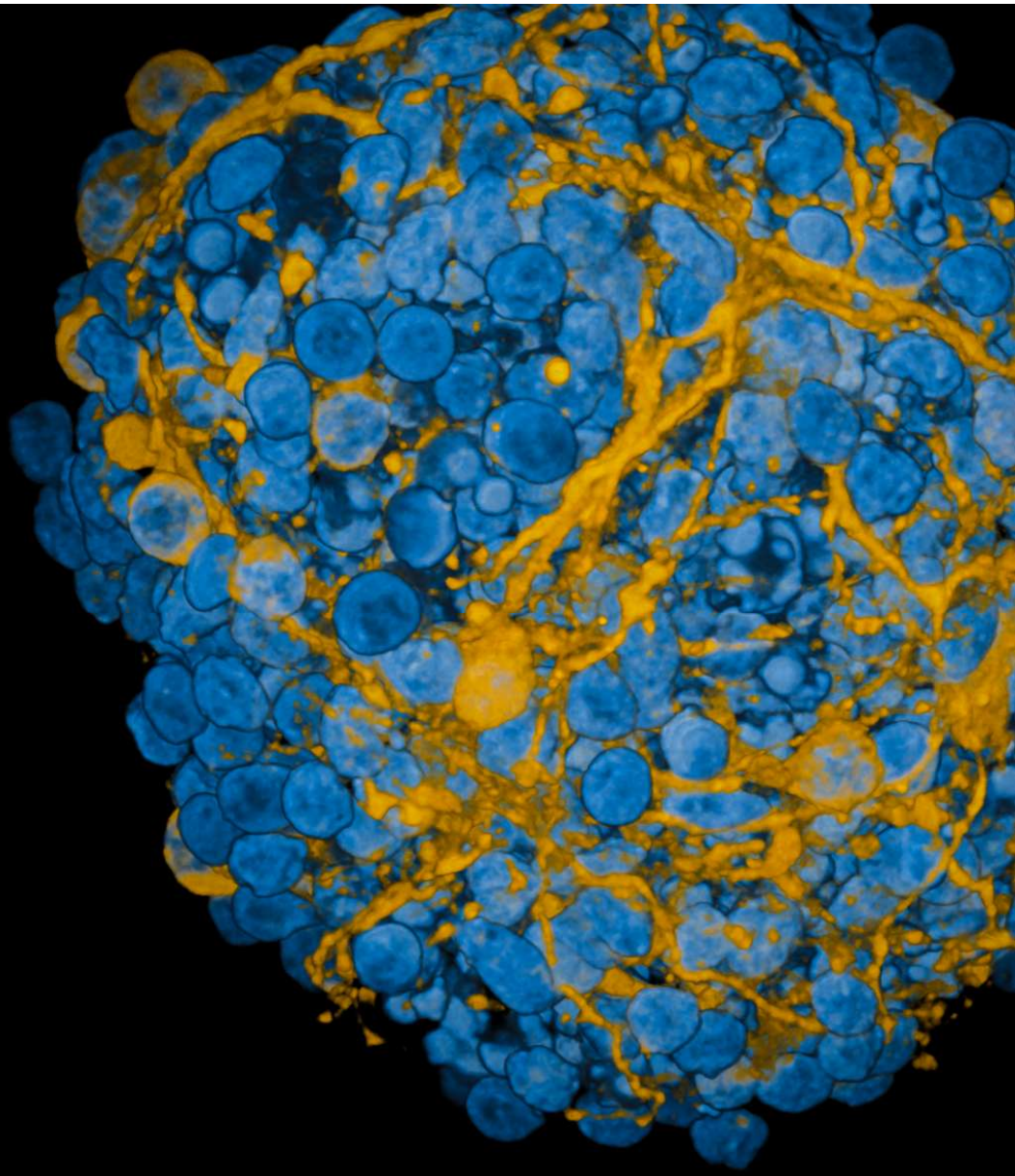
TreeFrog therapeutics

Unlocking iPSC-Derived Cell Therapies

Scaling Up Without Compromise. Delivering High-Quality Cell Therapy for All

Focus: the genomic integrity challenge



François Gianelli · CASSS October 2026

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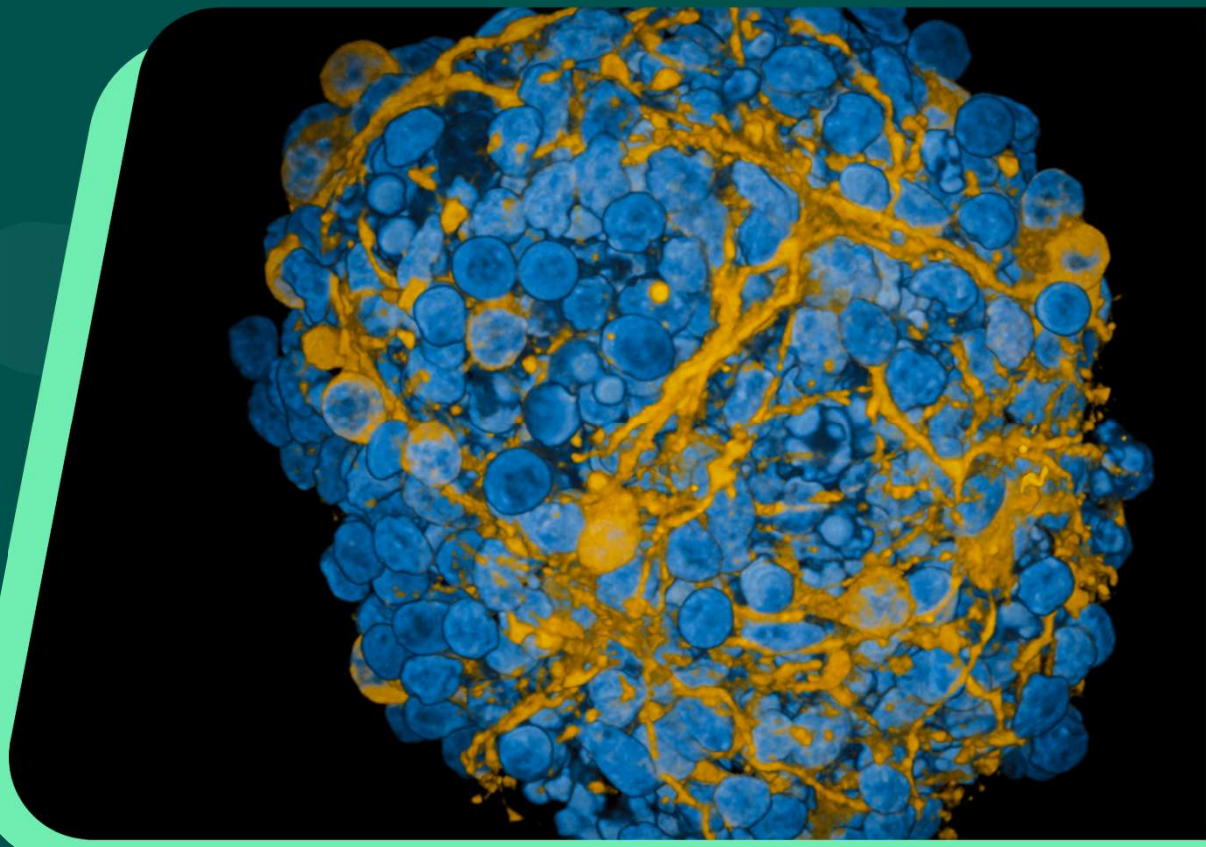
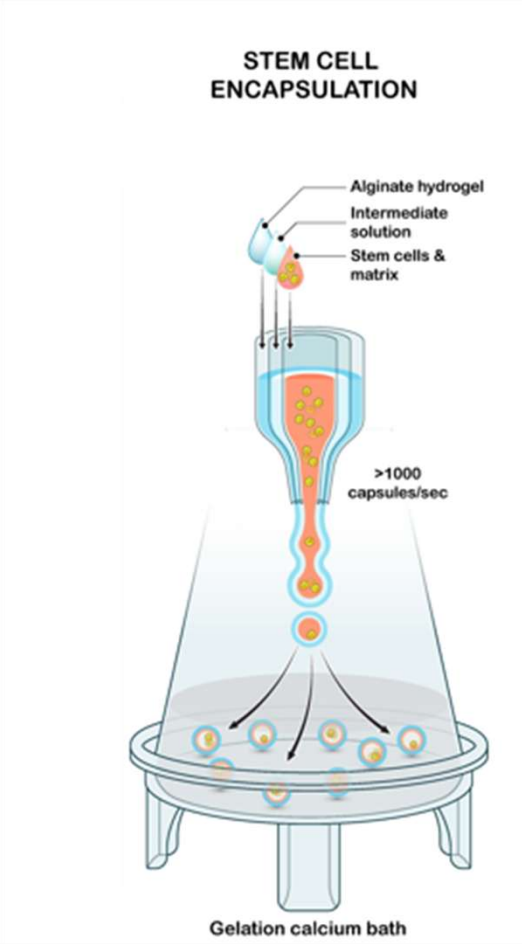
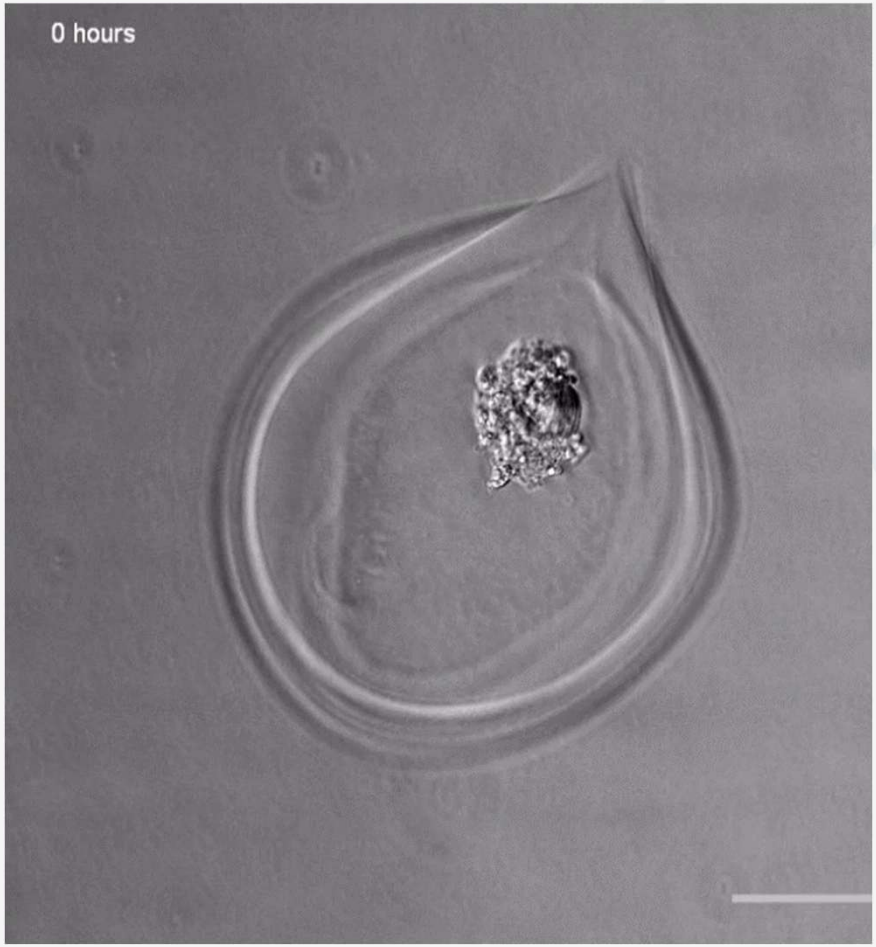
About TreeFrog

C-Stem™



Encapsulation solves 3D suspension limitations by offering a protective microenvironment

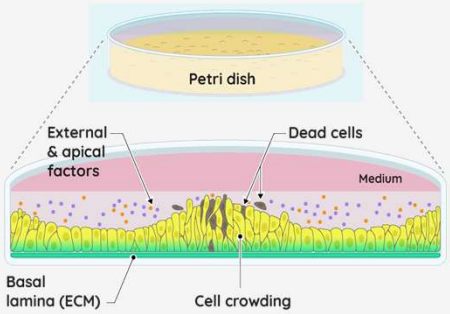


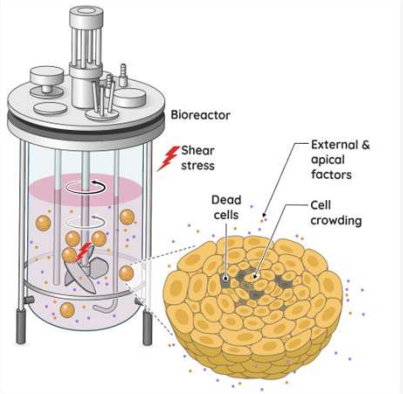


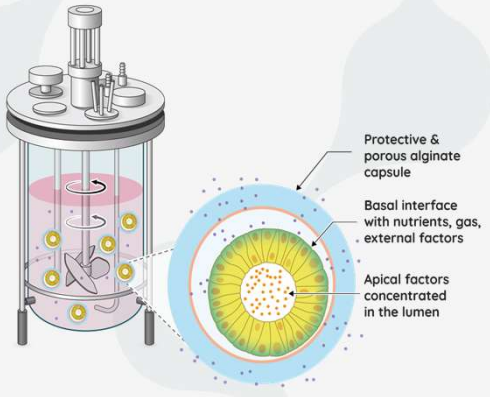
C-Stem™ addresses bottlenecks for human PSC growth

outperforming 2D and 3D approaches while maintaining *in vivo*-like cell quality & functionality

Current approaches | **TreeFrog Therapeutics**







	2D COLONIES	AGGREGATES IN BIOREACTORS	C-STEM™ IN BIOREACTORS
Yield (per week)	**30- to 100-fold**	**10- to 40-fold**	**100- to 300-fold**
Genomic risk	**High** Overcrowding and high mortality increasing selective pressure	**Critical** Overcrowding and cell death (necrotic and impeller-induced) driving selective pressure	**Contained** In vivo-like growth, low mortality and low selective pressure
Scalability	**Scale-out only** Labor-intensive, batch-to-batch variability; high QC costs	**Scale-up limited to 3L** Impeller-induced shear stress damages cells	**No limitation in bioreactor size** Cells protected by the capsule; culture parameters preserved across scales

Source: TreeFrog White paper "The Lumenized Rosette, mimicking the in vivo micro-environment of human pluripotent stem cells", 2023

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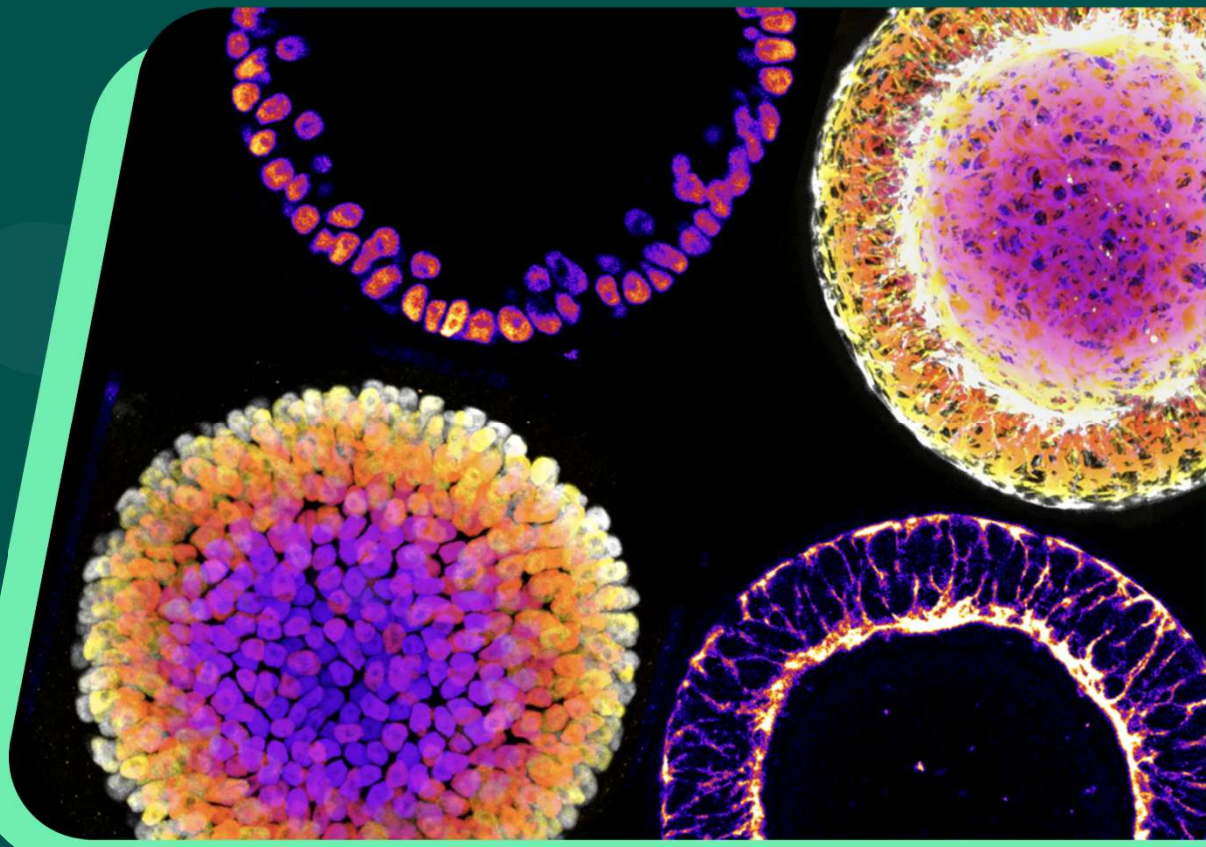
Genomic Integrity

Why it matters



Every dose may inherit the bank's genome

Why it matters

Allogeneic iPSC banks are made once and resulting medicinal products are administered many times. A variant that enters the bank, or emerges during culture, potentially reaches every patient.

2026

Japan approves the first two iPSC-derived products

100+

PSC-derived products in clinical trials worldwide

1.5 VS 3.5

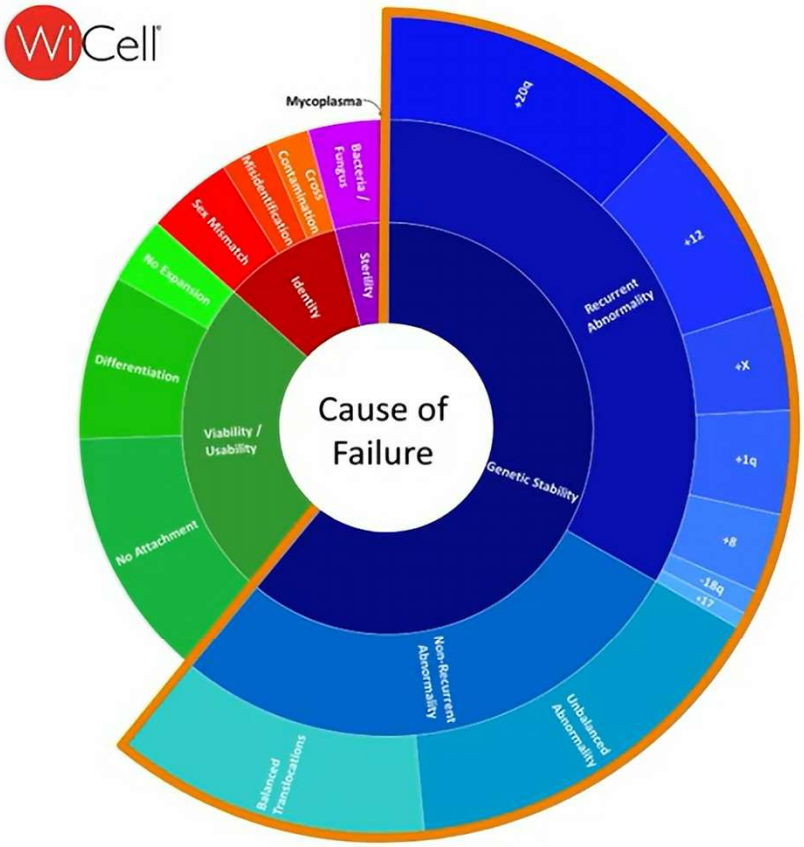
In vivo vs in vitro new mutations per genome per population doubling

5–10

passages for a variant with a growth advantage to take over a culture

One bank can treat hundreds or thousands of patients: it is iPSCs biggest asset, and a variant in it is the biggest liability.

How good are hPSC Lines ?



Study of 839 hPSC lines, 285 failed testing (34%)

61% - Genetic Stability (G-banding)

- Half of which were recurrent abnormalities

25% - Viability/Usability

- No attachment, expansion or excessive differentiation

10% - Identity (Short Tandem Repeats)

- Identity mismatch, sex mismatch or mixed cell population

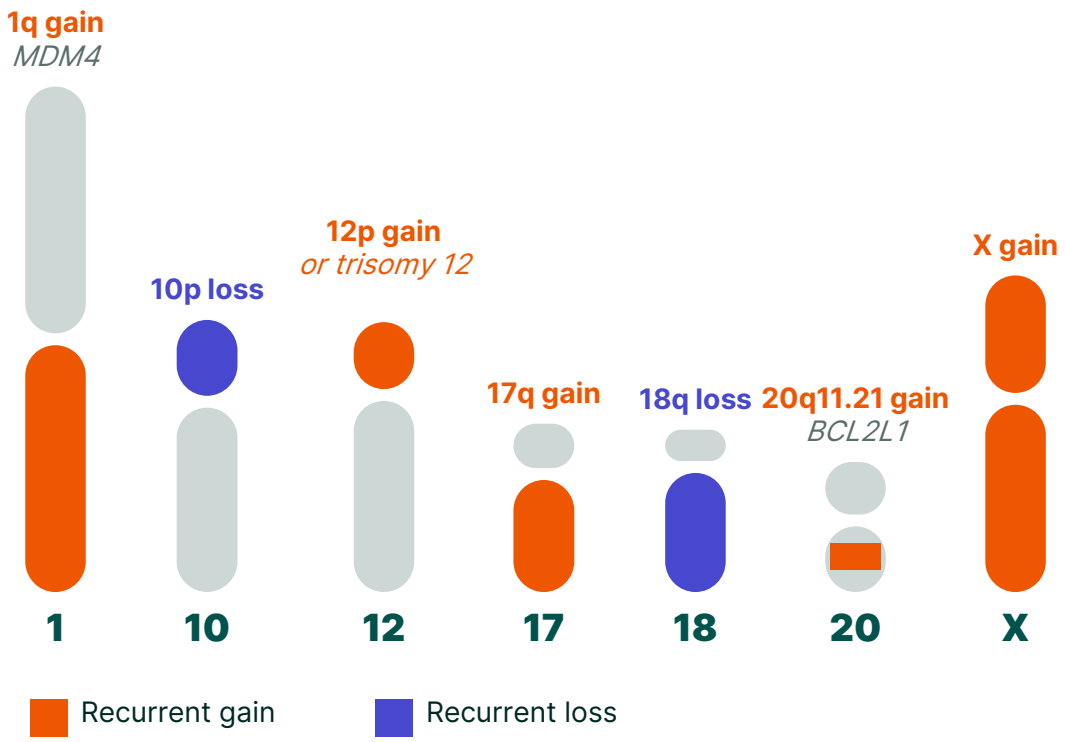
4% - Sterility

- Non-sterile or mycoplasma positive

D. Felkner., J. Brehm, E. McIntire, S. Minter, A. Paguirigan, K. Remondini, S. Taapken & T.E. Ludwig. "Human pluripotent stem cell quality: A scientific wake-up call." Poster presented at the ISSCR Annual Meeting; June 2019; Los Angeles, California.

Culture conditions select recurrent variants in a spontaneous and nonrandom manner

The biology



TP53

Dominant-negative variants expand in culture: the clearest disqualifying gene

BCOR

Acquired in 26.9% of blood-derived lines, with functional effects on differentiation

~20%

of hiPSC lines show aneuploidy

~72%

of fibroblast-derived lines carry UV damage

Sources: ISSCR Standards, Appendix 5, Table A5.2; Vales and Barbaric 2024; Madrid et al. (JSRM-ISCT), Cytotherapy 2024; Rouhani et al., Nat Genet 2022; Merkle et al., Nature 2017.

Signals so far: rare, but real

Clinical signals

1

Documented tumour among more than 1,200 recipients of hPSC-derived products, counted in 2025

It arose from cells that carried cancer-gene mutations.

2017

Mandai et al., NEJM

Second autologous iPSC-RPE graft not performed after new variants appeared in the cells

2022

Han et al., Stem Cells Dev

Immature teratomas after autologous iPSC-beta cells that carried cancer-gene mutations

2025

Hirayama et al., Cell Rep Med

De novo EP300 deletion in corneal endothelial cells; no adverse events so far

Detection is not always harm, but every event so far traces back to the cells' genome.

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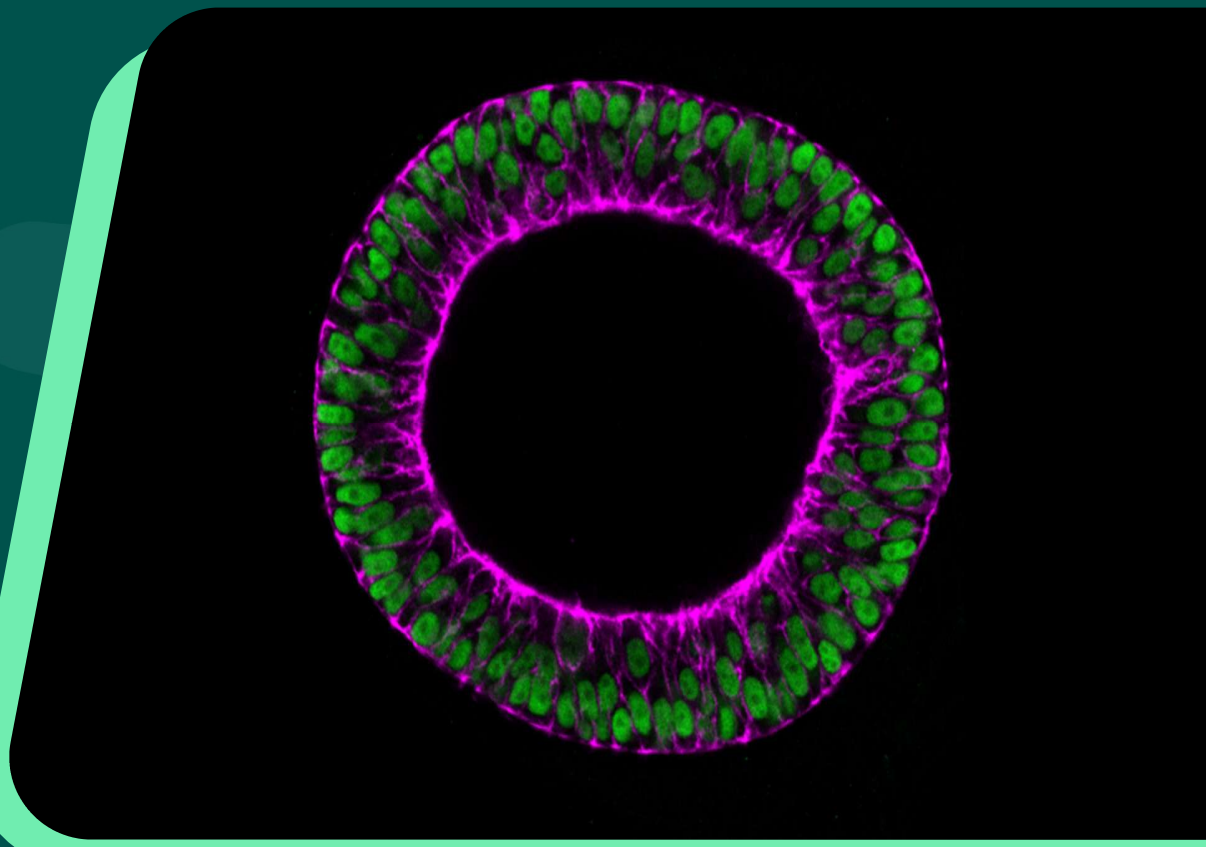
Regulatory Framework



Regulators converge: characterize every bank

No existing iPSC dedicated guideline

US
FDA

- iPSCs are continuous cell lines that can be in theory extensively cultured (draft, April 2024)
- WGS ≥50x on banks, compared with a cancer-mutation database
- April 2026 NGS draft

EU · UK
EMA · MHRA

- EMA/CAT/22473/2025, in force July 2025: orthogonal methods
- ICH Q5D master bank before exploratory trials
- MHRA: variant-by-variant risk assessment, no thresholds

JP
PMDA

- Karyotype, CNV and cancer genes vs COSMIC CGC and the Shibata list
- Reference characterization, not release (0627-1)
- Validated NGS may replace G-banding

CN · KR
CDE · MFDS

- CDE 2023: genetic and epigenetic stability; methods still evolving
- MFDS: no PSC-specific guideline yet
- Korean banks publish SNP-chip and exome data

Common ground: genome data qualify the bank and characterize the product.

Japan's first approvals set a precedent

AMCHEPRY (raguneprocel)

Sumitomo Pharma and RACTHERA · Parkinson's disease

Cells	Dopaminergic progenitors from a CiRA blood-derived stock
Genomics	Product genome analyses: no cancer-gene mutations (COSMIC CGC, Shibata list)
Safety	NOG mice for 39 and 52 weeks, with iPSC spiking at the specification limit
Approval	Conditional, 7 years; every patient followed by MRI and [18F]FLT PET

RiHEART

Cuorips · heart failure from ischaemic cardiomyopathy

Cells	Cardiomyocyte sheets from a CiRA blood-derived stock
Genomics	Clone chosen on karyotype and genome; bank screened for cancer genes; product vs working bank by array CGH
Safety	Soft agar, growth over 6 passages, NOG mice for 45 weeks
Approval	Conditional, 7 years; 75 patients targeted

Accepted: cancer-gene screening and CNV comparison as characterization, backed by long tumorigenicity studies, with no genome test on each lot.

Sources: PMDA review reports for AMCHEPRY (raguneprocel) and RiHEART (February 2026); JST Science Japan and Jiji Press (6 March 2026)

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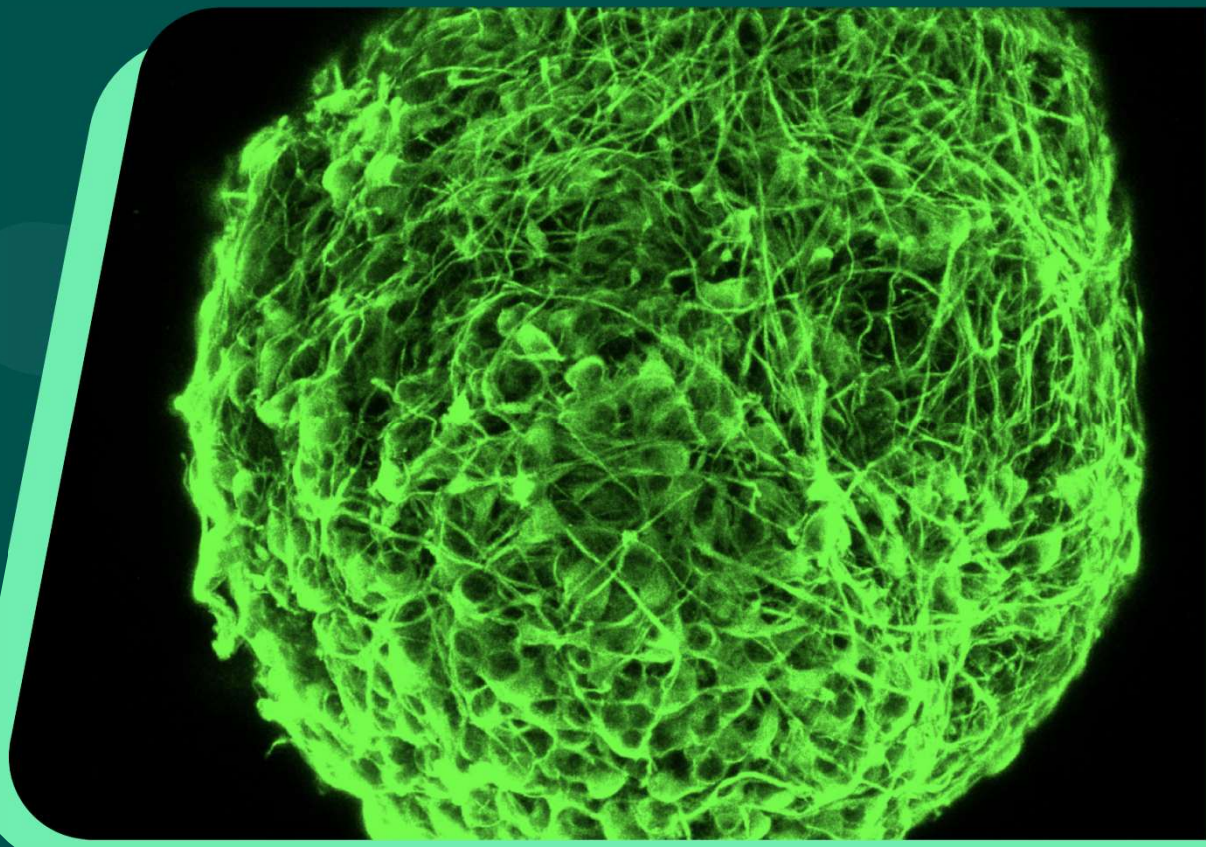
3 challenges

Detection, sensitivity, and interpretation



No single assay sees every variant class

Challenge 1 · Detection

Method	Aneuploidy	CNV	Balanced SV	SNV or indel	Copy-neutral LOH	Sensitivity
G-banded karyotype	**Yes**	**≥5–10 Mb**	**Yes**	—	—	≥15% (20 cells)
FISH	Probed loci	Probed loci	Probed loci	—	—	2–5%
qPCR or ddPCR panel	Known arms	Known loci	—	Known sites	—	<0.1% SNV, ~10% CNV
SNP array	**Yes**	**≥10–100 kb**	—	—	**Yes**	10–20%
Optical genome mapping	**Yes**	**≥0.5–5 kb**	**Yes**	—	—	5–10%
Short-read WGS, 50x	**Yes**	**Yes**	Many	**Yes**	**Yes**	25–35%
Targeted or duplex NGS	—	—	—	Panel genes	—	~2%
Long-read WGS	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	15-20%

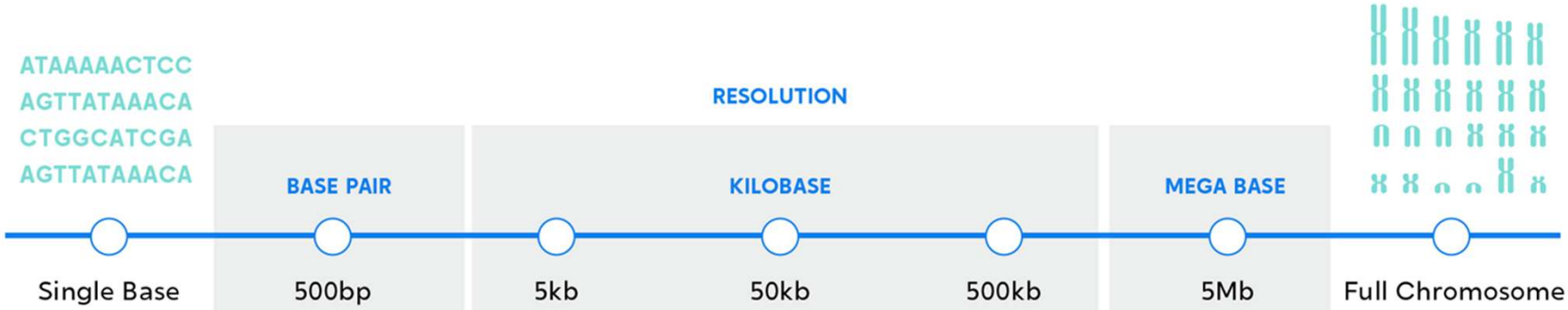
Detects genome-wide Only at chosen loci, or partly — not detected

Pair genome-wide sequencing with a cytogenomic method and sensitive targeted assays, each with a validated limit of detection.

Sources: ISSCR Standards Table A5.1, after McIntire et al. 2020; CAP review of optical genome mapping; Steventon-Jones et al., Curr Protoc 2022. Karyotype and WGS clone fractions computed (binomial, 95% detection).

QC strategy for Clinical use cell lines

Karyotyping
Aneuploidy and large SV



Short-read sequencing
SNV, short Indel and CNV detection

Long-read sequencing
SNV, short Indel, CNV and some SV detection

ANALYTICAL GAP COVERED BY OGM

OMG is able to detect large indels, inversions, duplications, balanced and unbalanced translocations commonly missed by karyotyping and NGS

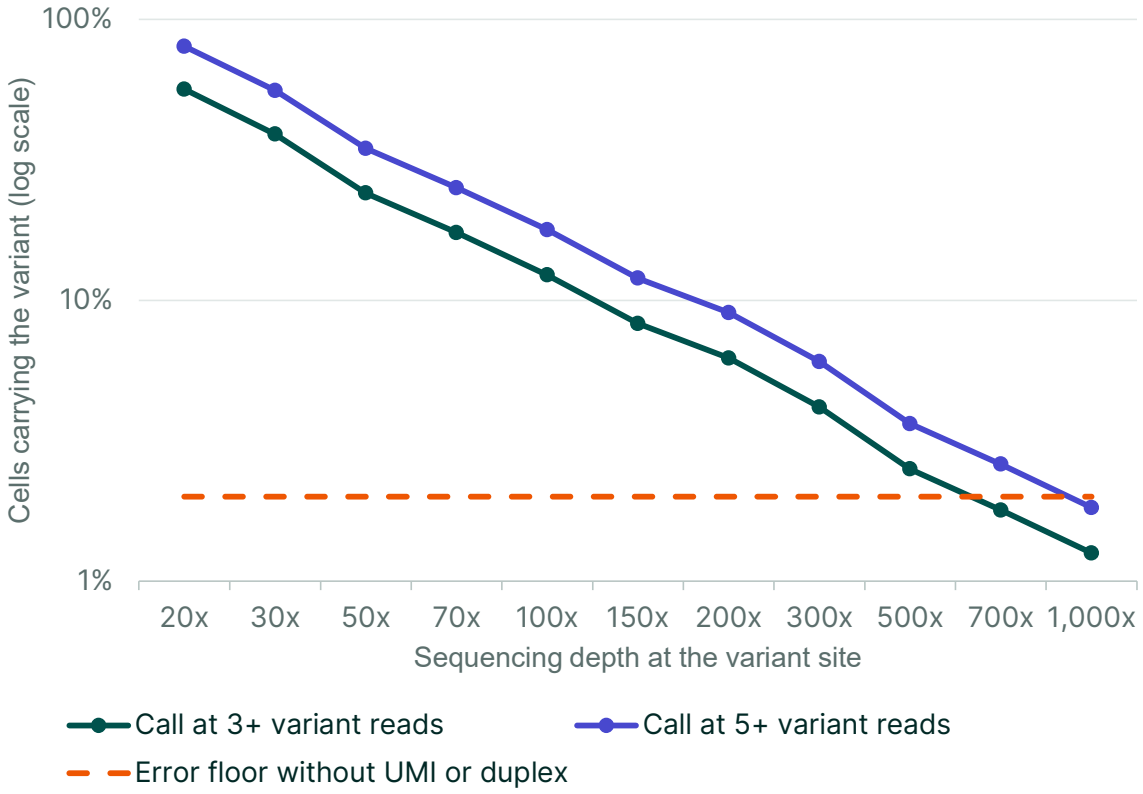
ANALYTICAL GAP COVERED BY NGS

NGS is able to detect SNVs, short indels, and other events <5 kb missed by OGM

OGM
Aneuploidy, CNV and SV detection

Depth decides which subclones you can see

Challenge 2 · Sensitivity



24–35%

of cells must carry a variant before 50x WGS, the FDA minimum, reliably calls it

250–360x

Min depth needed to see a heterozygous variant in 5% of cells

~2%

of cells: below this, standard sequencing errors cannot be distinguished

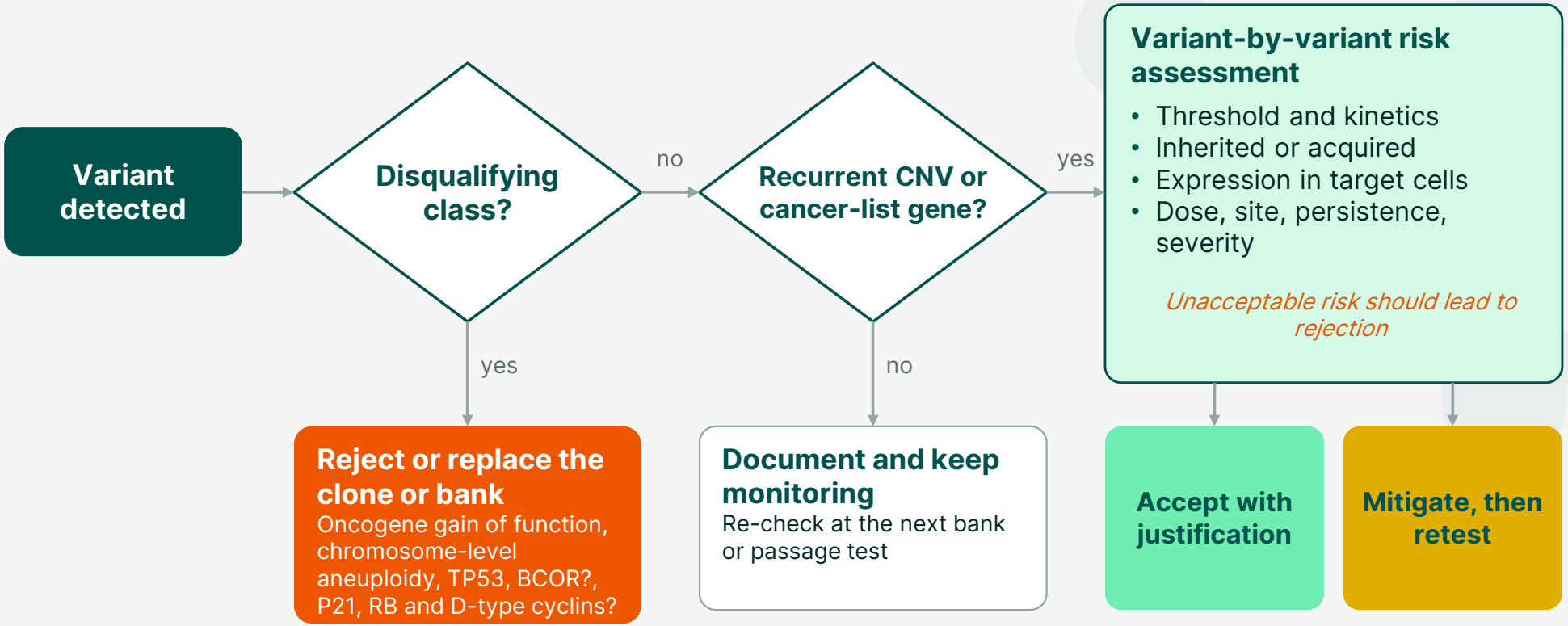
≥15%

mosaicism excluded by 20 metaphases; ruling out 1% takes more than 500

Binomial model computed for this talk: 95% detection, heterozygous variant, 3 or 5 variant reads, no sequencing error. Error floor: Kennedy et al., Nat Protoc 2014. Metaphase counts: same model; Baker et al., Stem Cell Rep 2016.

Only disqualifying variants stop a line

Challenge 3 · Interpretation



Threshold between a safe and an unsafe frequency for an undesirable variant is not known: track it against toxicology and clinical outcomes.

Synthesized from the ISSCR–MHRA scientific advice meeting (2023), and Dashnau et al. 2023. Not an official decision tree.

What is agreed, what is open

Agreed

- Test after every bottleneck and at every bank (ISSCR, GAiT, ISCBI, HESI)
- Use orthogonal methods with validated detection limits (EMA, MHLW)
- Genome-wide data for characterization. Bank screening to support risk assessment if needed
- Oncogene gain of function, chromosome-level aneuploidy and TP53 loss disqualify a line

Still open

- No allele-fraction threshold for an undesired variant
- No ranking for variants of uncertain significance
- Unknown effect of low-level contamination with variant cells
- Epigenetic variants; 3D and large-scale expansion impact
- FDA's final allogeneic guidance, on CBER's 2026 agenda

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What do we do

Scaling without compromise



Cut the risk before it takes over

Ideally before it reaches the bank

Fewer variants arising

- Consider including genomic screening for donor selection
- Screen several clones
- Select the best reagents yet anticipate GMP compatibility
- Improve processes and Limit the number of passages for MCB

Better detection and interpretation

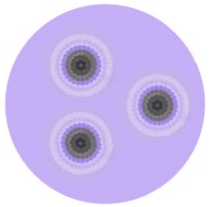
- Validate each assay's limit (spiking)
- Cover all alterations using orthogonal overlapping methods
- Track recurrent CNVs kinetics in process
- Work against gene-list versions, keep raw data, share variants in registries

Sources: Panther et al., bioRxiv 2021; Rouhani et al., Nat Genet 2022; Namvar et al., bioRxiv 2026; Dashnau et al. 2023, citing Kuijk 2020 and Thompson 2020; Zhang et al. 2026; Steventon-Jones et al. 2022; Laing et al. 2019; Assou et al. 2020; Benvenisty et al. 2025

Benchmarking Cell Culture Systems for iPSCs - Reduced passaging in C-Stem



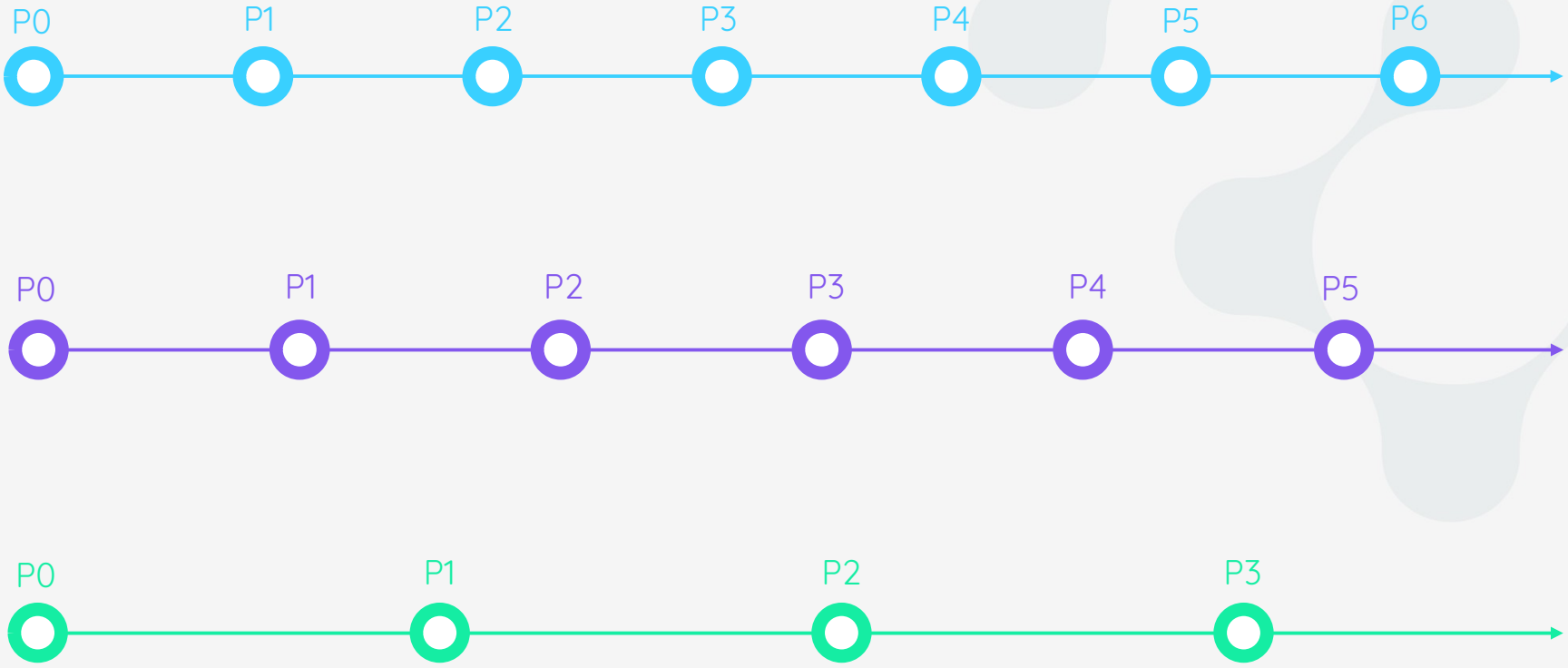
2D culture



Aggregate culture



C-Stem™



Continuous culture over 28 days

Preserving genomic integrity in C-Stem™

A) Expansion study performed using an iPSC cell line with undetectable levels* of genomic abnormalities. Cells were cultured in 2D, aggregate, and C-Stem™ conditions, and analyzed by ddPCR (Stem Genomics).

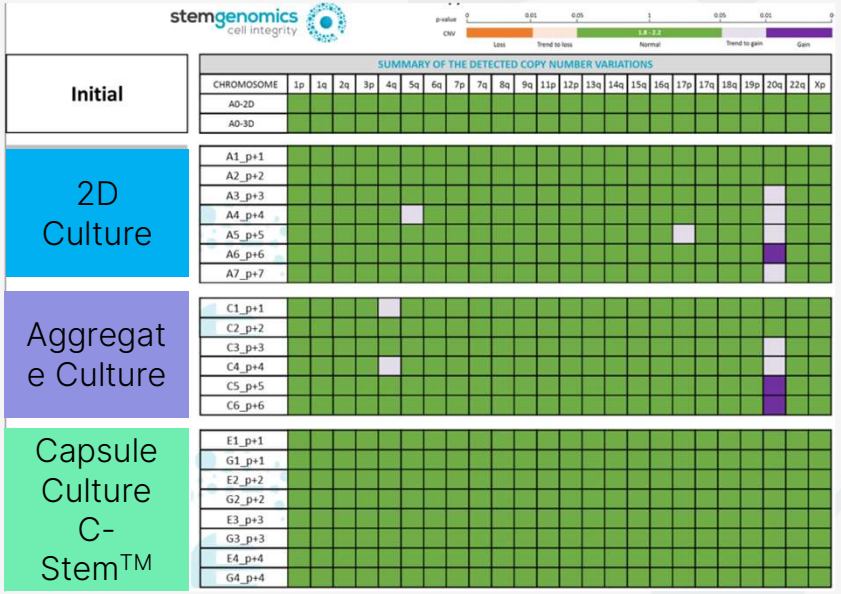
B) Kinetic study performed using isogenic iPSCs (95% normal karyotype; 5% harboring GFP with an additional copy of 20q11.21). The proportion of GMP+ iPSCs was compared across Aggregate and C-Stem™ cultures, with or without matrix, and analyzed by flow cytometry.

The microenvironment in C-Stem preserves the genomic integrity of iPSCs

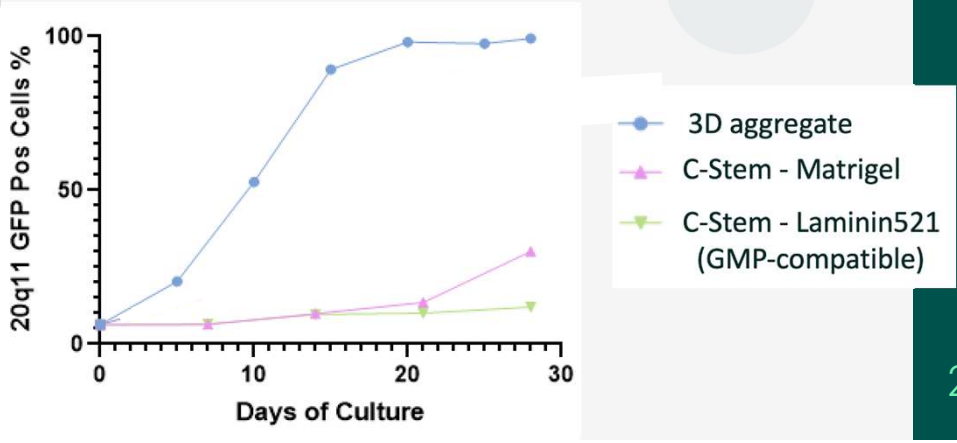
All experiments were performed at TreeFrog. Media, dissociation, and general cell handling conditions were kept consistent.

*below the limit of detection (LOD)

A)



B)



Delivering high-quality cell therapy for all

Takeaways

Test the Bank (Suppliers)

Screen clones, characterize the master bank, and prove stability over X passages (extended CB).

01

Test at Process Bottlenecks (Developers)

Improve process / reagents / protocols leveraging process development data, inc EoP

02

Know what the assays cannot see

Validate detection limits

03

Leverage Non-Clinical and Clinical Data

Support RBA with DP QC and nonclinical safety package.

04

Share data to globally define what works/fails

Variant registries and functional evidence set acceptance criteria for everyone.

05

Refine Guidance

Harmonize and review guidance and standards to support safe development of the field

06

This is a joint effort to provide high quality iPSCs to everyone


TreeFrog
therapeutics