Resuspending oligo plates for arraying

Goal: I use 70-mer oligos for printing our microarrays. I get them from a company (Illumina) and have them make aliquot plates for us of 400 pmoles of each oligo in each well. I have Illumina ship the plates dried down. (Side note: I also have them ship the remainders, since their aliquotting robots apparently have some lame limitations and so can't dispense the last few aliquots for us – but we can do it ourselves from the remainder plates…) So, the dried-down aliquot plates need to be resuspended before they can be used for printing.

1. Volumes:

First, determine what the resuspension volume will be. I typically resuspend a new oligo-aliquot-plate at 10ul per well, which gives 40pmol/ul of each oligo.

After a plate is used for printing, I dry it down in a speedvac vacuum centrifuge with a plate-holding rotor. The oligos store better dried-down, and then I don't need to worry about evap over time, etc.

Then in subsequent resuspensions, I add the volume that should cause the remaining oligo to be at ~40pmol/ul again. I do this calculation by assuming that for each inking, the wells lose 0.5ul of fluid (the pin takes ~0.3ul, but we assume 0.5ul total loss to account for evaporation – based on personal communication with Kevin Visconti, Schoolnik Lab, Stanford. However, it's **always** good to check a few wells at random after a printrun to see what is really left, since your loss will depend upon the ambient humidity, and how long your plates were in the stacker – e.g. if you put them all in at once they'd all be exposed (with lids but no Al-foil seals) a lot longer than if you put them in one or two at a time). Thus, if I had a new plate with 10ul/well and used it for one bed of slides that required two inkings, I would assume the new resuspension volume the next time I used the plate would be 9.0ul.

For the total volume of printing buffer required, I multiply the volume per well, by the plate size, by the number of plates. So, 9 ul/well * 384 wells/plate * 15 plates = 51840 ul = 51.84mls, just over a Falcon tube's worth.

2. Printing buffer:

For the first resuspension, I use 3X SSC. For subsequent resuspensions (after the plate has been used and dried down), I use 0.3X SSC, to account for the small amount of salt lost during evaporation (this is what I learned from the Schoolnik Lab). I have a 20X SSC stock (Ambion).

In addition, in recent printruns I have been adding the co-spot oligo to the print buffer. I make a 100pmol/ul co-spot oligo stock solution, in 3X or 0.3X SSC. Then I make 1pmol/ul working solution, so I dilute 1:100.

Primary Resuspension Fluid (first time a plate is used):

final concentration	recipe per 40ml
3X SSC	6mls 20X SSC
1pmol/ul co-spot oligo	400ul of 1nmol/ul co-spot oligo
water	33.6 mls Ambion water

Secondary Resuspension Fluid (each subsequent time a plate is used):

final concentration	recipe per 40ml
0.3X SSC	0.6mls 20X SSC
water	39.4 mls Ambion water

3. Using the *aliQuot* robot by Genetix

1. Sonicate the disassembled manifold:
        in 3% aQu clean for >15 minutes
        in MilliQ water for >10 minutes
        in 95% EtOH for >15 minutes

2. Replace manifold (see diagram in manual if necessary).

3. Run 50mls of 3% bleach (1 Chlorox : 1 MilliQ; Chlorox is 6%) through the robot, letting sit in the tubing for ~15", per the online recommendations for removing DNA.

4. Run 3 x 50mls of MilliQ through the system.

5. Rinse with 1 x 50mls of 80% EtOH.

6. See page 13 of the aliQuot instruction manual, for the section "Running a Filling Routine".

        Adjust the manifold's start position, dispense height, and tilt, in order to minimize splashes. Start position is adjusted in the software, the height and tilt are adjusted mechanically.

        Use a dummy plate of the same make as you'll be using to test the settings out to see if there's splashage.

        PCR mode dispenses volumes as multiple aliquots of smaller volumes, to decrease splashing. Accessible in software.

7. Before using real plates, test aliquotting accuracy in both of two ways:
        i. weigh a plate before and after aliquotting into it.
        ii. use a pipetteman to test the volume several wells of different rows, since each row is filled by a different pin.

8. Note: The bottle fill type is a 50ml Falcon-type tube, BUT Falcons, Fisher brand, and BD brand do not work. A Greiner tube is in there currently, and we have a limited supply of other Greiners. They don't all work smoothly either. In fact, if you undo the existing tube it can be very difficult to get back on. So, I clean the tube thoroughly and then use it for dispensing if I can. Also, I keep it screwed in to the black connector piece, and unscrew that piece instead from the arm, whenever possible.

The dead volume (volume taken up from the Falcon tube before it gets to the manifold) of the aliQuot is ~3mls, as stated in its instruction book.

Side note: I tested evaporation from a 384-well plate, unsealed but with plastic lid (I think), and it ended up averaging ~ 0.05ul per well per hour… with the caveat that perimeter wells evap. faster than internal ones, and that the evap process should be non-linear. This was calculated from an 18-hour benchtop exposure with 10ul per well of 3X SSC, and September humidity. (So the total loss over 18 hours was 0.9ul).