Sep 17, 2025

CRISPR knockout protocol

DOI
https://dx.doi.org/10.17504/protocols.io.yxmvmbpr5g3p/v1

Nicholas Pease[1]

[1]University of Pittsburgh

Nicholas Pease
University of Pittsburgh

DOI: https://dx.doi.org/10.17504/protocols.io.yxmvmbpr5g3p/v1

Protocol Citation: Nicholas Pease 2025. CRISPR knockout protocol. **protocols.io** https://dx.doi.org/10.17504/protocols.io.yxmvmbpr5g3p/v1

Protocol status: Working
We use this protocol and it's working

Created: September 16, 2025

Last Modified: September 17, 2025

Protocol Integer ID: 227460

Keywords: crispr knockout protocol, crispr knockout, sequencing, protocol, cell rna

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.