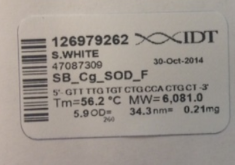
**End point oligo resuspension Protocol – Updated 1/4/23**

As of today (9/3/22) every stock primer tube in the fridge is resuspended incorrectly. We were supposed to use these instructions:

**To generate a 100uMolar concentration you need to take the number of nanomoles of oligo in your tube times a factor of ten, then the resulting number is the number of microliters of buffer or water to add to your tube to create a 100uMolar stock concentration.**

**For example, since you have 28.4nMoles, it would be: (28.4)(10) = 284 uLiters of buffer or water to add to your tube to create a 100uMolar stock concentration.**

But instead of using the nanomoles we used the mg. So for example for the primer below we should have added 343 µl (34.3 x 10 = 343 µl) but instead we added 210 µl. This led to the complicated spreadsheets we now must use to dilute primer stocks to working dilutions.



**So from now on (9/3/22) we’ll do this:**

1. Centrifuge at 1000 RPM’s for 30 seconds. Pushes pellet to bottom, otherwise you might lose it when you open tube.

2. Add µl based on the number of nMoles (34.3 \* 10 = 343 µl in the example in the picture)

3. Vortex for 30 seconds and of course vortex before removing liquid in Step #4.

4. This created a 100 µMolar stock solution, so to make a 2 µM working solution simply add 98 µl IDTE with a pH corresponding to what was used to resuspend the primers and 2 µl primer, or double this if needed.

- If F + R primers are diluted together then add 96 µl IDTE and 2 µl F primer and 2 µl R primer.

Notes:

- What to use: Always use IDTE pH 7.5, never water

- Labeling:

- Because of our previous errors we must label each primer suspended correctly as follows “RC – 9/3/22.”

- Also include “IDTE, pH 7.5.”

- Include initials: SAB, BFB

So: RC – 9/3/22, IDTE 7.5

BFB