

# Primer Design Considerations for Incorporating a T7 Promoter into a PCR Product

## Primer Design Considerations for Incorporating a T7 Promoter into a PCR Product for Subsequent *in vitro* Transcription/Translation.

### Forward Primer

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#### Required:

- T7 promoter sequence (5'-TAA TAC GAC TCA CTA TAG GG-3'). Required for transcription of the DNA template.
- ATG start codon (5'-ATG-3') if not present in the sequence being amplified. Needed for translation initiation.
- Gene-specific sequence. Needed to allow priming of the target gene.

#### Highly Desirable:

- Kozak consensus sequence (5'-CCACCATGG-3')  
OR  
Eukaryotic translation initiation sequences from sequence being amplified.  
Increases efficiency of translation initiation.
- 6–10 bases upstream of promoter. Improves efficiency of promoter.
- 3- to 6-base spacer between promoter sequence and Kozak sequence. Ensures transcription starts a few bases upstream of the Kozak sequence and allows better ribosome binding to RNA.

#### Completed Forward Primer Design:

5'-(N<sub>6-10</sub>)TAATACGACTCACTATAGGG (N<sub>3-6</sub>) CCACCATGG (N<sub>17-22</sub>)-3'

### Reverse Primer

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#### Required:

- Gene-specific sequence. Needed to allow priming of the target gene.

#### Desirable:

- Reverse complement of stop codon (TTA, CTA or TCA) if not present in the sequence being amplified. Terminates translation, allowing efficient release of ribosomes for further rounds of translation.
- Addition of a poly(A) tail results in greater RNA stability and higher levels of translation.

#### Completed Reverse Primer Design:

5'-T<sub>30</sub> stop anticodon (N<sub>17-22</sub>)-3'