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IDT Primer and gBlock Hydration and Aliquots SOP

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We use this protocol and it's working

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Abstract

This protocol describes the correct procedures on how to order primers, probes, and gBlocks; how to prepare them to a 100µM concentration; and how to aliquot them for future PCR applications.

Image Attribution

Stock Photo

Guidelines

Scope:

Primers are short, single strands of DNA (Deoxyribonucleic Acid) or RNA (Ribonucleic Acid), typically ranging from 18-22 bases that are used to initiate DNA synthesis. Primers are used because of their temperature stability in applications such as DNA sequencing and polymerase chain reaction (PCR). Probes are small sequences of DNA or RNA labeled with a reporter molecule (typically a fluorescent dye) used to detect the presence of a very specific DNA or RNA fragment.

PCR is a technique that enables researchers to rapidly and efficiently amplify (copy) a specific region of DNA or RNA into millions of copies. Primers are designed to be complementary to the beginning and end of the target sequence that will be amplified. In a PCR, it is the primers that dictate exactly what sequence of DNA gets copied. Primers are designed as pairs (forward and reverse) and enable DNA polymerase to attach and extend the new sequence. Probes, in conjunction with primers, are used for the detection of specific fragments in quantitative PCR (qPCR) reactions. Primers and probes can be designed using software and/or using known DNA sequences.

gBlocks Gene Fragments are double-stranded DNA fragments between 125-3000bp in length, designed for easy gene construction or modification. The gBlocks contain the primers (forward and reverse complement) of the gene of interest and serve as a synthetic positive control. gBlock sequences can either be obtained from literature research articles, or the NCBI (National Center for Biotechnology Information) nucleotide database.

NCBI website: **https://www.ncbi.nlm.nih.gov/**
IDT website: **Integrated DNA Technologies | IDT**

Responsibility:

This SOP applies to all staff members and students. These individuals must be knowledgeable about the requirements set forth within this document. The lab manager or designee shall ensure that all staff and students know the proper techniques.

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Appendix C - Calculations

To make a 10µM dilution:

V1∗C1 = V2∗C2

V1*100µM = 30uL*10µM
V1*100µM = 300uL*µM
V1=300µL*µM / 100µM
V1 = 3µL

Example: Make 30µL of the 10µM stock

27µL of molecular grade water
+ 3µL of the 100µM stock
30µL of the 10µM working stock

Amount Delivered to reach concentration of 10ng/µL:

V1*C1 = V2*C2
1µL * 500ng = V2 * 10ng/µL
500ng*µL/10ng = V2
50µL = V2

Manual Calculations:

New calculations will need to be done every time a gBlock is ordered

	A	B
	gBlock	**# of base pairs**
	GB3-sul1	344 bp
	GB4-intI1	494 bp
	GB5-erm(B)	370 bp
	GB6-ctxm32	296 bp
	GB7 -16S, sul1, intI1	438 bp
	GB8-bla-ctxm1, ermB	461 bp

1. To calculate copy number / µL of the gBlock stock tube

$(C)(M)(1 \times 10^{-15}$ mol/fmol$)(6.023 \times 10^{23}) =$ copy number/µL
C & M will come from the Properties section of the IDT form
C: current concentration of the gBlock Gene Fragment in ng/µL
(Should be the 10ng/µL from the newly hydrated gBlock)
M: molecular weight in fmol/ng

--Example: GB3-sul (from gBlock Specification Sheet - Appendix A, Step 44)

$(10\ ng/\mu L)(4.71\ fmol/ng)(1 \times 10^{-15}\ mol/fmol)(6.023 \times 10^{23}\ copies/mol) = 2.835 \times 10^{10}\ copies/\mu L$
$(1\ ng/\mu L)(4.71\ fmol/ng)(1 \times 10^{-15}\ mol/fmol)(6.023 \times 10^{23}\ copies/mol) =$ **2.835×10^{9} copies/µL**

2. To calculate concentration of gBlock to make 10^8 copies/5µL

$C_1V_1 = C_2V_2$
2.835×10^{9} copies/µL x V_1 = 5000 µL x (10^8/5µL) = **35.27 µL**

3. Add **35.27 µL** of gBlock stock to 4,964.73 µL TE buffer to create 5mL of 10^8 copies/5µL. Wet the pipette tip 5x before pipetting.

4. Wet pipette tip 5x, then aliquot the 10^8 copies/5µL standard into five 1mL tubes to keep as working stock to make serial dilutions. Clearly label and date.
- Can further aliquot 155µL into 0.2mL PCR strip tubes to prevent continual freeze/thaw

Materials

- Aerosol barrier no retention pipette tips (various volumes 1-1000µl)
- Pipettes for various volumes from 1-1000µl
- Microcentrifuge (Heraus Pico 21 Centrifuge, Thermo-Scientific, Waltham, MA)
- Fine-tip Sharpie Markers (black, blue, red ink)
- Cryo round labels (T-SPOTS, Microtube Tough-Spots, Dedham, MA)
- Vortex (Vortex Genie, Scientific Industries, Bohemia, NY)
- Molecular Biology Grade Water **HyClone Catalog #**SH30538.01
- Tris-EDTA buffer solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**93283-100ML
- 8-strip 0.2mL PCR tubes (T320-2N, Simport Scientific, Quebec, Canada)
- CRYOELITE 2ML **Andwin Scientific Catalog #**02-912-729
- Sterile Nitrile Examination Gloves
- Primers and Probes (Integrated DNA Technology, Coralville, IA)
- gBlocks (Integrated DNA Technology, Coralville, IA)
- Freezer boxes (71001-642, Wilson Dependable Services, Washington D.C.)
- -20°C Freezer
- 4°C Refrigerator
- 50°C Incubator (or dry/water bath)

Safety warnings

Wear proper personal protective equipment (PPE) at all times when handling DNA: sterile nitrile gloves and lab coat.

gBlocks must be hydrated in another space from where you set-up PCR reactions.

Before start

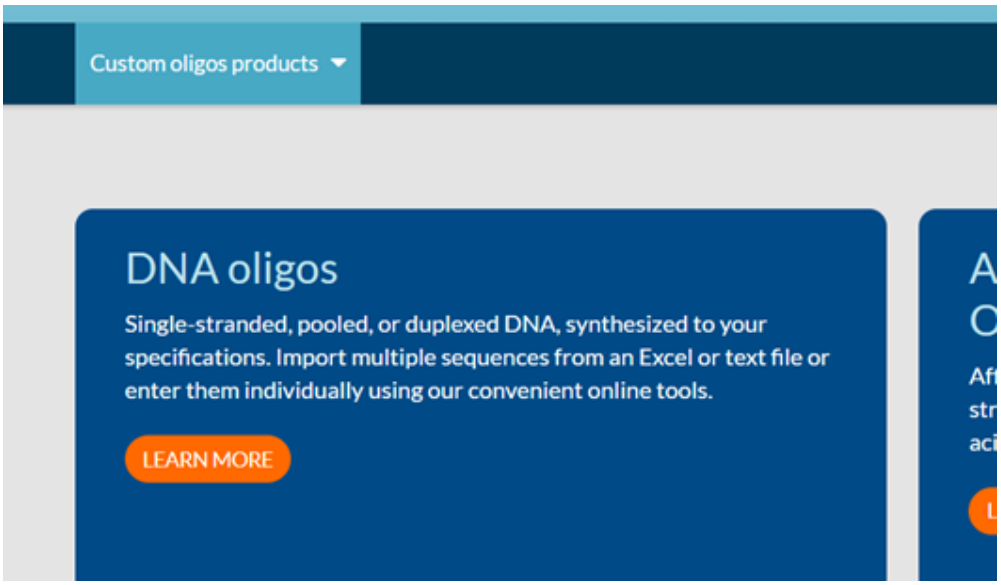
gBlocks must be hydrated in another space from where PCR reactions are set-up.

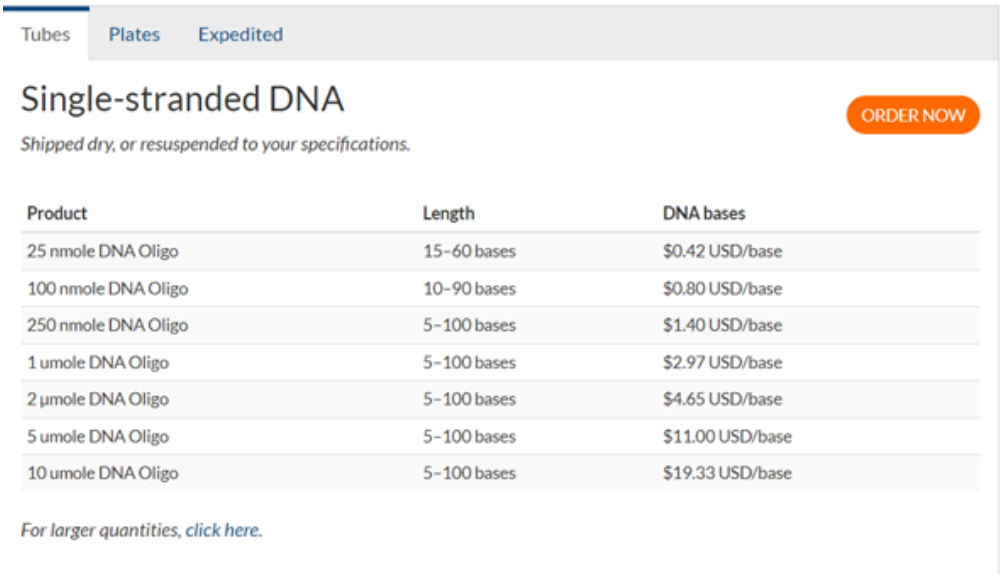
Procedure - Ordering Primers

1 Navigate to the Integrated DNA Technologies (**IDT**) website (**www.idtdna.com)**

2 In the menu under 'Products and Services' > 'DNA & RNA' select 'Custom DNA Oligos' > 'DNA Oligos.' Then 'Order Now.'

↓





Tubes Plates Expedited

Single-stranded DNA

ORDER NOW

Shipped dry, or resuspended to your specifications.

Product	Length	DNA bases
25 nmole DNA Oligo	15–60 bases	$0.42 USD/base
100 nmole DNA Oligo	10–90 bases	$0.80 USD/base
250 nmole DNA Oligo	5–100 bases	$1.40 USD/base
1 umole DNA Oligo	5–100 bases	$2.97 USD/base
2 µmole DNA Oligo	5–100 bases	$4.65 USD/base
5 umole DNA Oligo	5–100 bases	$11.00 USD/base
10 umole DNA Oligo	5–100 bases	$19.33 USD/base

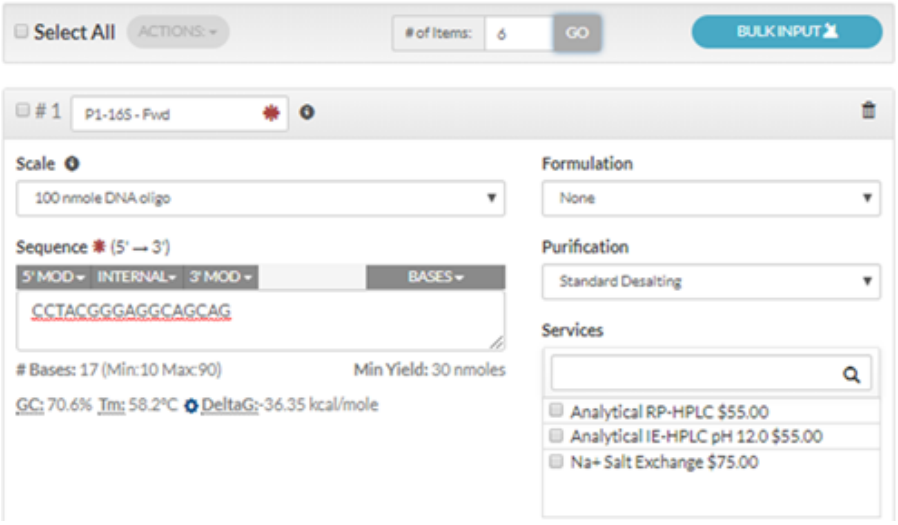
For larger quantities, click here.

3 Insert the information of the primers of interest (name, scale, sequence 5′-3′, formulation, purification, etc. (Name: **include primer lab ID #, gene name, and 'Fwd' or 'Rvs'**),

Example: LDP1-16S-Fwd, 100 nmol DNA oligo, CCTACGGGAGGCAGCAG, No Formulation, Standard Desalting.

Note

When inputting the sequence, be mindful not to have gaps or spaces between the nucleotide bases.

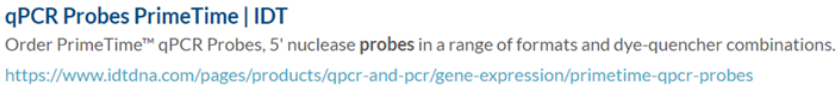


4 When ready, 'Check out.' The order should arrive in the mail within a few days.

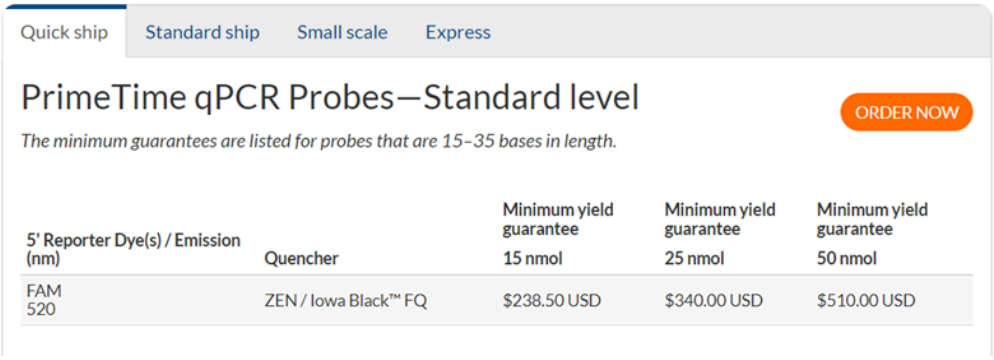
Procedure – Ordering Probes

5 Navigate to the IDT website (**www.idtdna.com**).

6 Search for 'PrimeTime Probes'.



7 The current lab probes use the FAM reporter dye and ZEN/Iowa Black Quencher.

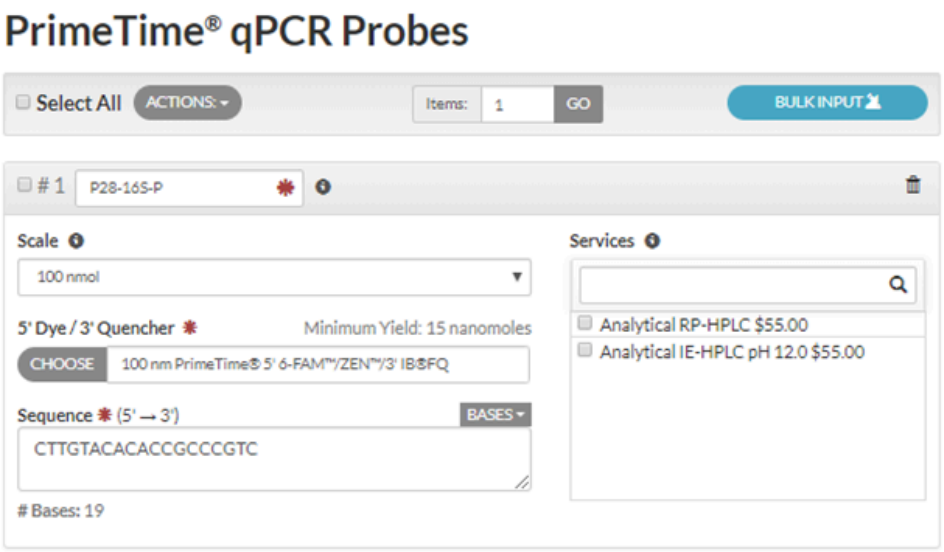


8 Input the ordering information: Name of probe (include primer lab ID #, gene name, and 'P' for probe), scale, type of dye/quencher, sequence.

Example: 16S: Name - **P28-16S-P** ; Scale – 100nmole ; Dye/Quencher - 5' 6-FAM/ZEN/3'IBFQ ; Sequence - CTTGTACACACCGCCCGTC

Note

When inputting the sequence, be mindful not to have gaps or spaces between the nucleotide bases.



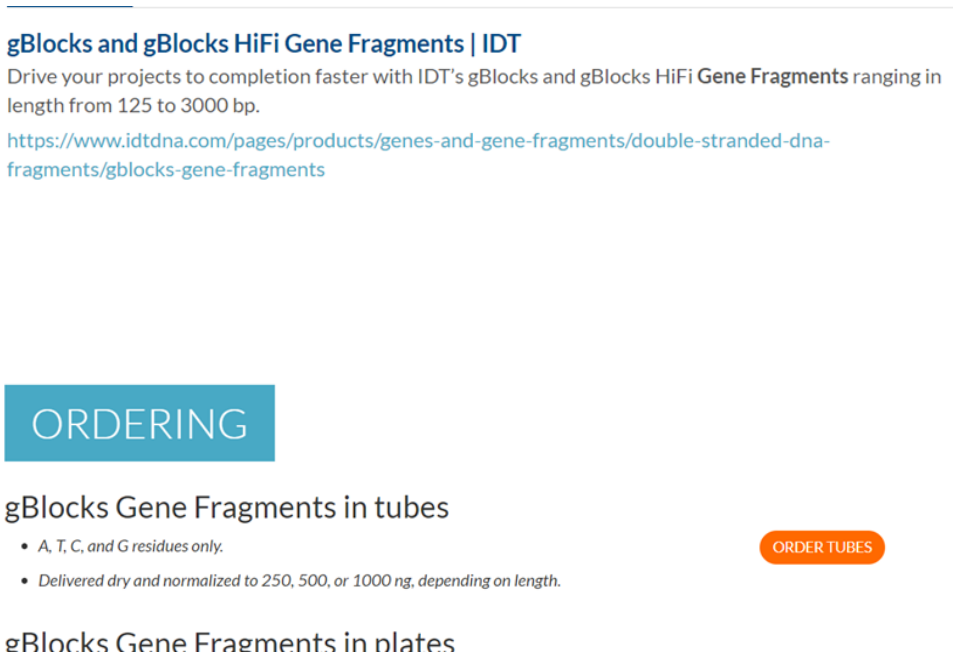
9 Check inventory first, but if low or out, order additional **PrimeTime Gene Expression Master Mix** (25mL, cat # 1055771). The PrimeTime mix contains a hot-start antibody, Taq polymerase, and other components needed for probe-based qPCR

10 When ready, 'Check out.' The order should arrive in the mail within a few days.

Procedure – Ordering gBlocks

11 Navigate to the IDT website (**www.idtdna.com**).

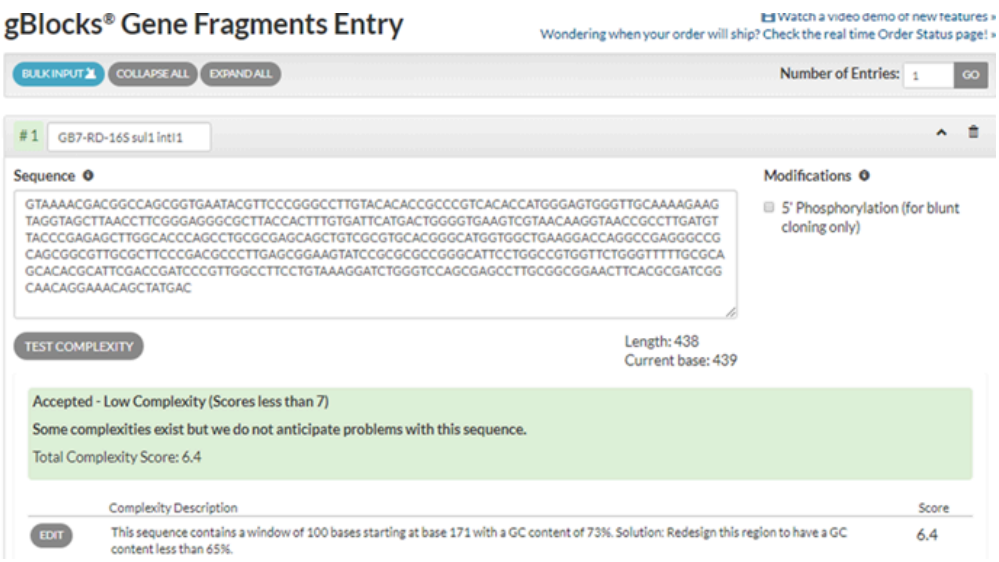
12 Search for 'gBlocks Gene Fragments,' then 'Order Tubes.'



13 Fill in the required name and sequence information. Then test the complexity of the sequence. This will let you know if there are any problems with the sequence and any suggestions on how to improve it.

Note

When inputting the sequence, be mindful not to have gaps or spaces between the nucleotide bases.



14 When ready, 'Check out.' The order should arrive in the mail within a few days.

Procedure – Preparation and Aliquots – Primers and Probes

21m

15

Note

Precise pipetting is important for all transfers.

Safety information

At all stages of this process, wear Sterile Nitrile Examination Gloves to avoid potential contamination.

16 Color Coding: Forward primer = **Blue**, Reverse Primer = **Black**, Probe = **Red**

17 When IDT packages arrive in the mail open the package containing the dry, lyophilized product and store at -20 °C until they need to be prepared for any project that requires them.

- With a sharpie, label the top of each tube with its ID, and write the received date and your initials on the side of each tube.

18 Before starting any DNA work for a project, remove the primers (or probe) from the freezer (-20 °C).

19 Centrifuge the tubes to get the dry flakes to the bottom.

20 Using the specification sheet (**Appendix A** go to step #44), look for the “Amount of Oligo” section (outlined in the red box).

21 Each specification sheet will indicate a different amount of molecular biology grade water that will be needed to produce primers (or probe) at a [M] 100 micromolar (µM) concentration.

22 Pipette the amount needed to produce the [M] 100 micromolar (µM) primers (or probe).

- Using a round, colored cryo label, label the top of each tube with the ID (ie P28-16S-Fwd) and color coding listed above in go to step #16 . This will be an indication that the tube has been hydrated.

23 Vortex the primers (or probe) for 00:00:30, and centrifuge briefly. 30s

24 Let the primers (or probe) hydrate for at least 00:20:00 (or Overnight) in the refrigerator. 20m

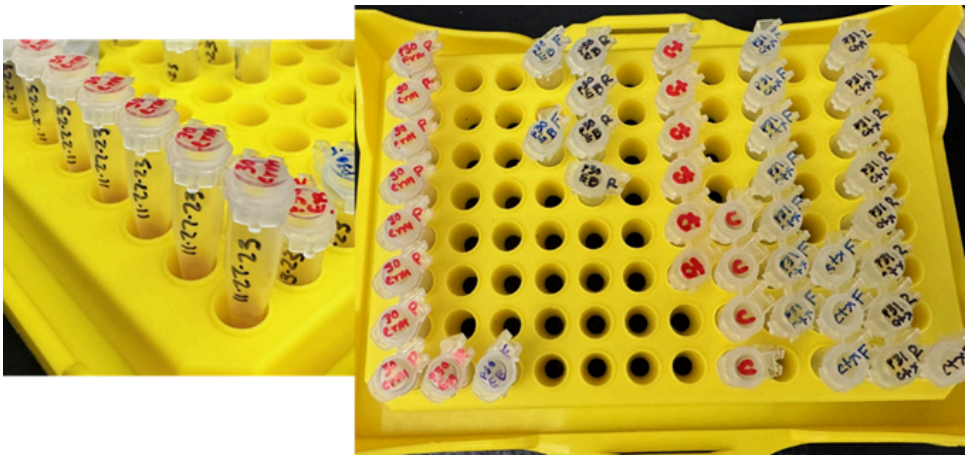
- The hydrated tube will be the **Stock tube**.
- Dilutions and aliquots will be the **Working Stock.**

25 When ready to use, vortex the Stock Tube (00:00:30) and centrifuge again before use. 30s

- The 4GU, 4GM, Tet, and invA assays use the [M] 100 micromolar (µM) concentration, so proceed to go to step #27 for aliquots.

26 The RD Probe-based qPCR uses 10µM concentrations. See **[Appendix C in the Guidelines tab]** for the calculation steps and equations to make 30µl aliquots, then follow go to step #27 - go to step #28 .

27 Label a PCR tube with a recognizable and unique identifier. Date the side of the tube. (See color coding in go to step #16).



28 Aliquot 30 µL of primer (or probe) into each PCR tube and prepare 16 tubes worth of aliquots at a time.

29 Seal the PCR tubes containing the primer (or probe) aliquots.

30 For Sample DNA Product: To make a working stock dilution of sample DNA that delivers at 1µl/rxn at a 5 µL volume: create a dilution at the desired volume in increments of 1 µL DNA + 4 µL PCR water.

30.1 **Example:** for 25 µL of working stock sample DNA, mix 5 µL DNA with 20 µL PCR water.

31 Place the aliquots into a labeled freezer box.

Note

Note: The labeling used for the freezer box should be specific to a given project or genes of interest.

32 Store the labeled freezer box of primer, probe, or sample aliquots at -20 °C .

33 Anytime a set of primers (or probe) is needed for an experiment, just pull one tube of required Forward and Reverse primers and/or probe from the freezer and thaw for use. Keep in a cold-block to keep cool while using.

Note

Use a razor to cut between the tubes – **DO NOT tear the tube off** – can cause breaks in the tube, or adjacent tube, leaving an open hole and leading to evaporation.

34 Discard any unused primer (or probe) from the pulled and thawed aliquot to avoid contamination of the reserve.

Procedure – Preparation and aliquots – gBlocks

8h 20m 5s

35

Safety information

gBlocks MUST BE HYDRATED IN A SEPARATE LAB to avoid contamination.

gBlocks must be hydrated in another space from where you set-up PCR reactions.

36

Note

Precise pipetting is important for all transfers.

Safety information

At all stages of this process, wear Sterile Nitrile Examination Gloves to avoid potential contamination.

37 When IDT packages arrive in the mail open the package containing the dry, lyophilized gBlock and store at -20 °C until they need to be prepared for any project that requires them.

- With a sharpie, label the top of each tube with its ID, and write the received date and your initials on the side of each tube.

38 Before starting any DNA work for a project, remove the gBlocks from the freezer (-20 °C).

39 Centrifuge the tubes for 00:00:03 - 00:00:05 at a minimum of 3000 x g to ensure the material is at the bottom of the tube. 5s

40 gBlocks need to be at a concentration of 10ng/µL.

- Using the specification sheet (See **Appendix A** go to step #45), look for the "Amount Delivered" in the 'Properties' section (outlined in the red box) stating how much dsDNA is in the tube.
- In the specification sheet example the starting amount is 500 ng , 50 µL will be added to get 10 ng/µL **(See Appendix C for the steps and equations in the Guidelines tab)**.

41 Wet the pipette tip 5x, and add Tris-EDTA (TE) to reach a final concentration of 10 ng/µL .

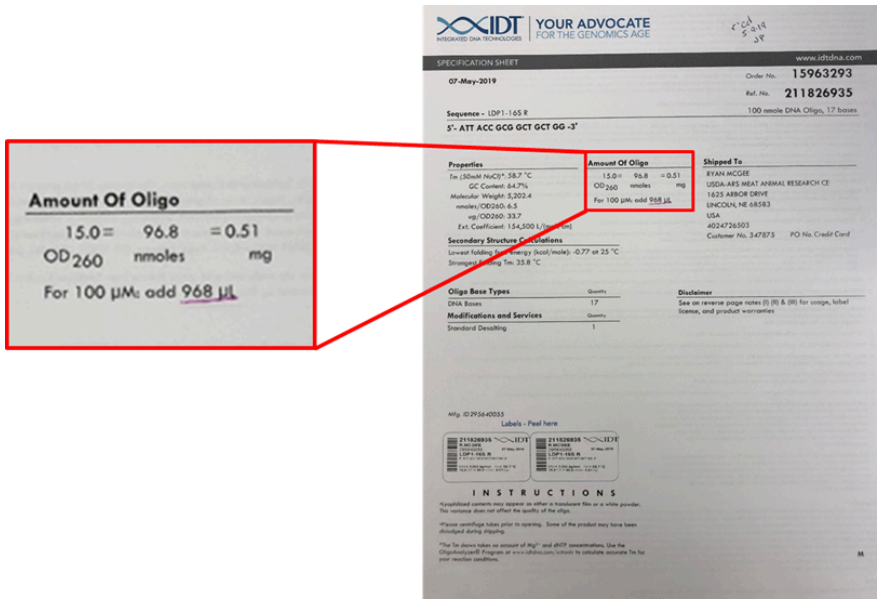
42 Vortex the tube, and incubate at 50 °C for 00:20:00 (can use a dry bath or water bath). 20m

43 Briefly vortex again, and centrifuge. Leave Overnight at 4 °C before aliquoting (See **Appendix B** go to step #46 for diluting to copy number and standards). 8h

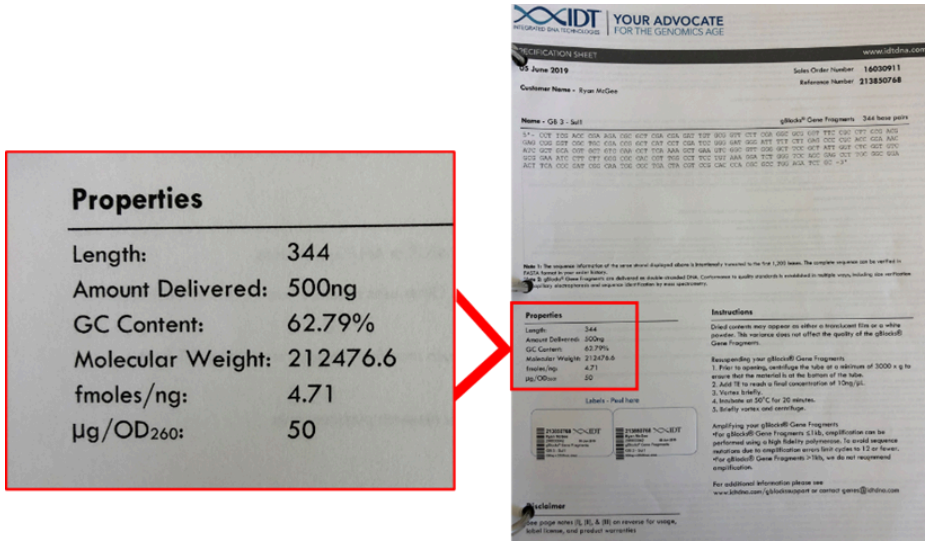
- The hydrated tube will be the **Stock tube**.
- Dilutions and aliquots will be the **Working Stock**.

Appendix A – Specification Sheet Examples

44 **Primer Specification Sheet**



45 **gBlock Specification Sheet**



Appendix B - gBlock dilute to copy number (10^1-10^8 copies) 15m 5s

46 **Serial Dilutions for standard curve** 15m

Label 0.2mL strip tubes (10^8 to 10^1) and UV for 00:15:00 .

47 Wet the pipette tip, and transfer 45 µL of TE into the tubes labeled 10^7 – 10^1 (this can be done in the biosafety hood), then spin briefly

48 Using the freezer stock of the 10^8 copies/5µl gBlock standard created. 5s

1. Wet pipette tip 5x, and transfer 50 µL of the 10^8 copies/5µl standard into the first tube labeled 10^8.
2. Continuing with the first strip tube, wet pipette tip 5x, and transfer 5 µL into the 10^7 tube. Then rinse the tip in the diluent 5x to expel all DNA. Vortex for 00:00:05 , spin briefly, and repeat steps for all remaining tubes to the 10^1. (Do not push on the bottom of the tube with pipette tip).

49 Keep fresh at 4 °C for one week, or freeze immediately for longer storage.

Protocol references

Dungan, R.S., C.W. McKinney, A.B. Leytem. 2018. Tracking antibiotic resistance genes in soil irrigated with dairy wastewater. *Science of the Total Environment* 635"1477-1483.

IDT website: **Integrated DNA Technologies | IDT**

NCBI website: **https://www.ncbi.nlm.nih.gov/**