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CRISPR knockout protocol

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Protocol status: Working

We use this protocol and it's working

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Abstract

This is a protocol used to perform CRISPR knockouts in primary human B cells to be subsequently profiled by single-cell RNA-sequencing. It has been adapted from Synthego and Lonza user manuals.

Materials

- sgRNAs (20uM stock, Synthego)
- P3 nucleofector solution (Lonza)
- Cas9
- PBS
- Nucleocuvette strips (Lonza)
- 24 well flat-bottom tissue culture plates

CRISPR KO protocol

- 1 Thaw sgRNAs (20uM stock, Synthego) at RT for 15-30 minutes.
- 2 Centrifuge sgRNA stocks for 5min at 1000xg.
- 3 Pre-warm culture media at 37C.
- 4 Add supplement 1 to P3 nucleofector solution (Lonza) at 4.5:1 ratio.
- 5 Assemble ribonucleoprotein (RNP) complexes by adding 60 pmol of sgRNA to 20 pmol of Cas9 diluted in supplemented P3 nucleofector solution (total volume of 20uL). Incubate for 10-60 minutes at room temperature.
- 6 Centrifuge 300,000 cells per nucleofection at 90xg for 10 minutes.
- 7 Remove supernatant and resuspend in 5mL of PBS and centrifuge at 90xg for 10 minutes.
- 8 Remove supernatant and resuspend cells in 5uL of supplemented P3 nucleofector solution per nucleofection.
- 9 Add 5uL of diluted cells to each RNP complex (total volume of 25uL).
- 10 Mix cell-RNP mixture and transfer to Nucleocuvette strips (Lonza). Nucleofect cells with CA-137 pulse code.
- 11 Add 75uL of pre-warmed culture media, mix and transfer cells to 24 well flat-bottom tissue culture plates containing 400uL of pre-warmed culture media.