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Reference pictures of hPSC cultured in defined conditions

 In 1 collection

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We use this protocol and it's working

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Abstract

Collection of reference images of hPSCs in different culture conditions and at different timepoints.

Example images of undifferentiated hPSCs in different culture conditions

- 1 Timepoints, matrix, and media used are labeled or indicated in Step description.

The **standard morphology of undifferentiated hPSC colonies** is distinctive and serves as an important criterion for assessing the pluripotent state of the cells. Here is a detailed description:

Shape and Edges:

- Colonies are typically **round or oval** with **well-defined, smooth edges**.
- Edges should be **tight and compact**, without cells protruding or migrating outward (which suggests differentiation or unhealthy colonies).

Cell Density and Arrangement:

- Colonies appear **densely packed**, with little or no visible space between cells.
- The central area of the colony is more **densely populated**, with cells tightly connected via intercellular junctions.

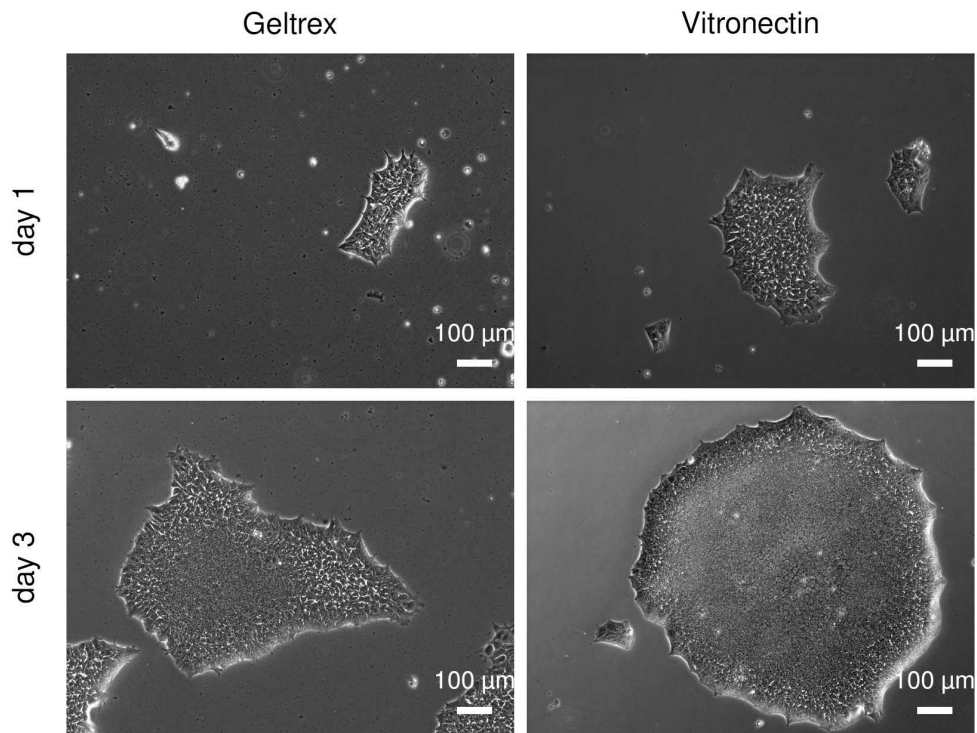
Cell Appearance:

- Cells within the colony are generally **small**, with a **high nucleus-to-cytoplasm ratio**.
- Nuclei are **large, round**, and **centrally located**, often with **prominent nucleoli** visible under phase-contrast or bright-field microscopy.
- The cytoplasm is **scant and difficult to distinguish**.

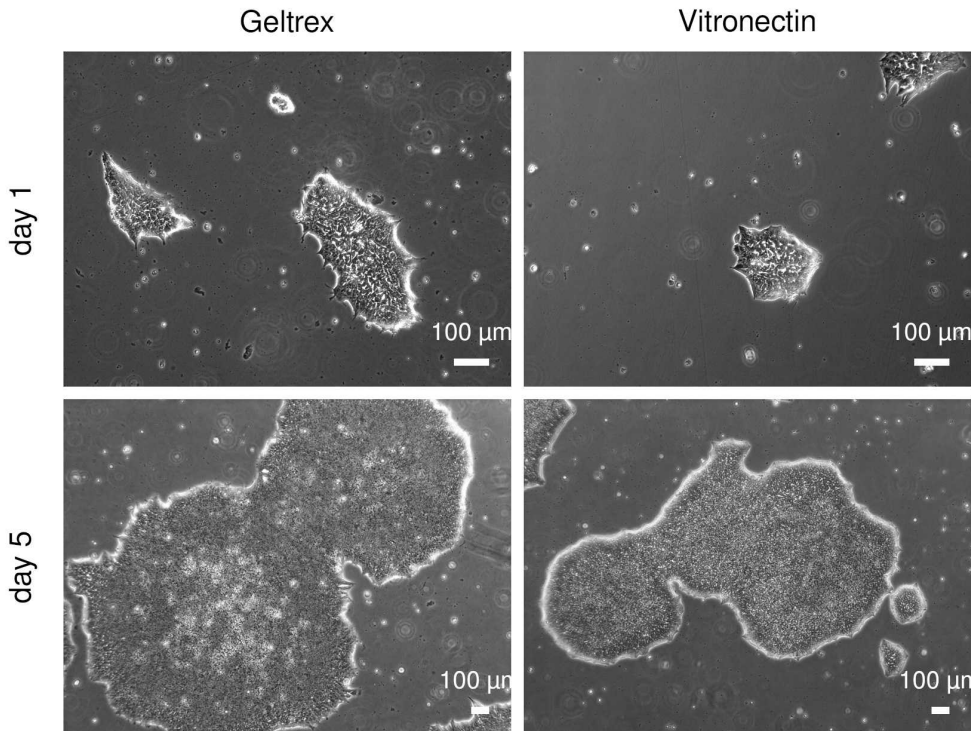
Color and Texture (in phase contrast):

- Colonies have a **refractive (bright) edge** under phase contrast, indicating tight cell-cell contact.
- The center may appear **slightly raised or dome-shaped** due to the dense packing of cells.

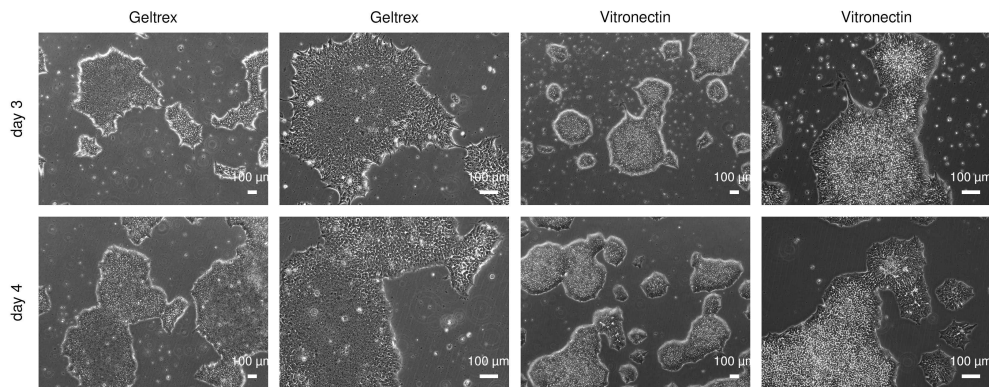
- 1.1 Morphology comparison for hiPSC cells cultured on either Geltrex or Vitronectin coated plates.



hiPSC line BIHi005-A cultured in E8 media



hiPSC line BIHi250-A cultured in E8 media

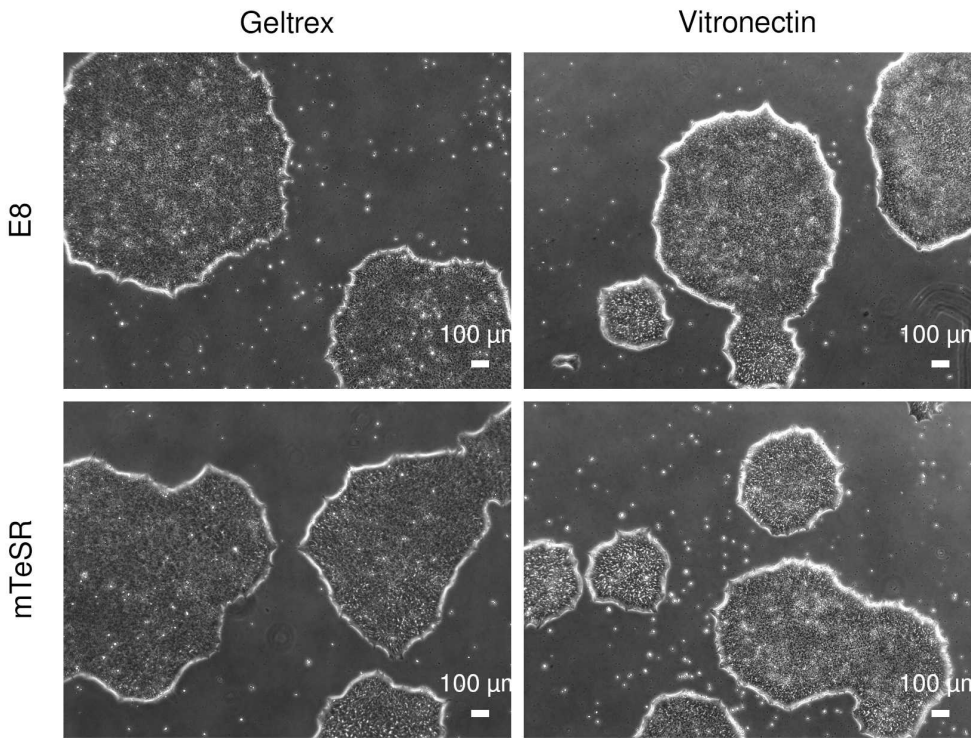


hiPSC line BIHi001-A cultured in E8 media.

Note

In addition to hPSC line intrinsic differences in growth kinetics, the difference in matrix used can also contribute to difference in cell proliferation rates.

Colony morphology differs between Geltrex and Vitronectin; notice more compacted colony appearance and sharper edges for cells cultured on Vitronectin.



hiPSC line BU3NG-G5 (day 4 in culture)

Note

The effect on the colony appearance is persistent in different media (Figure above E8 vs mTeSR)

- 1.2 Additional examples of healthy hPSC colony morphology.
- 1.3 Additional examples of healthy hiPSC colony morphology accquired with Oblique Coherent Contrast (OCC) Illumination (Stereo microscope).



Example colony images from Stereomicroscope

2 Confluency assessment

Evaluating **confluency** of hPSC cultures is essential for monitoring growth, timing passaging, and ensuring reproducibility in downstream applications.

Confluency refers to the percentage of the surface area of a culture vessel (e.g., dish or flask) covered by adherent cells. For hPSCs, this reflects the extent to which colonies occupy the culture surface.

How to Evaluate Confluency:

■ Visual Estimation

Confluency is estimated by looking at the culture under a microscope and judging what percentage of the surface is covered by cells. This method is quick and requires no equipment beyond the microscope, but it is subjective and depends on the observer's experience.

■ Image Analysis (Software-Based)

A more objective approach involves taking images of the culture and analyzing them using a software. This process typically includes converting the image to grayscale, applying a threshold to separate cells from the background, and calculating the area percentage covered by cells. Tools like ImageJ, CellProfiler, or custom Python scripts are well suited for this method and offer more consistent, reproducible results. This method is time consuming and requires image analysis expertise.

■ Live-Cell Imaging Systems

Automated systems such as IncuCyte or BioStation can track confluency in real time by capturing images over time inside an incubator and estimating confluency using built-in image analysis pipelines. These systems are ideal for long-term studies or high-throughput applications because they reduce manual work and provide

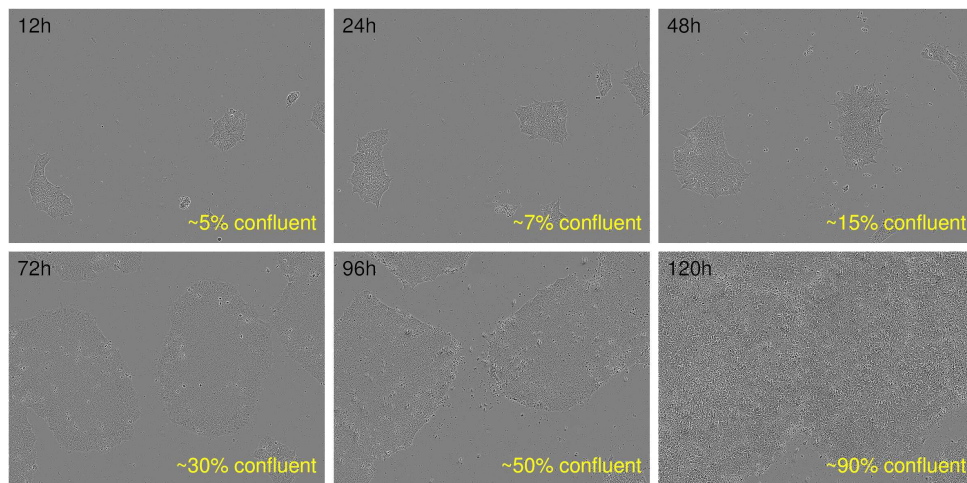
continuous, standardized data on culture growth. This method requires specialized equipment.

2.1 Example images of healthy hiPSC colonies acquired with Oblique Coherent Contrast (OCC) Illumination reflecting various confluency levels.



hiPSC line BIHi250-A cultured in E8 media on Geltrex coated plates. Images display confluency estimation.

2.2 Example images of healthy hiPSC colonies acquired with automated imaging system IncuCyte reflecting various confluency levels over time.



hiPSC line BIHi001-B-3 cultured in E8 media on Geltrex coated plates. Images display confluency estimation over time.

Example images of undifferentiated hPSCs cultured with survival factors

- 3 Timepoints, matrix, and media used are labeled or indicated in Step description.

When **hPSC** are cultured in media supplemented with a **survival factors**, their appearance undergoes notable changes, especially during the early phases after passaging. These are used to enhance cell survival—especially **after enzymatic dissociation into single cells**—and reduce apoptosis.

Immediate Post-Passage (0–24 hours)

Cell Shape:

- Cells tend to be **rounder and more spherical** compared to their flattened counterparts in non-ROCK conditions.
- These cells are **highly refractile** and may look **less adherent** initially.
- Cytoplasmic processes or spreading are minimal during this phase.

24–48 Hours Post-Passage

Colony Initiation:

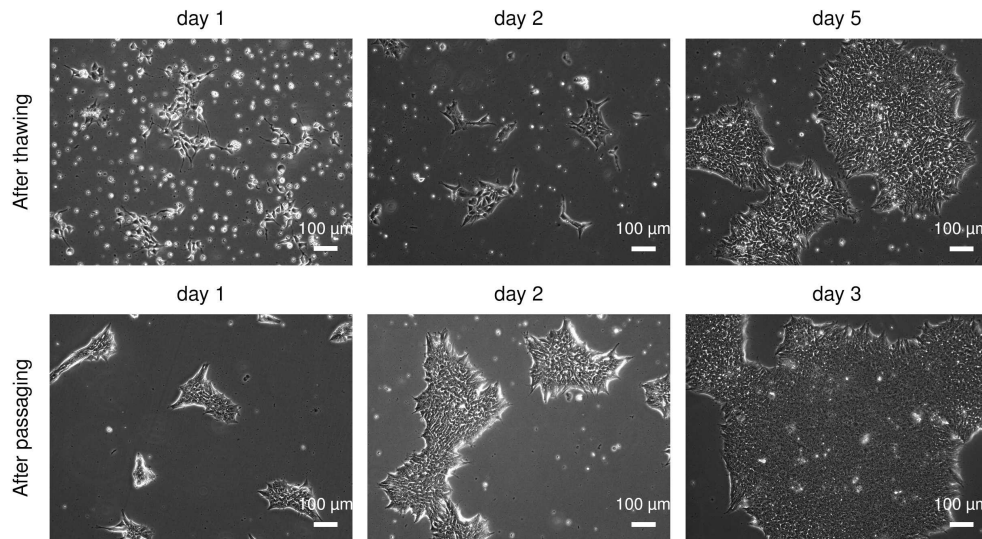
- Cells begin forming **tight, cohesive colonies**, resembling **compact epithelial-like clusters**.
- The cells become more **flattened** and start displaying **typical hPSC morphology** as they re-establish contacts.
- The classical appearance of hPSCs with **large, prominent nuclei** and scant cytoplasm re-emerges.

- Nuclei remain round and centralized, with visible nucleoli under high magnification.

Longer-Term Cultures (48+ hours)

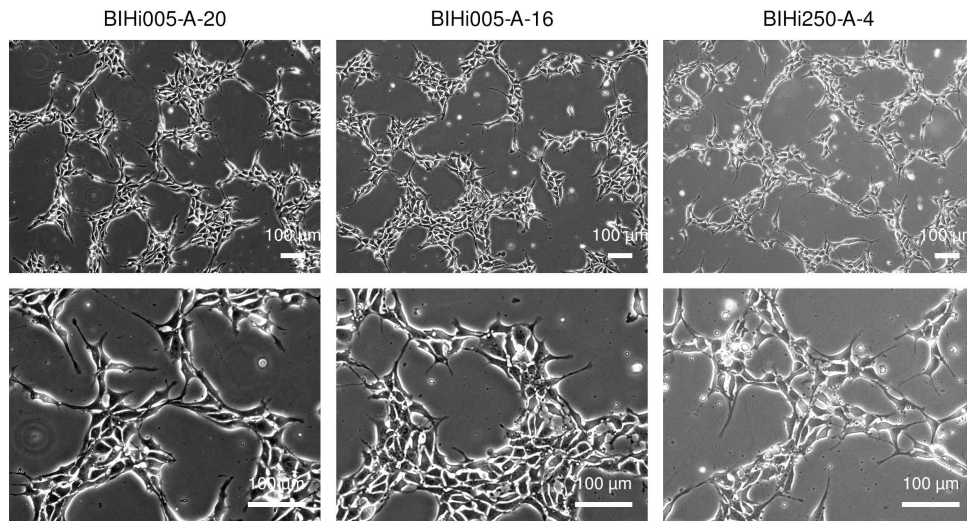
- Colonies regain **typical undifferentiated morphology**, as described in your previous section.
- Survival factors are typically removed after 24–48 hours.

- 3.1 Example images of undifferentiated hiPSC line after thawing and after first passage. Note the single cell morphology (due to **ROCK inhibitor**) and presence of floating cells in the culture 1 day after thawing. Cells show expected colony formation after 5 days in culture.



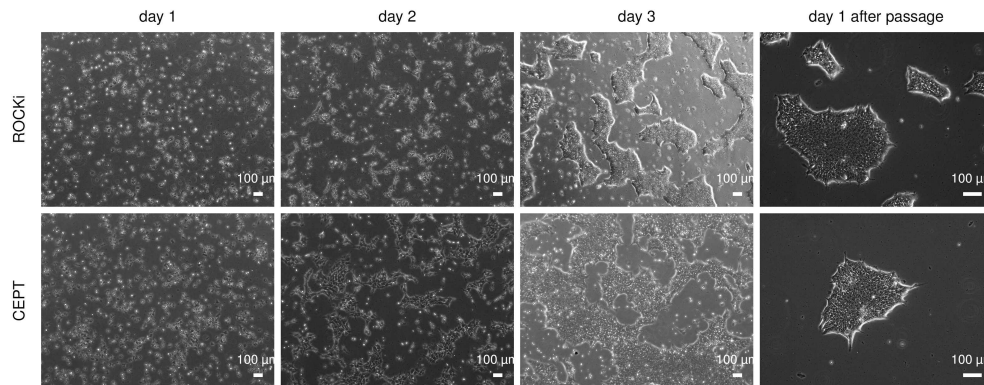
hiPSC line 250-A-4 cultured in E8 media on Geltrex. ROCK inhibitor added for the first 24h after thawing.

- 3.2 Example images of undifferentiated hiPSC lines one day after passaging with TrypLE Select and plated on Geltrex. Note the single cell morphology due to presence of **ROCK inhibitor**. This morphology disappears after media change to media without ROCK inhibitor. See examples in the next step.

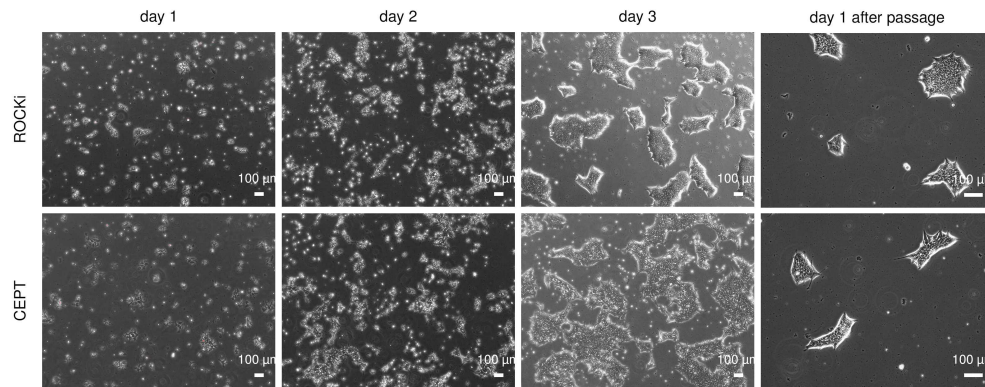


Indicated hiPSC lines 24h after plating on Geltrex in E8 supplemented with ROCK inhibitor.

- 3.3 Example images of morphology changes after single cell passaging of hPSCs, plating in media supplemented with indicated survival factors (**ROCK inhibitor** or **CEPT**), which were subsequently removed after 24h.



hiPSC line BCRTi005-A passaged with TrypLE and plated on Geltrex in E8 media supplemented with ROCK inhibitor (ROCKi) or CEPT; survival factors were withdrawn after 24h.

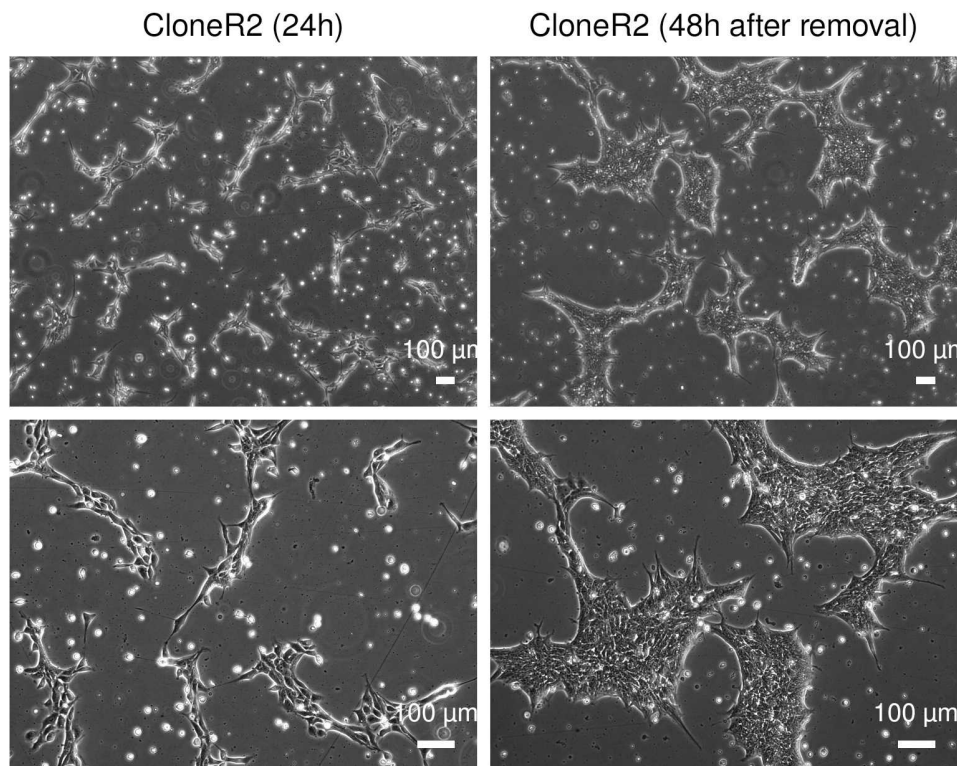


hiPSC line BHI004-B passaged with TrypLE and plated on Geltrex in E8 media supplemented with ROCK inhibitor (ROCKi) or CEPT; survival factors were withdrawn after 24h.

Note

hPSCs exposed to **ROCK inhibitor** and **CEPT** exhibit similar survival and morphological characteristics; however, cells cultured in CEPT display a delayed onset in colony reformation following withdrawal of the compound compared to those treated with ROCK inhibitor.

- 3.4 Example images of morphology changes after single cell passaging of hPSCs and plating in media supplemented with **CloneR2**.



hiPSC line BIHi292-A passaged with TrypLE and plated on Geltrex in StemFlex media supplemented with Cloner2. Survival factor Cloner2 was withdrawn after 24h.

Example images of spontaneous differentiation of hPSCs

4 Description of spontaneous differentiation of hPSCs.

Spontaneous differentiation of pluripotent stem cells is a common phenomenon that can occur in standard culture conditions, especially when colonies are overgrown, improperly maintained, or exposed to suboptimal media or environmental stress. Morphological cues are often the earliest indicators of this unwanted differentiation. Below is a detailed description of the **characteristics and patterns** of spontaneous differentiation in hPSC cultures:

Irregular, Flattened Cells:

- Differentiating cells at the colony periphery often become **flattened, spread out**, and **less refractile**.
- These cells lose the tight packing and cobblestone-like morphology typical of undifferentiated iPSCs.
- There is a visible **loss of junctional integrity**, leading to **gaps between cells** or **protrusions** extending from the colony edge.

- A mixed population of cells may emerge, with some appearing **epithelial-like**, while others may exhibit **mesenchymal traits**, depending on the differentiation trajectory.

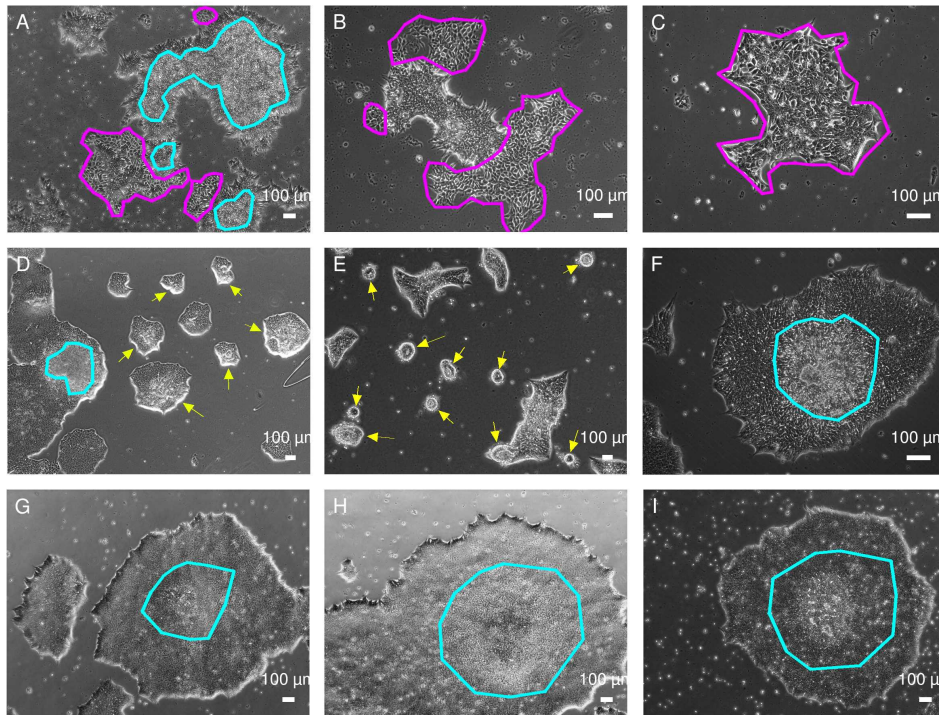
Raised, Dome-Like Areas:

- Overgrown colonies often develop **elevated central mounds or domes**, which may be indicative of spontaneous **differentiation initiation**.
- These dome regions may contain a mix of **larger cells with abundant cytoplasm** and cells forming **rosette-like** or **pseudo-epithelial structures**, suggesting early lineage commitment.
- The architecture within domes often shows **loss of planar polarity**, with cells oriented in various directions.

Color and Texture Variations:

- Differentiating regions appear **less shiny and more opaque** under phase-contrast microscopy.
- Colonies lose their uniform refractive edge and develop a **"ruffled" or scalloped appearance**.
- When subcultured, differentiated cells often fail to re-establish tight colonies and instead grow as **dispersed monolayers**.

4.1 Examples of spontaneous differentiation of hPSCs.



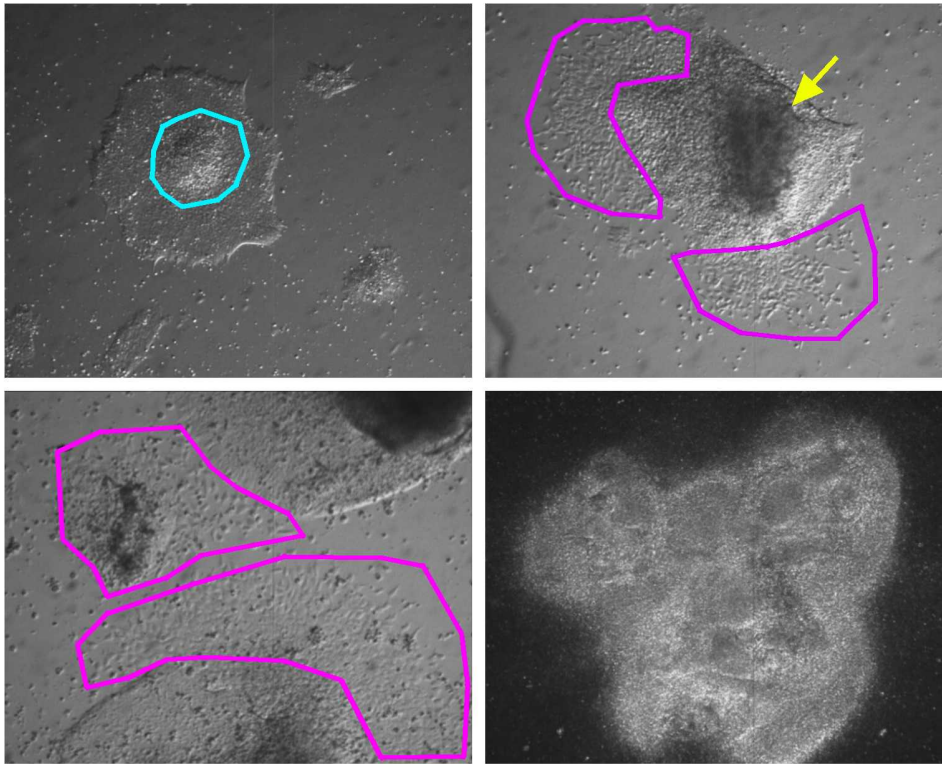
Examples of spontaneous differentiation of hiPSCs.

Teal polygons mark raised dome like areas (Panels: A, D, F, G, H, I).

Magenta polygons mark differentiating cells at the colony periphery (Panels: A, B, C).

Yellow arrows mark colonies with loss of proper shape and junctional integrity (Panels D and E).

4.2 Additional examples of hiPSC differentiation acquired with Oblique Coherent Contrast (OCC) Illumination.



Examples of spontaneous differentiation of hPSCs captured with Stereomicroscope.
 Teal polygons mark raised dome like areas.
 Magenta polygons mark differentiating cells at the colony periphery.
 Yellow arrows mark colonies with loss of proper shape and junctional integrity.