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# Poster P12

## The impact of Schwann cell differentiation on transgene expression in human stem cells.

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Human stem cells, including induced pluripotent stem cells (iPSCs) and dental pulp-derived stem cells (DPSCs), are powerful tools for disease modeling and regenerative medicine. Their ability to differentiate into specialized cell types makes them ideal platforms for studying cellular behavior under both physiological and pathological conditions. To facilitate such studies, genetic modification with reporter genes such as green fluorescent protein (GFP) is commonly used for cell tracking and visualization. However, the stability of transgene expression during differentiation remains an important consideration.

In this study, we examined the effects of Schwann cell (SC) differentiation on transgene expression following either CRISPR/Cas9 gene editing or lentiviral transduction. iPSCs were genetically modified using CRISPR/Cas9 to create a GFP-positive master line with a hygromycin resistance cassette under the constitutive CAG promoter, enabling FLPe-mediated cassette exchange. Separately, DPSCs were transduced with a lentiviral vector encoding GFP, firefly luciferase (fLUC), and a puromycin resistance cassette, driven by the EF1alpha promoter.

Both iPSCs and DPSCs were differentiated into SCs. Differentiation was confirmed by the upregulation of SC markers, including neurotrophin receptor P75 (P75 NTR), laminin-211, and laminin-411. GFP expression was evaluated before and after differentiation using fluorescence microscopy, qPCR, and western blotting, while luciferase activity was assessed using fLUC assays.

Our results show that differentiation into SCs significantly reduces transgene expression in both iPSCs and DPSCs. These findings highlight the need for protocol optimization to maintain or enhance reporter gene expression following differentiation. Despite this reduction, modified cells remain useful for research involving the peripheral nervous system requiring SC visualization, including co-culture systems and transplantation models.