Sequence of Kozak / Myc / Linker

M E Q K L I S E E D L L R K R
5' GA TCA GCC ACC ATG GAA CAA AAG TTG ATT TCT GAA GAT TTG TTA AGA AAG AGA
Kozak
G S A² E³
Myc-tag
GGA TCC GCG GAG 3'
BamHI mTRF1

Sequence of junction

S³¹⁰ Q³¹¹ A³¹⁷ P³¹⁸ ..... M³³⁷ E³³⁸ T³⁴³ S³⁴⁴
5'...TCG CAG GCT CCA........ ATG GAA ACC AGT... 3'

Sequence of Stop / Linker

f⁴²⁰ S⁴²¹
5' ATT AGC TGA GAA TTC TGC AGA TAT C CAT CAC ACT GGC GGC CGC TCG AGC ATG 3'
mTRF1 Stop EcoRI PstI EcoRV NotI XhoI

Cloning strategy
Second deletion in #7838 generated by PCR (see below) and PCR product cut by BamHI / EcoRI. Construct inserted into BamHI / EcoRI sites of #2409.

BamHI f1.

r2. f2. r1. EcoRI

mTRF1 cDNA
deletion

PCR 1: f1 + r2; #7838 as template
PCR 2: f2 + r1; #7838 as template
PCR 3: f1 + r1; mixed products of PCRs 1 and 2 as template