**FOOD SAFETY & MQIP LABORATORY**
**CORNELL UNIVERSITY**

***Name of Protocol***

**FILE NAME: P:\FOOD-Labs\BoorWiedmann Lab\LAB STUFF\PRIMER DATABASE\Primer_Ordering_Reconstitution_Storage.doc**

**Authored by: Barbara Bowen**
**Last Modified on: 3-14-2019 by Rachel Cheng**
**Approved by: Martin Wiedmann**

EFFECTIVE DATE: 12/3/2013

APPROVED BY:

_____ _____
Dr. Martin Wiedmann (date)

AUTHORED BY:

_____Barbara Bowen_____ _____10/03/2001_____

(Name) (date)

TABLE OF CONTENTS

## SECTION 1 INTRODUCTION

### 1.1 Purpose

The purpose of this document is to set forth standard guidelines for primer/probe ordering, reconstitution, and storage.

### 1.2 Scope

This SOP applies to the Food Safety Lab and Milk Quality Improvement Program.

### 1.3 Definitions

Primer – Short single stranded nucleic acid that serves as starting point for DNA synthesis.

Probe – A hybridization probe is a fragment of DNA or RNA of variable length (usually 100-1000 bases long) which is used in DNA or RNA samples to detect the presence of nucleotide sequences (the DNA target) that are complementary to the sequence in the probe.

Real-time PCR – A molecular biology technique based on polymerase chain reaction that is used to determine the presence and abundance of a particular DNA sequence in a sample. Real time PCR is frequently used in gene expression studies which rely on cDNA reverse transcribed from mRNA.

TaqMan probe – Hydrolysis probes used in real-time PCR.

### 1.4 Safety

## SECTION 2 MATERIALS

- 10mM Tris-HCL (pH 8.0)
- 1.5 mL microcentrifuge tubes
- Computer with Microsoft Access (lab computers have Access)

## SECTION 3 PROCEDURES

### 3.1 Primer Ordering and Naming Overview

Before ordering primers, check the primer and probe databases on the fileserver under P:\FOOD-Labs\BoorWiedmann Lab\LAB STUFF\PRIMER DATABASE\Primer Access database. Search the Access file by sequence or name (see protocol **8.1.1.1.11-Primer Database Search Protocol**). If the desired primer/probe sequence is not in the database, proceed to name and order new primers (see **ordering primers** below). If the desired primer/ probe sequence is in the database, check the chest freezer in 352C to see if the primer is in stock before ordering.

### 3.2 Naming of Synthesized DNA Oligonucleotides (including PCR Primers)

All synthesized oligonucleotides need to be named according to the following system. Exceptions to the naming system include primers that are already in the database or primers described in a publication. These "legacy" primers are considered "grandfathered in" even if they were not named according to the rules detailed here (as an exception, previously designed primers with misleading or redundant names should be renamed according to the convention detailed here). Assign every sequence you design with your NET ID and a sequential number. A brief description, as usual, may follow. The primer name should not have spaces, periods, commas, or special characters. Underscores may be used. Always put a dash (-) between your net ID and the sequential number.

The primer and probe names need to follow the following system:
Examples:

**AG67-8-artAB-F**
**AG67-9-artAB-R**
[*Net ID*]-[sequential #] [*brief description*]

These primers were designed by Ahmed Gaballa (AG67), were the eighth and ninth sequences he designed, and are the forward and reverse primers.

Notes:

1) This applies to all regular and Real Time PCR primers .

2) If a primer is redesigned (for example, the PCR didn't work so you're trying a new primer), the new primer *must* receive a new number.

### 3.3 Ordering Primers

Before ordering new primers, walk over to the freezer and write in the primer names on the next available lines for tower, box and slot. Include those locations in the following Excel

format attached to an email that must be sent to Nancy Carey:

| Primer Location (Tower-Box-Slot) | Primer Name | Sequence |
|---|---|---|
| A-09-57, e.g. | AG67-2-cdt-GFP-F2, e.g. | TAAGCAGAATTCGATAAGTGAATGTAGTTTTAAATT |

***Note: New primers will not be ordered unless accompanied by a location.***

**When you request primers to be ordered, please do <u>not</u> have extra spaces in your locations or sequences:**

e.g., A-03-19 not A - 03 - 19

ATATGTCGACCAAGCGAGATTTCAGGTGGTCAA not ATAT GTCGAC CAA GCG AGA TTT CAG GTG GTC AA

**Also, list locations as:**

e.g., A-<u>04</u>-<u>08</u> not A-4-8

This is so we can sort correctly by location to track that all slots are properly filled in the freezer towers. Otherwise, e.g., A-4-8 and A-4-9 <u>follow</u> rather than precede A-4-79 in the database when sorted (and the same will happen eventually with the box numbers, also).

If you personally need to have spaces in your sequences (to more easily check that they are correct, e.g.), please clean them up when you copy them into Excel to request your order as follows:

"Find & Select – Replace"
Enter a space (you won't see it) in the "Find What" box
Leave the "Replace With" box blank
Choose "Find All"
Choose "Replace All" – this will clean up the entire page

## <u>3.4</u> <u>**Primer/Probe Reconstitution and Storage**</u>

- Primers typically arrive in a lyophilized (freeze-dried) form. Briefly centrifuge the tube to drop any powder to the bottom of the tube and carefully open tube.

- Within the tube the primer arrives in, hydrate lyophilized primers or probes to a 100uM (100pmol/ul) concentration with 10mM Tris-HCL (pH 8.0). Tris is used rather than water to help prevent autocatalysis of the DNA during freeze-thaw cycles.
- All original vials containing 100uM stock primers are to be stored in the –20°C chest freezer in room 352C, NOT in personal boxes. Aliquots of the stock primers can be stored in personal boxes.

- When you need to dilute the 100uM primer stock to a working dilution, thaw the desired tube, make the necessary dilution **using 10 mM Tris-HCl (pH 8.0)** and return the 100uM stock tube to the primer/probe storage freezer.

- Give primer order sheets to Sherry Roof, but make a copy for yourself. You can also peel one of the primer stickers off the sheet and put it in your notebook.

## SECTION 4 TROUBLESHOOTING

GENERAL HINTS ON TRIS BUFFER USAGE:

- Primers should be reconstituted in Tris-HCL due to the following reasons. While they could be reconstituted in water or Tris-EDTA, if reconstituted in water, pH could vary, which leaves the DNA susceptible to autocatalysis during freeze-thaw cycles. Although both Tris-HCL and Tris-EDTA are prepared to a pH (8.0) optimal for DNA storage, and EDTA, a chelator, can help prevent primer degradation if contaminant nucleases are present, EDTA has the potential to inhibit downstream enzymatic processes.

- Do not use the 10mM Tris (pH 7.4) that is in in the PCR prep room for gel elution or with the Qiagen kit. Qiagen kit elution buffer is more basic than the Tris-HCL (pH 8.0) used for primer hydration.

- To make Tris buffer: Tris is basic. A Tris solution needs to be titrated to the desired pH with HCl.
    - **To prepare 200 mL of 0.5 M Tris stock solution:** 12.114 g TRIS brought to 200 mL in a volumetric flask with ddH20, autoclave at 121°C for 15 minutes and cool before proceeding
    - **To prepare 10 mM Tris-HCl for Primer Rehydration**: Add 4 mL of this stock solution to ~150 mL dH20 in a beaker with a stir bar. Place on stir plate and adjust pH to 8.0 using 1N HCl (should take ~1.1 mL). Bring to 200 mL in a volumetric flask. Filter-sterilize.
- If you do not know how to use the pH meter, or how to titrate a solution, please talk to one of the technicians.

## SECTION 5 REFERENCES

http://en.wikipedia.org/wiki/Hybridization_probe

http://en.wikipedia.org/wiki/TaqMan