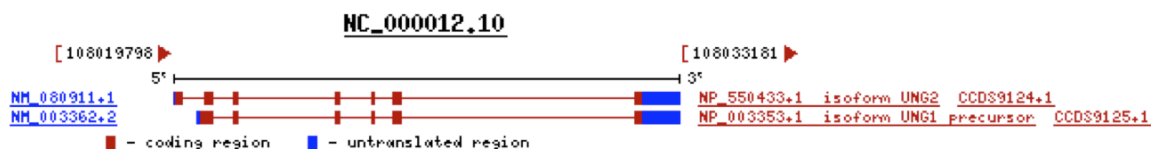


## Primer BLAST – NCBI’s Primer Designer and Specificity Checker

### 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 UNG2 splice variant, NM\_080911.

**Task #2:** Use Primer BLAST to design primers that will identify both splice variants.

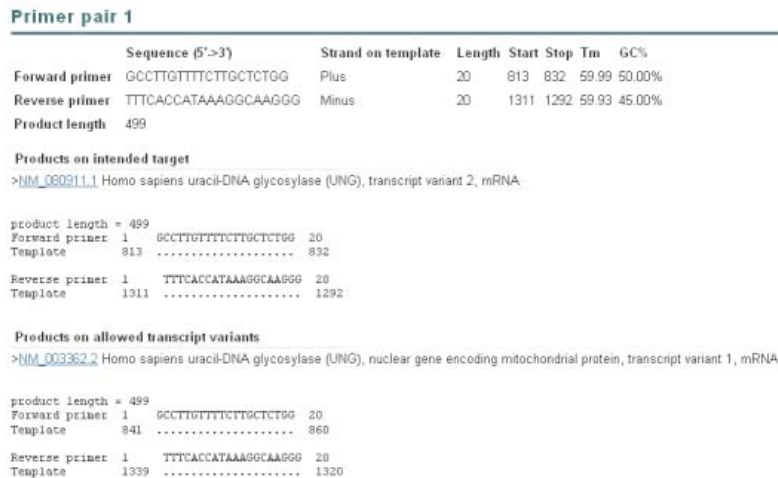
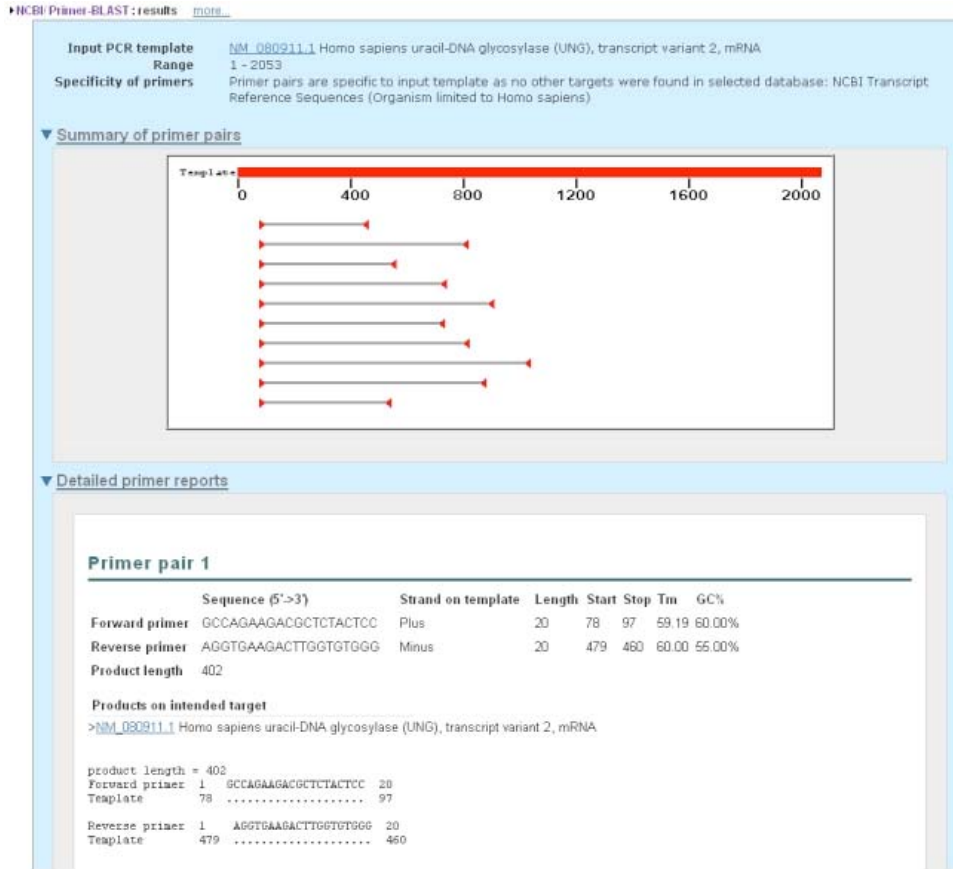
**Task #3:** Carry out a specificity check for one of your primer pairs. Will this primer pair (designed against the human UNG transcripts) also amplify transcripts from other primate species?

### Step-by-Step Instructions

Follow demonstration, 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 variant 2.** The NCBI Reference sequence NM\_080911 was used as a template. **Top panel:** Primers specific to the single splice variant are reported by default with the mRNA RefSeq database limited to human sequences. **Bottom panel:** Primers that amplify both splice variants are found with the option to allow splice variants.

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