Automated Induced Pluripotent Stem Cell 2D amplification

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Flask culture

Introduction

Stem biology embraces the evolving need for personalized medicine and more accurate investigation models. Although stem cells offer promising opportunities, their culture and maintenance **are** laborious, time-consuming, and prone to bias. On the one hand, stem cells' plasticity is a powerful tool to achieve near-to-any biological differentiation but, on the other hand, it requires meticulousness, daily handling and, sometimes, month-long protocols. As removing manual care has been described to promise reproducibility and scalability, it most often relies on complex protocols and costly machinery. At Cellaven, using our Nestor cell culture automation system, we sought to implement with minimal modifications our culture protocols using standard culture dishes. To this end, we cultured and amplified human induced pluripotent stem cells (HiPSC) over a month. Here, we demonstrate Nestor's ability to cope with iPSC culture.

Methods

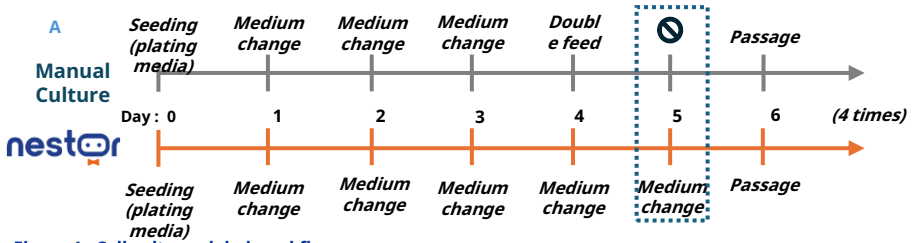


Figure 1 : Cell culture global workflow
Nestor autonomously cultured iPSC cells (SCTi003-A Stemcell Technologies) between each of the 4 manual passage. While Nestor allowed for weekend free ipsc culture, we had to ensure manual feeding everyday. Nestor daily delivered the cells with 15 mL eTeSR (Stemcell Technologies). However, manual culture was double fed (30 mL) every Saturday (day 5) to avoid wasting human resources on Sundays.

Results

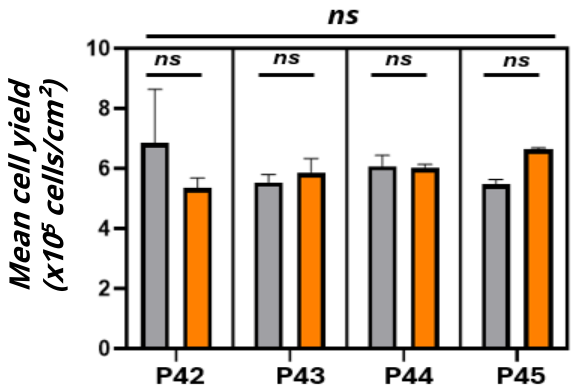


Figure 2 : Yield comparison
For each passage, cells were detached, isolated, counted and reseeded at a density of 7,3 x 10^3 cells.cm^{-2} . Counting was performed using Malassez's counting slides and trypan blue staining. No significant difference in yield was reported between culture techniques.
Nested 2 ways Anova resulted in a p-value = 0,9257, n=3

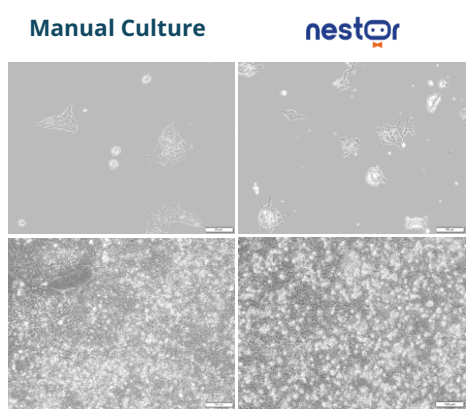


Figure 3 : Brightfield morphology
Brightfield pictures of iPSC cultured in eTeSR for 48 hours (top) and 144 hours (bottom)
(Scalebar = 100µm)

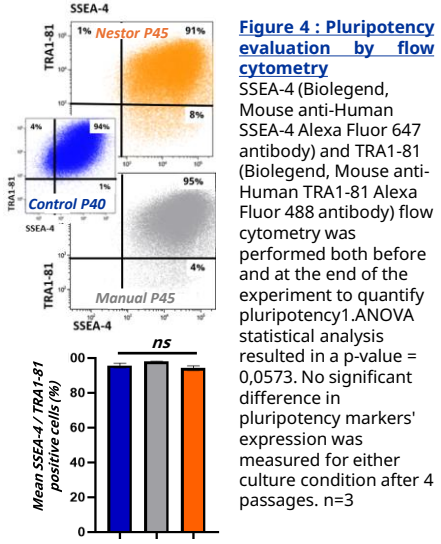
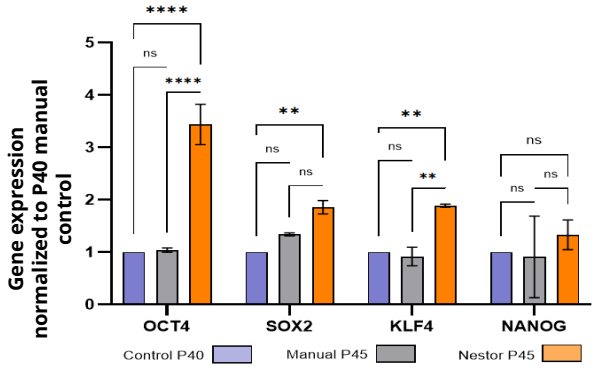


Figure 4 : Pluripotency evaluation by flow cytometry
SSEA-4 (Biolegend, Mouse anti-Human SSEA-4 Alexa Fluor 647 antibody) and TRA1-81 (Biolegend, Mouse anti-Human TRA1-81 Alexa Fluor 488 antibody) flow cytometry was performed both before and at the end of the experiment to quantify pluripotency1.ANOVA statistical analysis resulted in a p-value = 0,0573. No significant difference in pluripotency markers' expression was measured for either culture condition after 4 passages. n=3

Figure 5: iPSC pluripotency markers RTqPCR
Classic pluripotency-related gene expression was assessed using RTqPCR.

Two-way ANOVA resulted in various significance between expression levels (* p < 0,01, **** p < 0,0001, n=3)



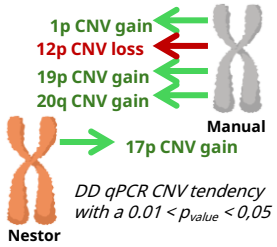


Figure 6: Genomic stability evaluation by dd qPCR
Genomic stability was assessed by detection of recurrent genetic abnormalities using the iCS-digital™ PSC test, provided as a service by Stem Genomics (https://www.stemgenomics.com/), as described previously[2]."
Stemgenomics' technology allows for the detection of Copy Number Variations (CNVs) that is, DNA segments of one kilobase (kb) or larger that are present at an abnormal copy number compared to a reference genome. Normal copy number should be equal or close to the value of 2 at all the 28 recurrently analyzed regions.

Discussion

After four passages comparing culture techniques, we are confident that Nestor succeeded in culturing induced pluripotent stem cells.
In terms of yield and morphology, Nestor did not significantly impact cells (fig 2). In terms of functionality, Nestor did not induce any loss of pluripotency potential compared to manual culture. Both culture conditions showed similar SSEA 4 / TRA1 81 expression (fig 4) levels after four passages. Surprisingly, while pluripotency expression and cell yield remained unchanged, the genetic profile differed between manually and Nestor-cultured cells. Indeed, cells whose environment was less disturbed during medium changes (Nestor) displayed increased expression of transcription factors related to lineage plasticity3 (fig 5) compared to manually handled populations.

Furthermore, regarding stability, it is important to note that Nestor performed slightly better than manual culture.According to Stemgenomics' ddqPCR report, automation led to fewer genomic aberrations in key stemness-associated genetic regions (fig 6), likely due to reduced handling stress compared to manually cultured iPSC, which are known to suffer from hypoxia and temperature variations.

We may soon strengthen these observations with multiple cell lines to underpin Nestor's benefits across line-to-line cell populations.

References

1 – Natunen S et al. The binding specificity of the marker antibodies Tra-1-60 and Tra-1-81 reveals a novel pluripotency-associated type 1 lactosamine epitope. Glycobiology. 2011 Sep
2 – Assou S et al. Recurrent Genetic Abnormalities in Human Pluripotent Stem Cells: Definition and Routine Detection in Culture Supernatant by Targeted Droplet Digital PCR. Stem Cell Reports. 2020 Jan
3 - Rizzino A. Concise review: The Sox2-Oct4 connection: critical players in a much larger interdependent network integrated at multiple levels. Stem Cells. 2013 Jun

nestor
Less cell work, more cell Science