WiCell Cytogenetics Laboratory
Guidelines for cytogenetic testing of pluripotent stem cell lines

www.wicell.org/cytogenetics

Assay	What it Detects	What it Doesn't Detect	TAT[i]	When to Use
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)	7-10 days	• 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
Spectral Karyotyping (SKY)	• Microscopic genomic abnormalities (>5-10 Mb) – Balanced and unbalanced translocations – Aneuploidies	• Submicroscopic abnormalities (<5 Mb) • Inversions • Duplications/deletions	10-15 days	• As an adjunct to g-banded karyotyping – To define complex rearrangements – To identify marker chromosomes • When publication-quality spectral karyotypes are needed
CGH + SNP Microarray **and** **CGH Microarray**	• Microscopic genomic abnormalities (>5-10 Mb) • Genomic gains and losses (>50 Kb) – Copy number variants – Duplications/deletions – Unbalanced translocations – Aneuploidies • >~10% mosaicism (for example: cultures where >1 of 10 cells are trisomy 12 • Loss of Heterozygosity (LOH) - detected by CGH + SNP	• Balanced translocations • Inversions • <~10% culture mosaicism (for example: cultures where 1 of 10 cells is trisomy 12) • Chromosomal position of genomic gains • Loss of Heterozygosity (LOH) - not detected by CGH alone	14-21 days	• As a baseline genomic screen – To detect submicroscopic (<5 Mb) abnormalities – To identify amplified or deleted genes of interest • 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
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 aCGH analysis • As a screen for microdeletions/duplications of known targets
Fast FISH	• Greater than 50% of recurrent trisomies in both human ES and iPS cell lines.[ii] – Trisomies of human chromosomes X, 8, 12 and 17.	• Aneuploidies of chromosomes other than X, 8, 12 and 17 • Structural chromosome abnormalities	1-2 days	• As a rapid screen for large numbers of clones • As a cost-effective tool for monitoring aneuploidy
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	10-15 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

[i] Turn-around times provided are based on provision of sufficient mitotically active hES or iPS cultures grown in matrigel/TeSR or MEF/hES media conditions.
[ii] Based on n=1220 hES/iPS cell cultures karyotyped by WiCell Cytogenetics Laboratory.

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