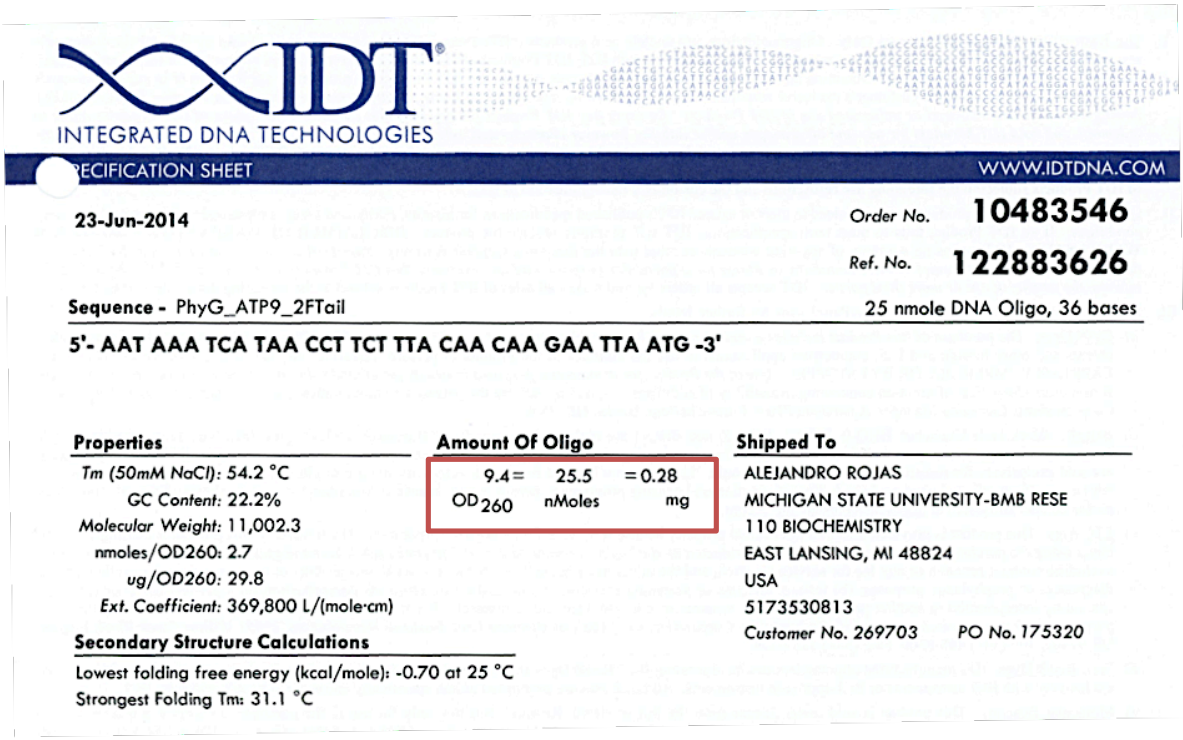
Primer resuspension protocol

I. Calculation for primer/probe resuspension

- For primer/probe resuspension, check the information sheet attached with your primers. On the sheet, find the amount of nanomoles (nMoles) synthetized for your primer, this is different from the scale of synthesis.
    - We are using the nanomoles quantity information to calculate the amount of water or 10 mM TE buffer (10 mM Tris pH 8.0; 0.1 mM EDTA) to resuspend your primers. The pictures below show where this information is located for two different companies.

IDT Primer information sheet



INTEGRATED DNA TECHNOLOGIES

PECIFICATION SHEET WWW.IDTDNA.COM

23-Jun-2014

Order No. 10483546

Ref. No. 122883626

Sequence - PhyG_ATP9_2FTail 25 nmole DNA Oligo, 36 bases

5'- AAT AAA TCA TAA CCT TCT TTA CAA CAA GAA TTA ATG -3'

Properties

Tm (50mM NaCl): 54.2 °C
GC Content: 22.2%
Molecular Weight: 11,002.3
nmoles/OD260: 2.7
ug/OD260: 29.8
Ext. Coefficient: 369,800 L/(mole·cm)

Secondary Structure Calculations

Lowest folding free energy (kcal/mole): -0.70 at 25 °C
Strongest Folding Tm: 31.1 °C

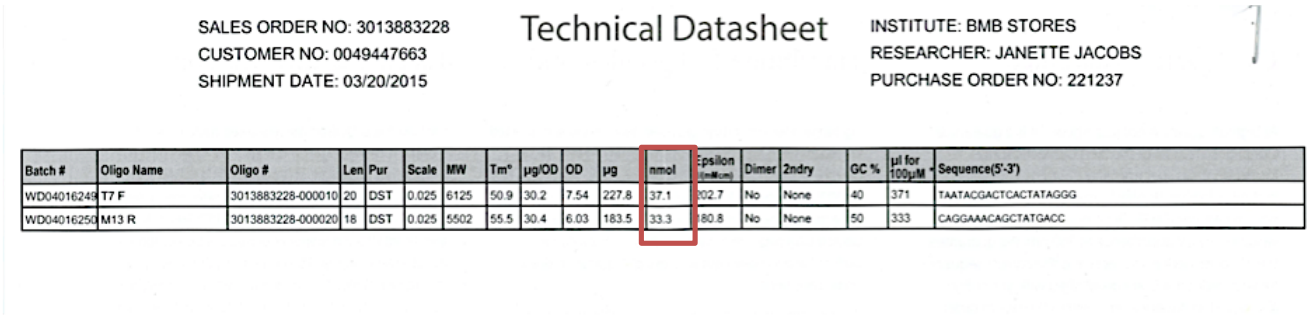
Amount Of Oligo

9.4 =	25.5	= 0.28
OD_{260}	nMoles	mg

Shipped To

ALEJANDRO ROJAS
MICHIGAN STATE UNIVERSITY-BMB RESE
110 BIOCHEMISTRY
EAST LANSING, MI 48824
USA
5173530813
Customer No. 269703 *PO No. 175320*

Sigma Primer information sheet



SALES ORDER NO: 3013883228
CUSTOMER NO: 0049447663
SHIPMENT DATE: 03/20/2015

Technical Datasheet

INSTITUTE: BMB STORES
RESEARCHER: JANETTE JACOBS
PURCHASE ORDER NO: 221237

Batch #	Oligo Name	Oligo #	Len	Pur	Scale	MW	Tm°	µg/OD	OD	µg	nmol	Epsilon (mM·cm)	Dimer	2ndry	GC %	µl for 100µM	Sequence(5'-3')
WD0401624	T7 F	3013883228-000010	20	DST	0.025	6125	50.9	30.2	7.54	227.8	37.1	202.7	No	None	40	371	TAATACGACTCACTATAGGG
WD0401625	M13 R	3013883228-000020	18	DST	0.025	5502	55.5	30.4	6.03	183.5	33.3	180.8	No	None	50	333	CAGGAAACAGCTATGACC

- To calculate the amount of water for the IDT example "PhytG_ATP9_2F", the total amount of nanomoles is 25.5, so these are the calculations:

  Note:
  - **1 mole = 10^3 mmoles = 10^6 µmoles = 10^9 nmoles = 10^{12} pmoles**
  - **µM = µmoles/L = pmole/µL**

  1. The nanomoles are converted to picomoles:

$$\boldsymbol{primer}\ nmoles \times \frac{10^3 pmoles}{1 nmoles} = \boldsymbol{primer}\ pmoles$$

$$25.5\ nmoles \times \frac{10^3 pmoles}{1 nmoles} = 25{,}500\ pmoles$$

  2. Since the stock solution will have a final concentration of 100 µM, we are going to use this value to calculate the water or TE buffer volume.

     Since,

$$100\ \mu M = \ 100 \frac{\mu moles}{L} = 100 \frac{pmoles}{\mu L}$$

     Then, we can calculate the volume for water or buffer:

$$\text{Buffer or water volume} = \frac{\textbf{primer}\ \text{pmoles}}{100\ \frac{pmoles}{\mu L}} = X \mu L$$

$$\text{Volume} = \frac{25{,}500\ \text{pmoles}}{100\ \frac{pmoles}{\mu L}} = 255 \mu L$$

     In this case, you need 255 µL to resuspend your primer. The same approach can be used to resuspend primers or probes.

II. Preparation probes and primers

1. Briefly centrifuge the tubes with primers and probes for 20 secs at 10,000 rpm before opening the tubes.

2. **100 µM stock solutions:** dissolve primers and probes by adding molecular grade water for a final concentration of 100 µM. Store probes in amber-colored tubes at -20ºC or -80ºC if possible.

3. **10 µM working solutions:** Prepare working solutions by making a ten-fold dilution from the stock solutions using molecular grade water and store in 100 µL aliquots at -20ºC or -80ºC if possible. Use amber-colored tubes for probes.
    a. For example, to make 500 µL of primer working stock, dilute 50 µL of the primer/probe and dissolve in 450 µL of molecular grade water.

4. **Plant Internal Control 1 µM working stocks:** a plant internal control for amplification is required to be at a lower concentration than the rest of the working stocks, hence a 100-fold dilution from stock solutions should be made using molecular grade water. Store 100 µL aliquots at -20ºC or -80ºC, using amber-colored tubes for probes.
    a. For example, to make 500 µL of plant internal control, dilute 5 µL of plant internal control primer/probe and dissolve in 495 µL of molecular grade water.