

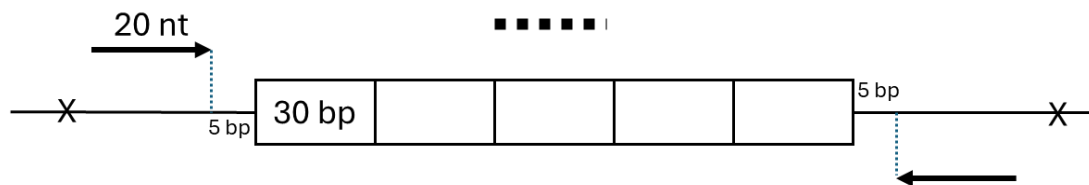
PCR exercises

Note: You can prepare a neat and organized solution sheet. Send it to me, I will give you a feedback and I will share it with others.

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?
2. How many VNTRs does a person have if the generated PCR product has a size of 140 bp?
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).
 - a. Which grandparent is homozygous and which one is heterozygous?
 - b. Draw a pedigree of the family and assign the alleles to the parents and children using different combinations of alleles to each individual.
 - 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)

4. For the Southern blotting part:
 - a. What is the name of the procedure when you performed restriction digestion and Southern blotting?
 - b. Draw an illustration of the Southern blot for each member of the family under the pedigree.