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IDT Primer and gBlock Hydration and Aliquots SOP

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Justine C. Condon¹, Lisa M. Durso¹

¹USDA-ARS



Justine C. Condon

USDA-ARS

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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 100 μ M 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 10 μ M dilution:

$$V1 \times C1 = V2 \times C2$$

$$V1 \times 100\mu\text{M} = 30\mu\text{L} \times 10\mu\text{M}$$

$$V1 \times 100\mu\text{M} = 300\mu\text{L} \times \mu\text{M}$$

$$V1 = 300\mu\text{L} \times \mu\text{M} / 100\mu\text{M}$$

$$V1 = 3\mu\text{L}$$

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:

$$V1 \times C1 = V2 \times C2$$

$$1\mu\text{L} \times 500\text{ng} = V2 \times 10\text{ng}/\mu\text{L}$$

$$500\text{ng} \times \mu\text{L} / 10\text{ng} = V2$$

$$50\mu\text{L} = V2$$

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-int11	494 bp
	GB5-erm(B)	370 bp
	GB6-ctxm32	296 bp
	GB7 -16S, sul1, int11	438 bp
	GB8-bla-ctxm1, ermB	461 bp

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

$$(C)(M)(1 \times 10^{-15} \text{ mol/fmol})(6.023 \times 10^{23}) = \text{copy number}/\mu\text{L}$$

****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)

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

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

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

$$C_1V_1 = C_2V_2$$

$$\mathbf{2.835 \times 10^9 \text{ copies/}\mu\text{L}} \times V_1 = 5000 \mu\text{L} \times (10^8/5\mu\text{L}) = \mathbf{35.27 \mu\text{L}}$$

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

4. Wet pipette tip 5x, then aliquot the 10^8 copies/5 μ L standard into five 1mL tubes to keep as working stock to make serial dilutions. Clearly label and date.

- Can further aliquot 155 μ L 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.'

**PRODUCTS & SERVICES** ▴**APPLICATIONS & TECHNOLOGIES****DNA & RNA**

Custom DNA oligos

Custom RNA oligos

Affinity Plus DNA & RNA oligos

DNA oligo pools

GENES & GENE FRAGMENTATION

Double-stranded DNA frag

Single-stranded DNA frag

Custom gene synthesis

DNA Origami



Custom oligos products ▾

DNA oligos

Single-stranded, pooled, or duplexed DNA, synthesized to your specifications. Import multiple sequences from an Excel or text file or enter them individually using our convenient online tools.

[LEARN MORE](#)



[Tubes](#) [Plates](#) [Expedited](#)

Single-stranded DNA

Shipped dry, or resuspended to your specifications.

[ORDER NOW](#)

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.



Select All ACTIONS: # of Items: GO BULK INPUT

1 P1-165 - Fwd

Scale 100 nmole DNA oligo

Sequence (5' → 3') 5' MOD INTERNAL 3' MOD BASES CCTACGGGAGGCAGCAG

Bases: 17 (Min:10 Max:90) Min Yield: 30 nmoles

GC: 70.6% Tm: 58.2°C DeltaG: -36.35 kcal/mole

Formulation None

Purification Standard Desalting

Services

- Analytical RP-HPLC \$55.00
- Analytical IE-HPLC pH 12.0 \$55.00
- Na+ Salt Exchange \$75.00

- 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'.

qPCR Probes PrimeTime | IDT

Order PrimeTime™ qPCR Probes, 5' nuclease probes in a range of formats and dye-quencher combinations.

<https://www.idtdna.com/pages/products/qpcr-and-pcr/gene-expression/primetime-qpcr-probes>

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



Quick ship Standard ship Small scale Express				
PrimeTime qPCR Probes—Standard level				
The minimum guarantees are listed for probes that are 15–35 bases in length.				
5' Reporter Dye(s) / Emission (nm)	Quencher	Minimum yield guarantee 15 nmol	Minimum yield guarantee 25 nmol	Minimum yield guarantee 50 nmol
FAM 520	ZEN / Iowa Black™ FQ	\$238.50 USD	\$340.00 USD	\$510.00 USD

[ORDER NOW](#)

- 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.

PrimeTime® qPCR Probes

☐ Select All ACTIONS: ▾ Items: 1 GO [BULK INPUT](#)

1 P28-16S-P

Scale ⓘ
100 nmol

5' Dye / 3' Quencher ⓘ Minimum Yield: 15 nanomoles
CHOOSE 100 nm PrimeTime® 5' 6-FAM™/ZEN™/3' IBFQ

Sequence ⓘ (5' → 3') BASES ▾
CTTGTACACACCGCCCGTC

Bases: 19

Services ⓘ

☐ Analytical RP-HPLC \$55.00
☐ Analytical IE-HPLC pH 12.0 \$55.00



- 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.'

gBlocks and gBlocks HiFi Gene Fragments | IDT

Drive your projects to completion faster with IDT's gBlocks and gBlocks HiFi Gene Fragments ranging in length from 125 to 3000 bp.

<https://www.idtdna.com/pages/products/genes-and-gene-fragments/double-stranded-dna-fragments/gblocks-gene-fragments>

ORDERING

gBlocks Gene Fragments in tubes

- A, T, C, and G residues only.
- Delivered dry and normalized to 250, 500, or 1000 ng, depending on length.

ORDER TUBES

gBlocks Gene Fragments in plates

- 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.

gBlocks® Gene Fragments Entry Watch a video demo or new features »
 Wondering when your order will ship? Check the real time Order Status page! »

BULK INPUT COLLAPSE ALL EXPAND ALL Number of Entries: 1 GO

#1 GB7-RD-16S sul1 int1

Sequence

```
GTAAACGACGGCCAGCGGTGAATACGTTCCCGGCCCTGTACACACCGCCCGTCACACCATGGGAGTGGGTGCAAAAGAAG
TAGGTAGCTTAACCTTCGGGAGGGCGCTTACCACCTTTGTGATTCATGACTGGGGTGAAGTCGTAACAAGTAACCGCTTGATGT
TACCCGAGAGCTTGGCACCCAGCCTGCGGAGCAGCTGTCGCTGCACGGGCATGGTGGCTGAAGGACCAAGCCGAGGGCCG
CAGCGCGTTCGCTTCCGACGCCCTTGAGCGGAAGTATCCGCGCCGGGCATTCTGGCCGTGTTCTGGGTTTTTGCGCA
GCACACGATTGACCGGATCCCGTTGGCCCTCTGTAAAGGATCTGGGTCCAGCGAGCCTTGGCGCGGAACCTCACGCGATCGG
CAACAGGAAACAGTATGAC
```

Modifications

☐ 5' Phosphorylation (for blunt cloning only)

TEST COMPLEXITY Length: 438
Current base: 439

Accepted - Low Complexity (Scores less than 7)
 Some complexities exist but we do not anticipate problems with this sequence.
 Total Complexity Score: 6.4

Complexity Description	Score
EDIT This sequence contains a window of 100 bases starting at base 171 with a GC content of 73%. Solution: Redesign this region to have a GC content less than 65%.	6.4

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

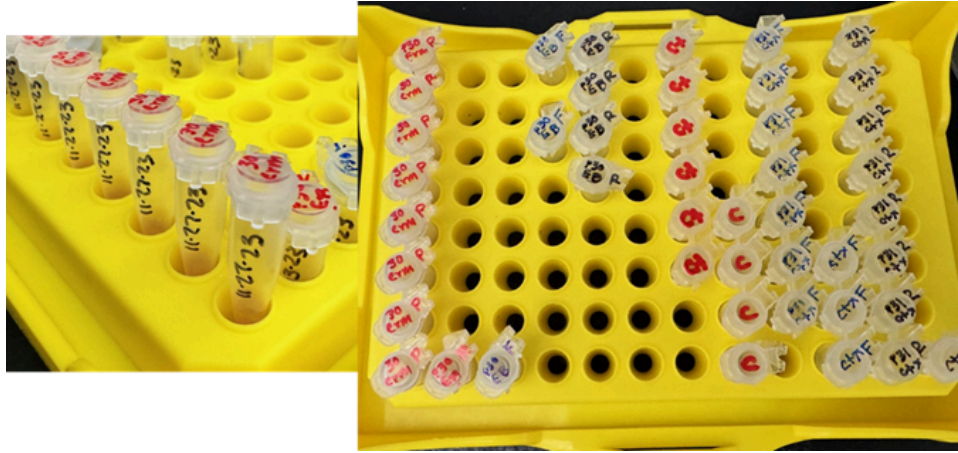
At all stages of this process, wear Sterile Nitrile Examination Gloves to avoid potential contamination.



- 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  100 micromolar (μM) concentration.
- 22 Pipette the amount needed to produce the  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.
- 24 Let the primers (or probe) hydrate for at least 00:20:00 (or Overnight) in the refrigerator.
- 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.
- The 4GU, 4GM, Tet, and invA assays use the 100 micromolar (μM) concentration, so proceed to [go to step #27](#) for aliquots.
- 26 The RD Probe-based qPCR uses 10 μM concentrations. See **[Appendix C in the Guidelines tab]** for the calculation steps and equations to make 30 μl 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 $1\mu\text{l/rxn}$ 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



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/5 μl gBlock standard created.

5s

1. Wet pipette tip 5x, and transfer 50 μL of the 10^8 copies/5 μl 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/>