Reconstituting and Diluting Primers and TaqMan® Probes

Introduction

TaqMan® primers are commonly shipped in a lyophilized state. TaqMan® probes are sometimes shipped in the lyophilized state, but more often are shipped in solution (1X TE) and their concentration is reported on your Applied Biosystems' oligofactory data analysis sheet. The units of a lyophilized primer or probe are given as a mass, in picomoles. To create a stock of primers or probe, one would reconstitute the primer or probe in sterile 1X TE (1mM Tris, 0.1mM EDTA, pH 8.0) or sterile, nuclease-free H_2O.

Determine the working stock concentration of primer or TaqMan® probe

First, determine the working stock concentration of primer or probe needed. It is important to take into account the volumes that will routinely be pipetted from the working stock of primer or probe when setting up 5' Nuclease assays using TaqMan® probes or real-time PCR assays using SYBR® Green dye. We recommend pipetting no less than 5 μl of any reagent into a PCR reagent cocktail or reaction itself because pipetting can be imprecise at lower volumes. A common range of primer working stock concentrations is from 10 μmolar (μM) to 100 μM. A common range of probe working stock concentrations is from 2 μM to 10 μM.

Reconstituting the primer or TaqMan® probe

To reconstitute a primer or probe, the volume of TE or sterile H_2O that is needed to achieve the working stock concentration must be calculated. In the following example a lyophilized primer with a mass of 119,000 picomoles (pmols) will be reconstituted to a working stock concentration of 50 μM.

Example:

Note: For the following calculations it is critical to understand these definitions.
Mole (mol) is defined as a fundamental unit for measuring the amount of a substance.
Molar (M) is defined as having one mole of solute in one liter of solution.

By definition a 50 μM solution would be $\frac{50\ \mu mol}{L}$

First convert the mass of the probe to μmols since the probe working stock will be at a μM concentration.

$$\left(119{,}000\ pmol\right)\left(\frac{1\ \mu mol}{1{,}000{,}000\ pmol}\right) = 0.119\ \mu mol$$

Then, solve the following equation for X to identify the volume needed to achieve the working stock concentration.

$$\frac{0.119\ \mu mol}{X} = \frac{50\ \mu mol}{L}$$

By cross-multiplying, X = 0.00238L

Liters can be converted to a more practical volume unit.

$$\left(0.00238\text{L}\right)\left(\frac{1000\text{mL}}{\text{L}}\right) = 2.38\text{mL}$$

To achieve a concentration of 50 μM, a lyophilized primer with a mass of 119,000 pmol will be reconstituted with 2.38mL of 1X TE or sterile H_2O.

Diluting already reconstituted TaqMan® probes to a working stock concentration

TaqMan® probes are commonly shipped in solution and their concentration is reported on the Applied Biosystems' data analysis sheet. The following example demonstrates how to dilute probes to a desired working stock concentration. A common range of probe working stock concentration is from 2 μmolar to 10 μmolar.

Example: Diluting already reconstituted TaqMan® probe with a concentration of 100 μM to a working stock concentration of 10 μM.

Please note that in this example Applied Biosystems reconstituted the TaqMan® probe with 50 μl of 1X TE to achieve an initial concentration of 100 μM.

Calculation used is $C_1V_1=C_2V_2$

Where
C_1 = Initial Concentration of solution
V_1 = Initial Volume of solution
C_2 = Final Concentration of solution
V_2 = Final Volume of solution

A Molar concentration is one mole of solute in one liter of solution. Therefore a 100 μM solution would be 100 μmol in one liter. And, there is 1000 μl in 1 L.

$$\left(\frac{100\ \mu\text{mols}}{1000\ \mu\text{l}}\right)\left(50\ \mu\text{l}\right) = \left(\frac{10\ \mu\text{mol}}{1000\ \mu\text{l}}\right)\left(V_2\right)$$

Solving for V_2 ,
$V_2 = 500\ \mu\text{l}$

To achieve a final probe volume of 500 μl, 450 μl of 1X TE would be added to 50 μl of the 100 μM TaqMan® probe solution.

Storage of primers and TaqMan® probes

Once the primers and probes are reconstituted and/or diluted, it is recommended that the primers and probes be distributed into single-use aliquots. Making single-use aliquots limits the freeze-thawing of primers and TaqMan® probes and therefore will extend their life. It is recommended to store both primers and TaqMan® probes at –20°C. It is also important to keep TaqMan® probes in the dark to prevent photobleaching, which can damage the probe.

Optimizing primer and TaqMan® probe concentrations

Consult with the appropriate Applied Biosystems' protocol to determine how to optimize primer and probe concentrations for a 5' nuclease assay or primer concentrations for a real-time PCR assay using SYBR® Green dye. Protocols can be obtained from the Applied Biosystems' Documents on Demand service at http://docs.appliedbiosystems.com/search.taf/ For instance, one could access the protocol for the TaqMan® Universal PCR Master Mix to find specific information about optimizing primers and probes. However, for the vast majority of quantitative 5' nuclease assays designed and run following ABI assay development guidelines, using a concentration of 900 nM forward and reverse primers and 250 nM probe will provide for a highly reproducible and sensitive assay when using cDNA or DNA as a substrate. Due to the non-specific nature of its detection, SYBR® Green I primer optimization should be eliminated only with caution. However, if all guidelines are followed, concentrations of 50 nM forward and reverse primer should provide robust amplification with a good level of specificity when using cDNA or DNA as a substrate.

Preparing a PCR reagent cocktail

It is recommended not to pipet primers and TaqMan® probes directly into your 5' nuclease reaction but rather to pipet the primers and probes into a PCR reagent cocktail. The cocktail can then be added to each of the wells of the reaction plate. The following example outlines how to calculate the volumes of primers and probe needed such that the primers and probe will be at a desired final concentration in a 5' nuclease reaction. This example is presented simply to demonstrate how to dilute TaqMan® primers and probes in a PCR reagent cocktail. Please consult with your specific Applied Biosystems' protocol in order to determine the specific recommendations for usage of your Applied Biosystems' reagents. Protocols can be obtained from Applied Biosystems' Documents on Demand service at http://docs.appliedbiosystems.com/search.taf/

Example: Preparing a PCR reagent cocktail with final reaction primer concentrations of 900nM and final reaction TaqMan® probe concentrations of 250nM.

Determine the number of reactions that the experiment will require and add an additional 10%-20% (ex. 50 reactions + 5) as it is likely that some cocktail volume will be lost due to reagents sticking to the sides of the tube. In this example the user will run 50 reactions and will prepare the PCR reagent cocktail as if for 55 reactions.

The reactions are being prepared with the TaqMan® Universal PCR Master Mix (supplied at a 2X concentration, p/n 4304437), which provides all of the necessary reagents for the 5' nuclease PCR process with the exception of primers and TaqMan® probe and DNA template. The working stock concentrations of primers are 50 μM and the working stock concentration of probe is 10 μM. 10 μl of DNA template will be added to each well. The reaction volumes are 50 μl.

Calculate the volumes of all reagents needed for a single reaction.

The TaqMan® Universal PCR Master Mix is at a 2X concentration and would therefore constitute half of the reaction or 25 μl in order to be at a 1X concentration.

To dilute the primer and probe, use the following calculation.
$C_1V_1=C_2V_2$

Where
C_1 = Initial Concentration of solution
V_1 = Initial Volume of solution
C_2 = Final Concentration of solution
V_2 = Final Volume of solution

Solve for V_1 to calculate the volume of each stock primer needed per reaction.

$$\left(\frac{50\ \mu\text{mols}}{1000\ \mu\text{l}}\right)\left(V_1\right) = \left(\frac{0.9\ \mu\text{mols}}{1000\ \mu\text{l}}\right)\left(50\ \mu\text{l}\right)$$

V_1 = 0.9 µl

Solve for V_1 to calculate the volume of stock probe needed per reaction.

$$\left(\frac{10\ \mu\text{mols}}{1000\ \mu\text{l}}\right)\left(V_1\right) = \left(\frac{0.25\ \mu\text{mols}}{1000\ \mu\text{l}}\right)\left(50\ \mu\text{l}\right)$$

V_1 = 1.25 µl

Solve for the volume of Sterile H_2O needed per reaction by subtracting the volumes of all other reaction components from 50 µl.

50 µl (final reaction volume) - 25 µl (TaqMan® Universal PCR Master Mix) - 0.9 µl (primer 1) - 0.9 µl (primer 2) - 1.25 µl (probe) – 10 µl (DNA template) = 11.95 µl Sterile H_2O

Multiply all components (except DNA template) by 55

Reagent	Stock concentration	Volume of single reaction		Volume needed for master mix cocktail	Final concentration
TaqMan® Universal PCR Master Mix	2X	25 µl	X 55	1375 µl	1X
Forward primer	50 µM	0.9 µl	X 55	49.5 µl	900 nM
Reverse primer	50 µM	0.9 µl	X 55	49.5 µl	900 nM
TaqMan® probe	10 µM	1.25 µl	X 55	68.75 µl	250 nM
Sterile H_2O	N/A	11.95 µl	X 55	657.25 µl	N/A

Please note that all components would be at their appropriate final concentration when 40 µl of the PCR reagent cocktail is mixed with 10 µl of DNA template in each sample well.

Ordering primers and TaqMan® probes

To order primers and TaqMan® probes from the Applied Biosystems' oligofactory, go to the Applied Biosystems' store at http://store.appliedbiosystems.com/ You must register to be able to login to order primers and probes via the web. Once the registration has been filled-out, Applied Biosystems' order administration will send an e-mail within 48 hours confirming your registration and you will then be able to place an order. Click on ABI PRISM® Primers/Probes in the catalog (left-hand column). Then, click on TaqMan Primers & Probes (left-hand column) to order. Ordering questions can be directed to the Applied Biosystems' oligofactory ordering group at 800-327-3002; option 2. Technical questions about TaqMan® primers and probes can be directed to the Applied Biosystems' PCR/SDS Technical Support group at 800-762-4001.