Microarray platform-independent detection of submicroscopic amplicons critical to genomic stability in human pluripotent stem cells

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Introduction
For accurate determination of a cell line's cytogenetic state, genotyping is a high-resolution adjunct to karyotyping. We compared copy number variants (CNVs) in three related subclones of WA09 (HB) cultures across three microarray platforms (Agilent, Illumina, and NimbleGen) regarding the ability to detect both karyotypic abnormalities and critical submicroscopic changes. These subclones are part of a series of cultures with related complex cytogenetic changes that occurred in WiCell Cytogenetics lab during culture. Based on this and previous microarray data from our lab and other's showing a recurring amplicon of chromosome 20q11.21, we tested the capability of all three platforms to detect this submicroscopic change. Utilizing the genomic positions identified by microarray, a custom SureFISH (Agilent) probe was designed to detect the minimal overlapping region. Given the findings from this study, WiCell Cytogenetics recommends higher resolution testing for genetic characterization of stem cell lines.

Methods and Materials
Test DNA was extracted from subclones that were part of a series of cultures with related complex cytogenetic changes that occurred in WA09 during culture. Microarray analysis was performed following each provider's protocol. For NimbleGen, we used the Human CGH 135k and 385k Whole Genome-Tiling Arrays with WA01 opposite gender hESC for reference material and followed NimbleGen Arrays User's Guide. For Agilent, we used SurePrint G3 Cancer CGH+SNP 4x180k Microarray Kit with Agilent Human Male Reference DNA for reference material and followed Agilent Oligonucleotide Array-Based CGH for Genomic DNA Analysis protocol.

Results/Conclusions
All three genotyping platforms proved effective at detecting the copy number changes found by karyotyping each of the WA09 subclones. Previous karyotypic analysis showed three acquired chromosome abnormalities, all of which were found by these microarray analyses (Fig 1). For example, duplication of 12p was not present in the initial WA09 karyotype, but did occur in both the DT and the TSF subclones; the microarray data is consistent with established karyotypes for all three cultures in all three platforms for this abnormality. Gains of two functional submicroscopic aberrations were detected in each platform: a long (q) arm amplicon of chromosome 20 at q11.21, thought to be related to growth advantage1, and a 5q35.3 gain that may function as a cap to stabilize the 12p rearrangement. The platforms differ significantly in overall frequency and type of CNV due to array and reference designs.

<table>
<thead>
<tr>
<th>Platform</th>
<th>Array</th>
<th>No. of Features</th>
<th>Number of CNVs</th>
<th>Length (kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agilent</td>
<td>Agilent (Male)</td>
<td>180k</td>
<td>34-79</td>
<td>1,953,845</td>
</tr>
<tr>
<td>Illumina</td>
<td>HapMap</td>
<td>220k</td>
<td>5-15</td>
<td>35-95,876</td>
</tr>
<tr>
<td>NimbleGen</td>
<td>WGSS (Male)</td>
<td>385k and 135k</td>
<td>26-46</td>
<td>31-132,249</td>
</tr>
</tbody>
</table>

In addition, Illumina and Agilent chips include probes for SNPs that allow for the determination of copy neutral changes (Fig 1). We found loss (or absence) of heterozygosity in all subclones by both SNP microarrays: Agilent contained one region (4468-4483kb) and Illumina contained 11-14 regions, (1072-2313kb). Based on the conclusive finding of the 20q amplicon in the WA09.TSF subclone by analysis with all three microarrays (Fig 2), we compiled our retrospective microarray data for this region from 235 human embryonic stem (HES) and induced pluripotent stem (IPS) cell cultures, 51 (22%) of which carried the duplication (Fig 3). We determined that the minimal overlapping region was 690kb, and regardless of amplicon size, the HM13, ID1, and BCL2L1 genes were always present. Based on these findings, we designed an oligo FISH probe (SureFISH, Agilent) to be used for rapid and inexpensive screening for the duplication of the 20q11.21 region. Initial testing of the first in the series of custom SureFISH probes (Fig 4) shows the ability to distinguish cells with two sequences of the 20q amplicon due to the structural abnormality (isochromosome(20)(q10)). We are currently refining the system to detect tandem duplications of 20q11.21. While g-banded karyotyping is regarded as the gold standard for assessing cytogenetic stability, this study clearly shows that both microscopic and submicroscopic abnormalities are consistently detected by microarray, independent of platform. Both karyotype and microarray should be utilized as part of a comprehensive quality system.

References

Figure 1. Three subclones of a WA09 abnormal cytogenetic progression compared using three different microarray platforms. The log-ratio (upper track) shows copy number variation across all chromosomes. The B-allele frequency (lower track) shows any regions of loss/absence of heterozygosity.

Figure 2. Critical amplicon 20q detected in three microarray platforms. Roche NimbleGen track shown in hg18, Agilent and Illumina tracks in hg19.

Figure 3. UCSC Genome Browser view of critical amplicon 20q in hiPSC (top, green) and hESC (bottom, green) detected by WiCell Cytogenetics: HM13, ID1, and BCL2L1 genes within the critical amplicon 20q region (red).

Figure 4. Custom SureFISH probe for the critical amplicon 20q (red) and CEP20 probe (green).

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