Introduction of Cas9 into target cell line

Reagents

Generation Cas9 lentivirus:

1. Seed 293T cells at 0.75/1.0/1.5 x 10^6 per 6 well and grow O/N at 37 ℃ in total 2 ml IMDM with 10% FCS and P/S (complete IMDM)
2. For transfection choose well were confluency of the 293T cells is about 70%
3. Prepare transfection mix (amounts for one 6-well):
    a. 100 µl Optimem containing:
        i. 0.44 µg pMD.G
        ii. 0.67 µg pCMV dR8.91
        iii. 0.93 µg Lenti-Cas9-2A-Blast
    b. 100 µl Optimem containing:
        i. 4 µl TransIT293 transfection reagent
4. Mix solution a and b together and mix well
5. Incubate 20 min at RT
6. Add transfection mix drop-wise to 293T cells
7. Remove supernatant from cells containing the virus 48 hours later
    a. Note: 293T cells should look very sick (membranes bubbly, etc)
8. Filter supernatant with 0.45 µm filter to remove 293T cells
    a. Virus can be stored at -80 ℃
9. Add 100 µl of virus to 4 x10^5 HAP1 cells in a 6-well in 5 ml complete IMDM
10. Spinfect:
    a. Centrifuge plate 1,800 RPM for 1 hour at 37 ℃
11. Transfer cells to incubator and grow for 48 hours at 37 ℃
12. Blasticidin selection:
    a. Add 20 µg/ml blasticidin for at least 48 hours
    b. Expand cells, freeze and determine Cas9 efficiency