WiCell Characterization

Guidelines for characterization testing of cell lines

Assay	What it Detects	What it Doesn't Detect	TAT[i]	When to Use
Fluorescence In Situ Hybridization (FISH)	• Genomic sequence of interest – Duplications or deletions >20 Kb – >2% mosaicism (for example: cultures where >2 of 100 cells are trisomy 12) – Chromosomal location of genomic gains – Chromosome fusions (breakaparts)	• Changes in regions other than the probe-specific sequence	10-15 days	• To confirm findings and refine breakpoints detected by g-banded karyotyping • To confirm findings and localize genomic gains detected by SNP microarray • As a screen for microdeletions/duplications of known targets
G-Banded Karyotyping	• Microscopic genomic abnormalities (>5-10 Mb) – Inversions – Duplications/deletions – Balanced and unbalanced translocations – Aneuploidies • >10% mosaicism (for example: cultures where >1 of 10 cells are trisomy 12)	• Submicroscopic genomic abnormalities (<5 Mb) • <~10% culture mosaicism (for example: cultures where 1 of 10 cells is trisomy 12)	7-10 days (4-6 days expedited)	• As a baseline genomic screen – At derivation of cell lines – At the start of experimental protocols – To assess and monitor genomic stability (for example: every 5-10 passages of cell culture) – At conclusion of experiments (prior to publication) – For cell line banking • When publication-quality karyotypes are needed
Mycoplasma Detection by PCR	• 96 species of mycoplasma contamination from stem cell cultures. – Sensitivity (5-100 CFU/ml)	• This system does not allow for the amplification of DNA originating from other sources, such as bacteria.	5-7 days	• To monitor the health of your cell line • To monitor for contamination in shared lab spaces • To assure that mycoplasma is not interfering with your experiments • To rule out mycoplasma as the culprit of chromosomal aberrations
Short Tandem Repeat Analysis (STR)	• STR polymorphisms for 15 loci plus amelogenin (Promega® PowerPlex® 16) • Probability of matching identity to an existing STR profile	• STR polymorphisms in areas other than those represented in Promega® PowerPlex® 16	10-20 days	• To monitor identity of a cell line • To confirm relationship of iPS cells to their parent line • To establish an STR profile of a newly derived or reprogrammed cell line • To rule out culture cross-contamination
Single nucleotide polymorphism (SNP) Microarray	• Submicroscopic genomic abnormalities (>5-10 Mb) • Genomic gains and losses (>50 Kb) – Copy number variants – Duplications/deletions – Unbalanced translocations – Aneuploidies • Copy neutral Loss of Heterozygosity (LOH) / Absence of Heterozygosity (AOH) (>5 Mb) • >~10% mosaicism (for example: cultures where >1 of 10 cells are trisomy 12	• Balanced translocations – Robertsonian • Balanced insertions • Inversions • <~10% culture mosaicism (for example: cultures where 1 of 10 cells is trisomy 12) • Chromosomal position of genomic gains	14-21 days	• As a baseline genomic screen – To detect submicroscopic (<5 Mb) abnormalities – To identify amplified or deleted genes of interest – To assess and monitor genomic stability (for example: every 5-10 passages of cell culture) • In conjunction with G-banded karyotyping – To define unbalanced translocation breakpoints • For research of genomic copy number change – To identify structural variation within populations or disease cohorts – To develop a cell line copy number variant profile
Spectral Karyotyping (SKY)	• Microscopic genomic abnormalities (>5-10 Mb) – Balanced and unbalanced translocations – Aneuploidies	• Submicroscopic genomic abnormalities (<5 Mb) • Inversions • Duplications/deletions	14-21 days	• As an adjunct to g-banded karyotyping – To define complex rearrangements – To identify marker chromosomes • When publication-quality spectral karyotypes are needed

[i] Turn-around-times (TAT) provided are based on provision of sufficient mitotically active hES or iPS cultures grown in Matrigel/TeSR or MEF/hES media conditions.

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