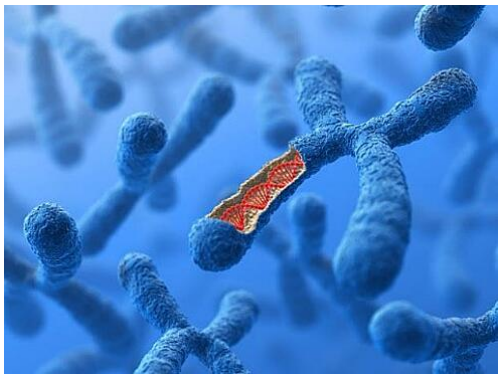
November 2021 newsletter

How to be sure that you can detect sub-karyotypical abnormalities, such as 20q amplicon in hPSC culture before it's too late

One of the downsides of the amazing abilities human pluripotent stem cells (hPSC) have to multiply indefinitely is their **propensity to develop genomic alterations during their time in culture**. For this reason, scientists working with hPSC are faced with the challenge of finding **the most suitable testing strategy** for their needs. In this November edition, we highlight the most important chromosomal abnormalities you should watch for and share our expertise in this complex field of integrity testing.

Chromosome 20q11.21 amplification: the most common genetic defect in hPSCs

Back in 2011, the International Stem Cell Initiative analysed 125 human embryonic stem (ES) cell lines and 11 induced pluripotent stem (iPS) cell lines from 38 laboratories worldwide looking for genetic changes over the duration of the cells' culture.



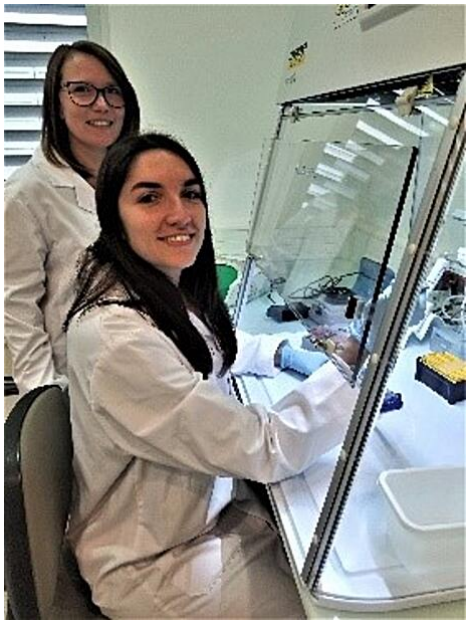
Most lines were analysed both at early and late passages. The comparison of the profiles revealed that the frequency of **genomic alterations tended to progressively increase during culture**. These defects concerned mainly

chromosomes 1, 12, 17, and 20 (Amps et al., 2011). Copy number gains at **chromosome 20q11.21 were the most frequent. This region includes the *BCL2L1* gene that encodes an inhibitor of apoptosis and that was identified as a driver gene** (Avery et al., 2013). Indeed, *BCL2L1* overexpression confers a survival advantage to cells and is often observed in human cancers (Beroukhim et al., 2010; Scotto et al., 2008; Tabach et al., 2011). More specifically, chromosome 20q amplification is strongly correlated with colorectal adenoma-to-carcinoma progression, and *BCL2L1* may have a functional role in this transition (Sillars-Hardebol et al., 2012). In addition, *BCL2L1* expression is inversely correlated with the chemosensitivity of cancer cell lines. This contributes to the cells' chemoresistance (Williams et al., 2005).

Gain of the chromosome 20q also leads to decreased differentiation potential of human ES cells toward neural lineages (Werbowetski-Ogilvie et al., 2009).

Therefore, it is essential to identify the potential presence of 20q abnormalities to eradicate their **detrimental impact at the clinical level,** but also for **basic research**, disease **modelling,** and **pharmaceutical efficacy and toxicity assays**.

At Stem Genomics, we have found very similar results in cell lines we have tested so far. Looking at the samples analysed, **26% showed recurrent abnormalities, with 50% of those found on the 20q chromosome**. According to Elena Hauser, Stem Genomics Platform Manager: "*Chromosome 20q alterations have been the most frequent recurrent abnormality found in the hPSC lines analysed at our facility since we started testing in 2018".*



According to Elena Hauser, Stem Genomics Platform Manager (left) and Marie Bouaud, Laboratory Technician

(right), 50% of the genome alterations found in the hPSC lines tested at our facility are on chromosome 20q.

Like for the 20q11.21 region, other recurrent abnormalities encountered in hPSCs are often associated with cancer risk. The functional consequences of these recurrent abnormalities are the ability to escape apoptosis (Avery et al., 2013), decrease differentiation capacity (Markouli et al., 2019) increase tumorigenicity (Ben-David, 2015) and faster cell-cycling (Barbaric et al., 2014).

This is where research stands today, however, with the continuous improvement of the hPSC characterisation methods as well as the adoption of new culture conditions, **it is to be expected that new abnormalities will get identified**. This means that tests to detect these abnormalities and their interpretation should be regularly updated.

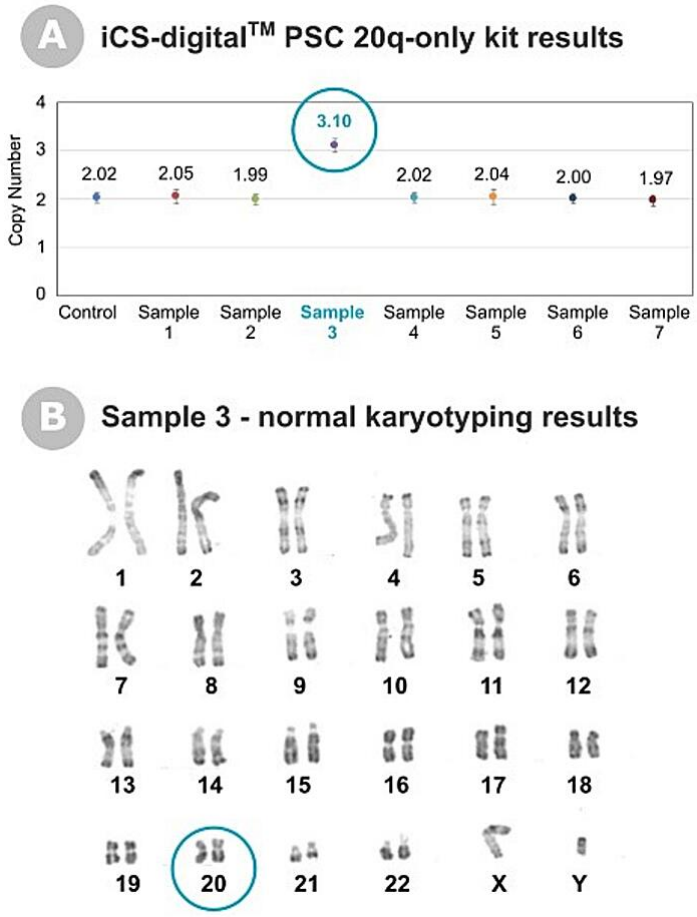
Identifying the most suitable test combination

The risk of detecting genomic alterations in hPSC lines increases with their time in culture. In their 2011 study, the International Stem Cell Initiative observed that **77% of the 20q gains were happening in late passages and only 23% in early passages.** Additionally, none of the abnormalities found in early-passage disappeared over time, reflecting the selective advantage of the acquired abnormalities. This supports the need of regular testing during culture.

The challenge for researchers is to find **the right test strategy** among the many technologies that are currently available, and also **the right testing frequency** by considering the risks, required time and costs.

To help you find the best solution, we present below the Stem Genomics Technical team's opinion on this issue.

*"The most frequent technique combination used for our clients is **karyotyping, to look at the whole genome,** combined with* Stem Genomics ***targeted digital PCR** tests (*iCS-digital™) *to identify specific hPSC genomic alterations smaller than 5Mb that cannot be detected by karyotyping. The FISH technology is another possible targeted approach, but it can be laborious particularly for the detection of tandem duplications (Mascarello et al., 2011).*



Digital PSC can detect sub-karyotypic abnormalities

Considering the speed at which a recurrent abnormality can take over a culture, we and others (McIntire et al., 2020) recommend to test cells ***ideally every 5 passages****.*

Although karyotyping is the right approach for cell line characterisation at early, late or banking stages, it usually takes too long and is therefore not suitable for in-process testing.

Due to the need of frequent testing and high-resolution detection, the ***digital PCR technology is an excellent technical choice.*** *With this method, targeted genomic fragments are amplified in thousands of individual reactions that allows the nucleic acid quantification and detection with high precision. In addition, this technology is* ***fast*** *(1 to 3 days) and* ***not too expensive*** *compared with other technologies. Therefore, it is* ***perfectly adapted to the requirement of regular testing****. which is why we've developed this technology to meet our clients' needs".*

Learn more about dPCR technology

Stem Genomics obtains ISO 9001:2015 certification!

We embarked on the ISO 9001:2015 journey with a willingness to strengthen our foundations by adopting global best-practice processes. We also recognised a lot of our clients were operating within this system and wanted the same level of commitment from us.

The ISO 9001:2015 certification will enable our biotechnology company to better meet your needs and better withstand the market fluctuations to remain your partner in the long run!

The Quality Management team. From left to right: Agnès Miermont, R&D Manager, Nicolas Chapal, CEO, and Elena Hauser, Platform Manager.

Thank you for taking the time to read this e-newsletter. We strive to share useful and relevant information to help you looking after your cells the best possible care treatment. If you think this may be useful to others, please share this e-newsletter with your colleagues!

Many thanks!

If you want to find out more about the routine tests you could put in place in your organisation, contact us on the link below.

Schedule meeting

Best,

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