5’ 6 nt clamp for RE:RE sequence: Kozak/SD sequence

Complimentary to sequence (~20nts) 3’

Example primer: 5’ AAAAAAGCGGCCGCACCATGGAGTCAACTACCGTATCCTC 3’
Amplified sequence 5’ NNNNNNATGGAGTCAACTACCGTATCCTC

Unfortunately you will always get homodimers of your primer here. With NotI being 8 bp long, you now have a large stretch of self binding. Use GC buffer or DMSO to fix primer dimers.

ACC is the Kozak sequence, needed for eukaryotic translation.

This can be switched out for the shine dalgarno for bacterial translation

ATG… Is the start of the open reading frame in this situation, but this can be any sequence that you are amplifying.

!!This sequence is where you will calculate the Tm for the PCR cycling!! (in this case it 55 ˚C for this PCR reaction)

C- is the CG clamp on the primers 3’ end, this aids in higher fidelity

Additional sequences (Such as RE sites) need to be at the 5’ end, adding at the 3’ is incompatible with polymerases