APTAMARKER PLATFORM

STANDARD OPERATING PROCEDURE APTAMARKER KIT

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KIT INFORMATION

The Aptamarker kit is shipped at room temperature. Upon arrival, all components should be stored at 2-8°C to guarantee maximal shelf life. The kit remains stable at 2-8 °C until the expiration date if stored as specified.

- Expiration date: 2 months from the date of shipment (see on tubes).

KIT CONTENTS AND STORAGE

The Aptamarker kit contains the following:

- Premixed Aptamarkers in solution (called Aptamarker library)
  - 25 samples/tube
  - Storage + 4°C
- Competitor antisense conjugated to resin solution (called Antisense)
  - 25 samples/tube
  - Storage + 4°C

➔ Important note: 3 extra sets of Aptamarker library and competitor antisense are provided as controls per NGS submission lane (called control library) and have to be processed as a sample (Part A: Translation of protein abundance to DNA abundance + Part B: NGS Submission). The maximum number of samples per NGS submission lane is 45 plus the three negative controls for a total of 48 samples.

EQUIPMENTS AND REAGENTS REQUIRED BUT NOT SUPPLIED

1. REGULAR LABORATORY EQUIPMENT

- 2, 1.5 and 0.5 mL sterile microfuge tubes
- Nuclease-free water
- Selection buffer recommended: PBS 10X
- Microcentrifuge
- Tube rotator
- Real-Time qPCR Detection System that acquires data for green channel protocol

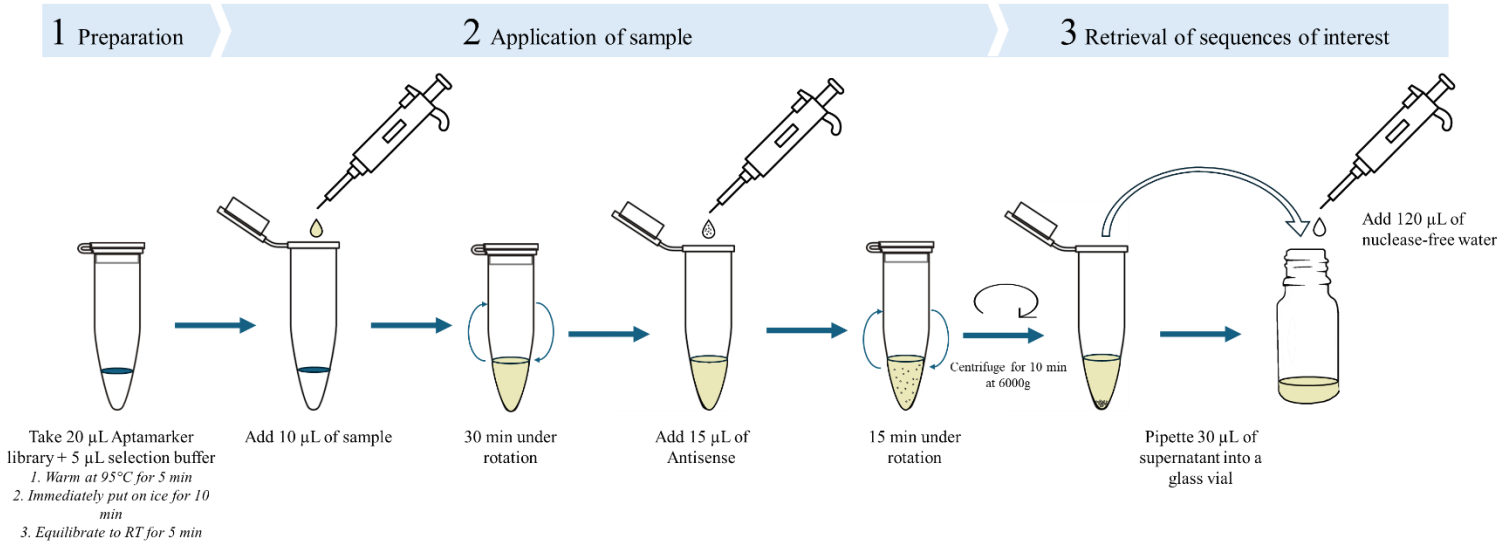
2. EQUIPMENT SPECIFIC TO APTAMARKER KIT

- Silane-treated glass vials and caps: 27029-U and Z188808 (Supelco).
- NGS primer sequences (see instructions and sequences provided in the annex): We recommend ordering primers from Integrated DNA Technologies (IDT) with the following minimum requirement: 25 nmole DNA Oligo with Standard Desalting Purification.
- Cleanup column: GFX™ PCR DNA and Gel Band Purification Kit 28903471 or 28903471 in function of the format ordered (Cytiva) – 1 Column required per NGS submission lane.
- qPCR Master Mix: SsoAdvanced Universal SYBR® Green Supermix: 1725270 to 1725275 (Bio-Rad).

PART A: TRANSLATION OF PROTEIN ABUNDANCE TO DNA ABUNDANCE

➔ Procedures within this section are performed at room temperature.

➔ The sequences recovered at the end of this section may be stored at 4 °C in a silane-treated glass vial for up to 3 months; however, it is preferable to proceed with Part B on the following day.



A.1. PREPARATION OF THE APTAMARKER LIBRARY

- Allow the Aptamarker library to equilibrate at room temperature for 5-10 minutes.
- Pipette, the following into a clean 0.5 mL microfuge tube:
  - 20 µL of Aptamarker library solution.
  - 5 µL of 10X selection buffer.
- Mix well and centrifuge briefly.
- Heat the solution at 95°C for 5 minutes.
- Snap cool the solution directly on ice for 10 minutes to make a temperature shock.
- Equilibrate the solution at room temperature for 5 minutes.

A.2. APPLICATION OF SAMPLE

- Add 10 µL of sample (for control libraries, sample is replaced with water) to the 25 µL of prepared Aptamarker library and buffer.
- Place the tube on a rotator to keep solution agitated. Incubate for 30 minutes.

- Add 15 µL of competitor Antisense resin to each tube containing 35 µL of Aptamarker library, sample and buffer solution.
  - Important: Stock tube should be mixed and vortexed to resuspend resin. Do not centrifuge and mix stock tube between each distribution.

- ❖ Vortex the tube containing library, sample, buffer solution and resin to mix resin. Ensure that the resin is well distributed in the tube. Do not centrifuge.
- ❖ Incubate for 15 minutes on a rotator.

A.3. RETRIEVAL OF SEQUENCES OF INTEREST BINDING TO THE SAMPLE

- ❖ Centrifuge the tube at 6000 x g for 10 minutes to pellet resin.
- ❖ Collect 30 µL of the supernatant and transfer it to clean glass vial. It is important not to disturb the resin at the bottom of the tube while collecting the supernatant.
- ❖ Add 120 µL of Nuclease-free water to the collected supernatant.
- ❖ Store at 4°C and proceed to the next part of the NGS preparation.

PART B: NGS PREPARATION

- ➔ This section proceeds in two steps. The first step is simply to recover a sufficient amount of each library and to add of a specific hex code for identification in the pooled NGS data while the second step is intended to enable equal balancing of the quantity of each library in a pool. All the steps in this section must be completed 48 hours apart, as the results from Step 1 are required to carry out Step 2.
- ➔ The forward primers listed in the Annex all have a different hex code that has been validated for use with this platform. The reverse primer is common for all reactions.
- ➔ Each selected Aptamarker library has to be amplified following processing against samples in order to obtain a sufficient concentration for NGS analysis. We recommend pooling the amplified library from up to 45 samples with the addition of 3 control libraries for each NGS channel processed (NGS submission lane). The pooling of samples requires that each amplified library be identified by a unique hex code for identification in the pooled NGS data.
- ➔ It is important to evaluate the total number of samples processed per project in order to create pools of equal depth for NGS submission lane. For example, if your project involves analysis of 100 samples, we recommend pooling 34 samples in one pool and 33 in the other two pools. In this case you would only need to use a total of 37 hex codes, 34 for pool A and three for the controls. The same hex codes can be used in different pools.

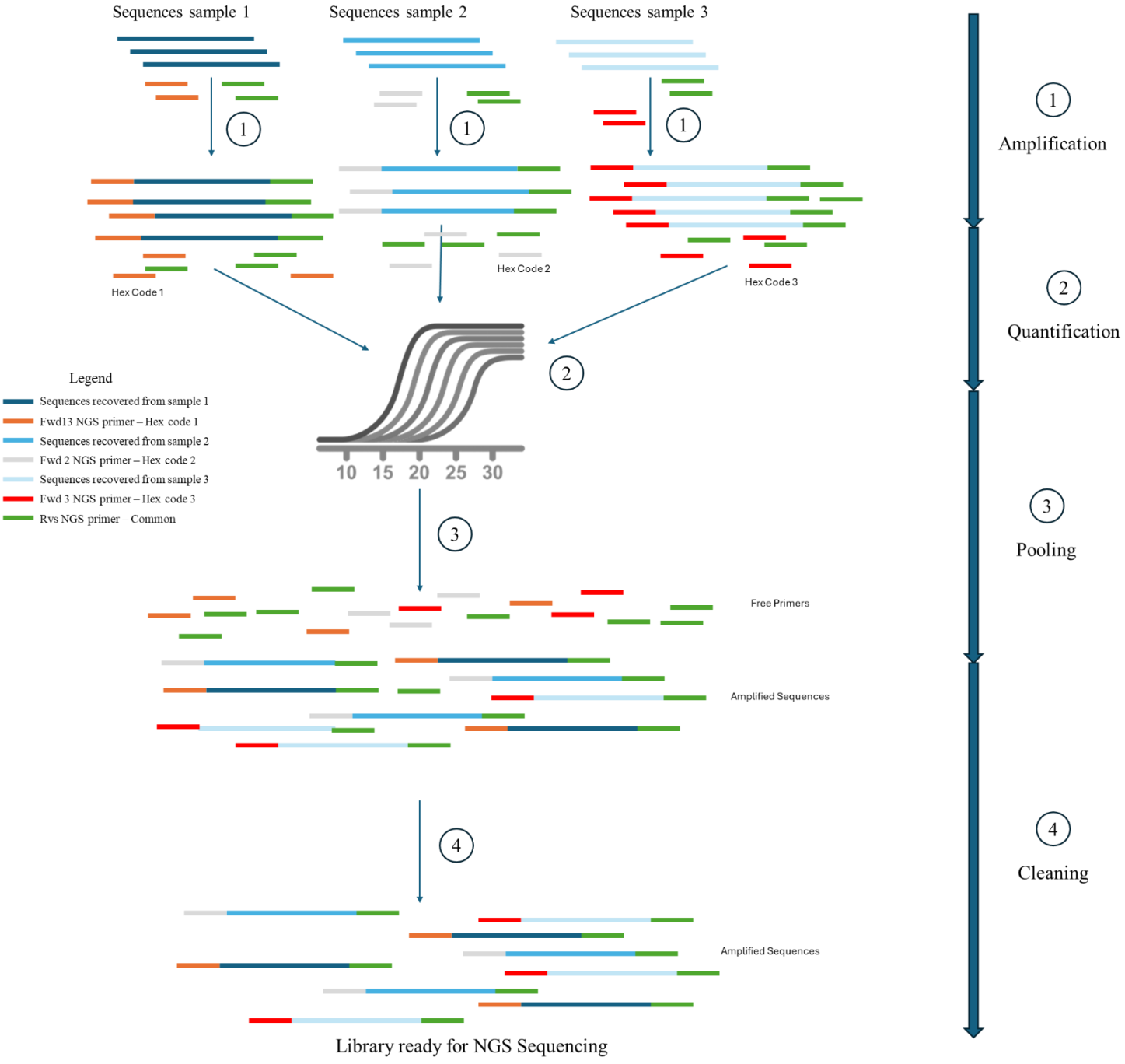
Outline of project plan for 100 samples:

NGS Subissions Lane 1	
Sample 1	Fwd 1
Sample 2	Fwd 2
Sample 3	Fwd 3
…	…
…	…
Sample 33	Fwd 33
Control 1	Fwd 34
Control 2	Fwd 35
Control 3	Fwd 36

NGS Subissions Lane 2	
Sample 34	Fwd 1
Sample 35	Fwd 2
Sample 36	Fwd 3
…	…
…	…
Sample 66	Fwd 33
Control 4	Fwd 34
Control 5	Fwd 35
Control 6	Fwd 36

NGS Subissions Lane 3	
Sample 67	Fwd 1
Sample 68	Fwd 2
Sample 69	Fwd 3
…	…
…	…
Sample 99	Fwd 33
Sample 100	Fwd 34
Control 4	Fwd 35
Control 5	Fwd 36
Control 6	Fwd 37

Example of Samples/Hex codes pairing for 100 samples + 3 control libraries per NGS submission lane.


Sequences sample 1
Sequences sample 2
Sequences sample 3
1
Amplification
Hex Code 1
Hex Code 2
Hex Code 3
2
Quantification
Legend
Sequences recovered from sample 1
Fwd13 NGS primer – Hex code 1
Sequences recovered from sample 2
Fwd 2 NGS primer – Hex code 2
Sequences recovered from sample 3
Fwd 3 NGS primer – Hex code 3
Rvs NGS primer – Common
10 15 20 25 30
3
Pooling
Free Primers
Amplified Sequences
4
Cleaning
Amplified Sequences
Library ready for NGS Sequencing

B.1. AMPLIFICATION OF LIBRARIES

➔ Each sample should be assigned to a different hex code which is included in the forward primer. Establish a record of the hex code assigned to each sample. Reverse primer is common for all reactions.

➔ Prepare a separate master mix for each hex code/sample pair. This step is a critical error-risk point.

- If 30 samples will be processed within a NGS submission pool/lane, 30 master mix have to be prepared, each containing a different forward primer.

Note: To ensure optimal performance, make sure all mix components are thawed and resuspended/homogenized before use.

❖ Prepare (on ice or at room temperature) the qPCR reaction mixture according to the table below.

Component	1 reaction volume	Volume to pipette per sample	Final Concentration
2X qPCR master mix SYBR Green	20 µL	25 µL	1X
10 µM Reverse Primer (common)	1 µL	1.25 µL	0.250 µM
10 µM Forward Primer (specific for each sample)	1 µL	1.25 µL	0.250 µM
Nuclease-free water	8 µL	10 µL	-

❖ Gently mix the qPCR reaction mixture. Centrifuge briefly to spin down the contents.

❖ Pipette 30 µL of the specific qPCR reaction mixture into 1 well of a qPCR plate/PCR tube, according to your experimental plate/strip/tube configuration.

❖ Pipette 10 µL of template (solution obtained in Part A) into each respective well.

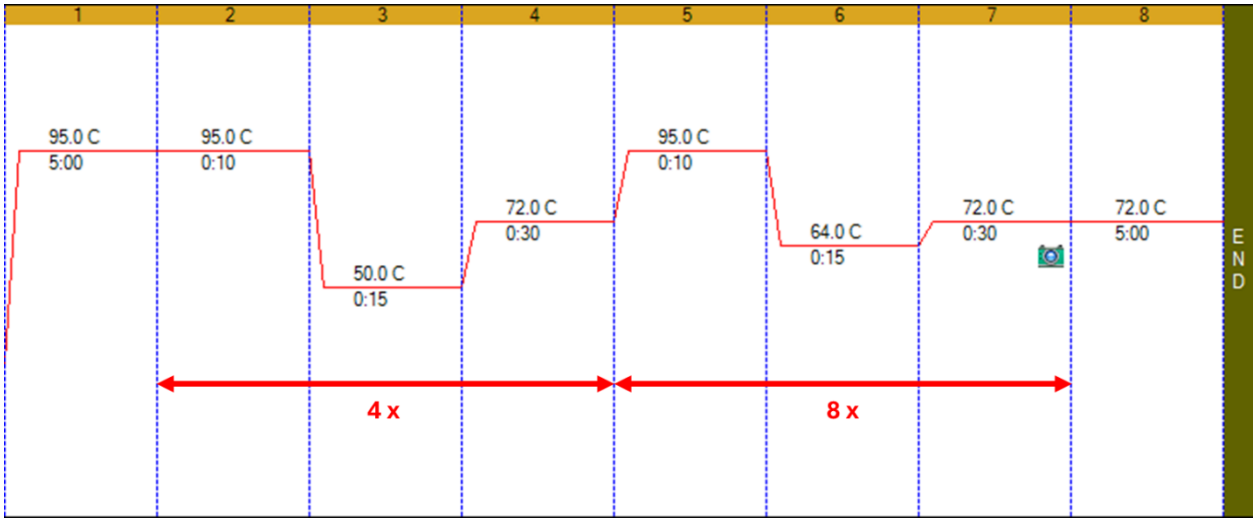
Note: To avoid cross-contamination, we strongly recommend pipetting the template last, preferably in a separate work area.

❖ Seal the plate/strip/tube with appropriate caps or optical adhesive film before proceeding with the real-time qPCR detection steps.

❖ Centrifuge the plate/strip/tube to spin down the contents and eliminate air bubbles.

❖ Place the reaction plate/strip/tube within the real-time qPCR instrument and run the protocol defined below:

STEP	TEMPERATURE	TIME
Polymerase Activation and Initial Denaturation	95°C	5 minutes
4 Cycles	95°C ***50°C*** 72°C	10 seconds 15 seconds 30 seconds
8 Cycles	95°C ***64°C*** 72°C + Plate Read (optional)	10 seconds 15 seconds 30 seconds
Final Extension	72°C	5 minutes
Hold	4-10°C	Infinite



qPCR protocol

❖ Load the PCR tubes or plate onto the real-time qPCR instrument and start the qPCR run with SYBR Green setting/mode.

❖ Once the amplification step is performed, keep tubes/plate stored at 4°C and keep them covered.

- Amplified products will be used for pooling the libraries per NGS submission lane.

B.2. QUANTIFICATION OF AMPLIFIED LIBRARIES FOR POOLING

➔ For Aptamarker use it is necessary that a minimum of 20 million reads per sample be obtained from NGS analysis. To achieve this, it is best if the amount of Aptamarker library per sample is as close to equal within each pool.

➔ For quantification use the same hex code primer for each sample that was used in the first amplification.

❖ Prior to qPCR mix preparation, dilute amplified DNA obtained after amplification step B.1. 1000-fold

- Take 1 µL of amplified DNA and add it to 999 µL of Nuclease-free water. Mix well.

➔ No template control: For verification of the absence of contamination, no template control (without DNA template) must be performed. Make a common qPCR master mix for libraries and no template control, split in 2 and set up 2 runs, one for libraries and one for no template controls. This step must be to be done for all hex codes.

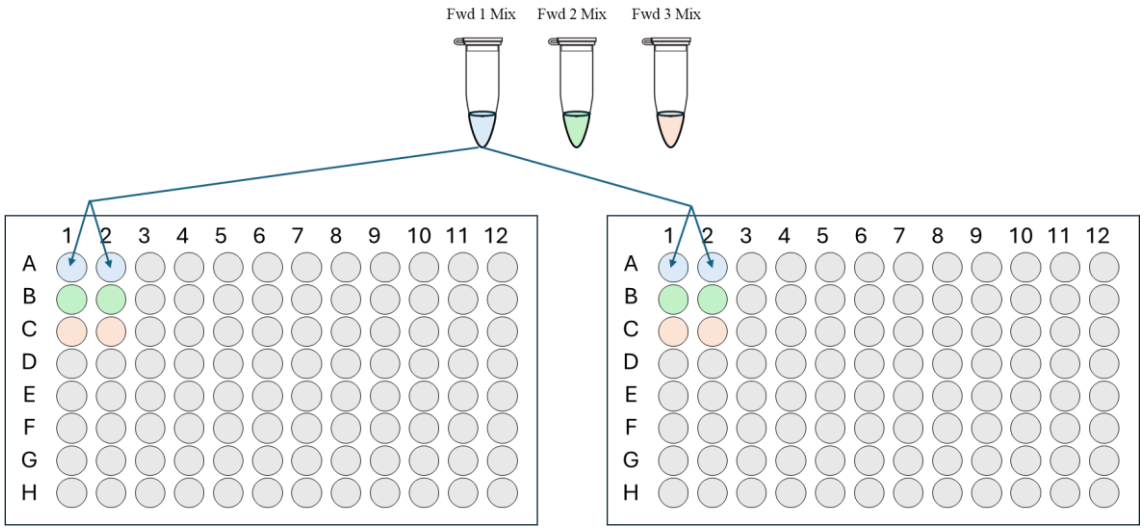
➔ Replicates: It is recommended that at least two replicates are performed per sample and per no template control.

❖ Prepare (on ice or at room temperature) the qPCR reaction mixture according to the table below.

Component	1 reaction volume	Volume to pipette	Final Concentration
2X qPCR master mix SYBR Green	10 µL	55 µL	1X
10 µM Reverse Primer (common)	0.5 µL	2.25 µL	0.250 µM
10 µM Forward Primer (specific for each sample)	0.5 µL	2.25 µL	0.250 µM
Nuclease-free water	4 µL	18 µL	-

❖ Gently mix the qPCR reaction mixture. Centrifuge briefly to spin down the contents.

❖ Pipette 15 µL of the specific qPCR reaction mixture into 2 wells of a qPCR plate/tube, according to your experimental plate/strip/tube configuration. Split in 2 runs for same mix created as shown in picture below:



Run 1 with DNA template from 1000X dilution of amplification step

Run 2 with water for no template control

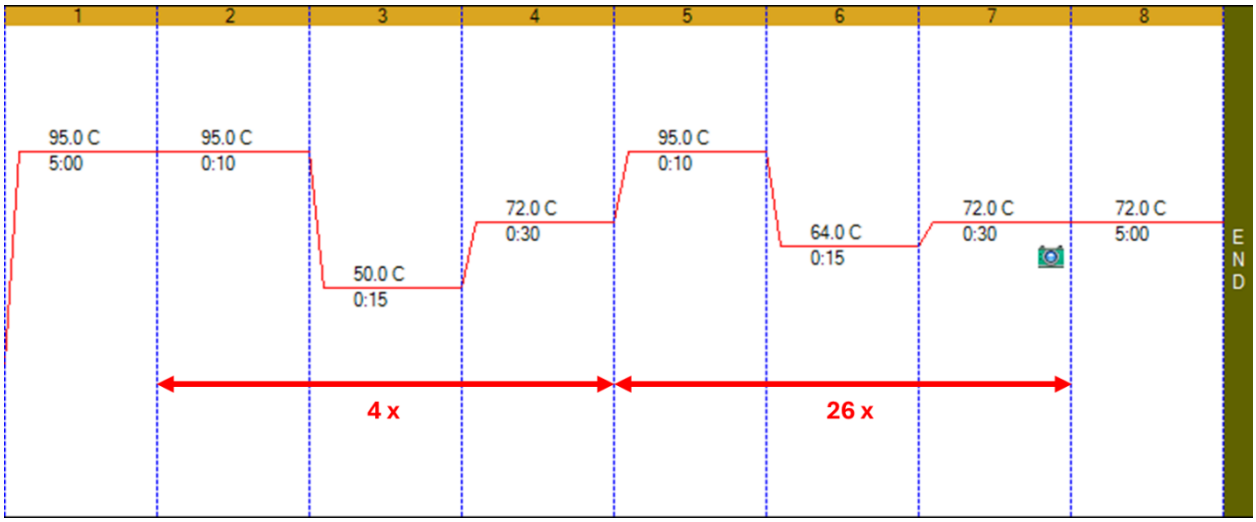
❖ Pipette 5 µL of template (either 1000-fold diluted solution from part B.1. or water for no template control) into each respective well.

Note: To avoid cross-contamination, we strongly recommend pipetting the template last, preferably in a separate work area.

➔ Seal the plate/strip/tube with the appropriate cap or optical adhesive film before proceeding with the real-time qPCR detection.

- Centrifuge the plate/strip/tube to spin down the contents and eliminate air bubbles.
- Place the reaction plate/strip/tube within the real-time PCR instrument and run the protocol defined below:

STEP	TEMPERATURE	TIME
Polymerase Activation and Initial Denaturation	95°C	5 minutes
4 Cycles	95°C ***50°C*** 72°C	10 seconds 15 seconds 30 seconds
26 Cycles	95°C ***64°C*** 72°C + Plate Read	10 seconds 15 seconds 30 seconds
Final Extension	72°C	5 minutes
Hold	4-10°C	Infinite



qPCR protocol

- Load the PCR tubes or plate onto the real-time PCR instrument and start the qPCR run with the SYBR Green setting/mode.

B.3. QPCR ANALYSIS AND SAMPLE POOLING

- Extract the Cq value obtained from automatic threshold of the qPCR software.
- Average the Cq value of replicated sample.
- To obtain an equimolar solution of each sample into a pool (of 48 samples maximum - we recommend 36 for a better read - including 3 controls per NGS submission lane), an equation is applied to averaged Cq obtained from previous qPCR run. The number obtained from this equation correspond to the volume per library, amplified in step B.1., to pipette into a 1.5 mL tube to make the pool for NGS submission lane.
    - if all the averaged Cq values range between 1 and 5, use this equation

$$\frac{10}{18exp^{-0.58779666490Cq}}$$ =10/(18*EXP(-0,5877866649*Cq))

        - Example, for a Cq of 5,256 the subsequent volume to pipette will be

$$\frac{10}{18exp^{-0.58779666490*5.256}} = 12.20\ \mu L$$

    - if all the averaged Cq values range between 4 and 11, use this equation

$$\frac{1}{18exp^{-0.58779666490Cq}}$$ =1/(18*EXP(-0,5877866649*Cq))

        - Example, for a Cq of 5,256 the subsequent volume to pipette will be

$$\frac{1}{18exp^{-0.58779666490*5.256}} = 1.05\ \mu L$$

    - if all the averaged Cq values range between 10 and 16, use this equation

$$\frac{0.05}{18exp^{-0.58779666490Cq}}$$ =0.05/(18*EXP(-0,5877866649*Cq))

        - Example, for a Cq of 11,38 the subsequent volume to pipette will be

$$\frac{0.05}{18exp^{-0.58779666490*11.38}} = 2.23\ \mu L$$

    - if all the averaged Cq values range between 13 and 19, use this equation

$$\frac{0.01}{18exp^{-0.58779666490Cq}}$$ =0.01/(18*EXP(-0,5877866649*Cq))

        - Example, for a Cq of 15,38 the subsequent volume to pipette will be

$$\frac{0.01}{18exp^{-0.58779666490*15.38}} = 4.68\ \mu L$$

B.4. CLEANUP AND PRIMERS REMOVAL

➔ This cleanup step is meant for the concentration and purification of libraries from qPCR reactions for NGS submission lane. This step is performed for each NGS submission lane. Example: if you have 3 NGS submission lanes, perform 3 cleanup reactions.

❖ Clean up the pooled libraries with 1 column per NGS submission lane. Use the GFX™ PCR DNA and Gel Band Purification Kit and follow the protocol for the purification of DNA from solution or an enzymatic reaction from the supplier.

❖ Elute the libraries from the column with 150-200 µL of Nuclease-free water.

PART C: NGS SUBMISSION

Recommendations for NGS submission:

- NextSeq 2000 "P4": 1.8B reads for 1 lane.
    - 1 lane correspond to what was called “NGS submission lane”.
- Low diversity library, 15% PhiX.
- No index reads. Users must inform the sequencing provider of this custom configuration and make sure they can deliver raw FASTQ with the full read for their custom demultiplexing.
- 1 x 50 bp kit with extra cycles (kits have 38 extra bases of reagents, we run 1 x 88 bp).

Please note: although 48 hex codes are available, it is recommended to use a maximum of 36 libraries per lane to ensure sufficient read coverage (> 20 million reads) when using the above specifications.

ANNEX: NGS PRIMERS SEQUENCES, RESUSPENSION AND CONCENTRATION

NGS PRIMER SEQUENCES

- ➔ Sequences should be dissolved as described below at a stock solution of 100 µM.
- ➔ The reverse primer is common to all PCR reactions.
- ➔ Each forward primer contains a unique hex code which enables sample identification (underlined in the following sequences). One unique forward primer must be assigned to one specific sample per NGS submission lane.
- ➔ Important notes: up to 48 hex codes can be used per NGS submission lane. The number of hex codes used is at your discretion. Three control libraries must be conducted per NGS submission lane.

Name	Sequence
Rvs	CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATGATGTGTACGGTTTG TCA
Fwd1	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTATCACGGACGAGTC TAGTTGCAC
Fwd2	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCGATGTGACGAGTC TAGTTGCAC
Fwd3	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTTAGGCGACGAGTC TAGTTGCAC
Fwd4	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTGACCAGACGAGTC TAGTTGCAC
Fwd5	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTACAGTGGACGAGTC TAGTTGCAC
Fwd6	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGCCAATGACGAGTC TAGTTGCAC
Fwd7	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCAGATCGACGAGTC TAGTTGCAC
Fwd8	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTACTTGAGACGAGTC TAGTTGCAC
Fwd9	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGATCAGGACGAGTC TAGTTGCAC
Fwd10	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTAGCTTGACGAGTC TAGTTGCAC
Fwd11	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGGCTACGACGAGTC TAGTTGCAC
Fwd12	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCTTGTAGACGAGTC TAGTTGCAC
Fwd13	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTAGTCAAGACGAGTC TAGTTGCAC

Fwd14	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTAGTTCCGACGAGTC TAGTTGCAC
Fwd15	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTATGTCAGACGAGTC TAGTTGCAC
Fwd16	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCCGTCCGACGAGTC TAGTTGCAC
Fwd17	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGTAGAGGACGAGTC TAGTTGCAC
Fwd18	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGTCCGCGACGAGTC TAGTTGCAC
Fwd19	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGTGAAAGACGAGTC TAGTTGCAC
Fwd20	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGTGGCCGACGAGTC TAGTTGCAC
Fwd21	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGTTTCGGACGAGTC TAGTTGCAC
Fwd22	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCGTACGGACGAGTC TAGTTGCAC
Fwd23	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCAGTGGGACGAGTC TAGTTGCAC
Fwd24	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGCTCCGGACGAGTC TAGTTGCAC
Fwd25	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTACCCGGACGAGTC TAGTTGCAC
Fwd26	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTCCGTAGACGAGTC TAGTTGCAC
Fwd27	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCAAGCTGACGAGTC TAGTTGCAC
Fwd28	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTACAACCGACGAGTC TAGTTGCAC
Fwd29	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTTACATGACGAGTC TAGTTGCAC
Fwd30	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTCGACGGACGAGTC TAGTTGCAC
Fwd31	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGCGGTTGACGAGTC TAGTTGCAC
Fwd32	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGATGCAGACGAGTC TAGTTGCAC
Fwd33	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCACGAAGACGAGTC TAGTTGCAC

Fwd34	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGCACGTGACGAGTC TAGTTGCAC
Fwd35	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTAACTTTGACGAGTC TAGTTGCAC
Fwd36	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTGCATCGACGAGTC TAGTTGCAC
Fwd37	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTAATGACGACGAGTC TAGTTGCAC
Fwd38	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTGGGTGGACGAGTC TAGTTGCAC
Fwd39	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTAAACGCGACGAGTC TAGTTGCAC
Fwd40	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTGCCGTGACGAGTC TAGTTGCAC
Fwd41	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCATTATGACGAGTC TAGTTGCAC
Fwd42	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGCCCTGGACGAGTC TAGTTGCAC
Fwd43	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTGGAATGACGAGTC TAGTTGCAC
Fwd44	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTAGCGGCGACGAGTC TAGTTGCAC
Fwd45	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTATTGGTGACGAGTC TAGTTGCAC
Fwd46	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCTGCACGACGAGTC TAGTTGCAC
Fwd47	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCCCACAGACGAGTC TAGTTGCAC
Fwd48	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTTCGCTGACGAGTC TAGTTGCAC

*Unique hex codes are underlined in the above table.

Most oligonucleotides synthesized and shipped from IDT are delivered lyophilized and often appear as a white flakey substance at the bottom of the tube. Dried DNA is quite stable and is easy to resuspend in an aqueous solution.

Resuspension calculations can be made using the yield information that is provided both on the oligo tubes themselves and on the IDT oligo product specification sheets provided with the order.

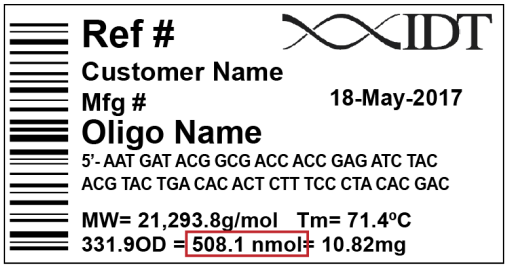
OLIGO RESUSPENSION

- Centrifuge lyophilized oligo for 30s at full speed. Check for lyophilized DNA at bottom of tube.
    - This ensures that dried DNA that may have become dislodged during shipping is brought down to the bottom of the tube.
- Add an appropriate volume of nuclease-free water, to achieve the desired stock concentration of 100 µM (IDT provides instructions regarding how much water must be added to prepare a 100 µM concentration of oligo. This information is found under the 'Amount of Oligo' section of the oligonucelotide's specification sheet).
    - Otherwise, the volume of water required to obtain a 100 µM stock is easily determined by multiplying the number of nanomoles listed for a particular oligo by a factor of 10 and then resuspending the dry DNA in that same number of microliters of water. For example, if the oligo specification sheet states that 20.3 nmol of oligo were delivered, add 203 µL water to obtain a 100 µM stock solution. This stock solution can be diluted as needed to appropriate working solutions.
- Vortex the DNA solution and solubilize the stock at RT for 1 hour.
- Vortex the DNA solution. Centrifuge for 30s at full speed.
- Measure the oligo concentration.

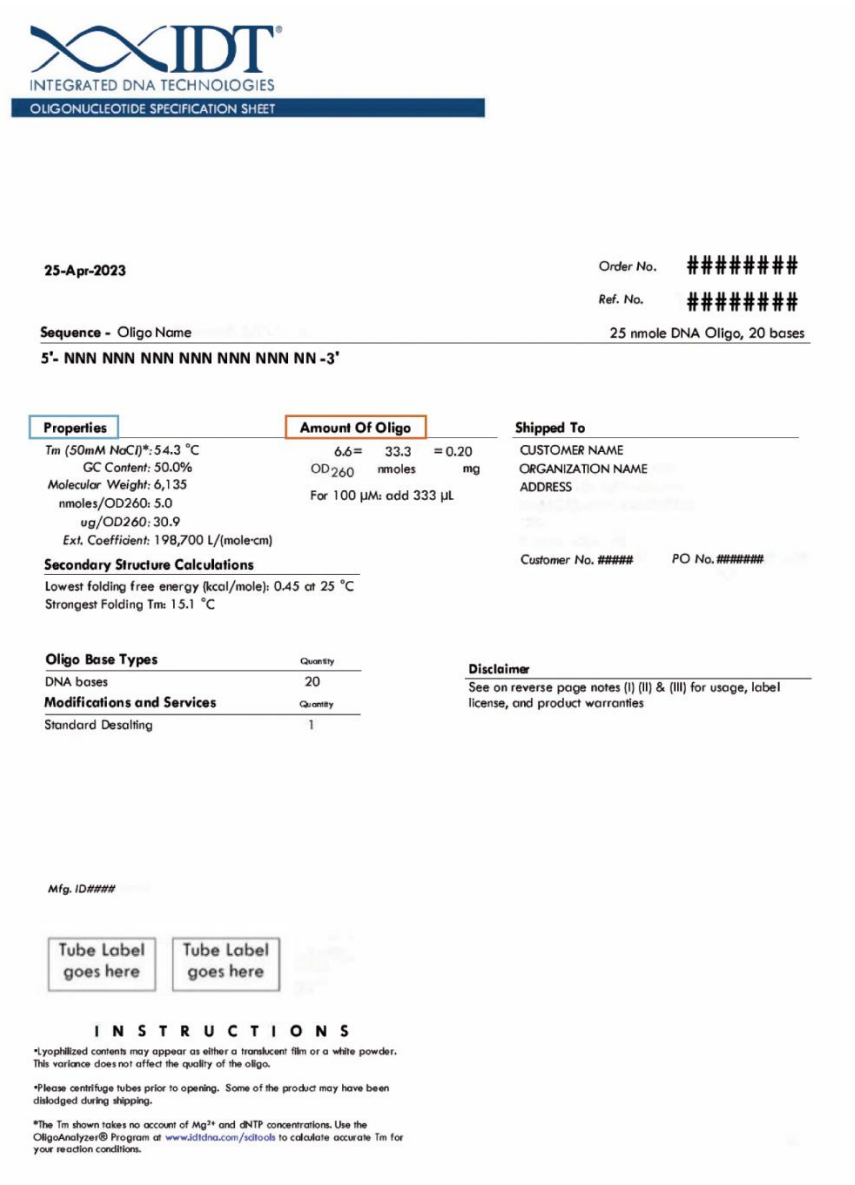
DETERMINING CONCENTRATION OF OLIGO USING NANODROP METHOD

- From the home screen select the 'Nucleic Acids' Tab.
- Select the desired nucleic acid sample to be measured: dsDNA (i.e., PCR products), ssDNA (i.e., primers, library, cDNA, aptamers) or RNA.
- Follow the instructions as prompted.
- Use 1.5-2 µL of water to blank.
- When prompted, place 1.5-2 µL of sample to measure the oligonucleotide concentration.
- Record the concentration of the oligonucleotide. Check the A260/A230 ratio to ensure purity:1.8-2.0 (minimum).

Standard Full Yield Oligo Label



Where to find oligo yield information in nmol on an IDT oligo tube label.



IDT
INTEGRATED DNA TECHNOLOGIES
OLIGONUCLEOTIDE SPECIFICATION SHEET

25-Apr-2023

Order No. ########
Ref. No. ########

Sequence - Oligo Name — 25 nmole DNA Oligo, 20 bases

5'- NNN NNN NNN NNN NNN NN -3'

Properties

Tm (50mM NaCl): 54.3 °C
GC Content: 50.0%
Molecular Weight: 6,135
nmoles/OD260: 5.0
ug/OD260: 30.9
Ext. Coefficient: 198,700 L/(mole·cm)

Amount Of Oligo

6.6 = 33.3 = 0.20
OD_{260} nmoles mg
For 100 µM: add 333 µL

Shipped To

CUSTOMER NAME
ORGANIZATION NAME
ADDRESS

Customer No. ##### PO No. #######

Secondary Structure Calculations

Lowest folding free energy (kcal/mole): 0.45 at 25 °C
Strongest Folding Tm: 15.1 °C

Oligo Base Types	Quantity
DNA bases	20
Modifications and Services	Quantity
Standard Desalting	1

Disclaimer

See on reverse page notes (I) (II) & (III) for usage, label license, and product warranties

Mfg. ID####

Tube Label goes here | Tube Label goes here

INSTRUCTIONS

*Lyophilized contents may appear as either a translucent film or a white powder. This variance does not affect the quality of the oligo.

*Please centrifuge tubes prior to opening. Some of the product may have been dislodged during shipping.

*The Tm shown takes no account of Mg^{2+} and dNTP concentrations. Use the OligoAnalyzer® Program at www.idtdna.com/sdtools to calculate accurate Tm for your reaction conditions.

Example of an IDT specification sheet with oligo properties and yield information. Oligo characteristics such as melting temperature (Tm), molecular weight, and extinction coefficient are included in the 'Properties' section (blue rectangle). Yield information in the 'Amount of Oligo' section (orange rectangle) is presented in optical density (OD) units, mass (mg), and nanomoles (nmol).

This product is intended for research purposes only. This product is not intended for therapeutic or diagnostic purposes in humans or animals.

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