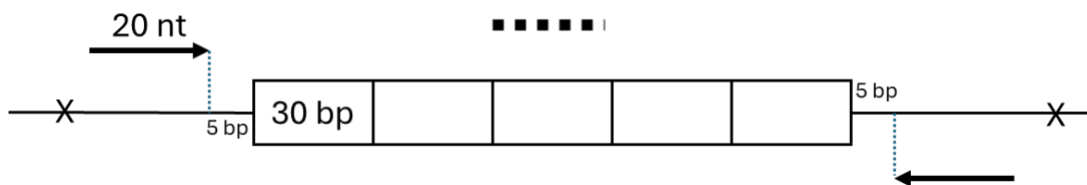


PCR exercises

The human genome contains a region of VNTR called (Ahramoccha). Each VNTR consists of 30 bp. This VNTR has different alleles. The most common is an allele that consists of 5 repeats within this VNTR.

You can perform PCR to amplify the VNTR region. The sizes of the forward and reverse primers you can use are 20-nucleotide long (shown as straight arrows). The forward primer precedes the beginning of the first VNTR by 5 bp and the reverse primer is located 5 bp downstream of the last VNTR.

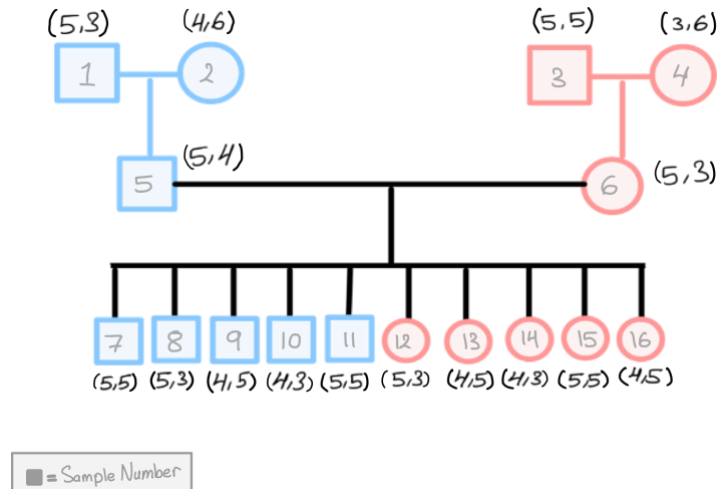
You have also used a restriction endonuclease to cut around the VNTR region (indicated by X) and performed Southern blotting using a probe (represented as a dashed line) that can hybridize to the VNTR. The distance between the restriction sites and the VNTR region is 50 bp on both sides.



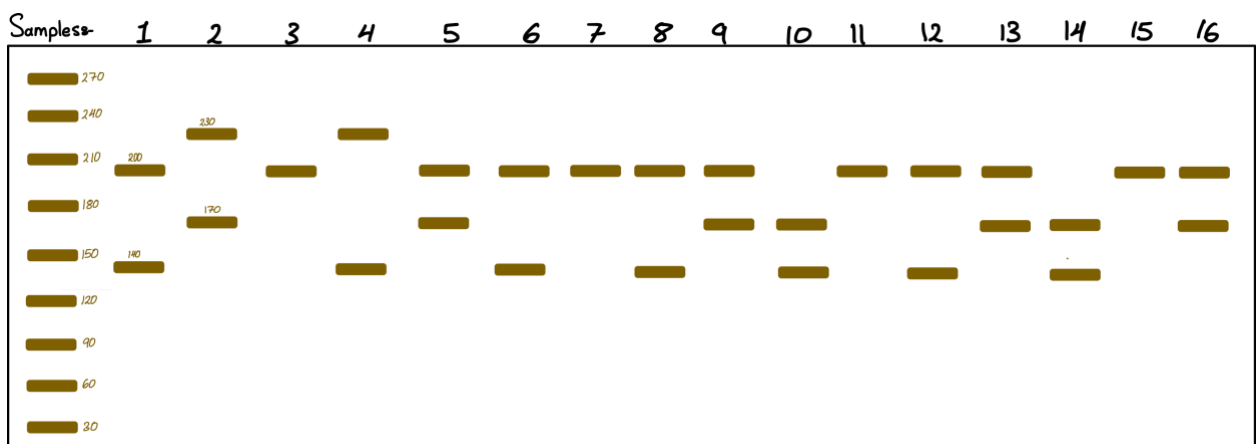
Answer the following questions:

1. What is the size of the PCR product generated from amplifying the most common allele of the (Ahramoccha) VNTR allele? **200bp**
VNTR region length $5 \times 30 = 150$
Each primer contributes 20bp
Between each primer and VNTR 5bp
 $150 + 40 + 10 = 200$
2. How many VNTRs does a person have if the generated PCR product has a size of 140 bp? **3 repeats**
 $140 - 40 (20\text{bp for each primer}) - 10 (5\text{bp between each primer and VNTR}) = 90$
 $90 / 30 (\text{length of 1 VNTR}) = 3$
3. The grandparents of 10 children (5 boys and 5 girls) have the following combinations of the VNTR alleles: paternal grandfather (5, 3), paternal grandmother (4, 6), maternal grandfather (5,5), and maternal grandmother (3,6).

- Heterozygous: maternal grandmother, paternal grandfather, paternal grandmother



- c. Draw an illustration of an agarose gel (meaning you have performed gel electrophoresis on the PCR products of all 16 samples). Indicate the positions of each band relative to a molecular weight standard sample containing DNA fragments of the following sizes (30, 60, 90, 120, 150, 180, 210, 240, and 270 bp)



-
- | Samples | Time Slots |
|---------|---|
| 270 | 2, 3, 4 |
| 250 | 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16 |
| 240 | 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16 |
| 210 | 2, 3, 4, 5, 9, 10, 13, 14, 16 |
| 190 | 1, 2, 3, 4, 6, 8, 10, 12, 14 |
| 180 | 3, 4, 6, 8, 10, 12, 14 |
| 150 | 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 |
| 120 | 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 |
| 90 | 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 |
| 60 | 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 |
| 30 | 1, 2 |