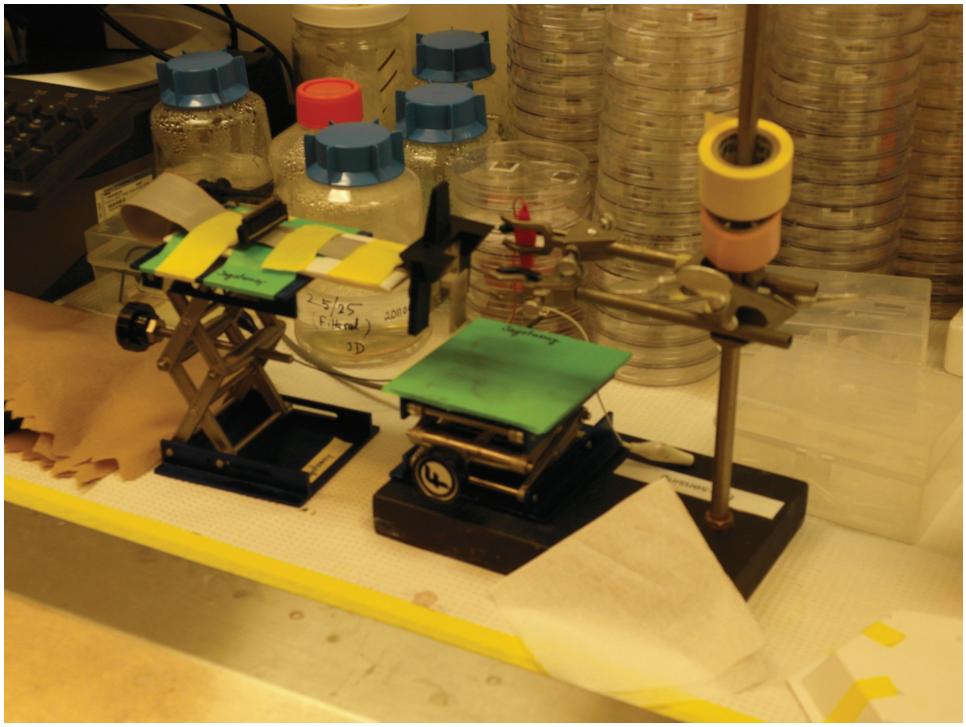
Blocking Assay on chip with EpCAM antigen

Thursday, 12 May, 2011

Day 1

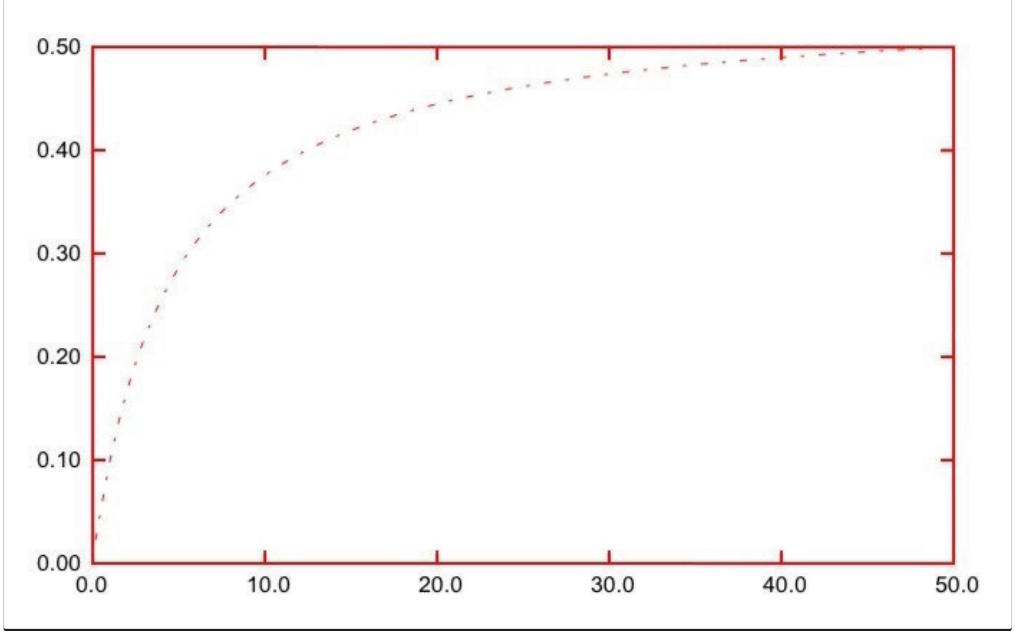
1. Cut CPFC chip down the middle leaving 4 electrodes for each chip
2. Place chip in 15 mL falcon tube and immerse in acetone
3. Sonicate chip for 5 minutes
4. Rinse with IPA then with DI H2O
5. While chips are sonicating make gold plating solution
    - 69 uL of gold stock solution
    - 2.5 mL of 1 M HCl solution
    - Fill H2O to a total of 5 mL
6. Wash platinum wire and reference electrode
7. Setup plating experiment (see pictures below)



8. Dry clean chips with N2 after DI H2O rinse
9. Place chip in solution so just the apertures are inside and the least amount of area is covered as possible
10. Leave in solution for 2 minutes – might not be a necessary step at all
    - clean another chip during this time
11. Plating condition on epsilon potentiostat

- mode: chronocoulometry (chronology potentiometry)
- current: 30
- unit: nA
- time: 50
- unit: sec
- potential range +/- 1 v
- sample interval: 0.05 sec
- applied current polarographic
- applied noise filter 100 Hz
- rest of checkboxes are unchecked

12. Start plating, graph should look roughly as seen below (y axis in mV and x axis in seconds)



13. After lead is done switch to next lead and continue until chip is fully plated

14. After the while chip is done, dip in H2O bath and plate in clean petrie dish
15. Plate all other chips
16. Prepare cystamine solution 100 mM
17. Aliqoute into 10 mM cystamine
18. Prepare H2O bath for incubating overnight
19. Pipette 20 uL of 10 mM cystamine and incubate for 12 hours
20. Seal Petrie dish with parafilm

Day 2

1. Remove parafilm and solution from chip
   - either remove solution using the side of the chip and touching a kimwipe
   - use pipette to remove solution
2. Pipette 100 uL of H2O

3. Wash for 2 minutes twice
4. Prepare GA soution 2.5 %
5. Pipette 20 uL of GA on the surface and incubate for 1 hour
6. Wash with 100 uL DI H2O
    - wash 2 minutes twice
7. Remove solution with kimwipe and dry surface as best as possible using the kimwipe
8. Unfreeze antibody aliquote
9. Dry chip using blue blower
10. pipette 20 uL of 10 ug/mL antibody on the surface
    - incubate for 1 hour
11. Remove solution and pipette 100 uL of PBS
    - wash for 5 minutes twice
12. Make BSA solution 1%
13. pipette 30 uL of BSA on the chip and incubate for 1 hour
14. Remove solution
15. Pipette 100 uL of PBS
    - wash for 5 minutes 3 times
16. Dry chip using blower
17. Pipette 20 uL of the following:
    - **Chip 1**: 20 uL of EpCAM (10 ug/mL works, so does 1 ug/mL and 0.1 ug/mL)
    - **Chip 2**: 30 uL of BSA 1% for specificity control
    - **Chip 3**: 20 ug of PBS as negative control
18. Incubate at 37 degrees celcius for 40 minutes with parafilm and in a water bath
19. remove Petrie dish from incubator and settle at room temperature for 2 minutes
20. Remove solution from chips and wash 3 times with PBS for 5 minutes with 100 uL PBS
21. Make ferro/Ferri scanning solution: scanning solution
22. Dry chip before scanning
23. Wash for 1 minute in H2O
24. Dry chip
25. Do DPV scan twice for each lead and save the second scan
    - initial potential: 0 mV
    - final potential: 400 mV
    - quiet time: 2 seconds
    - step E: 5 mV
    - pulse width: 50 mSec
    - pulse period: 100 mSec
    - pulse amplitude: 50 mV
    - scale: 1uA
    - pulse type: volt merry
    - sample period: check mark
26. Save data