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DISPENCELL reaches 98.4% probability of clonality for robust cell line development

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Introduction

Industrial cell line development requires cell lines to be derived from a single progenitor cell. Thus, regulatory agencies request an assessment of the **probability of clonality (PoC)** to guarantee safety for commercial manufacturing. The PoC estimates the **likelihood that a production cell line is derived from a single cell progenitor**. *Chen et al.* rigorously compared cloning protocols and assessed their regulatory acceptance[1] in an article entitled "Methods for Estimating the Probability of Clonality in Cell Line Development"[1]. Their work led to the development of a gold standard assay that is used by many companies to estimate the PoC of their cell line development workflow.

- Two rounds of cloning are traditionally performed using limiting dilution cloning (LDC) to obtain a high PoC, adding weeks to the process. Thus, there is a rush to move away from LDC to reduce the timeline while maintaining a high PoC.
- DISPENCELL is a seamless alternative to LDC. It is a reliable, easy-to-use, and cost-effective single-cell dispenser based on impedance technology[2].
- In this study, we used the Chen et al.[1] assay to estimate the PoC of clones isolated with the DISPENCELL compared to those isolated with the traditional LDC (Fig.1).

Methods

CHO cells expressing GFP were mixed with RFP-expressing cells and then cloned using DISPENCELL and LDC as a control (N = 4 × 96-well plates, each). The PoC of the resulting clones was estimated using Chen's model [1]:

$$Yg = Y \times (1 - b) \times a + Y \times b \times (1 - (1 - a^2)) \quad (1)$$

$$Ygm \times 2 = Y \times b \times a^2 \quad (2)$$

with the following variable parameters (Fig.1):

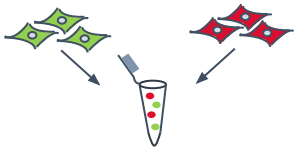
Y = wells defined as having 1 cell by the DISPENCELL
Yg = wells among Y showing cell outgrowth
Ygm = wells among Yg that are positive for both green and red fluorescence
a = single-cell recovery rate
b = probability of having 2 cells per well

(1) and (2) were combined and the experimental data were added to obtain the variables *a* and *b* to compute the PoC :

$$PoC = (Y \times (1 - b) \times a + Y \times b \times 2 \times a \times (1 - a)) / Yg \quad (3)$$

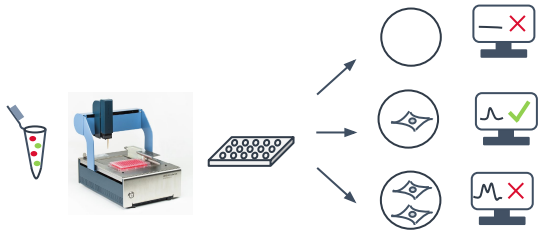
Gold standard PoC assay:

- CHO cell lines expressing a green or red fluorescent protein with similar doubling times were mixed in a ratio of 1:1



- Day 0: Cell cloning using DISPENCELL and LDC as a control
- Immediate clonality assessment using the DISPENCELL software (a feature that LDC does not offer)

1a



- 14 days of culture
- Green and red fluorescence imaging for the identification of single and mixed colonies

1b

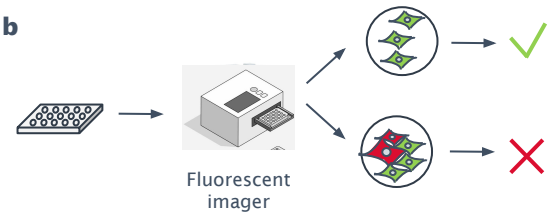


Figure 1: *Experimental design of the PoC assay*

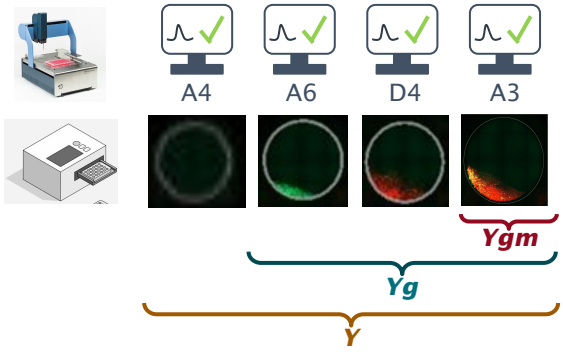


Figure 2: *PoC assessment schematics for each well condition*

Results

- Cloning on Day 0:
  The clonality check performed by the DISPENCELL software enabled the selection of 316 wells that exhibited a single-cell signature. In Fig. 3, the selected wells are marked green and the rejected ones red. For the plates dispensed by LDC, this selection was not possible. The number of wells containing one cell in LDC was estimated to be 124 using the Poisson distribution (0.6 cells/well).

- Fluorescence imaging of the colonies on Day 14:
  Of the 316 wells selected by the DISPENCELL, 255 colonies were observed; of these, only 2 were bi-coloured. The computed single-cell recovery rate (a) and the probability of having two cells per well (b) were 80% and 2%, respectively. Of the 380 wells used for the LDC, 155 colonies were observed; among them, 23 were bi-coloured. The computed single cell recovery rate (a) and the probability of having two cells per well (b) were 90% and 26%, respectively.

- Based on Chen et al[1] model, the PoC of each method was calculated by considering the single-cell recovery rate (Fig. 4). **The PoC calculated for the DISPENCELL was 98.4%, while the PoC for the LDC was 76.1%.**

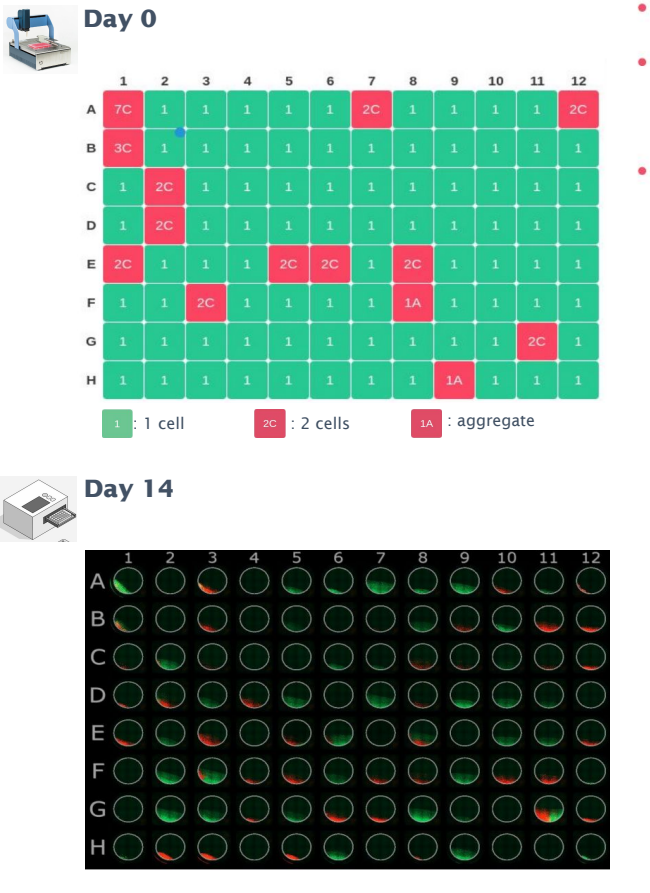


Figure 3: *Clonality check on Day 0 using the DISPENCELL analysis software (left panel) and imaging of colonies on Day 14 using fluorescent imaging (right panel)*

- **A probability of clonality of 98.4% was obtained with DISPENCELL, compared to 76.1% with the limiting dilution cloning (Fig. 4).**
- **DISPENCELL provided not only a much higher PoC than limiting dilution cloning, but also twice as many monoclonal colonies.**
- **Coupling DISPENCELL's impedance-based clonality assessment with a visual confirmation at Day 0 using a high-resolution imager (e.g., CloneSelect® Imager, Molecular Devices) would provide an even higher PoC, supporting a fast, seamless and confident IND filling.**

	DISPENCELL	LDC
Total wells	372	380
Monoclonal wells-Day 0	316 (impedance-based)	124* (estimated)
Total wells with colonies-Day 14	255 (observed)	155 (observed)
Wells with mixed colonies-Day 14	2 (observed)	23 (observed)
Probability of clonality (PoC)	**98.4%**	**76.1%***
Monoclonal colonies	**251**	**118*** (estimated)

Figure 4: *Experimental data analysis for PoC calculation. * Computed using the Poisson distribution-based model with 0.6 cells/well* and taking into account cell survival statistics.

SEED Biosciences thanks Mural Oncology for performing the experiments and providing the data.

References

1. *Chen et al., 2020. 'Methods for Estimating the Probability of Clonality in Cell Line Development', Biotechnol J. 15(2):e1900289.*
2. *Hannart et al., 2022. Traceable Impedance-based single cell pipetting: from a research set-up to a robust and fast automated robot', SLAS TECHNOL. 27(2):121-129.*

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