ORDERING SUPPLIES FOR qPCR MARKERS

1. INTRODUCTION

1.1. This method provides instructions for ordering qPCR reagents, including primers, probes, and gBlock DNA standards. These supplies are ordered for routine sample analysis and new marker validation.

2. ORDERING PRIMER/PROBE

2.1. Always purchase primers/probe together as a set. In most instances, primers should be ordered dry and salt free, and probes should be dry and HPLC purified. However, when ordering minor groove binder (MGB) probes from IDT, both the primers and probe should be ordered resuspended. MGB probes are not stable when dry.

2.1.1. Determining which reporter dye and quencher to order –

2.1.1.1. Probes are ordered with the 5' reporter dye listed in the publication and the same or comparable quencher. FAM or fluorescein amidite is a dye used in synthesis for the detection of oligos. All current probes use the 5' 6-FAM dye.

2.1.1.2. The quencher for each probe will determine which company to order from. Some primer/probe sets are ordered from Integrated DNA Technologies (IDT) with the 3' Zen-Iowa Black FQ quencher. This quencher is ideal only for use with FAM dye. Some assays use minor groove binder (MGB) probes which better stabilize the probe-target bond. MGB probes are generally ordered from Eurofins, but they can also be ordered via email from IDT.

2.1.1.3. Current Primers/Probe Sets –

Assay	Reporter	Quencher	Typically Ordered From
HF183	6-FAM	MGB	Eurofins
BacR	6-FAM	MGB	Eurofins
DG3	6-FAM	MGB	Eurofins
GFD	6-FAM	Iowa-Black FQ	IDT
Gull2	6-FAM	Iowa-Black FQ	IDT
SKETA	6-FAM	Iowa-Black FQ	IDT
HumM2	6-FAM	Iowa-Black FQ	IDT
CowM2	6-FAM	Iowa-Black FQ	IDT

2.1.2. Determining which scale to order –

2.1.2.1. Primers and probes are typically ordered at a 1 µmol scale but can be ordered in smaller quantities (250 nmol) for markers that historically produce a larger yield, testing a new marker, or for markers that are used infrequently.

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2.1.2.2. The yield of a primer/probe is dependent on several factors including the quencher and dye used. For instance, probes with the Iowa-Black FQ quencher tend to produce higher yields at the same scale than MGB quenched probes. Based on the historical yields received for each probe and frequency of analysis it is recommended to order the assays at the following scales:

2.1.2.2.1. For HF183, BacR, DG3, and Gull2: 1 µmol scale

2.1.2.2.2. For GFD, SKETA, CowM2, and HumM2: 250 nmol scale.

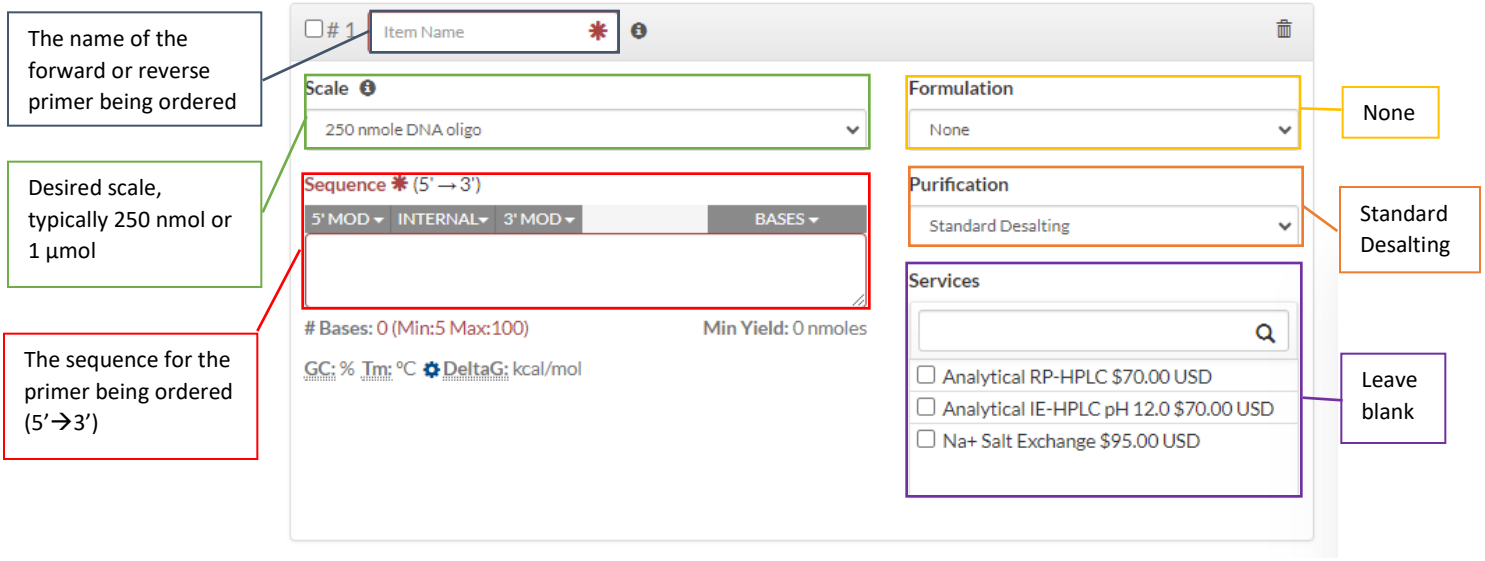
2.1.3. Order requests should be submitted to the supervisor for approval.

2.2. Ordering from IDT:

NOTE: Prior to ordering from IDT, an account must be created. The cart is then filled on the IDT website and a PCard request is placed. The current PCard form is found here \\Floridadep\data\DEAR\Labs\Lab5\Biology\MolecularBiology\Orders\Ordering\Pcard Orders.

2.2.1. Ordering Primers: On the IDT website (www.idtdna.com) under Products & Services select Custom DNA. Next click DNA oligos, then order single-stranded DNA oligos in tubes.

2.2.1.1. In the Oligo Entry screen add the following:

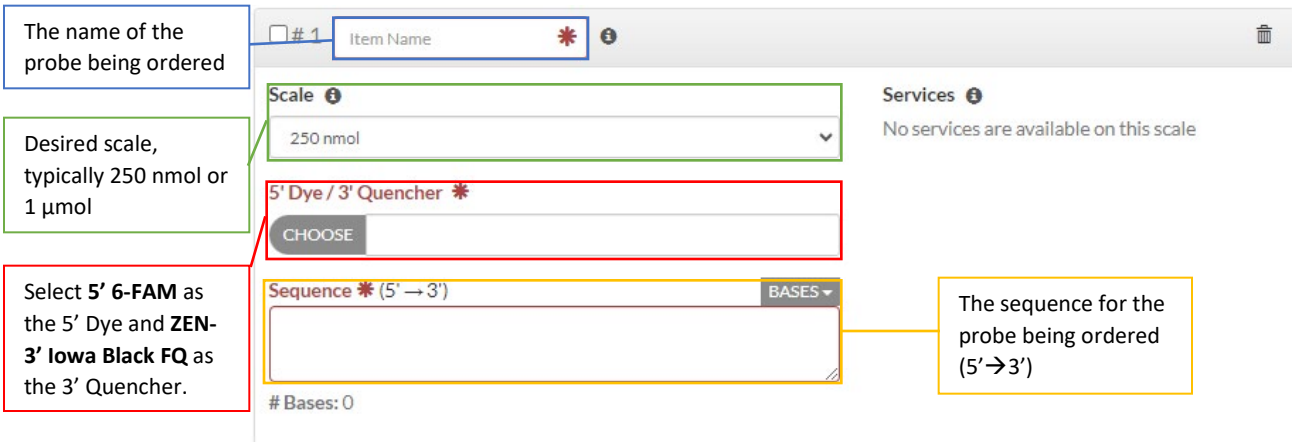


2.2.1.2. Double check that the sequences are entered correctly, then add primers to the cart.

2.2.2. Ordering Probes: On the IDT website under Products & Services select Custom qPCR probes and choose PrimeTime qPCR Probes.

2.2.2.1. In the PrimeTime qPCR Probes entry screen add the following:

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2.2.2.2. The default purification method is HPLC and no additional services or reagents are needed.

2.2.2.3. Double check that the sequence is entered correctly, then add the probe to the cart.

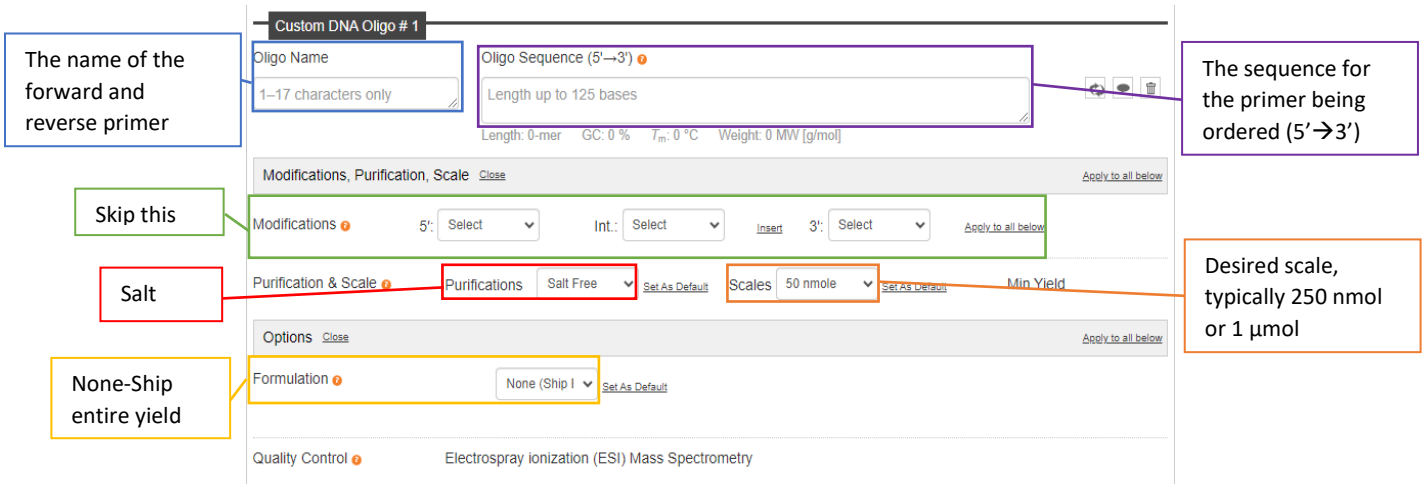
2.2.3. Once the cart is filled, email it to the analyst. Fill out a PCard request form with cost and details of order and submit by email to supervisor for approval. The supervisor will save the file as a PDF and send to the Program Administrator and copy the administrative assistant.

2.3. Ordering from Eurofins:

NOTE: Prior to ordering from Eurofins, an account must be created. The cart is then filled on the Eurofins website and a PCard request is placed.

2.3.1. Ordering Primers: On the Eurofins Genomics website (www.eurofinsgenomics.com) select Order Now the select Custom DNA under Oligos. Select Custom DNA Oligos then Order DNA in Tubes. Choose the number of oligos and manual entry, then press next.

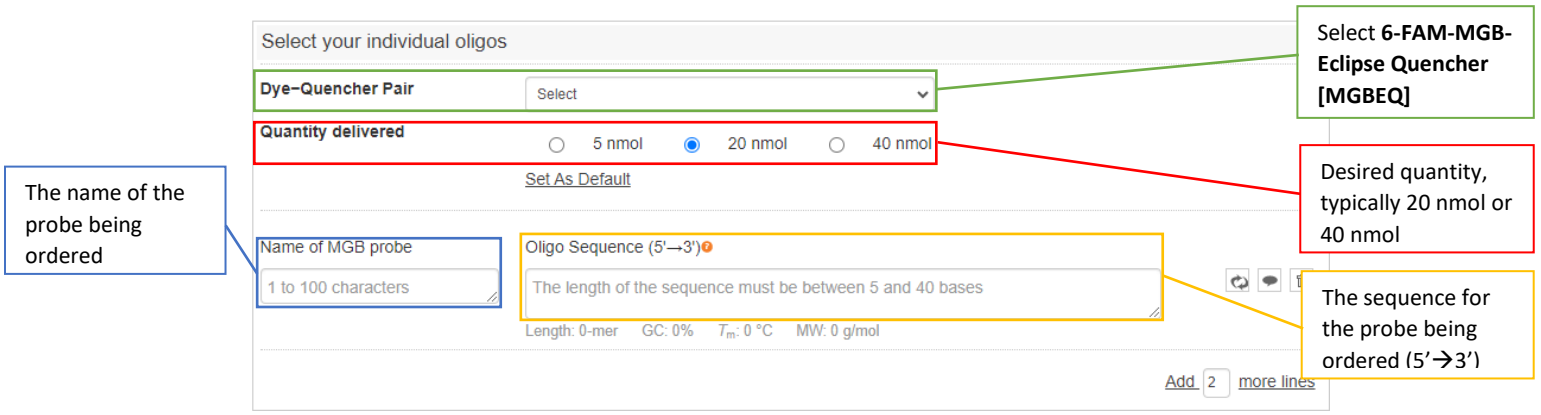
2.3.1.1. In the entry screen add the following:



2.3.1.2. Double check that the sequences are entered correctly, then add primers to the cart.

2.3.2. Ordering Probes: On the Eurofins website under Order Now, select MGB Probes from under Oligos. Choose the number of MGB probes and manual entry, then press next.

2.3.2.1. In the MGB Probes entry screen add the following:



2.3.2.2. The default purification method is HPLC.

2.3.2.3. Double check that the sequence is entered correctly, then add the probe to the cart.

2.3.3. Once the cart is filled, click PDF to save a copy of the cart. Fill out a PCard request with cost and details of the order and submit by email to supervisor for approval. The supervisor will send the PDF to the Program Administrator and copy the administrative assistant.

NOTE: The cart cannot be emailed so items must be entered manually at the time of the order.

3. ORDERING STANDARDS

3.1. All current assays except SKETA use a gBlock fragment for spikes and standards. gBlocks are double-stranded DNA fragments that are designed to contain the target sequence for one or more assays. They can be ordered from IDT alone or in combination with other target sequences if the fragments are compatible. These can be tested in various combinations. They are delivered dry in a centrifuge tube and normalized to 250, 500, or 1000 ng depending on length.

3.2. To order gBlock fragments, a cart is filled on the IDT website then a Pcard request is placed.

3.2.1. On the website www.idtdna.com under Products & Services select Double-Stranded DNA fragments. Order gBlocks gene fragments in tubes. Add in the gBlock name and sequence. No modifications are needed. Test complexity and add to cart if sequence passes initial screening. Refer to

the published method for each marker for sequence information. Double check to ensure the sequence is entered correctly.

3.2.2. Once cart is filled, email cart to analyst. Fill out Pcard request with cost and details of order and submit by email to supervisor for approval as described in 2.2.3.

4. ADDITIONAL MATERIALS

4.1. Order any additional reagents if a marker requires a unique master mix, purification kit, or bacterial culture/genomic DNA standard. Use published methods to determine if any new materials are needed.

Appendix of Significant Changes

07/29/2024 Added detailed information to Sections 2.1., 2.2 and 2.3.

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