BioLamina

Efficient gene editing

On Biolaminin® 521 substrate

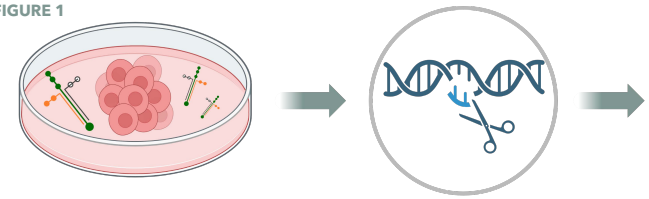
Single cell expansion of human pluripotent stem cells on Biolaminin 521

Biolaminin 521 is a chemically defined and animal origin-free system for robust and long-term expansion of human pluripotent stem cells (hPSCs) that provides a highly reliable and reproducible culture system. It increases the single cell survival and cloning efficiency, compared to other extracellular matrices (ECMs). Additionally, the purity and adhesive properties of Biolaminin 521 proves advantageous for imaging purposes on glass. Biolaminin 521 can coat a variety of surfaces including plastic, glass and metal, at every scale from microtiter plates to microcarriers. The protocol for derivation of hPSCs is reproducible, highly quality-controlled, and applicable for cell therapy applications.

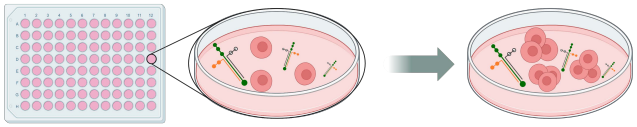
Biologically relevant culture environment with Biolaminin 521

Laminin 521 is a key ECM protein of the natural stem cell niche, expressed already at the inner cell mass of the preimplanted embryo.
Laminin 521 has a strong interaction with the α6β1 integrin, a key mediator of signaling pathways that regulates adhesion, proliferation, migration, and promotes their survival and efficient long-term self-renewal.

FIGURE 1



Recreation of the cellular microenvironment reduces the stress for cells *in vitro*.



Applicable for automation platforms

Increased single cell survival

Improved single cell cloning and transduction efficiency

Biolaminin 521, a biorelevant substrate for gene editing, incl. CRISPR/Cas9

Features and specifications:

- Supporting genome editing technologies, like CRISPR/Cas9
- Increased single cell survival, without ROCK inhibitor
- Improved single cell cloning and high transduction efficiency
- Long-term silencing efficiency
- Advantageous in automated imaging systems
- Biologically relevant cell culture environment
- Defined and xeno-free culture substrate

Direct link to Biolaminin products

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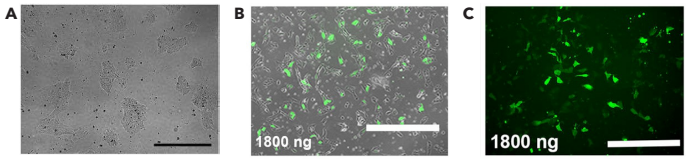
Efficient gene editing

On Biolaminin 521 substrate

Biolaminin 521 improves gene editing technology

FIGURE 2

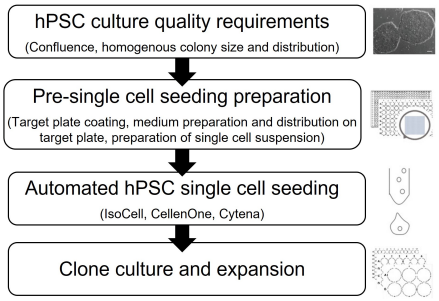
Single hPS cell survival and different transfection methods supported on Biolaminin 521 for xeno-free CRISPR-based genome editing



Biolaminin 521 allows clonal survival of hPSCs without apoptosis inhibitors, e.g. ROCK inhibitor as well as the use of different transfection methods.
A) Example image of hESC passaged as single cell suspension 48h after plating on Biolaminin 521. Scale bar 500 µm[1]. B,C) CRISPR-based genome editing of hiPSCs cultured under xeno-free conditions. Observed reporter expression after 24 hours. Both B) electroporation and C) Lipofectamine were efficient for transfection on Biolaminin 521 [5].

FIGURE 3

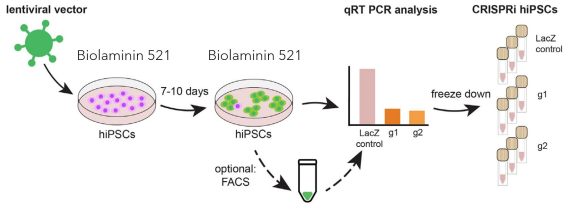
In automated systems, Biolaminin 521 increases single cell cloning efficiency and imaging quality



Vallone et al. 2020 described the use of three different automated cell isolation and dispensing devices to enhance the single-cell cloning process for hPSCs, after CRISPR/Cas9-mediated genome editing. BioLaminin 521 was shown to increases single cell cloning efficiency compared to commonly used ECMs such as Matrigel or Geltrex. In automated workflows the purity of Laminin 521 is an advantage for imaging purposes to facilitate high-throughput hPSC clonal selection and expansion [2].

FIGURE 4

Up to 95 % CRISPRi-mediated transduction efficiency and long-term silencing efficiency on Biolaminin 521

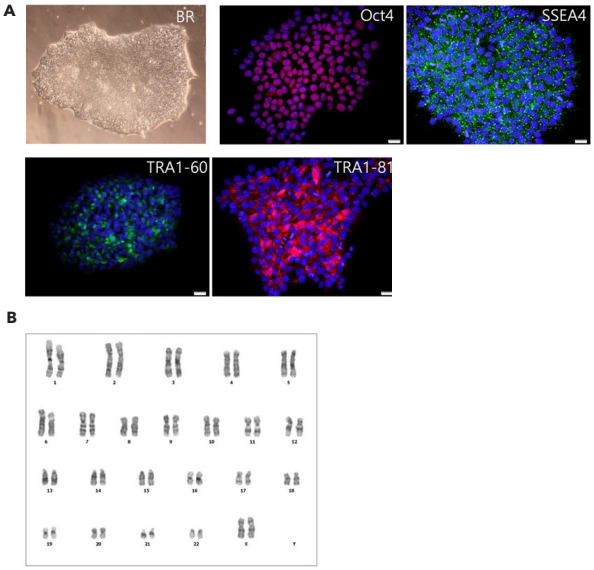


Johansson et al. 2022 demonstrated that Biolaminin 521 provides very high transduction (60-95%) and silencing efficiency for hiPSCs and fibroblasts. CRISPRi silencing efficiency in iPSC was tested using qRT-PCR for all three RNA guides. Edited cells were differentiated on Biolaminin 111 and stable transcriptional silencing was achieved in 4-month-old cerebral organoids. Functional studies in brain developmental research can utilize this CRISPRi-mediated transcriptional silencing in human iPSCs [3].

Biolaminin 521 as optimal substrate to generate CRISPRi-edited cell lines

FIGURE 5

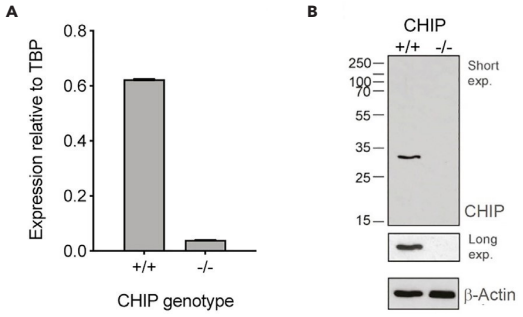
Generation of a knockout human embryonic stem cell line with CRISPR/Cas9 on Biolaminin 521



Kim et al. 2017 used CRISPR/Cas9 genome editing to generate a Nrf2 homozygous knockout human ESC line (Nrf2 -/-) [6].
A) Biolaminin 521 effectively maintained pluripotency as shown with the markers Oct4, SSEA4, TRA1-60 and TRA1-81 as well as B) a normal karyotype.

FIGURE 6

Generation of isogenic iPSCs on Biolaminin 521 for cortical neuron model



Dias et al. 2022 presented a protocol using a patient-derived iPSC line to produce gene-edited cells isogenic for the neuroprotective E3-ubiquitin ligase CHIP [4].
A) Biolaminin 521 supported high knockout efficiency of CRISPR/Cas9-edited CHIP KO clones, validated by PCR and B) western blot to obtain CHIP KO lines. Additionally, Biolaminin 521 improved single cell survival and pluripotency during the development of PSCs isogenic for CHIP. Further, neurodegenerative diseases models were efficiently created by differentiating iPSC lines into cortical neurons on Biolaminin 111.

REFERENCES

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[4] Dias et al. STAR Protocols, 2022. Generation of a CHIP isogenic human iPSC-derived cortical neuron model for functional proteomics.

[5] Giacalone et al. Curr Protoc Stem Cell Biol, 2018. CRISPR-Cas9 Based Genome Editing of Human Induced Pluripotent Stem Cells

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[7] Carlson-Stevermer et al. Stem Cell Research, 2016 High-Content Analysis of CRISPR-Cas9 Gene-Edited Human Embryonic Stem Cells.

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