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Gene editing of YIPF4 in hESCs V3

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol describes the creation of YIPF4 knockout hESCs.

Materials

anti-YIPF4 (Sino Biological 202844-T46)



Cell line maintenance

- 1 Maintain H9 human embryonic stem cells (AAVS1-TRE3G-NGN2 hESCs) in complete (E8 media).

Targeted knock-out of YIPF4

- 2 For YIPF4 knock-out, the following sgRNA sequences was designed and ordered from Synthego (5' AAGAGGTTATGGCTGGCTTC 3').
- 3 0.6 μ g sgRNA was incubated with 3 μ g SpCas9 protein for 10 mins at room temperature and electroporated into 2×10^5 H9 cells using Neon transfection system (Thermo Fisher Scientific).
- 4 Deletions were verified by DNA sequencing with Illumina MiSeq (F: GTTCAATAGCATCCCCAGATGT and R: TCATCCCACAGACTAGCTCAAA) and by immunoblotting with a YIPF4 antibody (Sino Biological 202844-T46).