Pluripro® is a Fully Defined System for Confluent Human Pluripotent Cell Culture in the Absence of bFGF

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Abstract: The Pluripro® system**,** a fully defined medium partnered with fully defined matrix, enables enzymatic, single cell passaging of human pluripotent stem cells (hPSCs). The Pluripro® system provides consistency of hPSC phenotype both within and between passages. Following extended passage in Pluripro®, hPSCs retain an undifferentiated phenotype, as evidenced by expression of markers for the undifferentiated state, and capacity for in-vitro differentiation into the three germ lineages.

Pluripro® defined feeder free culture system
Cell populations form confluent monolayers that are universally positive for pluripotency markers and negative for differentiation markers (Figure 1). The pluripotent culture is established as single cells (figire 2) and differentiates readily on withdrawal from the culture system either through spontaneous or directed differentiation to tissues representative of the three germ layers (Figures 3 and 4).

When passaging is performed enzymatically as single cells, rapid, highly efficient expansion (Fig 4) from 2x10^6 cell frozen vials to bulk quantities suitable for experimental, manufacturing, or high throughput screening applications, is enabled.

Formulation: Pluripro® Medium is supplied as a complete formulation containing BSA, amino acids, glutamine, lipids, cytokines, and trace elements optimised for hPSC cell culture and manufactured and tested under strict quality control criteria to ensure batch to batch uniformity. *For IP reasons, some modifications have recently been made to Pluripro®. The figures shown here are being updated.

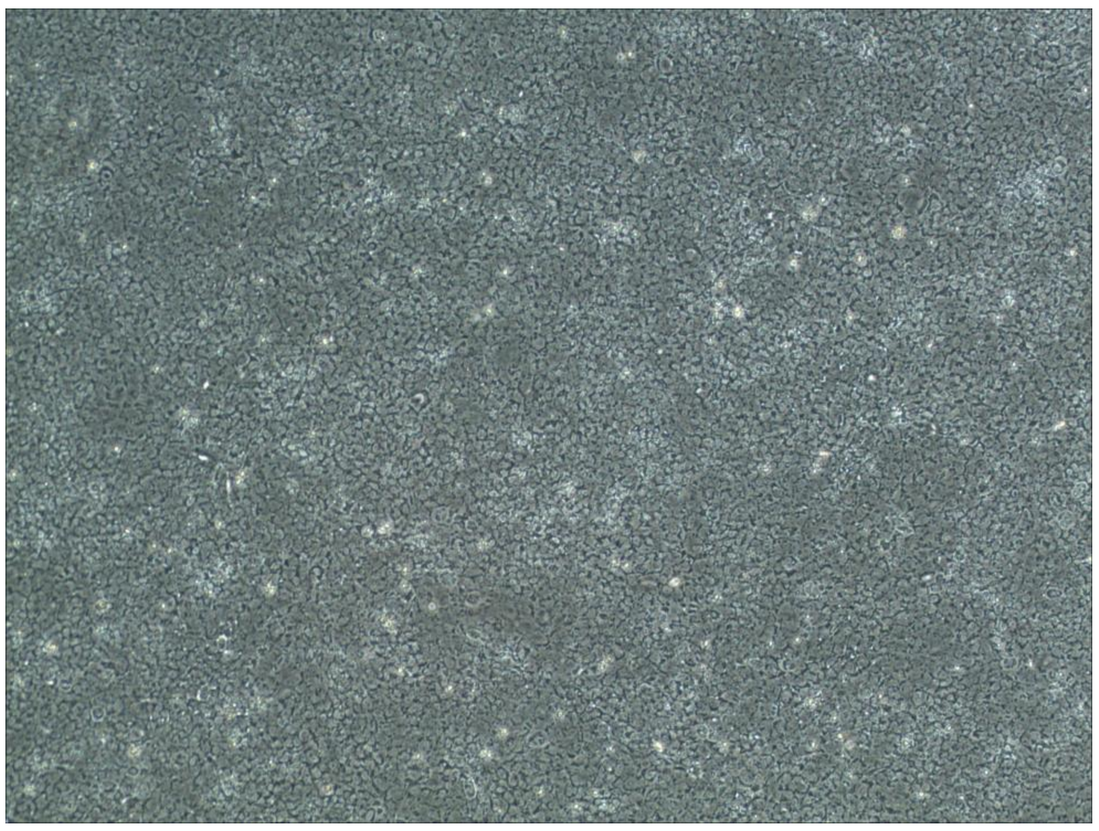

Figure 1: Confluent NCL5 P15 culture. immunofluorescence for OCT4 (A), NANOG (B), and SOX2 (C) and SSEA1 (D) in NCL5 at P13 in standard Pluripro® culture.

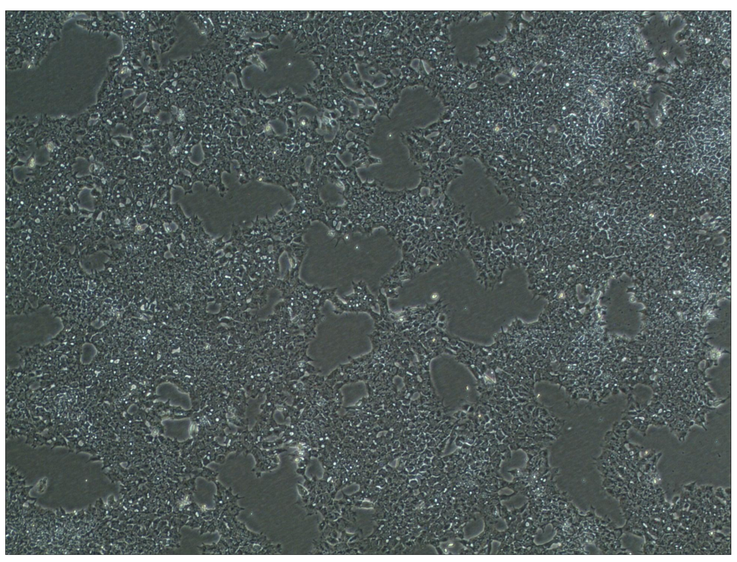

Figure 2: Cells cultured in Pluripro® medium shown 1 day post seeding (NOTT2 Passage 3).

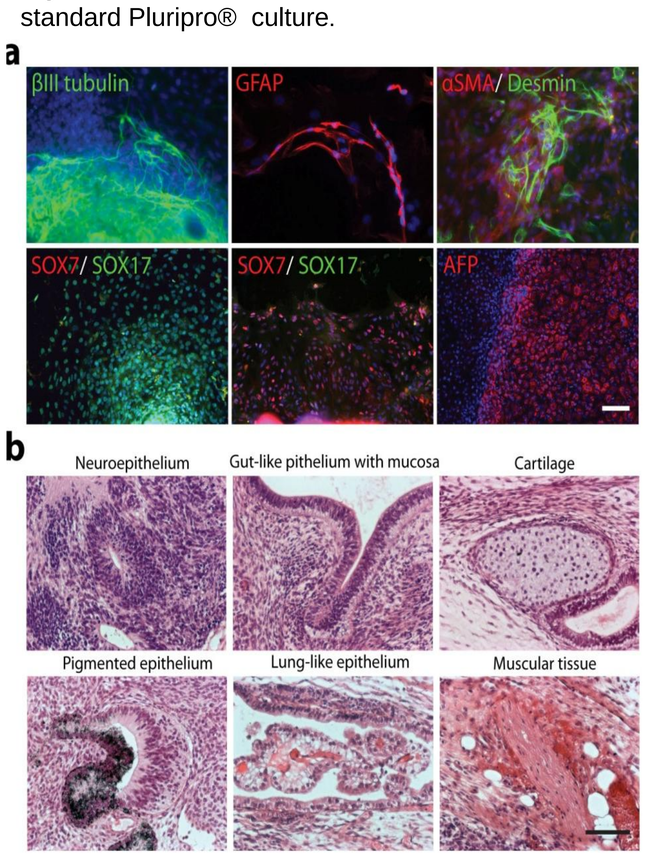

Figure 3: Teratoma formation (above) demonstrating immunohistochemistry for the 3 germ layers.

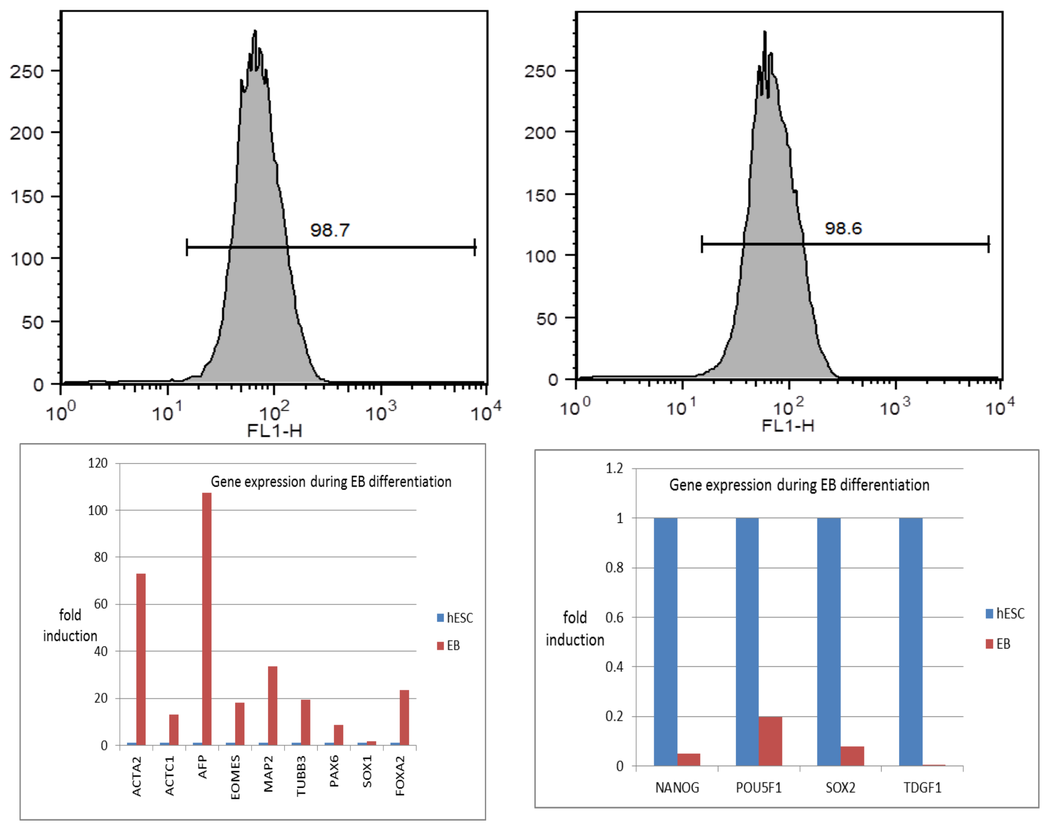

Figure 4: Top Cells cultured in Pluripro® in feeder free format. Oct4 flow cytometry for NOTT2 P13 and NCL5 P13. Bottom: Gene expression patterns after embryoid body based differentiation for 3 weeks in 10% FBS following culture in Pluripro® medium (NOTT2 hESC line)

Results: Cell lines cultured using Pluripro® medium in the absence of bFGF include NOTT2 and NCL5. Pluripotency markers were assayed by immunocytochemistry (Fig 1). Cells cultured in Pluripro® and seeded as single cells show characteristic morphology (figure 2). Teratoma formation in Pluripro® further demonstrates pluripotency (figure 3). OCT4 expression in NOTT2 and NCL5 has been confirmed at P13 by flow cytometry (Figure 4). Embryoid body formation using NOTT2 and NCL5 shows induction of differentiation related genes by qPCR and accompanying down-regulation of pluripotency related genes (Figure 4).

Summary: The Pluripro® culture system promotes efficient propagation of hPSCs, demonstrating excellent growth potential, and allowing rapid expansion within 5-6 passages. Cultures are uniformly positive for markers of pluripotency and, importantly, demonstrate efficient induction of differentiation associated genes (figures 4 and 5) following random *in-vitro* differentiation via embryoid body formation. These results indicate that the Pluripro® system is an ideal choice for maintaining and expanding hPSCs cells.