Transfection Protocols:

I use Lipofectamine 2000 (Invitrogen) for transfection of both siRNA and plasmid DNA into these 293 cell lines. I follow the manufacture's recommendations. I use 24-well plates for transfections in triplicates.

- For 293/A658 gene targeting assays: I use 2 µL of Lipofectamine 2000 in 50 µL OPTI-MEM (tube 1) and 5 µL of 20 µM duplexed siRNA + 2 µL of 100 ng/µL A979 plasmid in 50 µL OPTI-MEM (tube 2). I wait 5 min and then combine the two tubes and wait 20 min. After incubation, I add the mixture (~109 µL to cells containing 0.5 mL normal media. I change the media the next day and incubate the transfected cells for a total of 3 days before FACS analysis for GFP+ cells.
- For 293/DRGFP HR assays: Same protocol as above except use A961 plasmid instead of A979 plasmid. Wait 3 days before FACS analysis for GFP+ cells.
- For 293/1040 NHEJ assays: Same protocol as above except use A961 plasmid instead of A979 plasmid. Wait 5 days before antibody labeling (anti-CD8-PE) and FACS analysis for GFP– CD8+ cells.

In all cases I transfect a separate well for each siRNA condition to test for transfection efficiency (total of 4 wells per siRNA). In 293/A658 or 293/DRGFP assays I use a GFP expression plasmid + siRNA instead of A979 or A961 + siRNA. In 293/1040 I use a RFP expression plasmid + siRNA instead of A961 + siRNA since this cell line is stable GFP+. The transfection efficiency is then used to normalize the gene targeting/HR/NHEJ results for each siRNA.