**Introduction of Cas9 into target cell line**

*Reagents*

*Generation Cas9 lentivirus:*

1. Seed 293T cells at 0.75/1.0/1.5 x 106 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):
   1. 100 μl Optimem containing:
      1. 0.44 μg pMD.G
      2. 0.67 μg pCMV dR8.91
      3. 0.93 μg Lenti-Cas9-2A-Blast
   2. 100 μl Optimem containing:
      1. 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
   1. Note: 293T cells should look very sick (membranes bubbly, etc)
8. Filter supernatant with 0.45 μm filter to remove 293T cells
   1. Virus can be stored at -80 ℃
9. Add 100 μl of virus to 4 x105 HAP1 cells in a 6-well in 5 ml complete IMDM
10. Spinfect:
    1. 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:
    1. Add 20 μg/ml blasticidin for at least 48 hours
    2. Expand cells, freeze and determine Cas9 efficiency