PCR Lab Activity

In a PCR experiment, a student extracted DNA from her hair. The targeted sequence that she wanted to amplify is the following (framed gray sequence; *only the non-coding strand is shown*):

3' … TATAAAGACTTACAAATTTGTCCCCATTTTGC … 5'

1- She designed a pair of primers that recognize the DNA sequence above. What would the sequence of these primers be (indicate the 5' and 3' ends)? (3 pts)

Forward primer (forms complementary base pairs with the template DNA):

5'…ATATTT 3'

Reverse primer (forms complementary base pairs with the non-template DNA):

5'…CGTTTT 3'

2- Draw the annealing step in the first cycle of the PCR reaction for the sequence above, indicating the primers and the DNA template strands. Indicate with an arrow the direction of replication for each DNA strand. (4 pts)

3' … TATAAAGACTTACAAATTTGTCCCCATTTTGC … 5'
5'… ATATTT 3' →

← 3'TTTTGC …5'
5' … ATATTTGTGAATGTTTAAACAGGGGTAAAACG … 3'

3- The DNA sample was then put in a thermal cycler and subjected to 25 cycles of PCR amplification. Assuming she started with 0.1 pg DNA/µl, what would be the concentration of the *target duplex DNA sequence only* after 25 PCR cycles? Show your calculations. (3 pts)

$X(2^n—2n)= 0.1\ (2^{25}—2 \times 25)= 3.36 \times 10^6$ pg/ µl