

Abstract

By knocking in reporter lines such as GFP and RFP with CRISPR-Cas9 technology, developmental markers for hepatocyte differentiation markers could be labeled. One of the markers, MixL1, was used to verify this reporter system. Two homology arms were cloned into a donor plasmid, while four different sgRNAs were cloned into a Cas9 plasmid. The cloning procedures involved many repeated trial-and-error processes to optimize the protocols. Once the constructs were all made and their sequences were verified, the constructs were transfected into HEK cells and iPS cells. After 48 hours of transfection, the fluorescence activities were checked using microscopy. All the transfections were successful, as RFP or GFP expressions were observed from the homology arms and sgRNAs transfected cells. While the functionality of the Cas9 plasmids was verified through checking endonuclease activity, due to time restraints further testing should be done on iPS cell lines. Such a reporter system would be beneficial in characterizing different stages of differentiation and eventually improving stem cell differentiation protocols in research labs, and the system can be further developed to be used as a tracking system for diseased cells or cell-based treatments in the clinic.