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Efficient method for generating reporter based human hematopoietic stem cells for high throughput screening platform

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Pluripotent stem cells (PSCs) represent an alternative hematopoietic stem cell (HSC) source or treating hematopoietic disease due to their ability to both self-renew and differentiate. Here we generated induced pluripotent stem cells from a specific subtype of T cells, T stem cell memory (TSM) cells and characterized their pluripotency. Further, we have developed a step wise differentiation method for hemogenic endothelium CD34+ cells. We have employed tightly controlled optimized protocol using cytokines, growth factors and cellular signaling to generate authenticate CD45+ hematopoietic stem cells. Runt-related transcription factor 1 (Runx1) is a master hematopoietic transcription factor essential for hematopoietic stem cell (HSC) emergence. To monitor the onset of HSC generation we had targeted mCherry and GFP reporters under the RUNX1 primitive (P1) and definitive (P2) promoter elements respectively. Our initial targeting strategies were resulted in none of the reporter gene expression upon the differentiation into HSCs due to promoter silencing issue. We have circumvented the promoter silencing via use of site specific targeting into AAVS1 locus using CRISPER-Cas9 system. Overall, we have produced an efficient method to generate hematopoietic CD34+ followed by CD45+ definitive HSCs with the GFP+ reporter gene expression. This robust system could be utilized for small chemical compounds, macro molecules high throughput screenings

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