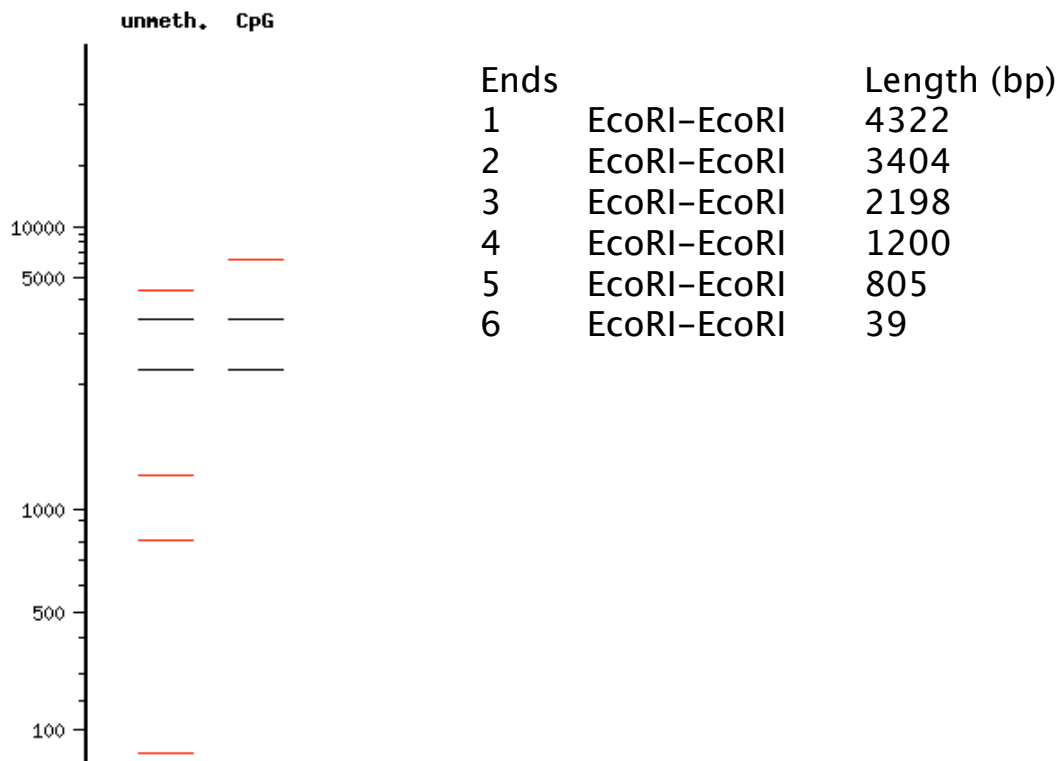


## pCMV-2xMyc-LRRK2

Tips for growing pCMV-2xMyc-LRRK2 plasmids (\*):

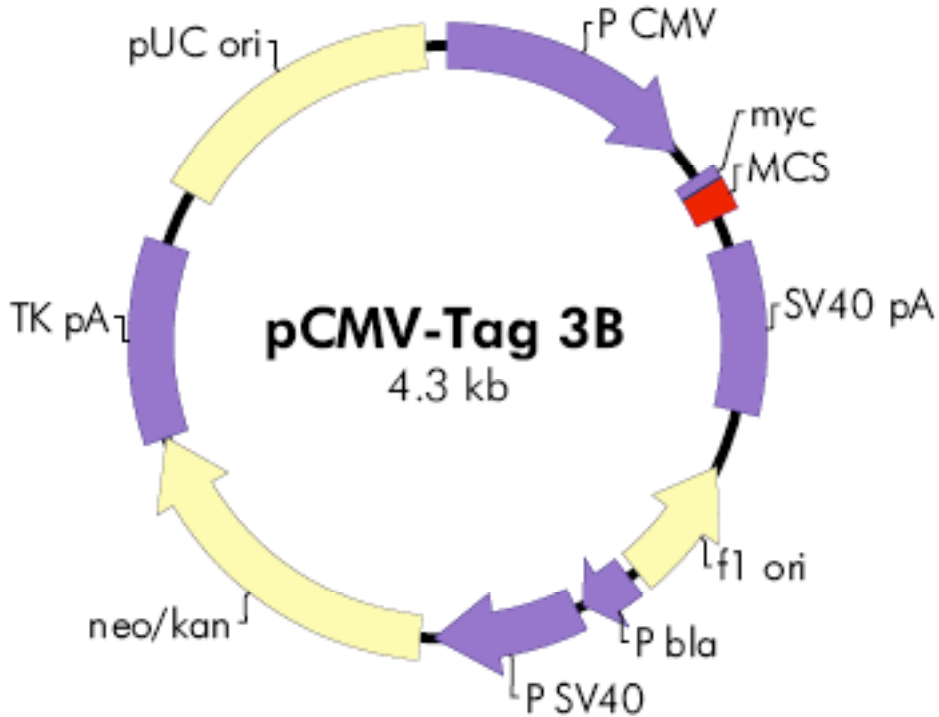
1. Use one tube of One-shot Stlb3 competent cells (Invitrogen) per construct
2. Follow the protocol of transformation. After heat-shock grow them for 1 hr at 37C.
3. Spin down the cells at low speed. Resuspend them in ~50  $\mu$ l of media and plate out everything in Kanamycin plates.

(\*) 2xMyc-LRRK2 usually gives only few transformants. It's important that you plate everything. Always check the integrity of your constructs by EcoRI digestion (see expected patten below). We have occasionally experienced recombination with LRRK2 plasmids.



## pCMV-Tag3B-2xMyc-LRRK2 derives from pCMV-Tag3B.

An additional Myc tag has been added to the original vector. The 2xMyc vector was adapted to the invitrogen gateway system by insertion of a gateway cloning cassette. LRRK2 was cloned by gateway recombination.



The vector has been modified with an additional Myc tag

### Multiple Cloning Site Region pCMV-3B-2xMyc:

**KOZAK** **myc-tag** |Srf I |BamH I  
gcc gcc atg gag cag aaa ctc atc tct gaa gag gat ctg agc ccg ggc gga tcc

**myc-tag** |Pst I |Eco RI |Eco RV  
gag cag aaa ctc atc tct gaa gag gat ctg ctg cag gaa ttc gat atc aca agt

LRRK2  
ttg tac aaa aaa gca ggc tcc gaa ttc gcc ctt **atg ---- taa** gag aga agg gcg

**multiple stop codons**  
aat tcg aaa gtg gtg atc ggt acc tta att aat taa ggt

## pCMV-2xMyc-LRRK2 complete sequence

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