iCS-digital™ PSC test report

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ORGANIZATION		
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PURCHASE ORDER (DATE)	**REMAINING TEST(S)**	
QUOT231213-5 (19/01/2024)	**8**	

TEST DATE	CONTROL VALIDATION
July 16, 2025	**Normal DNA control for the 28 probes: Passed**

SUMMARY OF THE DETECTED COPY NUMBER VARIATIONS

Samples	Cell line	Passage	dsDNA (ng/μL)	dsDNA quantity	CNVs	Sex**
P1	P1C26	P23	30.7	Good	Not detected	M
P2	P2C27	P23	102	Good	Not detected	F
C1	C1C1	P20	45.2	Good	Suspected	M
C2	C2C14	P22	72	Good	Not detected	F

The results of this test are for research use only.
** If unknown, sex is deducted from the ChrX and the ChrY copy number.

Note: a trend to loss or to gain corresponds to a position detected with a p-value between 0.01 and 0.05. Trends are not anomalies but are defined as "suspected anomalies". This could be due to the quality of the samples or of the test's sensitivity limit (<25% mosaicism). If the suspected CNV is an emerging recurrent variant, it will progressively takeover the culture. For this reason, we advise to closely monitor the suspicious samples and test them again a few weeks or passages later.

HOW TO CITE the iCS-digital™ test

"Genomic stability was assessed by detection of recurrent genetic abnormalities using the iCS-digital™ PSC test, provided as a service by Stem Genomics (https://www.stemgenomics.com/), as described previously (Assou et al., 2020)."

Elena HAUSER
Platform Manager

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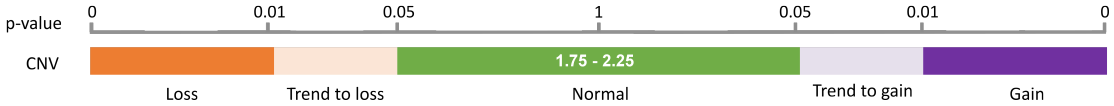
Copy number values:



SUMMARY OF THE DETECTED COPY NUMBER VARIATIONS																											
CHROMOSOME	1p	1q	2q	3p	4q	5q	6q	7p	7q	8q	9p	9q	11p	12p	12q	13q	14q	15q	16q	17p	17q	18q	19p	20p	20q	22q	Xp	Yp
P1																												
P2																												
C1																												
C2																												

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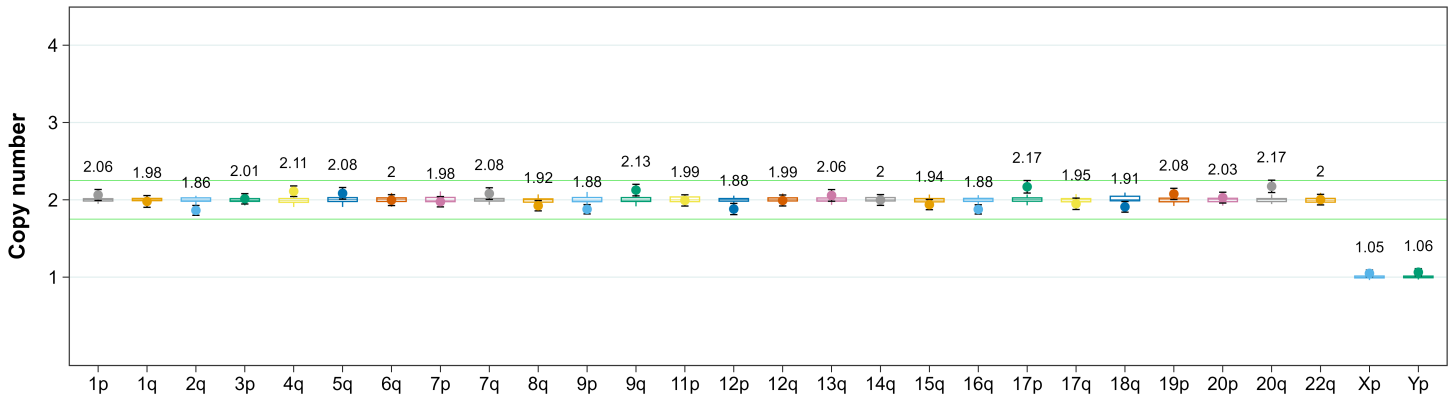
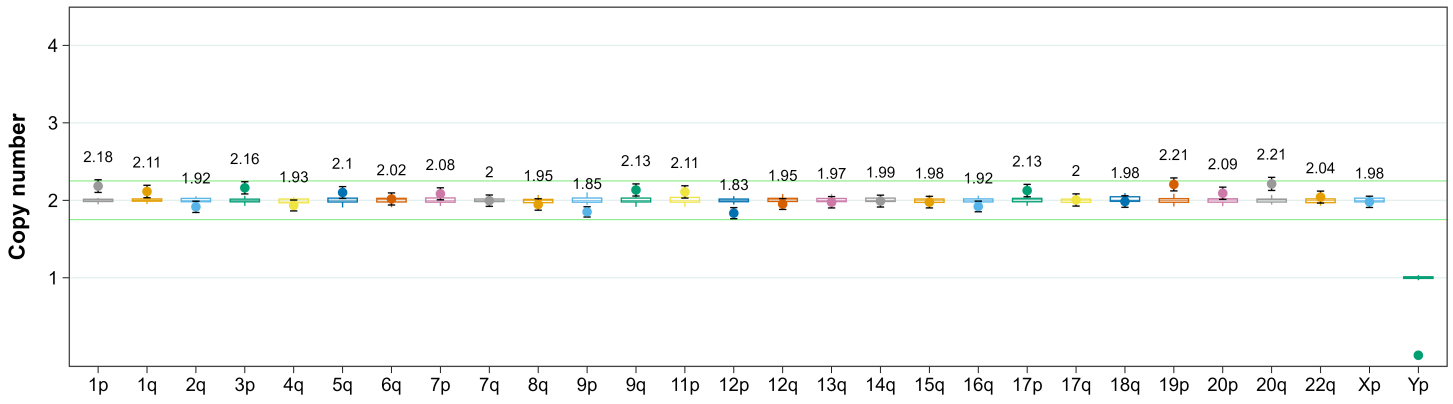
COPY NUMBER VARIATION

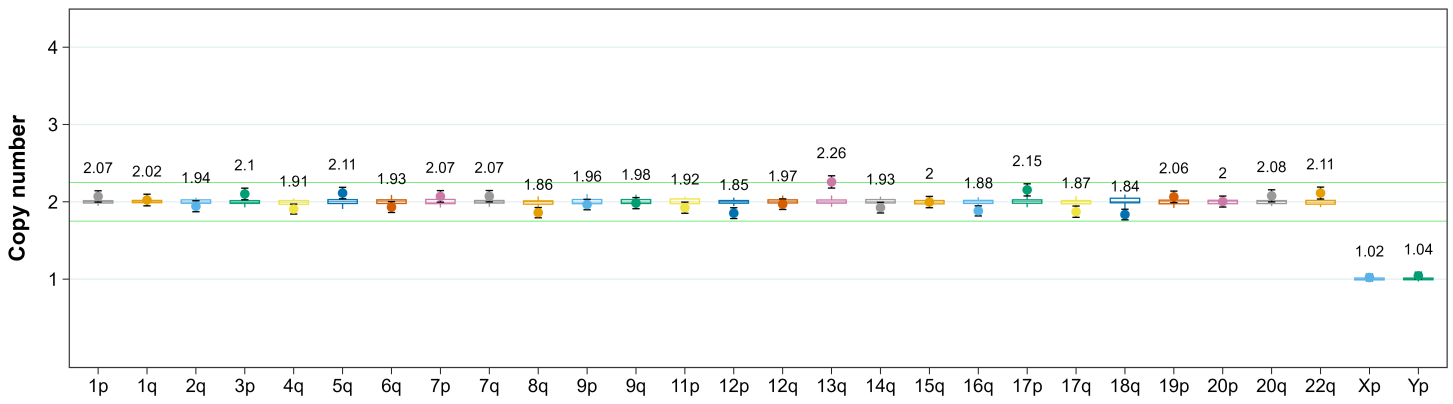
P1



P2



C1



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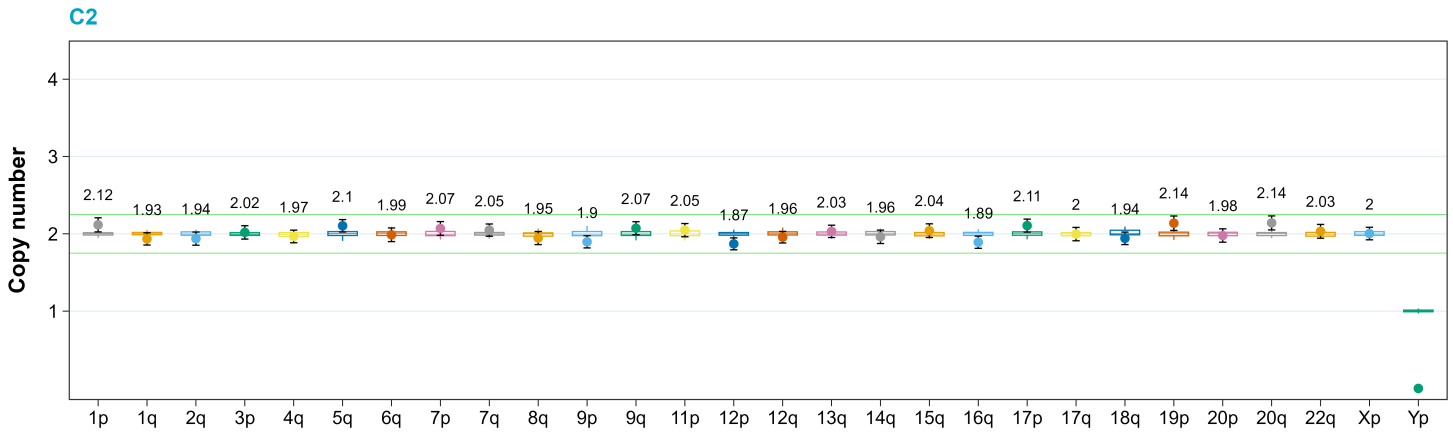
COPY NUMBER VARIATION



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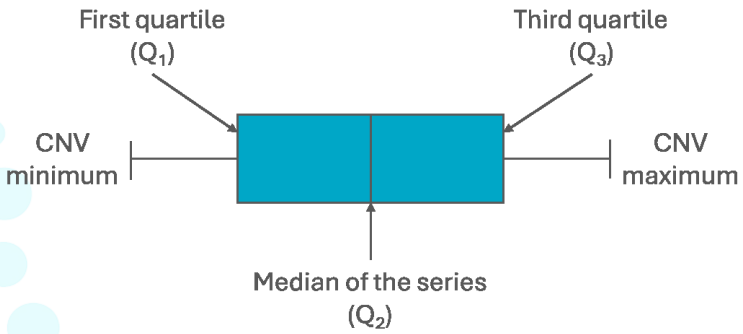
Appendix: iCS-digital™ interpretation method

Our technology allows for the detection of Copy Number Variations (CNVs) that is to say, DNA segments of one kilobase (kb) or larger that are present at an abnormal copy number in comparison with a reference genome. Normal copy number should be equal or close to the value of 2 at all the 28 recurrent regions that we analyze (except for the Xp and the Yp positions since they depend on the sex of the cell line studied: XX or XY). However, due to intrinsic variation caused by multiple factors (DNA concentration, quality, etc.), some samples will present higher copy number fluctuation than others over the 28 positions.

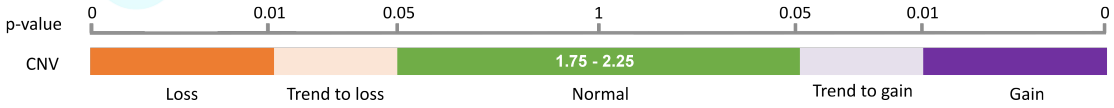
Our interpretation method takes into account the difference in CNV fluctuation observed among samples. More specifically, our statistical analysis is based on normal distribution and is adapted to the overall variability of each sample in an independent manner. P-values are then assigned to each probe and the detection of anomalies is calculated based on their specific p-values and CNVs. For each probe, a boxplot is generated based on the statistical analysis of the historical CNV values.



Visually, a non-significant value will be inside of the boxplot (indicating a similarity to a normal value) while a significant value will be outside of the boxplot (indicating a deviation from a normal value).

Green lines are displayed at 1.75 (lower limit) and 2.25 (upper limit) and represent the limit of detection of our test that is (<25% mosaicism). This means that our test can detect a minimum of 25% of abnormal cells among normal cells.

Also, a visual is shown describing the p-value and CNV thresholds detected at each position:



A sample is considered normal by default if its copy number values are strictly between 1.75 and 2.25, or if its p-values are strictly above 0.05. A trend (Trend to loss or Trend to gain), corresponds to a position detected with a p-value between 0.01 and 0.05. Trends are not anomalies but are defined as suspicion of anomalies. It could be linked to the quality of the samples, to the run, or the limit of sensitivity of the test (<25% mosaicism). In these cases, we advise to keep an eye on the samples involved and potentially re-test them few weeks/passages later.

An anomaly (CNV = Loss or Gain) is detected if a position presents a p-value strictly below 0.01.

The CNV value gives an information on the proportion of abnormal cells. For example:

- CNV = 2.2 means that 20% of the clonal population has acquired a third copy of the target region.
- CNV = 2.3 means that 30% of the clonal population has acquired a third copy of the target region.
- CNV = 3.0 means that 100% of the clonal population has acquired a third copy of the target region.
- CNV = 3.2 means that 100% of the clonal population has acquired a third copy of the target region and 20% of them has acquired a fourth copy.

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