The following list includes, respectively, cell line, media, and amount of puromycin, blasticidin and polybrene added:

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| --- | --- | --- | --- | --- |
| Cell line | Media | Puromycin | Blasticidin | Polybrene |
| A375 | RPMI + 10% FBS | 1 μg/ml | 5 μg/ml | 1 μg/ml |
| 293T | DMEM + 10% FBS | 1 μg/ml | 5 μg/ml | 1 μg/ml |
| HT29 | DMEM + 10% FBS | 1 μg/ml | 5 μg/ml | 1 μg/ml |
| MOLM13 | RPMI + 10% FBS | 2 μg/ml | 5 μg/ml | 4 μg/ml |
| BV2 | DMEM+10%FBS+1%HEPES | 2.5 μg/ml | 4 μg/ml | 0 |
| HCT116 | McCoy's 5A+ 10% FBS | 1 μg/ml | 5-10 μg/ml | 6-8μg/mL |

293T cells were plated at a density of 1.5e6 cells per well (2 ml volume) 24 h pre-transfection in a 6-well dish. Transfection was performed using TransIT-LT1 Transfection Reagent (Mirus) according to the manufacturer's protocol. Briefly, two solutions were prepared for each well. One solution contained 8.25 μl of LT1 diluted in 66.75 μl of Opti-MEM (Corning) and incubated at room temperature for 5 min. The second solution contained 250 ng pCMV-VSVG (Addgene 8454), 1,250 ng psPAX2 (Addgene 12260), and 1250 ng transfer vector (e.g., lentiGuide) in a final volume of 75 μL with Opti-MEM. The two solutions were combined and incubated at room temperature for 20–30 min. During this incubation period, the media on the 293T cells was changed. The transfection mixture was added dropwise to the cells, and then plates were centrifuged at room temperature at 1,000*g* for 30 min and returned to 37 °C. 6–8 h post-centrifugation, transfection media was removed, leaving ∼0.5 ml in each well, and replaced with 5.5 ml viral harvest media (DMEM + 10% FBS + 1% BSA). Virus was harvested 24 h post-transfection, the media was replenished, and a second harvest occurred at 48 h post-transfection.

Optimal infection conditions were determined for each batch of virus prep in each cell line in order to achieve 30–50% infection efficiency, corresponding to a multiplicity of infection (MOI) of ∼0.5 – 1. Infections were performed in 12-well plate format with 3.0 × 106 cells per well for adherent lines and 2.5 × 106 cells per well for suspension lines. Optimal conditions were determined by infecting cells with different virus volumes (0, 50, 100, 300 and 500 μl for lentiGuide virus; 0, 100, 200, 400 and 600 μl for lentiCRISPRv2 virus) followed by trypsinization and replating equal numbers of cells per each virus volume into 2 wells of a 6-well plate, each with complete medium, one supplemented with the appropriate concentration of puromycin. Cells were counted 3–5 d post selection to determine the infection efficiency, comparing survival with and without puromycin selection. Volumes of virus that yielded ∼30 – 50% infection efficiency were used for screening.