A marker-free co-selection strategy for high efficiency homology-driven and NHEJ-based gene editing in human cells

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Method Article

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Abstract

Genome editing using designer nucleases has revolutionized molecular biology by allowing DNA to be inserted, deleted or replaced in the genome of several model organisms. This protocol describes a marker-free co-selection strategy for high efficiency homology-driven and NHEJ-based gene editing in human cells by generating dominant cellular resistance to ouabain, a highly potent plant-derived inhibitor of the Na+,K+ ATPase \(1, 2, 3). This scarless coconversion strategy is highly efficient and can yield a stable population of modified cells in 14 days. Techniques relative to sgRNA cloning, cell nucleofection, selection using ouabain and validation of gene disruption/insertion are described.

Introduction

See attached Article file: "Agudelo et al. Prot. Exchange 2017":http://www.nature.com/protocolexchange/system/uploads/5365/original/Article_file_-_Agudelo_et_al._Protocol_Exchange_2017.pdf?1491228319

Reagents

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Equipment

See attached Article file

Procedure

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Timing

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Troubleshooting

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Anticipated Results

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References

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Acknowledgements

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