Primer and DNA Dilution

Making Primer and DNA Dilutions

To prepare;

1. *Making primer solutions:*

Measure the concentration of the primers reverse and forward with the spectrophotometer and calculates like this:

$$Vi = \frac{CfxVf}{Ci}$$

Vi = initial volume
Cf = final concentration
Vf = final volume
Ci = initial concentration

Forward primer:

$$Vi = \frac{10ng/\mu l \times 100\mu l}{866{,}35ng/\mu l}$$

Vi = 1,15 µl primer
in 98,85 µl milliQ water

Reverse primer:

$$Vi = \frac{10ng/\mu l \times 100\mu l}{231{,}45ng/\mu l}$$

Vi = 4,32 µl primer
in 96,68 µl MilliQ water

2. *Making DNA samples of 10ng/µl;*

Measure the concentration of the stock with the spectrophotometer and dilute the samples to 10ng/µl. Example; the concentration of sample RAM002 is 1969,5 ng/µl. You want a solution with a total volume of 100µl.

$$Vi = \frac{10ng/\mu l \times 100\mu l}{1969{,}5\ ng/\mu l}$$

Vi = 0,51µl Stock
in 99,49µl MilliQ water

Concentrations for PCR reaction

- DNA 10ng/µl
- Buffer 10x
- $MgCl_2$ 2 mM
- Primer Reverse 50 ng
- Primer Forward 50 ng
- dNTP's 0,2 mM
- Taq polymerase 2,5U

Primer and DNA Dilution

Primer concentration conversion

1 Molar (1M) = (Molecular Weight)g/Liter = 1 mol/Liter

1 µg = 1000ng 1 mg = µg

Ng/ µl = concentration (nmol/ µl) x MW

1 µM = 1 picomoles/ µl = 0.001 nmol/ µl 1 pico = 1000 nano

100 µl = 100 pmol/ µl = 0.1 nmol/ µl

Ng/ µl = 0.1 nmol/ µl x MW