Gene technology

The size of typical bacteria genome is about 4×1000. 000 base pairs and a typical bacterium might have 5 ribosomal RNA operons. The primers you are using(called 27f and 1525r) should amplify a target sequence of approximately 1500 base pairs from 16S rRNA gene.

a- What proportion of the total DNA of this typical bacterium does the target sequence constitute?

Proportion of genomic DNA= Total number of base pairs of target sequence ÷ number of base pairs in the genome.

Answer = 5 x 1500/4000000 = 0. 001875

b- The amplification reaction uses approximately 100 ng of genomic DNA. What mass of target sequence dose 100 ng of genomic DNA contain? Mass of target sequence= mass of genomic DNA × target sequence proportion of genomic DNA.

e. g (if the target sequence were 1% proportion 0. 01 of genomic DNA, 100 ng of genomic DNA would contain 1 ng of target sequence).

Ans. = 100 X 0. 001875 = 0. 1875 ng

c- the relative molecular mass (molecular weight) of DNA base pair is approximately 660. This means that I mol of DNA base pairs has a mass of 660 g; this is called the molar mass. How many nanomoles of the target sequence are there in the 100 ng of genomic DNA?

Molar mass of DNA sequence= number of base pairs× molar mass of 1 bp

Answer: 5 X 1500 X 660 = 4950000 daltons

Number of moles of DNA sequence = mass of DNA sequence÷ molar mass of DNA sequence.

Answer: 0. 1875 / 4950000 = 3. 7879 X 10-8 nM

d- One nanomole contains 6. 02× 100. 000. 000. 000. 000 molecules. How many molecules target sequence are there in 100 ng of genomic DNA?

Number of molecules= number of nanomoles × 6. 02× 100. 000. 000. 000. 000

Answer: [3. 7879 X 10-8] X [6. 023 X 1014] = 22814394

e- After 35 cycles of amplification, what mass of target DNA would you obtain if amplification were unlimited? You can assume that the DNA mass doubles every cycle.

Final mass= starting mass × 2n(n is the number of cycles)

Answer: 6442450944 ng

f- From your answer to 4. e, how many nanomoles of the target DNA would you obtain after 35 cycles if amplification were unlimited?

Answer = 6442450944 ng / (7500 X 660) = 1301. 5 nM

g- After 35 cycles, what would the concentration of the target DNA in the reactions mix be in millimoles per litre (mM) if amplification were unlimited?

Concentration (mM)= amount (mmol) ÷reaction mix volum(1)= amount (nmol) ÷reaction mix × vol. µl

Answer : Concentration (mM) = 1301. 5 / 24 = 24. 2 mM

Why is the concentration in millimoles per liter the same as the concentration in nanomoles per microliter?

Answer: Because both the units are divided by 106 (i. e. 1 million)

h- What factor is the most likely to limit the extent of amplification possible in your PCR reaction? Hint: compare the answer to 4. eg with the concentration of dNTPs and primers in the reaction from question3. Be careful to take into account any differences in the units of concentration.`

Answer:

i) In any Polymerase Chain Reaction, the enzyme is never a limiting factor since it can easily sustains the activity till 35 cycles and is recycled in the subsequent cycles.

ii) Primers are also recycled in the cycles but as the number of cycle increases the total number of polymerization chains is very high and requires a lot of primers to bind with each strand. So Primer may become limiting if they are not added in adequate concentrations.

iii) The most likely limiting component is the concentration of dNTPs, since exponentially increasing number of dNTPs are utilized in each subsequent cycle and they are not recycled.

Actual concentrations of the primers or dNTPs in the master mix of the kit is not given so actual limiting factor is difficult to calculate.