

**Biology 324
Winter 2000**

Leaf/Moyer

**PLEASE NOTE THAT THERE WILL BE A MAKE UP PCR QUIZ ON
THURSDAY FEB. 21.**

Sample PCR Quiz1

Name

1) PCR reaction - 20 points

Set up a PCR master mix for determining the sensitivity of a PCR reaction. You want to compare the efficiency of PCR when amplifying varying numbers of templates including: 10^6 , 10^5 , 10^4 , and 10^3 copies.

Reaction conditions:

final volume - 200 μ l

<u>Stock solutions:</u>	<u>final concentration or amount</u>
10X PCR buffer	1X
25 mM MgCl ₂	0.5 mM
20 mg/ml BSA	100 μ g/ml
Primer 1 (25 μ M)	1.0 μ M
Primer 2 (25 μ M)	1.0 μ M
2.5 mM dNTPs	250 μ M
Taq Polymerase (20 U/ μ l)	5 U

The initial DNA concentration is 10 μ g/ml.

Remember that 10 pg of 10 kb plasmid is 1.54×10^{-15} moles or 9.3×10^5 molecules. (To simplify calculations, you can assume that 9.3×10^5 is equal to 10^6 molecules). Please note that the template is a plasmid which has a total length of 5 kb.

- 1) Show how you to set up one PCR reaction with 10^6 molecules of the plasmid template.
- 2) Then show how you will set up the PCR reactions for 4 different template numbers (ie. 10^6 - 10^3) and a control. Be very clear in showing exactly what you will do. If it is garbled, you won't get full credit.

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Sample PCR Quiz2

Name

1) PCR reaction - 20 points

Set up a PCR master mix for determining the sensitivity of a PCR reaction. You want to compare the efficiency of PCR when amplifying varying numbers of templates including: 10^6 , 10^5 , 10^4 , and 10^3 copies.

Reaction conditions:

final volume - 50 μ l

<u>Stock solutions:</u>	<u>final concentration or amount</u>
10X PCR buffer	1X
25 mM MgCl ₂	2.5 mM
10 mg/ml BSA	100 μ g/ml
Primer 1 (10 μ M)	1.0 μ M
Primer 2 (10 μ M)	1.0 μ M
2.5 mM dNTPs	250 μ M
Taq Polymerase (20 U/ μ l)	5 U

The initial DNA concentration is 100 μ g/ml.

Remember that 10 pg of 10 kb plasmid is 1.54×10^{-15} moles or 9.3×10^5 molecules. (To simplify calculations, you can assume that 9.3×10^5 is equal to 10^6 molecules). Please note that the template is a plasmid which has a total length of 10 kb.

- 1) Show how you to set up one PCR reaction with 10^6 molecules of the plasmid template.
- 2) Then show how you will set up the PCR reactions for 4 different template numbers)ie. 10^6 - 10^3) and a control. Be very clear in showing exactly what you will do. If it is garbled, you won't get full credit

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Sample PCR Quiz 3

Name

1) PCR reaction - 20 points

Set up a PCR master mix for determining the sensitivity of a PCR reaction. You want to compare the efficiency of PCR when amplifying varying numbers of templates including: 10^3 , 10^2 , and 10^1 copies.

Reaction conditions:

final volume - 150 μ l

<u>Stock solutions:</u>	<u>final concentration or amount</u>
10X PCR buffer	1X
25 mM MgCl ₂	0.5 mM
10 mg/ml BSA	100 μ g/ml
Primer 1 (20 μ M)	1.0 μ M
Primer 2 (20 μ M)	1.0 μ M
2.5 mM dNTPs	250 μ M
Taq Polymerase (20 U/ μ l)	5 U

The initial DNA concentration is 0.10 μ g/ml.

Remember that 10 pg of 10 kb plasmid is 1.54×10^{-15} moles or 9.3×10^5 molecules. (To simplify calculations, you can assume that 9.3×10^5 is equal to 10^6 molecules). Please note that the template is a plasmid which has a total length of 50 kb.

- 1) Show how you to set up one PCR reaction with 10^6 molecules of the plasmid template.
- 2) Then show how you will set up the PCR reactions for 3 different template numbers)ie. 10^3 - 10^1) and a control. Be very clear in showing exactly what you will do. If it is garbled, you won't get full credit.