Aptamer binding test

1. Aim of the experiment

To determine the ability of two anti-thrombin aptamers to form a sandwich complex in the presence of serum

a. Hypothesis / expected results

We expect to observe a fluorescent signal in the Cy3 channel after washing indicating the formation of a sandwich complex between the Cy3 labelled aptamer, a component of the serum – hopefully thrombin -, the biotinylated aptamer, and neutravidin.

b. Team / TAs / schedule

iGEM student	TA	Scheduled TA help time
Matteo	Ekaterina	

c. Consumables / lab equipments

		Reference	Supplier	Amount required
	Native human a-thrombin, (storage buffer, 50% water, 50% glycerol), Catalog nb RP43100 150 umol		life technologies, Lot SE2378811	
	/Cy3/27B-G15D aptamer 500 uM		IDT	
	5'-/Biosg/TBD aptamer 500 uM		IDT	
	PBS		Gibco, life technologies	~60ul
	Neutravidin 500[ug/ml]			~30ul
	Biotin-BSA			~ 60ul
	Tris-HCl	[2]		1ml for 20ml buffer
	NaCl	[2]		2ml for 20ml buffer
	MgCl2	[2]		0.2ml for 20ml buffer
	MITOMI chip			
	BSA			
	Tween 20			

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d. Detailed description / protocol

Aptamer Binding Buffer

Binding buffer consists of (as in [2])

1. 50 mM Tris-HCl (pH 7.5)
2. 100 mM NaCl
3. 1 mM MgCl2

For 5 min incubation at 37C

Neutravidin surface chemistry

1. Set pressure for control layer at 15 psi, flow layer at 3 psi
2. Fill in the control lines with dH_2O one at a time, check that all lines are working. Close chamber valves (neck valves).
1. Flow 2 [mg/mL] biotin-BSA with the general outlet open until BSA approximately reaches the outlet. Close the outlet and allow any air inside the chip to escape. Then flow the BSA for 20 minutes (15min) in order to passivate the glass surface.
2. After this point, each time a new reagent is to be flowed through the chip, make sure **no air enters the chip**. Close the general inlet and open the waste and reagent lines. Allow the reagent to flow through the waste line for 30 sec to let air escape. Then close the waste line and open the general inlet and allow the reagent to flow.
3. Wash chip with PBS for 5 minutes
4. Flow 500 [µg/mL] Neutravidin for 20 minutes (15min) in order to bind to biotin, buttons up.
5. Wash chip with PBS for 5 minutes, buttons up
6. Flow 2 [mg/mL] biotin-BSA for 20 minutes (15min) in order to block Neutravidin sites outside button area, buttons down
7. Wash chip with PBS for 5 minutes, buttons down

Chip now has biotin-binding neutravidin sites blocked everywhere by biotin-BSA but in the button area

Sandwich test in serum

1. Flow the biotinylated aptamer 2 [uM] into the chip for 2min buttons down, then 15 min buttons up.
2. Close the button valves.
3. Wash with aptamer binding buffer for 3 minutes with buttons closed, then 5 minutes with buttons open.
4. In the **upper half** of the chip, flow serum for 3 min buttons closed, then 10 min buttons up.

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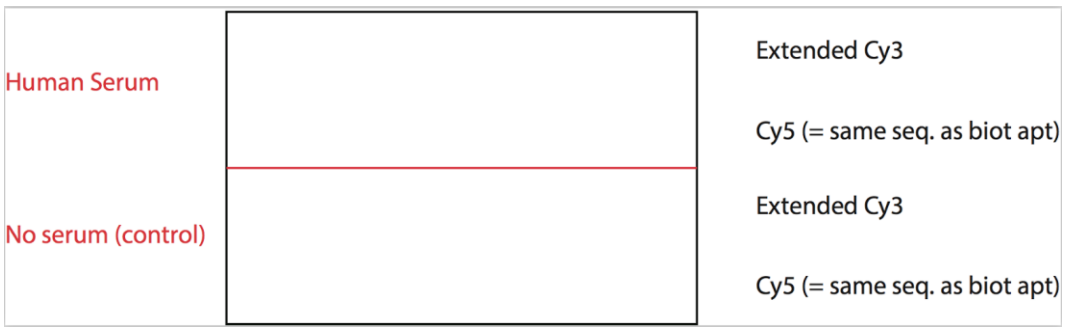
5. Close the buttons and then wash with (PBS+2%BSA+0.01%Tween 20) for 10 min
6. **Multiplex the chip.** In the first and third quarter of the chip, flow Cy5 labeled aptamer for 3 mins buttons closed then open the buttons for 10 min. Wash with (PBS+2%BSA+0.01%Tween 20) for 5 min.
7. **Multiplex the chip.** In the second and fourth quarter of the chip, flow Cy3-27B-G15D (modified Cy3) aptamer for 3 min buttons closed then open the buttons for 10 min. Wash with (PBS+2%BSA+0.01%Tween 20) for 5 min.
8. Remove the flow line from the flow manifold (but not from the chip).
9. Image the chip
10. Close buttons valves.
11. Image the chip
12. Wash with aptamer binding buffer for 5min, buttons down.
13. Image the chip

Imaging on flurescent microscope

1. Use the NIS program
2. Align the chip on the microscope with the grid in the software. Make sure that every chip cell is in the field of view when selected from the grid in the software. Set the upper left chip cell as the reference point
3. Choose the appropriate laser/channel for imaging
4. Choose the exposure time in order to maximize the signal:noise ratio, not to saturate the camera, and not to photobleach the fluorophores.
5. Choose a filename
6. Press “run now”. The program should take an image of each cell in the chip.
7. When the image is created, select it and export it to a new folder using the “export ND document” option, under “export” in the “file” menu
8. Select “mono image” and the name, then click “export”
9. Open ImageJ. Go to “plugins”, “stitching”, “grid”. Choose the grid size, no overlap, , make sure “display fusion” is selected, and select the folder where you saved the image from the microscope. Choose a file name, but it must end in “xy{iii}”
10. Once the stitched image is generated, scale it down by pressing ctrl-E.

Note :



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Results

2. Conclusion

We highly suspect that there is unspecific binding of the aptamers to a residual protein present in the blood and will investigate it further.

References

1. MITOMI : A Microfluidic Platform for In Vitro Characterization of Transcription Factor-DNA Interaction, Rockel Sylve, Geertz Marcel, Maerkl Sebastian J., 2009, Springer

2. Oligonucleotide Inhibitors of Human Thrombin that Bind Distinct Epitopes, Tasset Diane M., Kubik Mark F., Steiner Walter, Journal of Molecular Biology 272, 1997, p.688-698

3. Maerkl, S. J. & Quake, S. R. A Systems Approach to Measuring the Binding Energy Landscapes of Transcription Factors. *Science (80-.).* **315,** 233–237 (2007).

Appendix

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