Updated 9/10/2014 by Michelle R. Stuart

**To order oligos:**

Go to IDT website (www.idtdna.com), log in (Pinsky, larvae) and view past orders.

Click on the order and then hit the plus sign to see which oligos were purchased.

Hit edit to update or change the oligo (like dropping the biotinylation from P2.2 or adding a phosphotioated )

Double check the guaranteed nmole that you will be getting back.

**To rehydrate oligos:**

**Resuspend Oligos**

IDT says to multiply # of nmoles on vial by 10 and add the answer µL of buffer to bring concentration to 100µM. We want 200µM so we are multiplying by 5.

9 x 10^-9mol = 0.045 x 10^-3 L = 45µL of buffer to resuspend

200 x 10^-6mol

9 x 5 = 45

Go to the google doc "Oligo Construction and Resuspension” in the Pinsky Lab folder

Click on the “Resuspension” Tab

Scroll to the bottom, enter the date of resuspension, name of adapter on tube, nm of adapter on tube, and drag down the “amount of TE…” and “Final mol” cells

Example:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Date | Adapter Name | Sequence Name | nmoles | Amount of TE buffer to add (µL) (per IDT, multiply nmoles by 5 for buffer volume, will bring to 200µm) | Final molarity (µM) | Notes |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| 9/9/2014 | P2.1\_bond | P2.1\_bond | 10.8 | 54 | 200 | phosphorothioated bond |
| 9/9/2014 | P2.2\_bond | P2.2\_bond | 14 | 70 | 200 | phosphorothioated bond |

Spun dried tubes 30 sec @ 20,000g

Add TE buffer

Vortex 5 sec

Incubate at 4˚C overnight

**To anneal adapters:**

To make 200µL of 40µM adapters

* 40µL 1.1
* 40µL 1.2
* 20µL of 10x annealing buffer
* 100µL pH2O
* incubate @ 97.5˚C 2.5 min; cool @ 3˚ per minute to 21˚C, hold @ 4˚C

**To create working stocks:**

* UV sterlize pH2O and 10x annealing buffer 15 minutes
* Make 1x annealing buffer solution:
  + **final: 200µL @ 1x = initial 20µL @ 10x**
  + **Combine 180µL pH2O + 20µL 10x annealing buffer - use TE buffer instead of pH2O? - no**
  + **Update ligation molarity calculator spreadsheet**
  + **Prep adapter based on** [**ligation molarity calculator sheet**](https://docs.google.com/a/scarletmail.rutgers.edu/spreadsheet/ccc?key=0As4-rNyOzK7CdFY3VXRCUlJIS1hWZXk3RXM1NTd3SEE&usp=drive_web#gid=8) **volumes**
* **Made new working stocks using spreadsheet**
* **P1 adapters (P1\_bond and the rest regular)**
* **XX µL annealed adapter - from spreadsheet**
* **XX µL 1x annealing NOT 10X - from spreadsheet**
* **Made in plate, see map**
* **P2 adapter (P2 regular) - made in tube (moved rest of tube to plate after ligation on 7/16/2014)**
* **XXµL annealed adapter - from spreadsheet**
* **XX µL 1x annealing NOT 10X - from spreadsheet**

To make plate map worksheet, I dragged numbers down (1-48), in the next column I concatenated “P1\_”, cell to the left with number, resulting in P1\_1, P1\_2…

I then went to my plate map and in the first row, first cell typed “transpose(B12:B23)” to paste the entire row horizontally into my plate. I then copy, pasted as values to get rid of the transpose formula.

Loading plate horizontally because that is the direction our plate spinner (big centrifuge) needs

**TE buffer recipe** from IDT initial final

* Tris-HCl (ours is pH 9) 1M \* 500µL 10mM \* 50mL
* EDTA pH8 0.5M \* 10µL 0.1mM\*50mL

bring pH to 8 w/ HCl

fill to 50mL w/ dH2O

**10x annealing buffer recipe - 50mL**  initial final

* Tris-HCl 1M \* 5mL 100mM \* 50mL
* EDTA pH8 0.5M \* 1mL 10mM\*50mL
* NaCl 5M \* 5mL 500mM\*50mL

fill to 50mL w/ dH2O