

Quantitative Analysis of the PCR Reaction

PCR Conditions for Lambda Control:

- 200 μM (each) dNTPs; total [dNTPs] = 0.8 mM
- Total $[\text{MgCl}_2]$ = 1.5 mM
- Free $[\text{MgCl}_2]$ = 0.7 mM
- 2.5 Units AmpliTaq® DNA Polymerase per 100 μL
- 1 micromolar (each) primers [PCR01, PCR02]
- Bacteriophage Lambda DNA template (Template ds DNA = 48,500 bp)
- Target = 500 bp
- Number of cycles = 25
- Buffer: 10 mM Tris-HCl, pH 8.3 (at 25 °C); 50 mM KCl

	Before PCR				After PCR			
	Weight	Moles	Molarity	Molecules	Weight	Moles	Molarity	Molecules
Template (48,500 bp)	1 ng	3.10×10^{-17}	3.10×10^{-13}	1.86×10^7	1 ng	3.00×10^{-17}	3.00×10^{-13}	1.81×10^7
Target (500 bp)	10 μg	3.00×10^{-17}	3.00×10^{-13}	1.81×10^7	1 μg	3.00×10^{-12}	3.00×10^{-8}	1.81×10^{12}
Primers (25-mers)	1623 ng	2.00×10^{-10}	2.00×10^{-6}	1.20×10^{14}	1574 ng	1.94×10^{-10}	1.94×10^{-6}	1.17×10^{14}
dNTPs	39 μg	8.00×10^{-8}	8.00×10^{-4}	4.82×10^{16}	37 μg	7.70×10^{-8}	7.70×10^{-4}	4.64×10^{16}
Magnesium Ion	3.6 μg	1.50×10^{-7}	1.50×10^{-3}	9.03×10^{16}	3.6 μg	1.50×10^{-7}	1.50×10^{-3}	9.03×10^{16}
AmpliTaq® DNA Polymerase	12.5 ng	1.33×10^{-13}	1.33×10^{-9}	8.01×10^{10}	12.5 ng	1.33×10^{-13}	1.33×10^{-9}	8.01×10^{10}

Assumptions and Data:

- AmpliTaq® DNA Polymerase specific activity = 250,000 Units/mg
- Average MW of a dNTP is 487 Daltons
- Average MW of a dNMP is 325 Daltons
- Achieve at least 10^5 -fold amplification
- AmpliTaq® DNA Polymerase half-life is not considered

Spectrophotometric Conversions:

Double-stranded DNA (ds DNA):
 $A^{260} = \text{OD}^{260} = 1$ for a 50 $\mu\text{g}/\text{mL}$ solution

Single-stranded DNA (ss DNA):
 $A^{260} = \text{OD}^{260} = 1$ for a 33 $\mu\text{g}/\text{mL}$ solution

RNA: $A^{260} = \text{OD}^{260} = 1$ for a 40 $\mu\text{g}/\text{mL}$ solution

Reference: Freifelder, D., *Physical Biochemistry: Applications to Biochemistry & Molecular Biology*, W.H. Freeman and Company, CA, 1982, p. 494-536.

Useful Equations and Nucleic Acid Molecular Weight Data:

Absorbance =
 Molar Extinction Coefficient x Concentration x Pathlength

500 bp of double-stranded DNA = 325,000 Daltons
500 nt* of single-stranded DNA = 162,500 Daltons
Average MW of dNMP is 325 Daltons
(*nt = nucleotide)

Oligomer Quantitation:

For a 20-mer, a stock solution with $A^{260} = 1$ contains 5 nmol
 $5 \text{ nmol} = 33 \mu\text{g}/(20 \times 325)$

For a 40-mer, a stock solution with $A^{260} = 1$ contains 2.5 nmol
 $2.5 \text{ nmol} = 33 \mu\text{g}/(40 \times 325)$

Conversion of pmoles of primer to μg of primer:

Multiply pmoles by $(\text{length} \times 325)/1,000,000$

Example: 10 pmoles of a 25-mer
 $(10 \times 25 \times 325)/1,000,000 = 0.081 \mu\text{g}$ primer

Conversion of μg of primer to pmoles of primer:

Multiply by $1,000,000/(\text{length} \times 325)$

Example:
0.1 μg of a 20-mer
 $(0.1 \times 1,000,000)/(20 \times 325) = 15.4$ pmoles primer

Calculating Primer Concentrations for PCR Amplification:

Micromolar concentration of primer = pmoles/ μL

Example 1:
20 pmoles of primer in 100 μL PCR mixture =
0.20 micromolar

Example 2:
Primer is 24 nucleotides in length and is dissolved in 0.1 mL
of water

A 10 μL aliquot is diluted to 1.0 mL for A^{260} measurement:
 $A^{260} = \text{OD}^{260} = 0.76$

The stock solution has an absorbance at 260 nm (A^{260}) of 76

The stock solution (0.1 mL) contains 7.6 A^{260} units

The base composition of the primer is A=6, C=6, G=6, T=6

The Molar Extinction Coefficient at 260 nm for the primer = $a(16,000) + b(12,000) + c(7,000) + d(9,600)$

where: a is the number of A's, b is the number of G's, c is the number of C's, d is the number of T's

The Molar Extinction Coefficient of the PCR primer is:
 $6(16,000) + 6(12,000) + 6(7,000) + 6(9,600) = 267,600$

The Molar Concentration of the PCR primer stock solution is:
 $76/267,600 = 284$ micromolar

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