

Cloning of sgRNAs into:

pU6-(BbsI)_CBh-Cas9-T2A-BFP-Ad4E4orf6 (#64220)
pU6-(BbsI)_CBh-Cas9-T2A-BFP-P2A-Ad4E1B (#64218)
pU6-(BbsI)_CBh-Cas9-T2A-mCherry-Ad4E1B (#64221)
pU6-(BbsI)_CBh-Cas9-T2A-mCherry-P2A-Ad4E4orf6 (#64222)

Please note that due to the presence of BbsI sites in the Ad4 coding regions it is unfortunately presently NOT possible to directly clone new sgRNA sequences into the BbsI sites downstream of the U6 promoter. Therefore, precloned U6-sgRNA cassettes, isolated as 1.7 kb PvuI-XbaI fragments from the plasmids #64323 or #64324 of this deposit (or any other plasmid derived from pX330 (#42230) , must be ligated into the PvuI-XbaI digested backbone of the Ad4 carrying plasmids.