Analytical Performance of the iCS-digital™ PSC test

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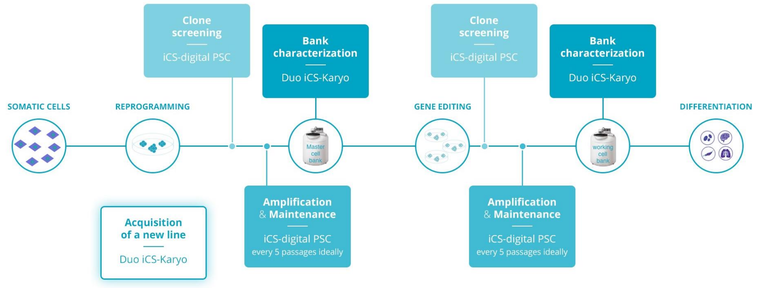
Introduction

The iCS-digital™ PSC 24-probe test is a multiplexed digital PCR assay for the identification of the most recurrent Copy Number Variants (CNVs) occurring in cultured human Pluripotent Stem Cells (hPSC)[1-2]. This test is based on digital PCR technology which enables sequence-specific detection and absolute quantification of nucleic acids. Use of the iCS-digital™ PSC test for routine testing purposes requires validation to ensure proper quality control of hPSC genome integrity. Herein, we present the results of an analytical performance study of the iCS-digital™ PSC test.

Graphical Abstract



Precision

Variability measurements were performed in independent tests for the same sample under stipulated conditions to test whether multiplex assays produce precise results. Both the repeatability (within-run variation) and intermediate precision (between-run variation) were investigated. Repeatability was tested on 24 replicates by the same operator. Intermediate precision was performed on 25 replicates on different days and two different operators were involved. Precision was assessed based on the target copy number concentration (copies/µL).

The results presented in Table 1 show that the relative standard deviation (RSD), which provides the precision of the method, is below the minimum performance criteria of ≤ 25% [3-4].

	Repeatability (copies/µL)	Intermediate precision (copies/µL)
Mean		
RSD (%)	7.37%	17.9%

Table 1. Repeatability and intermediate precision data.

Specificity

Specificity was demonstrated by the lack of amplification in the absence of DNA (No Template Control: NTC) and was compared with a positive control (Fig. 1)[3]. The false-positive rate of the iCS-digital™ PSC test is 0.098%. It was measured by collecting data on 23 NTC replicates. Digital PCR false positive rates ≤ 5% are generally expected [4].

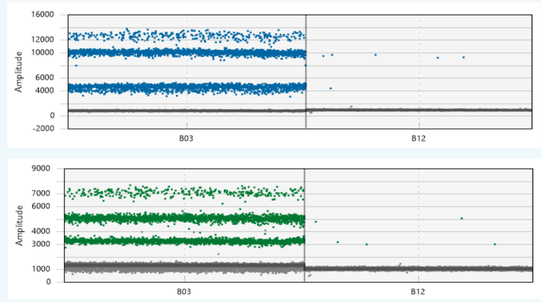
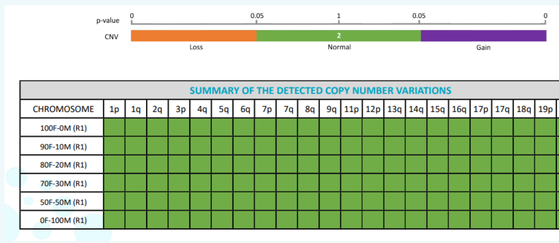


Figure 1. Comparison of a 1D scatterplot well for standard DNA (left) and NTC (right). FAM (top) & HEX (bottom) amplitude for 3 targeted sites and the reference gene are shown.

Mosaicism

To determine the minimum detectable percentage of abnormal cells by the iCS-digital™ PSC 24-probe test, female and male control DNA were mixed at different ratios to achieve a range of targeted percentage mosaicism, mimicking a loss on the X chromosome. Each combination was tested in duplicate. The ChrX loss is significantly detected for each replicate from 20% mosaicism (Figure 2).



Sample name	ChrX mosaicism (Loss)	CNV	p-value
100F - 0M	0 %	2	1
90F - 10M	10 %	1.91	0.2224
80F - 20M	**20 %**	**1.81**	**< 0.01**
70F - 30M	**30 %**	**1.66**	**< 0.001**
50F - 50M	**50 %**	**1.49**	**< 0.001**
0F - 100M	**100 %**	**1.06**	**< 0.001**

Figure 2. Mosaicism experiment. Top: color table representation of the iCS-digital™ PSC test mosaicism experiment. Bottom: summary table of the results. F: Female DNA. M: Male DNA.

Limit of detection (LOD)

The LOD is defined as the minimum PCR copy number concentration that can be distinguished from zero, with a 95% confidence level. The LOD results (Table 2) was obtained based on the limit of blank (LoB) value and Standard Deviation (SD)[5] of 23 No Template Control (NTC) test replicates.

LOD (95%) No. of positive partitions	LOD (95%) Copies/µL
17	1.20

Table 2. LOD results

Limit of Quantification (LOQ)

The LOQ is the lowest PCR copy number concentration that can be reliably quantified with an acceptable level of precision and accuracy. The LOQ of the iCS-digital™ PSC 24-probe test was measured by defining the lowest copy number within the dynamic range for which a 20% mosaicism anomaly is detectable. Two replicates of each reaction were performed on a DNA mixture consisting of 20% male and 80% female DNA over a range of DNA amounts from 1 ng to 66 ng. The abnormality (ChrX loss) was significantly detected for each replicate with a minimum DNA quantity of 15 ng, which corresponds to an LOQ of 214.5 copies/µL (Table 3).

DNA concentration	Copy number concentration Copies/µL	p-value
1 ng	4	0.4743
3 ng	39.3	0.2673
5 ng	70.8	0.3072
10 ng	143.5	0.4517
15 ng	**214.5**	**< 0.05**
66 ng	**1339.6**	**< 0.001**

Table 3. LOQ results.

Conclusion

This method validation study demonstrates that the precision (RSD < 25%) and the specificity (false positive rate < 5%) of the iCS-digital™ PSC test meet the specification criteria. The mosaicism experiment establishes that the lower clonal population detectable with the iCS-digital™ PSC test is 20%. Overall, this method validation study confirms that the iCS-digital™ PSC test is fit for the purpose of CNV detection in cultured hPSCs.

References

1.Assou S, et al. Stem Cell Reports. 2020;14(1):1-8. **2.** Assou S, et al. Stem Cells. 2018;36(6):814-821. **3.** Ma H, et al. Mol. Ther. Methods Clin.Dev.2020; 20:152-168.
4. Holst-Jensen A, et al. DECATHLON deliverable report D6.1. 2014. **5**. Armbruster DA, Pry T. Clin Biochem Rev. 2008;29 Suppl 1(Suppl 1):S49-S52.