Spotting

1. Aim of the experiment

To spot Cy3/Cy5 labelled aptamer dilution samples from a microwell plate onto glass slides for MITOMI experiments

a. Hypothesis / expected results

We expect to observe spots in a regular grid on our glass slides onto which we can align the MITOMI chips

b. Team / TAs / schedule

iGEM student	TA	Scheduled TA help time
John	Ekaterina	2017/07/12 10:30-18:00

c. Consumables / lab equipments

		Reference	Supplier	Amount required
	Epoxy glass slides, according to protocol (refer to experiment 1002)		Made in house on 10.07.2017	
	/Cy5/60-18[29]a oligo, 500 umol		IDT	5 dilutions of 0.3, starting from 2 umol
	/Cy3/G15D oligo, 500 umol		IDT	5 diltuions of 0.3, starting from 2 umol
	Cy3-labelled primer, negative control, 2umol		Ekaterina	5 diltuions of 0.3, starting from 2 umol
	Transparent microwell plate, NUNC plate 384 265196F		(??)	1
	Spotting machine, *Qarray2*		Genetix	1

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d. Detailed description / protocol

Dilutions

Spotting

Prepare the spotter

1. Sonicate the tip, submerged in ethanol in its storage tube, with the water bath in the spotting room for 15min. Make sure it is always facing up in its storage tube so as not to damage it.
2. Centrifuge the plate with the samples at 4000RPM for 10min (?)
3. Wash the glass slides with toluene and dry them with N2. Place them back in their storage box. Make sure you remember which ones they are, and which side of the slides are of better quality.
4. Dry the tip with N2 and check it under the microscope. Make sure it is not bent and that there is no dust.
5. Make sure the spotter has enough water (distilled) and ethanol (if using. Not the case in this experiment). If there is too much waste, empty it.
6. In the spotter program, choose to change the tip. Make sure you place the tip in the position set in the program (usually in the bottom left corner).
7. Align the glass slides in the spotter. Use the bottom and right edges of the slides to align. Never touch the surface of the slides !
8. Turn on the nebulator, and plug in the vacuum generator (plug is on the floor, on the left side of the spotter).
9. Set the spotting program. For this experiment :
    a. Head : 1-pin (96-well plate) head, laid out in the bottom left corner
    b. Plate Holder : stacker source plate holder
    c. Use stacker : yes
    d. Plate type : NUNC plate 384 265196F
    e. Max stamps per ink : 4
    f. Number of stamps per spot : 1
    g. Stamp time (ms) : 20
    h. Inking time (ms) : 100
    i. Print depth adjustment : 120 microns
    j. Same sample sterilize : yes
    k. Inks before sterilize : 4
    l. Number of touch-offs : 0
    m. Sterilization : water, 1500 ms wash time, 1500 ms dry time, 0 ms wait
    n. Slide layout (48x16) : 3x1” MCB area 65MMx20MM (1-Plate)
        i. 24 24 24 24 24 24 24 24 24 24 24 24 24 24 24 24
        ii. 24 24 24 24 24 24 24 24 24 24 24 24 24 24 24 24
        iii. …
        iv. 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
        v. 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
        vi. 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
        vii. 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
10. Place the sample plate in the rack
11. Launch the program, and never open the spotter while it is running. Always pause it and wait for the prompt from the program. Otherwise use the emergency stop button in a case of a problem, or use the master switch on the bottom right side of the machine to reboot. Always wait 30 sec after switching off the master switch before switching it back on.
12. Once the spotter is done, remember to unplug the vacuum generator.
13. Remove the tip and place it back in ethanol in its storage tube
14. If there is still sample left in the plate cover it, and label it.

e. Data analysis

Experimental design by: Felix Faltings, 2017/07/12
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f. Results

2. Conclusion

References

Appendix

Experimental design by: Felix Faltings, 2017/07/12
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