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 primer/probes together as a set. Primers should be dry and salt free, and probes should be dry and HPLC purified.

2.1.1. Primers and probes are typically ordered at a 1 µmol scale but can be ordered in smaller quantities for testing a new marker or for markers that are used infrequently or larger quantities if available.

2.1.2. Probes are ordered with the 5' reporter dye listed in the publication and the same or comparable quencher. All current probes use the 5' 6-FAM dye. FAM or fluorescein amidite is a dye used in synthesis for the detection of oligos. The quencher for each probe will determine which company to order from. Most 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. These are ordered along with their corresponding primers from Eurofins Genomics.

2.1.3. After an order request has been submitted to the supervisor for approval and administrative assistant for ordering, add the requested reagents to the Inventory List Excel file under the tab 'To Order" found in: \\FLDEP1\DEAR\Labs\Lab5\Biology\MolecularBiology\Administrative\Orders\

2.2. 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 X:\Labs\Lab5\Biology\MolecularBiology\Administrative\Orders\Pcard orders.

2.2.1. On the website www.idtdna.com under Products & Services select Custom DNA oligos to order primers. Order single-stranded DNA oligos in tubes. Add in the primer name and sequence. Choose the desired scale, typically 250 nmol or 1 µmol depending on frequency of use. For example, 250 nmol might be ordered for infrequently used reagents such as CowM2 and HumM2 markers. Select Formulation: None and Purification: Standard Desalting. No modifications or mixed bases are needed. Add primers to the cart.

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2.2.2. To order the probe, under Products & Services select Custom qPCR probes and choose PrimeTime qPCR Probes. Add in the probe name and sequence. Choose the desired scale (250 nmol or 1 µmol). Select 5' 6-FAM as the 5' Dye and ZEN-3' Iowa Black FQ as the 3' Quencher. The default purification method is HPLC and no additional services or reagents are needed. Add to cart. Refer to the published method for each marker for sequence information. Double check to ensure all sequences are entered correctly.

2.2.3. Once cart is filled, email cart to analyst. Fill out 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. Make sure to forward IDT cart to administrative assistant for ordering using DEP account and Pcard.

2.3. 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. On the Eurofins Genomics website www.eurofinsgenomics.com select Order Now Oligo Synthesis on the right side of the screen to order primers. Select Custom DNA Oligos then Order DNA in Tubes. Choose the number of oligos and enter the name and sequence. No modifications are needed. Select purification: Salt Free and Scale, either 250 nmol or 1 µmol. Under Normalization & Delivery, select None-Ship entire yield dry. Add primers to cart.

2.3.2. To order the probe, select Order Now Oligo Synthesis from the main screen. Select qPCR Probes then Order MGB Probes. Choose the number of MGB probes and enter the name and sequence. Select 6-FAM-MGB-Eclipse Quencher under Dye-Quencher Pair. Choose the Quantity delivered, either 20 or 40 nmol. The default purification method is HPLC. Add to cart. Refer to the published method for each marker for sequence information. Double check to ensure all sequences are entered correctly.

2.3.3. Once 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, then to the administrative assistant. After approval, the administrative assistant can place the order using DEP account and Pcard. The cart cannot be emailed so items must be entered manually by the analyst 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. Forward cart to administrative assistant for ordering using DEP account and Pcard.

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

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