Primer dilutions for PCR amplifications

Order primers from any vendor (i.e. Integrated DNA Technology http://www.idtdna.com/site)
Request primers with scale: 25 nmole (15-60 bases)
purification: standard desalting
formulation: lab ready (normalized to 100 µM in IDTE pH 8.0), **OR** none (dehydrated)

Note: If the centrifuge used is NOT a fixed 6 cm rotor (i.e. Centrifuge 5418, Eppendorf, US), convert all RPM to × g.

- **If primers are normalized,** vortex and centrifuge the primers (30 seconds at 10,000 rpm).
- **If primers are dry,** centrifuge (30 seconds at 10,000 rpm) and rehydrate to **100 µM** in molecular grade (MG) water.
- The amount of MG water is determined using the value of the **nmoles** provided by the vendor. For example, if label lists 47.54 nmoles of primer, then add 475.40 µL of water to make a final primer concentration of **100 µM.**
- Vortex briefly, centrifuge (30 seconds at 10,000 rpm) and **chill on ice** for **30 min.**

To prepare primer for use (working concentration)

- Keep your primers on ice during the preparation process.
- UV (15 min) the number of tubes to be used for the working concentration of the primers.
  Suggestion: use different color caps if preparing multiple sets of primers.
- The concentration of primers depends on the test. It is preferred to use concentrations of 5 µM for general tests because the same working concentration can be used for PCR amplifications and for submission to a sequencing facility (i.e. McLab http://www.mclab.com/home.php , GENEWIZ Inc http://www.genewiz.com/).
- The formula for primer concentration calculations is as follows:

(concentration 1) (volume 1) = (concentration 2) (volume 2)
C1 V1 = C2 V2
example: require 500 µL of a primer at 5 µM
(100 µM) (X) = (5 µM) (500 µL)
X = (5 µM) (500 µL) / (100 µM)
= **25 µL** of stock (100 µM) and **475 µL** MG water

- In the above example, 475 µL of MG water would be pipetted into an empty tube. Then 25 µL of the stock primer (100 µM) would be added for a final concentration of 5 µM (total final volume 500 µL).
- Labels should include the following information:

TEST:	PHY
PRIMER:	ITS5
CONCENTRATION/AMOUNT:	5µM/500µL
DATE:	8.3.09

- Store all primers at -20°C.

Citation
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Prepared by: Z. Gloria Abad, Ph.D. ZOILA ABAD Digitally signed by ZOILA ABAD Date: 2019.08.23 12:32:34 -04'00'

Submitted by:
KURT ZELLER Digitally signed by KURT ZELLER Date: 2019.08.27 15:15:21 -04'00'
Kurt Zeller, Ph.D.
Mycology Section Supervisor, Beltsville Lab

Approved by:
ASHLEE BARTH Digitally signed by ASHLEE BARTH Date: 2019.09.04 13:12:30 -04'00'
Ashlee Barth
Quality Manager, Beltsville Lab

MARK NAKHLA Digitally signed by MARK NAKHLA Date: 2019.09.05 10:17:46 -04'00'
Mark Nakhla, Ph.D
Laboratory Director, Beltsville Lab