2. Primer-Probe Assays Display Desirable Amplification Efficiencies

(A) Amplification curves showing each Genetic Assay using serially diluted Genomic DNA Control. Primer efficiencies and amplification factors were determined using the slope calculated from a standard curve plotting DNA concentration (log10) against Ct value. **(B)** Results from an average of three experiments to determine primer efficiency using pooled male (H1, STiPS-M001, WLS-1C), and female (H7, H9, STiPS-F016) cell lines, and the Genomic DNA Control provided with the kit.

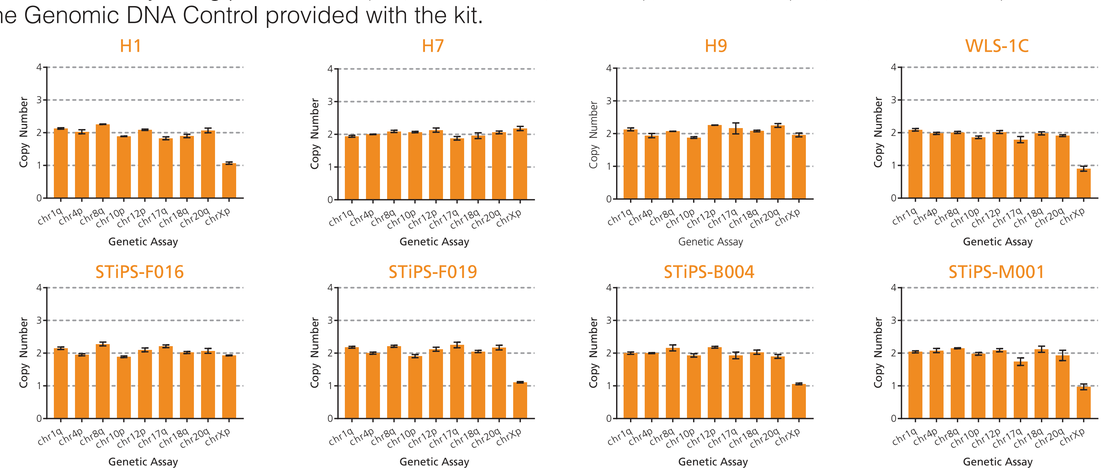


FIGURE 3. The hPSC Genetic Analysis Kit Shows Copy Number Consistency Across Multiple hPSC Lines

The hPSC Genetic Analysis Kit has been tested on a number of human embryonic stem cell (hESC) (H1, H7, and H9) and human induced pluripotent stem cell (hiPSC) lines (WLS-1C, STiPS-F016, STiPS-F019, STiPS-B004, and STiPS-M001). Copy number is consistent across all diploid cell lines tested (error bars indicate SD of three replicates).

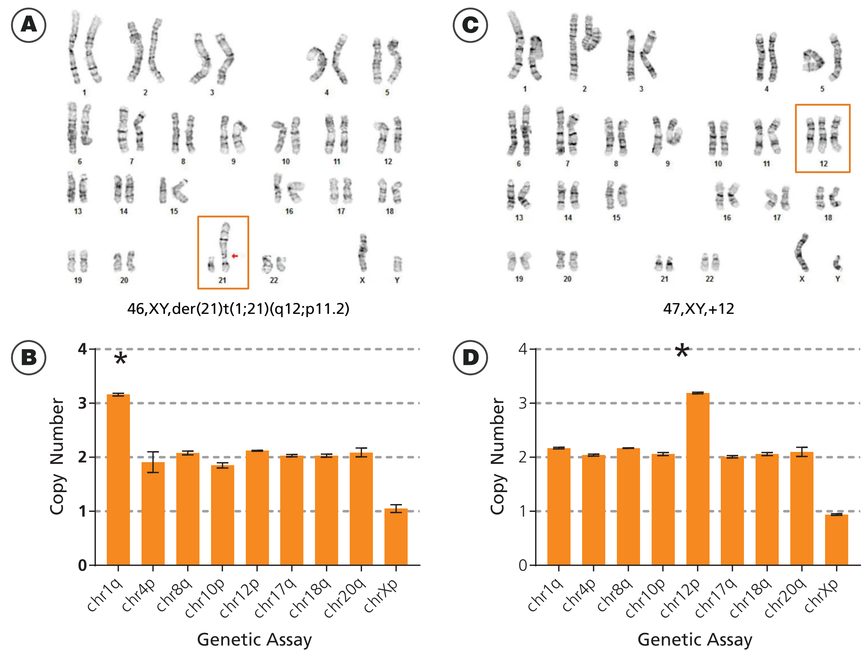


FIGURE 4. hPSC Genetic Analysis Kit Detects Karyotypic Abnormalities in hPSC Lines

The hPSC Genetic Analysis Kit has been verified on known abnormalities identified in hPSC cultures using G-banding. An unbalanced translocation between chromosome (chr) 1 and 21 resulting in a chr 1q gain was confirmed **(A,B).** The kit also detected the presence of a chr 12 trisomy **(C,D)**. Error bars show standard deviation of three replicates; asterisks indicate p-values < 0.05.

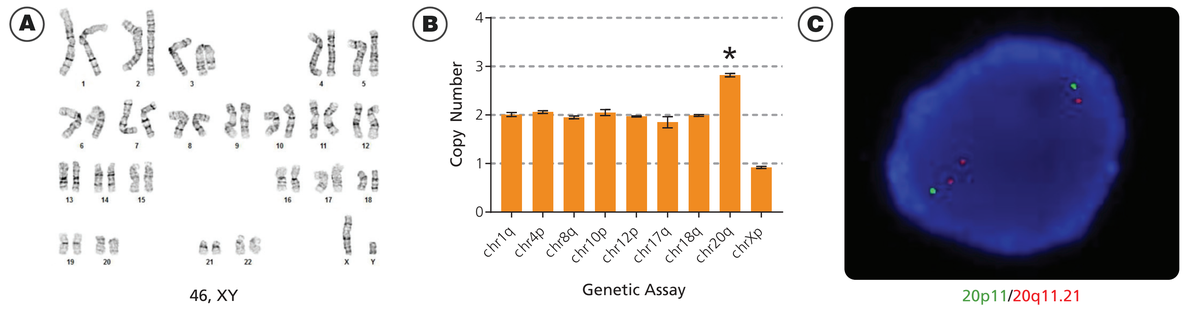


FIGURE 5. 20q11.21 Duplication Can Be Detected Using The hPSC Genetic Analysis Kit

The chr 20q11.21 duplication is frequently observed in hPSC lines and has been shown to confer a strong selective advantage in culture. This duplication is often undetected using conventional methods such as G-banding due to the varying size of the duplication. The hPSC Genetic Analysis Kit is able to detect this duplication. **(A)** shows that the WLS-4D1 hiPSC line was reported as karyotypically normal; the kit detected the chr 20q11.21 duplication **(B)** which was later confirmed using FISH **(C)**.

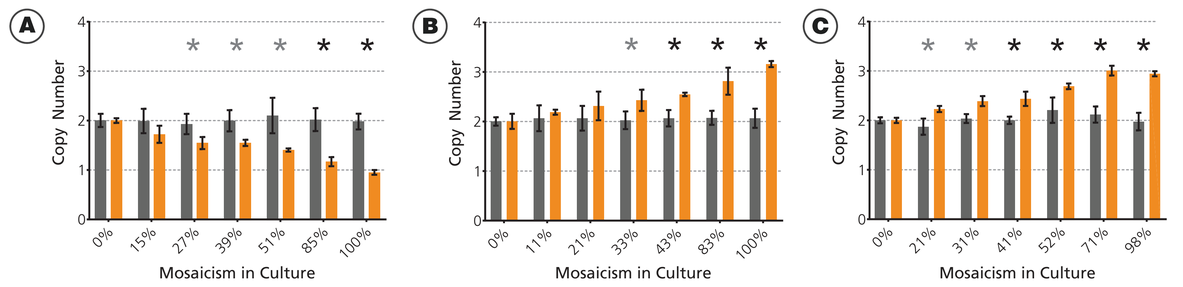


FIGURE 6. hPSC Genetic Analysis Kit Detects Approximately 30% Mosaicism in hPSC Cultures

Fluorescently labeled genetically variant hPSC lines harboring a 10p deletion **(A)**, 12 trisomy **(B)**, and 20q duplication **(C)** were mixed with diploid cells to mimic mosaicism in culture. A portion of the sample was analyzed using flow cytometry to determine exact percentages of abnormal cells; genomic DNA was extracted from the remaining population. The hPSC Genetic Analysis Kit detected approximately 30% mosaicism within the culture. Orange bars display copy number of the region of interest, and grey bars display the average copy number of all remaining Genetic Assays. Error bars represent standard deviation of all replicates. Black asterisks indicate a p-value < 0.05 and grey asterisks indicate samples identified as potentially abnormal using the new Genetic Analysis Application available at www.stemcell.com.

Summary

- The hPSC Genetic Analysis Kit can be used to screen multiple hPSC lines and monitor genomic integrity more frequently throughout culture in a rapid and cost-effective manner
- Recurrent abnormalities observed in hPSCs can be detected including the 20q11.21 duplication
- The kit can detect approximately 30% mosaicism in hPSC cultures enabling earlier detection of karyotype abnormalities
- The Genetic Analysis Application available at www.stemcell.com offers a simple, intuitive tool to analyze and interpret data