SCIEX

BECKMAN COULTER
Capillary Electrophoresis

# *Sequencing Template Conversion: Length to ng/fmole*

## CEQ™ Series Genetic Analysis System


### DNA Quantitation

After verification of the template purity and concentration by agarose gel electrophoresis, determine the number of nanograms of template required for the cycle sequencing reaction by using the graphs on other side.

Using the appropriate graph, on the X – axis select the total length of the template (vector length plus insert length) in base pairs, move up to the graphed line, then move to the Y – axis. The Y – axis location indicates the nanograms / femtomole for that length of template.

Calculate the number of nanograms of template required to maintain a primer to template ratio between 30:1 – 50:1 in the reaction mix. The suggested primer concentration is 3.2 picomoles per the 20 microliters of the reaction mix.

### Spectrophotometric Conversions for Approximation of Nucleic Acid Concentration

- 1 $A_{260}$ unit of dsDNA = 50µg/ml = 0.15mM (in nucleotides)
- 1 $A_{260}$ unit of ssDNA = 33µg/ml = 0.1mM (in nucleotides)
- The average MW of a deoxyribonucleotide base = 324.5 Daltons

### Approximation of Oligonucleotides Primer Concentration

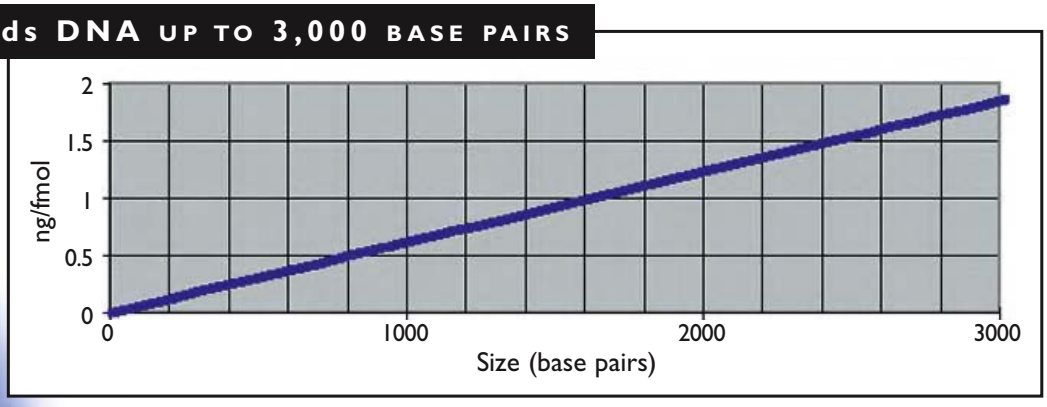
- nanomoles/mL of Primer = ($A_{260}$ units × 90)/(length of the Primer)

### Reference

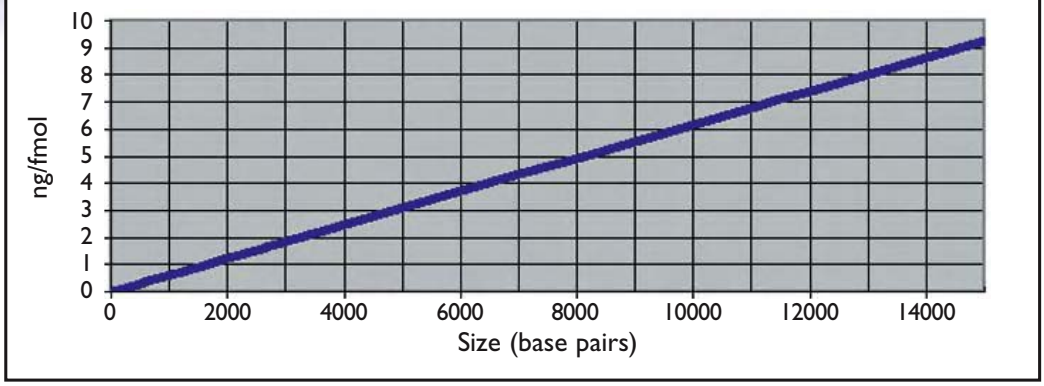
*Sambrook, J. et al,. (1989) Molecular Cloning. A Laboratory Manual, Cold Spring Harbor Laboratory. Cold Spring Harbor, N.Y.*

## Template Conversion

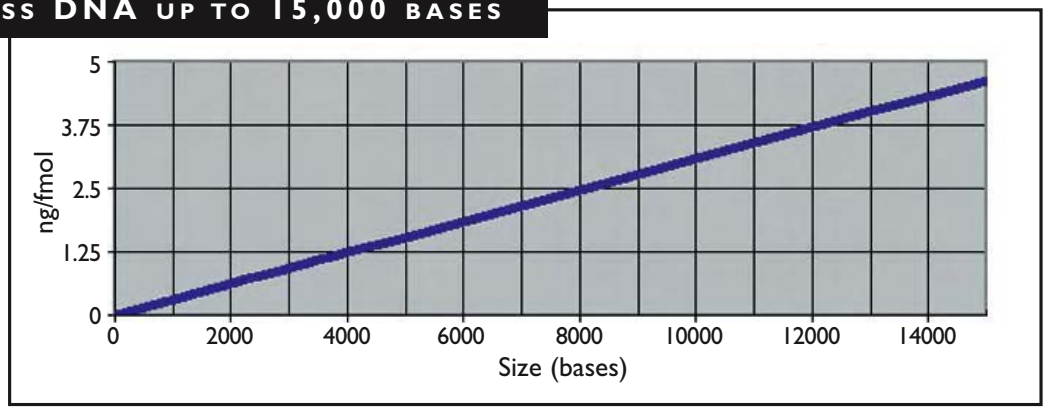
### ds DNA up to 3,000 base pairs




### ds DNA up to 15,000 base pairs




### ss DNA up to 15,000 bases



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