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TECHNICAL REFERENCE

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

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′-CCACC**ATG**G-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′- ($N_{6–10}$)TAATACGACTCACTATAGGG ($N_{3–6}$) CCACC**ATG**G ($N_{17–22}$)-3′

Reverse Primer

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_{30} stop anticodon ($N_{17–22}$)-3′

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