

A high-throughput digitalMLPA assay for routine characterisation of chromosomal integrity and cell line identity in induced pluripotent stem cells

Joery den Hoed¹, Cansu Yanginlar¹, Marieke Otten¹, Sander Palit¹, Fatma Uzbac², Vera Bejczy², Jean Corbally², Lisette Stolk¹, Simon E Fisher², Suvi Savola¹, Maggie MK Wong²

1. MRC Holland, Amsterdam, the Netherlands

2. Language and Genetics Department, Max Planck Institute, the Netherlands

Chromosomal characterisation and authentication of cell lines and primary cells

Cellular models, including patient-derived primary cells and induced pluripotent stem cells (iPSCs), provide a valuable tool for studying the molecular mechanisms underlying genetic conditions. iPSC reprogramming, cell culturing and passaging, as well as gene editing can result in the accumulation of chromosomal aberrations and cell line cross-contamination or mislabelling. Frequent characterisation of cell cultures is therefore crucial to ensure their quality for downstream applications (Figure 1). However, existing techniques such as karyotyping have long turnaround times and are costly, while qPCR/ddPCR assays can miss relevant abnormalities, and are therefore not ideal for routine cell culture quality control.

Here, we tested if the probe-based digitalMLPA assay **NXtec D024 KaryoProfiler** (Figure 2) could be a promising tool for high-throughput cell line quality control.

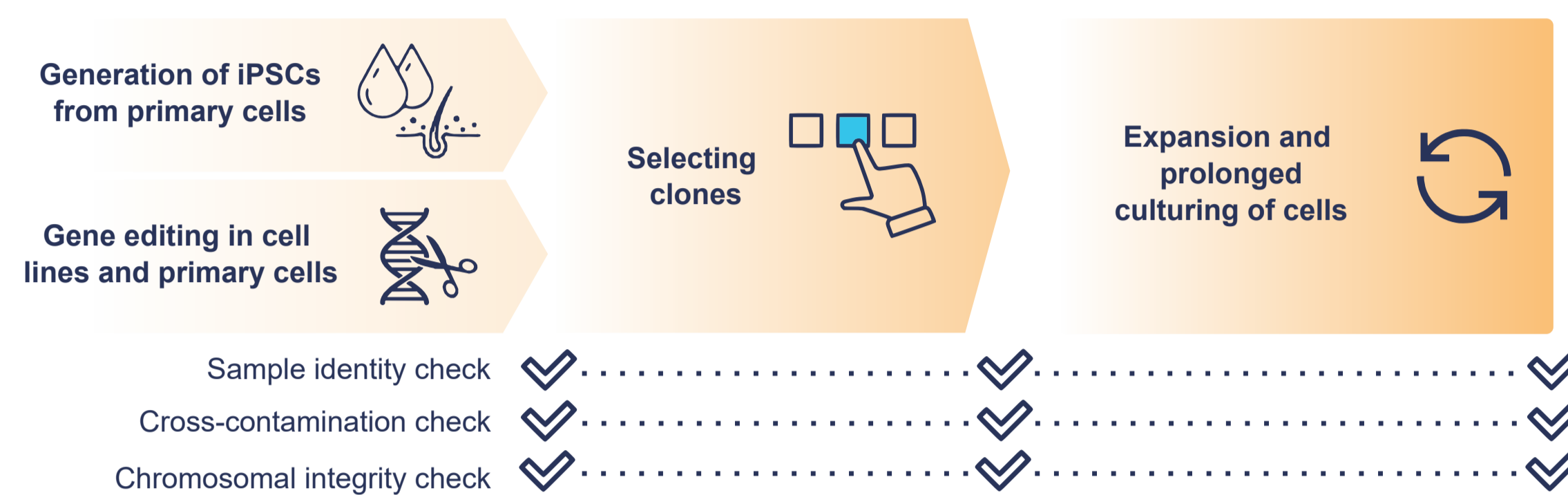


Figure 1. Quality control of cell cultures to ensure chromosomal integrity and sample identity.

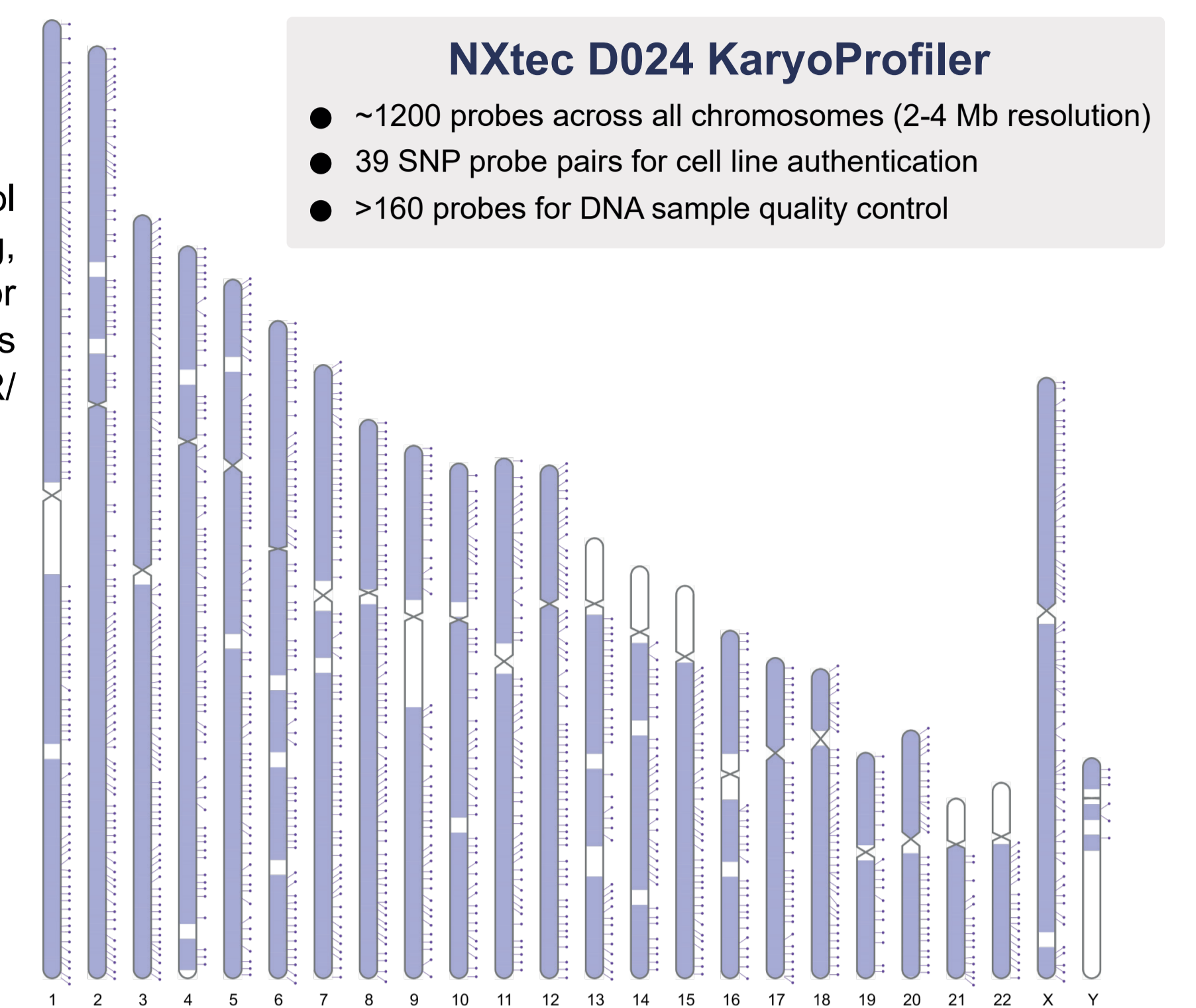


Figure 2. Ideogram showing the locations of the ~1200 probes included in NXtec D024 KaryoProfiler (blue pins) and covered regions at a resolution of 4 Mb shaded in blue.

Testing 70 iPSC-derived DNA samples

We analysed 70 cell culture-derived DNA samples with NXtec D024 KaryoProfiler, including iPSC lines before and after gene-editing as well as iPSC-derived neural progenitor cells. The resulting PCR amplicons were sequenced on an Illumina NextSeq 1000 and analysed using Coffalyser digitalMLPA (Figure 3).

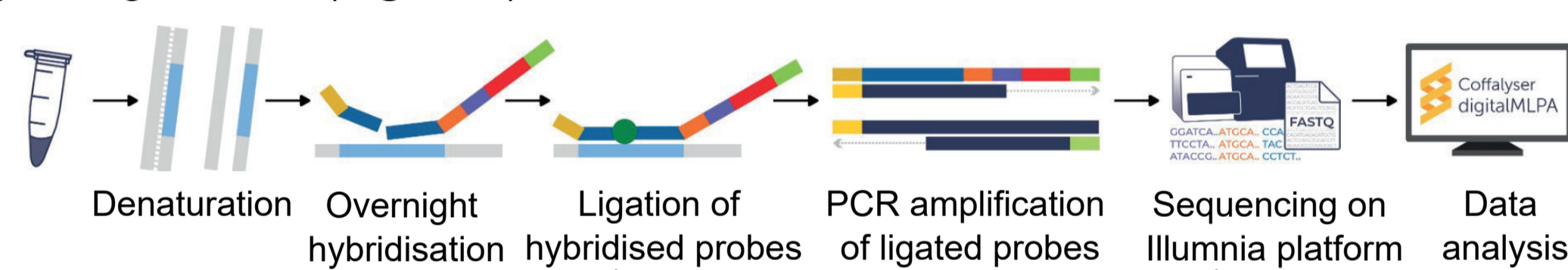


Figure 3. digitalMLPA workflow.

The assay identified one iPSC sample with multiple large-scale gains and losses (Figure 4), including mosaic aberrations on chromosomes 12 and 18.

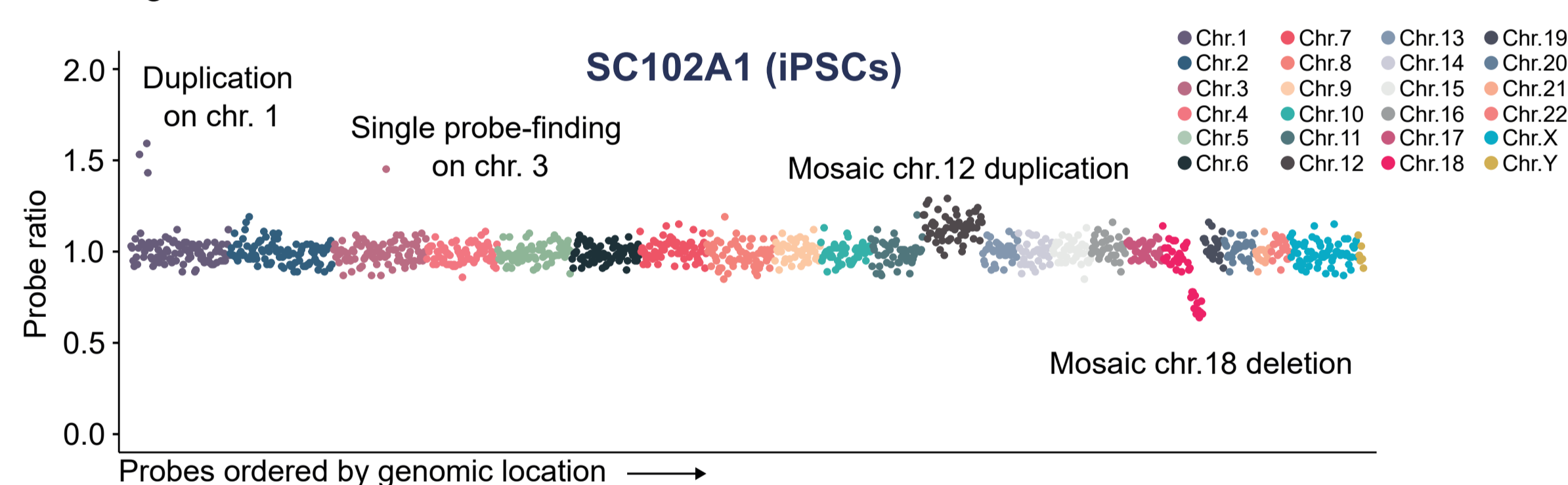


Figure 4. Probe ratio chart displaying the normalized probe ratios on the y-axis, ordered by chromosomal position on the x-axis. Copy number variations detected with D024 KaryoProfiler are consistent with those previously identified with aCGH.

A 39 SNP profile to ensure cell line identity and check cross-contamination

NXtec D024 KaryoProfiler contains probe pairs for 39 SNPs, allowing the generation of a unique SNP profile for each test sample (Figure 6A). This SNP profile can help with cell line authentication during cell culture workflows.

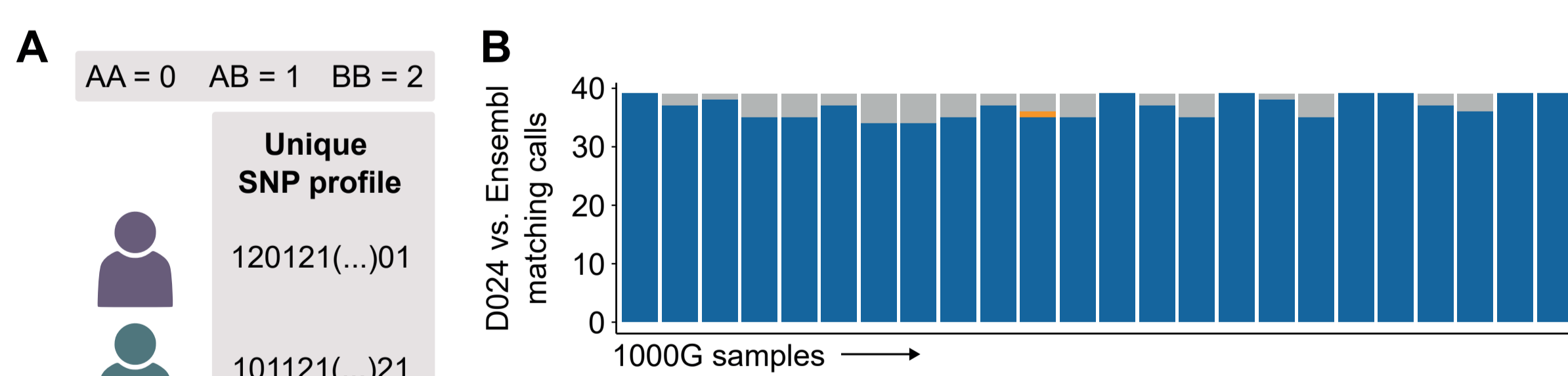


Figure 6. A) Schematic of the digitalMLPA-generated 39 SNP profile. Samples from distinct individuals or cell lines generate a unique SNP profile. B) Stacked bargraph of 24 1000G samples, with the number of digitalMLPA-generated SNP calls matching the 1000G project data shown in blue. Mismatches with the 1000G data are shown in orange and SNPs that could not be confidently determined in the digitalMLPA experiment are shaded in gray.

SNP profiles were generated for 24 samples from the 1000G project, all showing >30 SNPs matching Ensembl (1000G phase 3; Figure 6B). As few as 25 unique SNPs are already well over the required number of SNPs to confidently distinguish unrelated individuals within a sample set (random match probability $p < 4.0 \times 10^{-11}$). In addition, the SNP-targeting probes also allow the detection of potential cross-contamination within a sample (Menezes K et al. 2020, PMID: 32603764).

Characterisation across multiple stages of neuronal differentiation

Differentiation protocols require to maintain cells in culture for extended periods (Figure 5A). We checked the chromosomal integrity of a cell line at multiple stages during forebrain differentiation (Figure 5). The original iPSCs had a normal karyotype. We detected a potential mosaic 1q gain acquired at the neural progenitor cell stage which was found again later on during differentiation in neural precursors (Figure 5C-D).

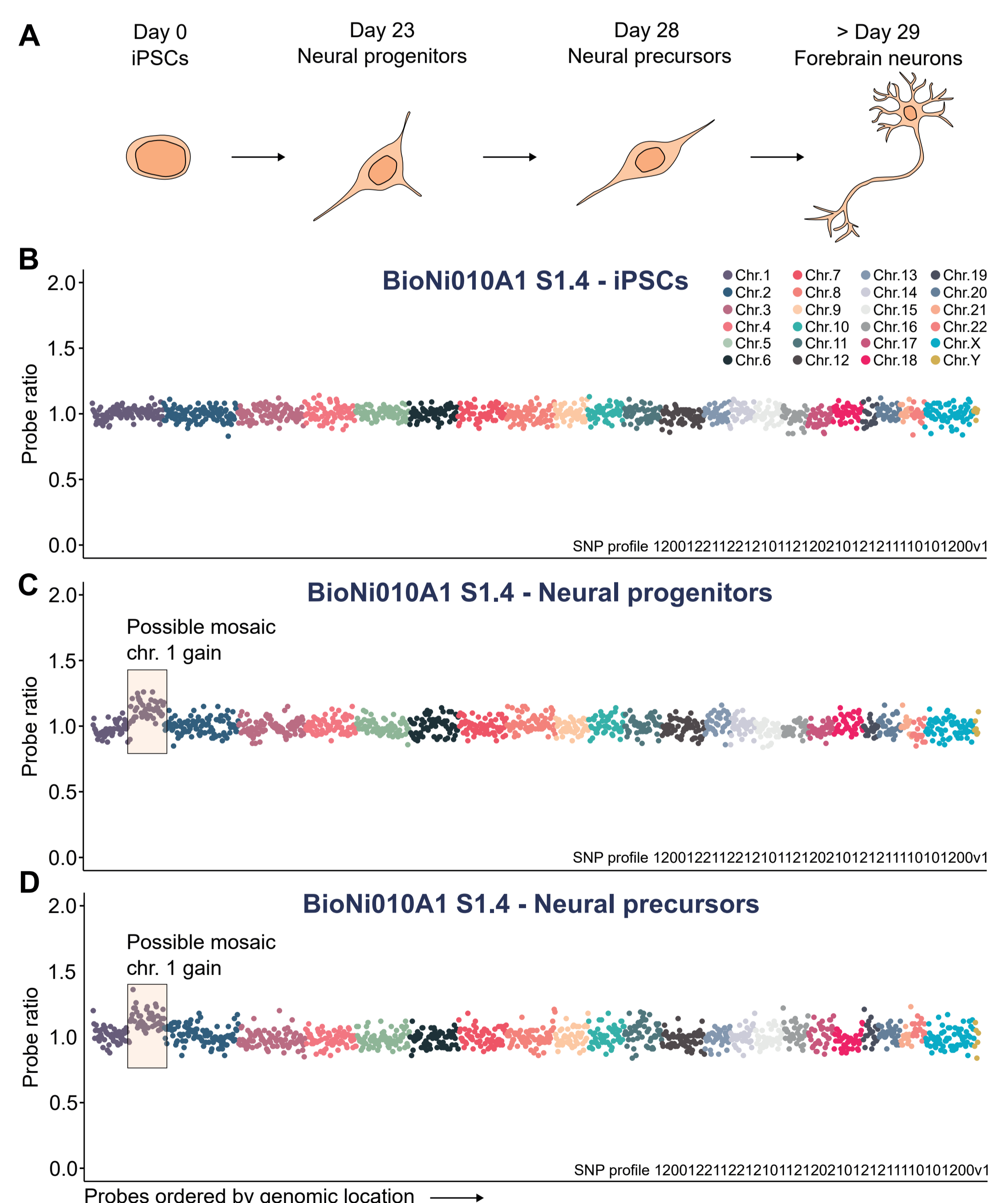
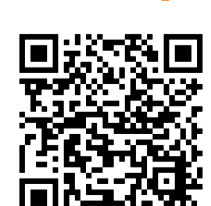


Figure 5. A) Schematic representation of neuronal differentiation (Stemcell Technologies, StemDiff Forebrain differentiation), B-D) Probe ratio charts displaying the normalized probe ratios on the y-axis, ordered by chromosomal position on the x-axis. For each sample the identified SNP profile for 39 SNP locations is shown.

D024 KaryoProfiler results at the iPSC stage were concordant with Karyostat data derived from the same sample. SNP profiles show that the samples at the different time points are from the same cell line. The emergence of a potential mosaic aberration during neuronal differentiation shows the importance of frequent monitoring of genomic integrity during long-term experiments, even when the cell line is well characterized at the iPSC stage.

For further information, please contact:
Joery den Hoed at j.denhoed@mrcholland.com or
Cansu Yanginlar at c.yanginlar@mrcholland.com

Download the poster here:



digitalMLPA is for research use only. Not for use in diagnostic procedures.

CONCLUSIONS

- ✓ The digitalMLPA assay can reliably detect chromosomal aberrations in cell culture samples at a resolution of 2-4 Mb, with results consistent with aCGH and Karyostat data.
- ✓ The assay was able to identify a putative mosaic chr. 1 gain in iPSC-derived neuronal cells, highlighting its applicability for routine chromosomal integrity monitoring in cell cultures.
- ✓ In addition, the assay reliably generates unique SNP profiles that can aid cell line authentication.

Taken together, NXtec D024 KaryoProfiler could serve as a useful tool for routine cell line characterisation and authentication.