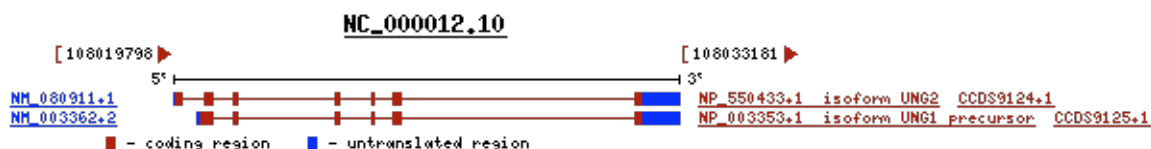


Primer BLAST – NCBI’s Primer Designer and Specificity Checker

Practical Exercise

Use Primer-BLAST to design primers to distinguish the two transcripts of the human uracil-DNA glycosylase genes (UNG, GeneID: 7374).



This gene encodes one of several uracil-DNA glycosylases. One important function of uracil-DNA glycosylases is to prevent mutagenesis by eliminating uracil from DNA molecules by cleaving the N-glycosylic bond and initiating the base-excision repair (BER) pathway. Alternative promoter usage and splicing of this gene leads to two different isoforms: the mitochondrial UNG1 and the nuclear UNG2.

Task #1: Use Primer BLAST to design primers specific to the UNG1 splice variant, NM_003362.

Task #2: Use Primer BLAST to design primers specific to the UNG2 splice variant, NM_080911.

Task #3: Carry out a specificity check for one of your primer pairs from either of the tasks above. Will this primer pair (designed against the human UNG2 transcript in Task #2, for example) also amplify transcripts from other primate species?

Step-by-Step Instructions

Follow demonstration, see over for example of expected results.

Space for your additional notes here:

Adapted from NCBI News, November 2008:

<http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=newsnbci&part=nov08>

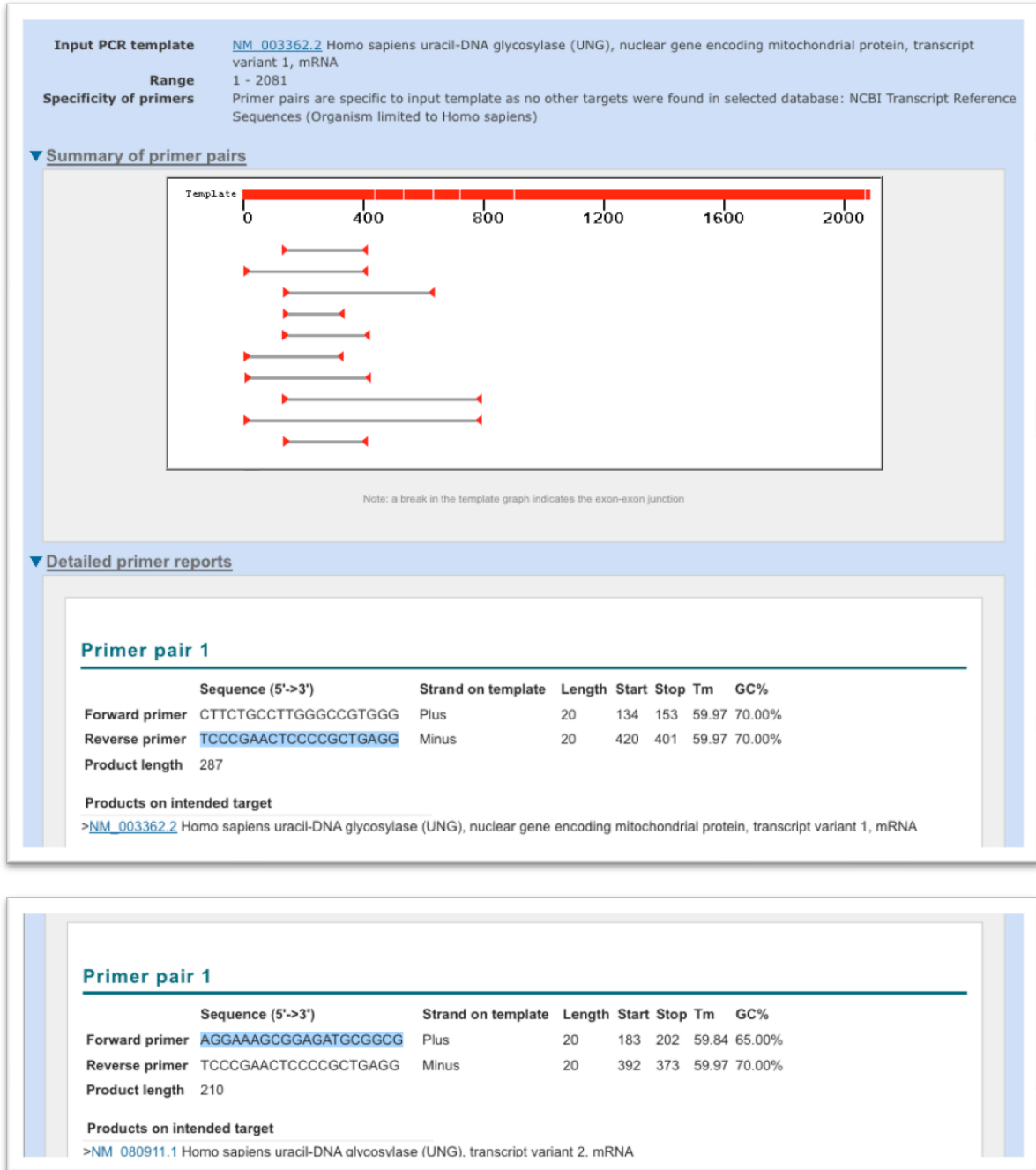


Figure 1. Primer-BLAST results for UNG transcript variants. The NCBI Reference sequences NM_003362 and NM_080911 were used as templates. **Top panel:** Primers specific to the UNG1 single splice variant are reported by default with the mRNA RefSeq database limited to human sequences. **Bottom panel:** An example of a primer pair specific to the UNG2 splice variant found using the same strategy is shown.

Adapted from NCBI News, November 2008:
<http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=newsnabi&part=nov08>