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Handling Tips

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How to Transfer hiPSCs from a Feeder-Dependent to a Synthetic Culture System on Eppendorf CCCadvanced® FN1 Motifs Surface

The transition of pluripotent stem cells (PSCs) from a traditional feeder-dependent to a completely synthetic culture system requires the change of both the growth surface and culture medium. The FN1 motifs surface is suitable to support such a change to an animal-component-free culture system. Due to sensitivity of certain PSC lines, a transition in two steps is highly recommended to reduce the cellular stress during the transition process (Figure 1). During step 1, PSCs are transferred to FN1 motifs using a highly nutritive feeder-free growth medium to allow PSC adaptation on the new surface within up to 5 passages. During step 2, the supportive medium can be substituted with a synthetic medium of choice to establish a completely defined PSC culture system.

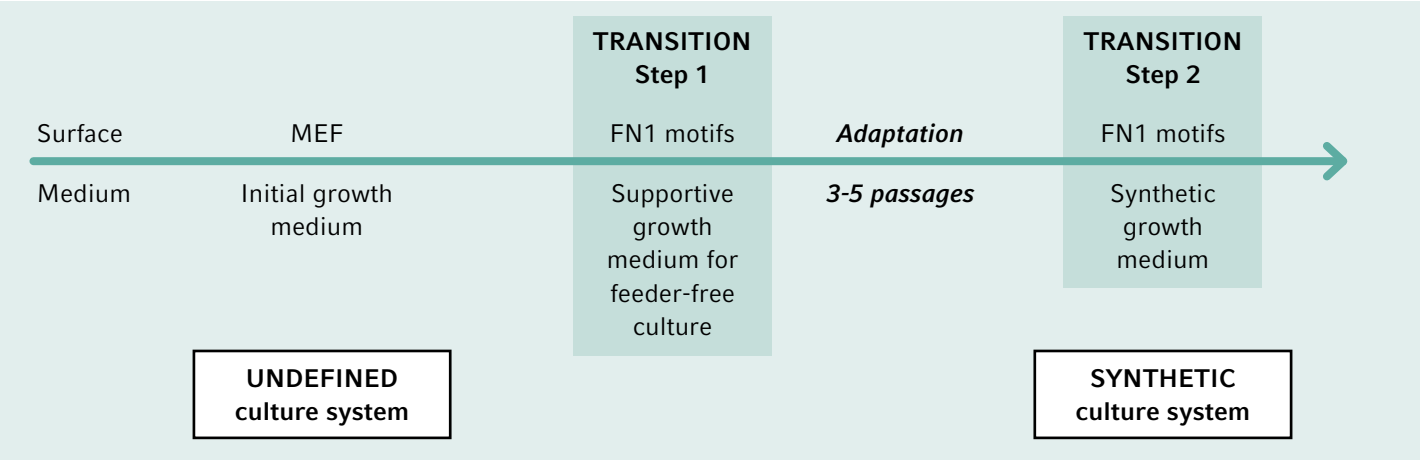


Figure 1: Two-step transition from feeder-dependent to a completely synthetic culture system on FN1 motifs surface.

Tips for successful transition step 1:

> Use a stable cell population of high quality, without spontaneous differentiation. Ideally, colonies are not too large, nor in contact with each other.

> If using a cryopreserved cell stock, only initiate a surface transition after full recovery from thawing in the original culture system.

> Choose a higher initial cell density than usually used during your routine culture (split ratio of 1:1 to 1:4). Decrease it progressively until complete cell adaptation on FN1 is reached (Figure 2).

> Using ROCKi for 24h post-seeding during this transition step can increase cell survival.

> Initiate the first passage on FN1 motifs latest 5-6 days post-seeding, even if colonies are not abundant to avoid overgrow in colony centers.

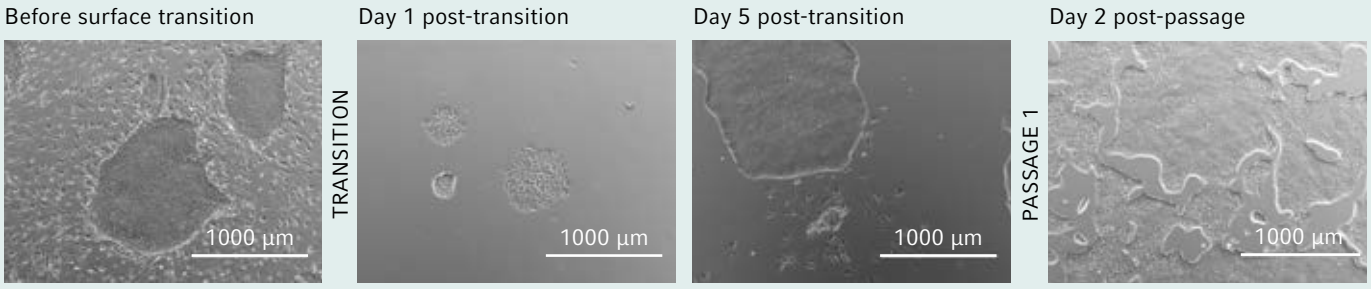
> Maintain your initial cell detachment technique for the surface transition on FN1 motifs. Once the culture is stable on FN1 motifs surface, use Versene®, EDTA or Gentle solutions for further passages.

> Some feeder cells could be carried over during surface transition. These mitotically inactivated cells will progressively disappear after the first passages on FN1 motifs.

> Perform a daily feeding and microscopical check of your cells. First days post-transition could be associated with more cell debris and slower cell growth.

> If spontaneous differentiation is observed, remove differentiated areas manually before passaging.

> Avoid changing any other culture conditions during this phase. Let cells adapt to the new surface before initiating another culture condition change. Consider 3-5 passages for full cell adaptation.



Transition of hiPSC from feeder layer-dependent culture to FN1 motifs surface

> **Initial culture surface:** Feeder layer of Mouse (ICR) Inactivated Embryonic Fibroblasts seeded 24h prior use (at 600.000 cells per well) on Eppendorf 6-well plates (TC treated)
> **Initial culture medium:** D-MEM/F-12 supplemented with 20 % KSR, MEM Non essential AA, bFGF (4 ng/ml final), b-mercaptoethanol
> **Initial cell detachment technique:** mechanical detachment
> **Split ratio for transition:** from 1:1 to 1:4 depending on the density of the initial culture on MEF
> **Culture medium for transition:** Gibco® StemFlex™ + RevitaCell™ supplementation for 24h post-seeding with daily medium refreshment
> **Split ratio for the first passage:** from 1:6 to 1:8 depending the growth rate of cells
> **Cell detachment solution post-transition:** Clump passaging using Gibco® Versene

Figure 2: hiPSC morphology following surface transition from feeder layer to FN1 motifs surface

Tips for successful transition step 2:

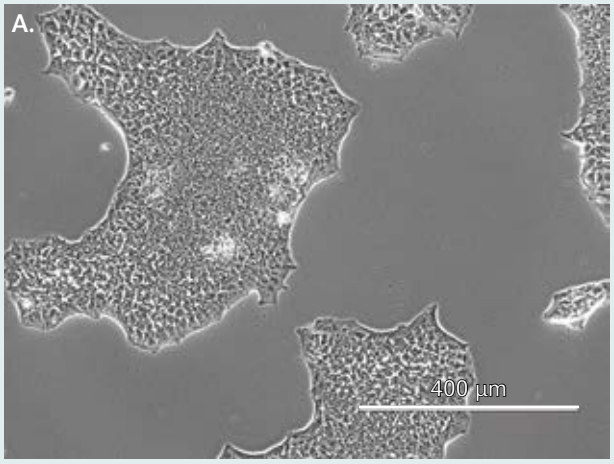
> In order to achieve a completely synthetic culture system, the highly nutritive feeder-free growth medium can be replaced by a synthetic medium of your choice several passages after surface transition (exact time point depends on cell recovery).

> Progressively substitute the former with new medium (Figure 3). Timeline as well as substitution can be adapted according to the sensitivity of cells.

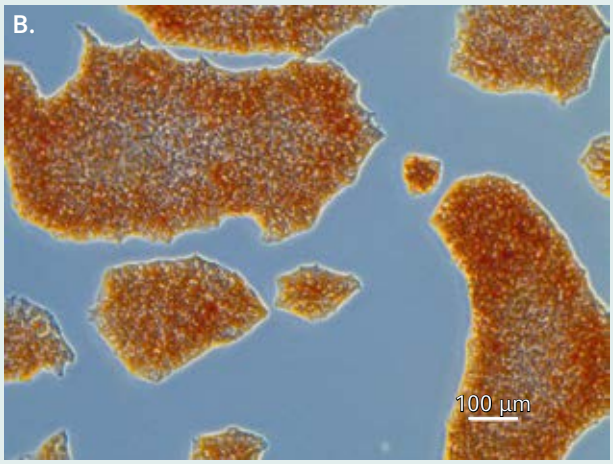
> Cells recover progressively during culture, achieve a stable proliferation rate and maintain a typical cell morphology and pluripotency status (Figure 4).

Timeline	Medium A	Medium B
START	100%	0%
d1	50%	50%
d2	50%	50%
d3	0%	100%

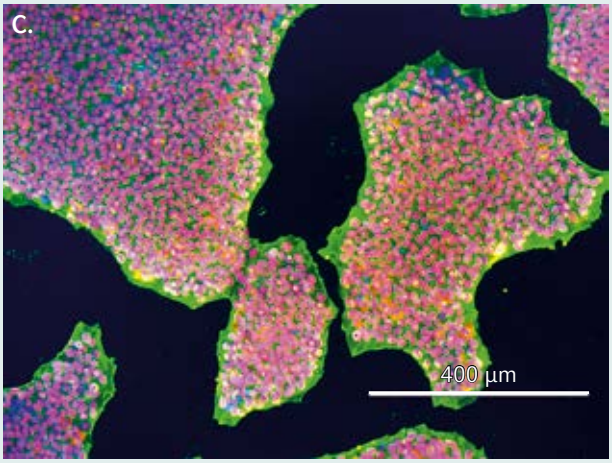
Figure 3: Progressive medium substitution.



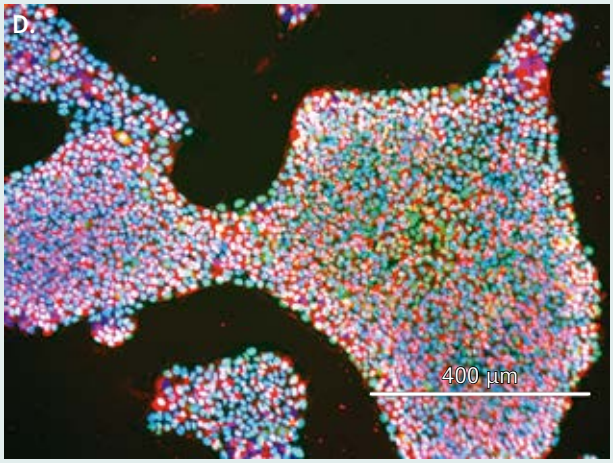
A. Cell morphology



B. Alkaline phosphatase staining



OCT4 – SSEA4 – DAPI



TRA-1-60 – SOX2 – DAPI

C./D. Key marker expression through immunostaining

Figure 4: Maintenance of typical hiPSC morphology and key pluripotency marker expression 5 passages after transition from a traditional feeder-dependent to a completely synthetic culture system based on FN1 motifs.

Interested in more tips for successful long-term expansion on FN1 motifs?
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