The backbone of the construct is pcDNA3.1(+) and was not changed (also confirmed by sequencing into the vector; although vector backbone was not fully sequenced). The boundary cloning sites of the complex protein synthesis reporter insert in the plasmid pcDNA3.1(+) are HindIII at the 5‘ end and XhoI at the 3‘ end (right next to XhoI follows 3‘ the XbaI site of the pcDNA3.1 MCS). HindIII, XhoI and XbaI are indeed intact as indicated in the addgene description. However, the insert was cloned in a multistep procedure. Due to the complex cloning strategy the HindIII and XbaI sites are both not unique in the final construct and the insert is NOT cut out as a whole by combination of neither HindIII – XbaI nor HindIII - XhoI. Release of the whole insert from the plasmid can be achieved by digestion with PmeI which cuts in the 3‘ and 5‘ part of the pcDNA3.1 MCS. Note that the XbaI sites might be methylation sensitive which complicates the XbaI digestion pattern. The cloning procedure is described in Aakalu et al., (2001) Neuron 30(2): 489-502.

unique sites according to restriction digests and sequencing results:
XhoI 3‘ cloning site; between CamK2a 3‘UTR and pcDNA3.1 MCS
NotI between mouse ornithine decarboxylase PEST seuquence from destabilized EGFP vector and CamK2a 3‘UTR
SacI 3‘ end of CMV promoter in pcDNA3.1
BamHI between CamK2a 5‘UTR and sequence encoding myristoylation signal from p10 N-terminal amino acids