Long UTR

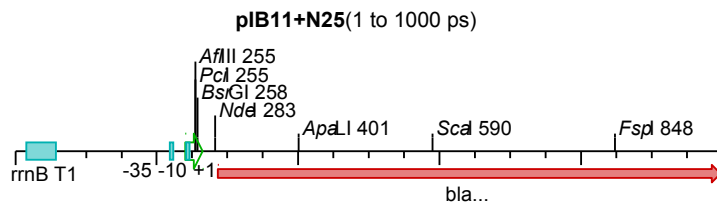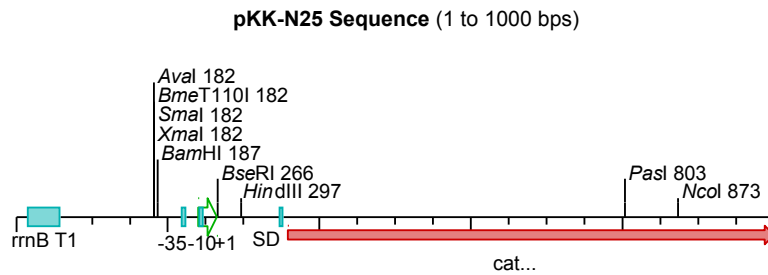**pKK-N25 Sequence** (1 to 1000 bps)

pIB11+N25(1 to 1000 ps)

mRNA features:

+1 - +32 ... UTR1
+33 - +49 ... spacer (can be shorter, but should contain one restriction site (enzyme) of the other 5 alternatives in the list below)
+50-+84 ... UTR2

mRNA sequence (WT in bold? – Jørgen):

  PciI   AgeI
AACATGTACAATAATAATGGAGTCATGAACATACATAT<u>ACCGGT</u>ACTGGAACTAGTACAATAAT
AATGGAGTCATGAA<u>CATATG</u>
                    NdeI

start codons E.coli:

 ATG, GTG, TTG, ATT, CTG

enzymes to be used:

AgeI ACCGGT (not optimal buffer with PciI and NdeI)
SacI GAGCTC (not optimal buffer with PciI) cuts within synluc
FseI GGCCGGCC (not optimal buffer with PciI)
PacI TTAATTAA (not optimal buffer with PciI)
SalI GTCGAC (not optimal buffer with NdeI) cuts within synluc

alternative sequences UTR1: (+1 - +32)

SI-r11 AACATGTACCATAATAACGGAGTCATGAACAT

alternative sequences UTR2: (+50 - +84)

SI-n2 AACTAGTAGTTTAATATGGAGTCATTAACAT
SI-n3 AACTAGTACTAAACTAATGGAGAAATGAACAT
SI-n15 AACTAGTACAACAATAGTGGAGTCATTAACAT
SI-n18 AACTAGTAAAAAAGTAATGGAGTCATTAACAT
LV-1 AACATGTACCATTATAACGGAGTAATGAACAT
LV-2 AACATGTACCATAATACAGGAGTTATGAACAT