For higher density arrays, (approximately 13 µ spot size), fluorescent signal from 3 µ resolution scanning typically provides 16 pixels per feature which translates to improved confidence in the resulting data and increased number of reference CNVs detected when compared to 5 µ resolution scanning. When scanning the same arrays at 2 µ resolution, at least 36 pixels per feature are generated; which greatly enhances the fluorescent data leading to more accurate calls and increased confidence in the final examination and reporting of chromosomal aberrations.

Ordering information

For more information on testing services or to order stem cells from WiCell®, go to: http://www.WiCell.org/

For more information on high resolution scanning or to order an INNOPSYS InnoScan® microarray scanner go to: http://www.innopsys.com/

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References

1. Stem cell lines which were formerly available for federally funded research need to be re-certified to ensure compliance with current regulations. Cell line WAO1 (H1) is an approved cell line available from WiCell®. See http://grants.nih.gov/stem_cells/registry/current.htm?id=29 for details.
For more information, refer to: http://stemcells.nih.gov/

2. Genome Technology Magazine, Genetic Variation Technical Guide, February 2010

Disclaimers

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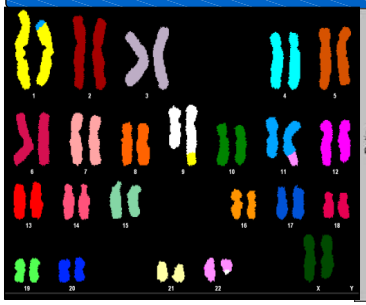
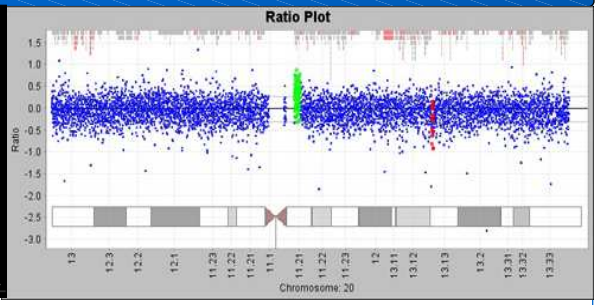
Applications note

Characterization of Stem Cell Copy Number Variation by aCGH at the Wisconsin International Stem Cell Bank





Fast Accurate Results Accelerate Stem Cell Science

INNOPSYS

WiCell® Research Institute

Introduction

Following isolation of human embryonic stem cells (hESC) at the University of Wisconsin-Madison in 1998 and subsequent approval of specific cell lines by the NIH, efforts are underway to thoroughly characterize these cell lines including Gene Expression Profiling, Karyotyping, Comparative Genomic Hybridization (CGH), Flow Cytometry analysis for ESC Marker Expression, Growth Characteristic (Doubling Time) analysis, Post-Thaw Viable Cell Recovery analysis, and Identity by Single Tandem Repeat (STR) analysis. These detailed characterization studies facilitate identification of cell line best suited for specific applications and provides information on the relationship between the genetic characteristics of each stem cell line and their functional properties.

Founded in 1999, the WiCell® Research Institute established its cytogenetics laboratory in 2005 to meet the needs of researchers to monitor karyotypic stability and copy number variation in hESC cultures. Starting in February of 2010, WiCell® provides cultures of the formerly federally approved lines[1] which were previously available from the National Stem Cell Bank (NSCB) under US government contract funding in effect from 2006 to 2010. WiCell's contract with the NIH ended on February 28, 2010. Cell lines previously available from the NSCB can now be ordered from the Wisconsin International Stem Cell Bank (WISC Bank).

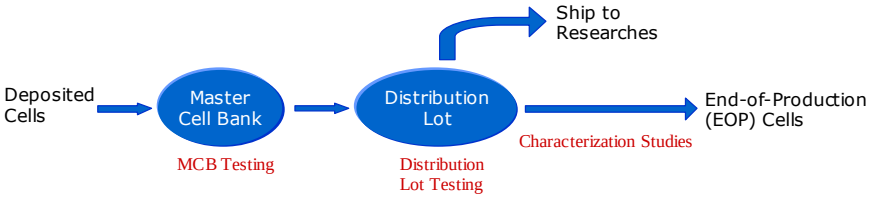


Figure 2. The overall process for banking cells involves production of banks, or lots of cells, with multiple tests performed at several points during the process.

Motivation

Given the urgency of identifying cell lines that enable translational medicine, fast and accurate testing procedures are essential for identification of instability in the stem cell lines. WiCell® is continually seeking advanced methods and instrumentation to remain on the forefront of the technology curve to best supply stem cells and testing for the benefit of humanity. For this reason, new technologies that improve confidence, accuracy and speed, concurrently reducing cost and footprint, are needed to provide array Comparative Genomic Hybridization (aCGH) testing services.

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Figure 1 (Cover page). Examples of Spectral Karyotyping (panel A) and array Comparative Genomic Hybridization (aCGH) ratio plot data (panel B) generated at WiCell® Research Institute in Madison, Wisconsin, USA.

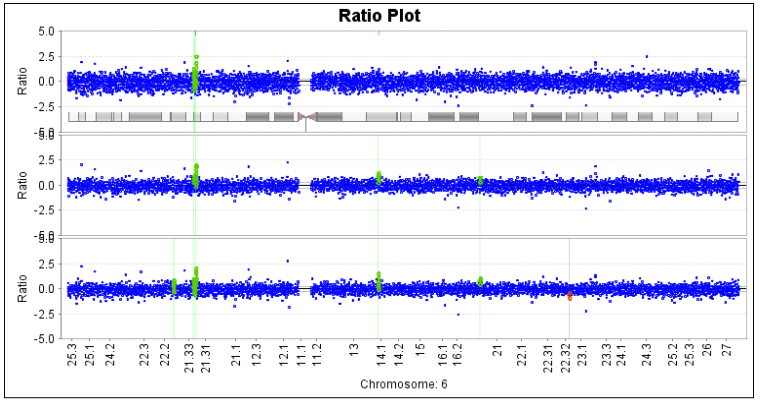

Figure 6. Zoomed-in image of Chromosome 6 from Figure 5 comparing 5 µ resolution (top) to 3 µ resolution (middle) and 2 µ resolution (bottom) images of the same microarray. Note one amplification detected at 5 µ resolution, three amplifications at 3 µ, and four amplifications plus one deletion at 2 µ resolution.

Quality Control

Each microarray processed at WiCell® is reviewed for the presence of known reference CNVs (23 in this example) which serve as positive controls. In the table below, QC data from an example array is listed. A plus sign denotes an expected insertion and a minus sign denotes an expected deletion. More reference CNVs on the exact same array were detected with higher resolution scanning.

Type: + is insertion - is deletion	+	-	+	+	+	-	-	-	-	+	+	+	+	+	+	-	-	-	+	-	+	-	-	Total # of expected aberations for QC: 23	
Chromosome #	**1**	**4**	**6**	**6**	**7**	**8**	**8**	**8**	**10**	**11**	**12**	**14**	**14**	**14**	**14**	**15**	**16**	**17**	**17**	**22**	**X**	**X**	**X**	**PAR**	**Total Ref CNV**	**Total Aberrations**
5 µ resolution		*	*		*				*	*	*	*	*	*	*	*	*	*	*	*	*		*	2	**17**	52
3 µ resolution	*	*	*	*	*		*		*	*		*	*	*	*	*	*	*	*	*	*	*	*	2	**20**	85
2 µ resolution	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	2	**22**	84

Conclusion

Array CGH analysis to determine Copy Number Variation is becoming a routine laboratory technique to better define breakpoints, identify or confirm aberrations under 5 Mb, and expand upon traditional g-banded karyotype analysis. Rapid and accurate analysis of banked stem cell lines is required to facilitate stem cell research worldwide. Better fluorescent signal leads to better data acquisition. According to Wei Wang at the Microarray Core Lab at Cornell University, "Microarray scanners with high resolution, low noise, and autofocusing can obtain better fluorescent signal from [an] array." [2]

Although adequate fluorescent signal is gathered from standard density arrays (>15 micron diameter spot-size) when scanned at 5 micron resolution, higher resolution scanning combined with autofocusing produces more reliable data due to higher number of pixels.

WiCell® Research Institute

Feature Extraction and Data Analysis

Feature extraction and segmentation were performed with NimbleScan® v2.5 software (Roche NimbleGen). Data analysis was performed with CGH Fusion® software (InfoQuant).

Typical aberration detection settings:
Algorithm: RBSv1.0 (robust circular binary segmentation)
p-value: 0.0010 or 0.010
Min. aberration length (probes): 5
Min. aberration length (Mbp): 0.0
Average log-ratio threshold: 0.0 or 0.2

Ratio plots were generated using CGH Fusion® software. A ratio plot is a visual representation of differences in the presence of hybridization to the microarray of the test sample and the reference sample. If there are equal amounts of test and reference sample, the dot is blue. If there is more test than reference (amplification), the dot is green. If there is more reference than test (deletion), the dot is red.

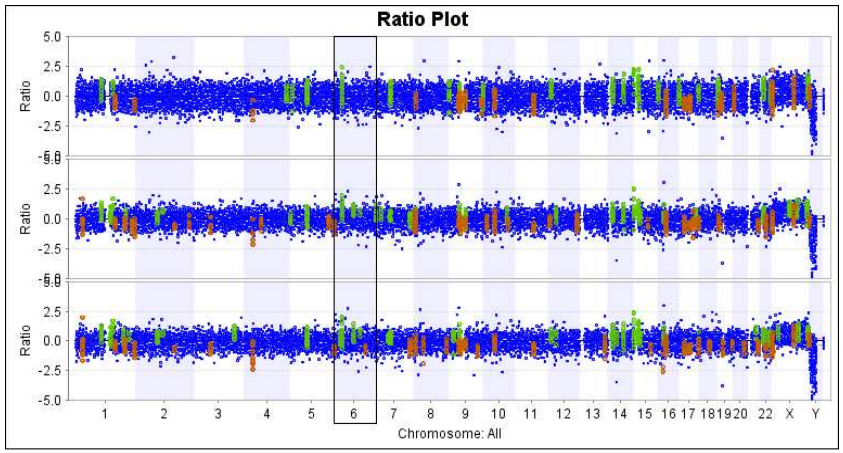

Figure 5. Comparison of ratio plots of the same microarray scanned at 5 µ (top), 3 µ (middle) and 2 µ (bottom) resolution on the InnoScan®900AL. Note, for example, that in chromosome 6 (in box) successively more aberrations (in green or red) were detected at 3 µ resolution than at 5 µ resolution; and still more were detected at 2 µ resolution than at 3 µ resolution.

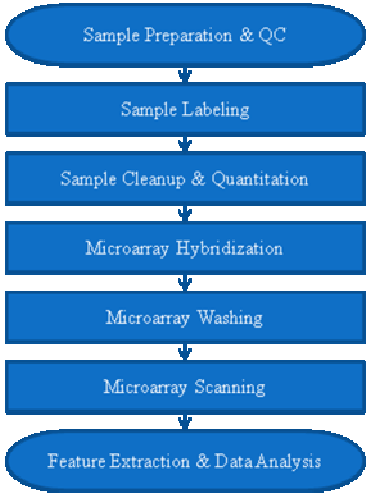

Table 1. Flow-chart of protocol for aCGH sample preparation and analysis

Procedure

Cells are removed from cryogenic storage, allowed to come to room temperature, and propagated in a 6-well plate for 5-10 days per WiCell® Standard Operating Procedure SOP-CC-005. DNA is extracted using DNeasy® Blood & Tissue Kit (QIAGEN) per WiCell® SOP-CH-014. DNA quality and quantity are measured using the Nanodrop-1000 spectrophotometer (Thermo Scientific). Sample integrity and absence of RNA contamination is measured using DNA gel electrophoresis and imaging per SOP-CH-007. Samples are then labeled with Cy-3 and Cy-5 fluorescent dyes (Tri-Link) followed by overnight Klenow (New England Biolabs) amplification. DNA is isopropanol precipitated, dried in a SpeedVac® (Thermo Scientific) and reconstituted. Alexa Fluor® (Life Technologies) dyes are added followed by another isopropanol precipitation and SpeedVac® centrifugation until dry. Dried pellets are then rehydrated in VWR water.

Hybridization solution, including alignment oligo, is made, sample quantified using the nucleic acid feature on the NanoDrop® (Thermo-Fisher) spectrophotometer and the mixture adjusted to ensure optimal concentration for hybridization. Equal amounts of test and reference sample are combined in VWR water; after which the appropriate amount of hybridization solution master mix is added for a total volume of 18 µL (385K array) or 20 µL (3x720K array).
Samples are then labeled with Cy-3 and Cy-5 fluorescent dyes (Tri-Link) followed by overnight Klenow (New England Biolabs) amplification. DNA is isopropanol precipitated, dried in a SpeedVac® (Thermo Scientific) and reconstituted. Alexa Fluor® (Life Technologies) dyes are added followed by another isopropanol precipitation and SpeedVac® centrifugation until dry. Dried pellets are then rehydrated in VWR water.
Hybridization solution, including alignment oligo, is made, sample quantified using the nucleic acid feature on the NanoDrop® (Thermo-Fisher) spectrophotometer and the mixture adjusted to ensure optimal concentration for hybridization. Equal amounts of test and reference sample are combined in VWR water; after which the appropriate amount of hybridization solution master mix is added for a total volume of 18 µL (385K array) or 20 µL (3x720K array).
The sample/hybridization solution is added to the NimbleGen array/hybridization chamber assembly and incubated with active mixing on the NimbleGen Hybridization System for 40-72 hours at 48°C.
After hybridization is concluded and the array/hybridization chamber assembly is removed from the hybridization system, the hybridization chamber is disassembled and the arrays are carefully washed and dried via centrifugation in a low-light, low-ozone environment followed by immediate scanning.*

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Fluorescent Scanning

In the images below, standard resolution (5μ) scanning and high-resolution (3 μ and 2μ) scanning was performed using the INNOPSYS InnoScan®900AL per the manufacturer's instructions

InnoScan®900AL 5 μ resolution | InnoScan®900AL 3 μ resolution | InnoScan®900AL 2 μ resolution

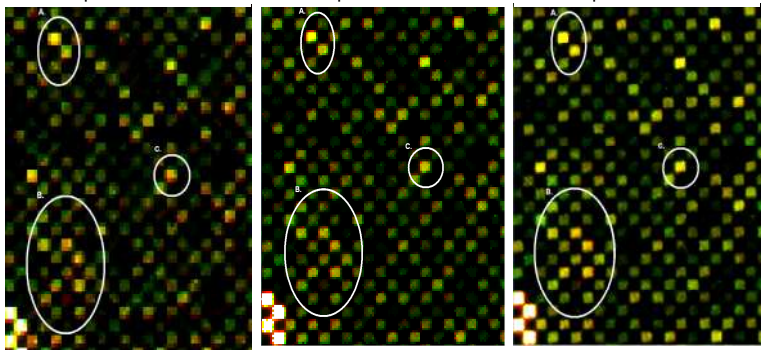

Figure 3a. Examples of **1x385K** (**16 μ spot size**) Roche NimbleGen array images at 5 μ, 3 μ and 2 μ resolution from the INNOPSYS InnoScan®900AL microarray scanner. Note circled areas of corresponding features on each array with successively better resolution as you view the images moving left to right.

InnoScan®900AL 5 μ resolution | InnoScan®900AL 3 μ resolution | InnoScan®900AL 2 μ resolution

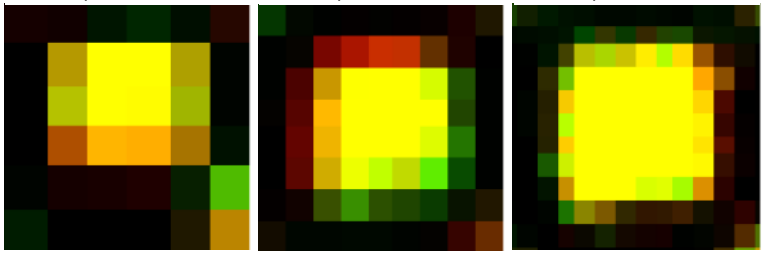

Figure 3b. Zoomed-in image of the upper left feature from Circle A in Figure 3a showing increased number of pixels per feature for a 16 μ diameter feature as resolution increases. Note 12 pixels for 5 μ resolution image, 38 pixels for 3 μ image, and 56 pixels for 2 μ images.

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Fluorescent Scanning
(continued)

InnoScan®900AL 5 μ resolution | InnoScan®900AL 3 μ resolution | InnoScan®900AL 2 μ resolution

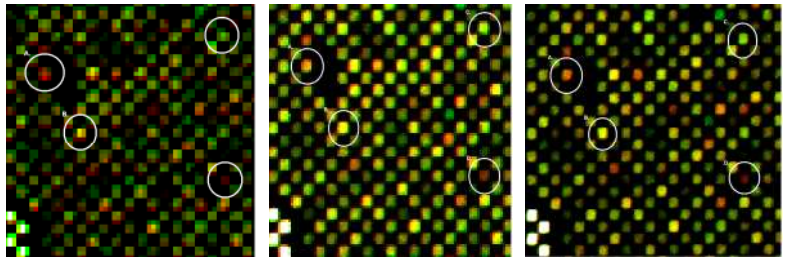

Figure 4a. Examples of **3x720K "HD2"** (**13 μ spot size**) Roche NimbleGen array images at 5 μ, 3 μ and 2 μ resolution from the INNOPSYS InnoScan®900AL microarray scanner. Note circled areas of corresponding features on each array with successively better resolution as you view the images moving left to right

InnoScan®900AL 5 μ resolution | InnoScan®900AL 3 μ resolution | InnoScan®900AL 2 μ resolution

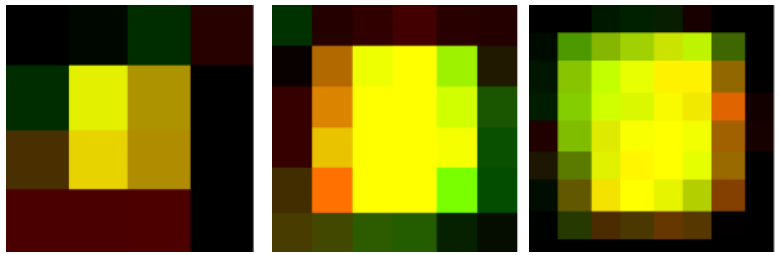

Figure 4b. Zoomed-in image of feature from Circle B in Figure 4a showing increased number of pixels per feature for a 13 μ diameter feature as resolution increases. Note 4 pixels for 5 μ resolution image, 16 pixels for 3 μ image, and 36 pixels for 2 μ images.

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