Gel and Electric Field-Based Desorption of DNA from PMMA-Coated Silicon Surfaces to Optimize Sequencing Accuracy

Category EGMT

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Research Plan

A. Rationale

Following the success of the Human Genome Project in 2003, DNA sequencing has been applied successfully to whole genome genotyping, mutation detection, carrier screening, detection of inherited disorders, and DNA library preparation.[1] Unfortunately, current sequencing methods are limited to DNA fragments at most a few kilobases long and result in many inaccuracies due to random cutting and the repetitive nature of DNA.[2] In one practice called molecular combing, a substrate is slowly pulled out of a DNA solution, depositing DNA molecules linearly on its surface. This offers the advantage of controlled cutting of DNA to then be sequenced in an orderly fashion.[3] Poly(methyl methacrylate) (PMMA)-coated silicon wafers have been effectively used for both DNA combing and cutting; however, researchers have not achieved complete and efficient removal of DNA fragments for subsequent replication and sequencing.

B. Research Questions

1. To what extent does the use of an agarose gel and/or an electric field aid in the desorption of DNA from PMMA-coated silicon wafers?

2. What is the optimal voltage and running time with an electric field to remove the most DNA unharmed?

C. Hypotheses/Expected Outcomes

1. The addition of an electric field will cause DNA desorption to increase significantly.

2. The lower voltage for a longer amount of time will result in less evaporated buffer, and thus create more stable conditions for DNA and minimize damage.

D. Engineering Goals

The goals of this study are to develop a more efficient method to remove cut DNA fragments from PMMA-coated silicon wafers for sequencing purposes.

E. Procedures

1. Cleaving Silicon Wafers

Note: The silicon should be on a Texwipe Cleanroom Paper during the entire process to protect the silicon and to prevent damage to the instruments.

a. Etch 6 1cm x 1cm squares on the silicon wafers with the scribe. The flat edge of the silicon should be lined up parallel and perpendicular with the bottom blocks on the scribe, and the blocks should be screwed in on top of the silicon wafer to keep it stable when etching. The position of the diamond cutter can be adjusted by twisting the knob at the top of the machine or with metal blocks.
b. The etched lines will make the silicon easier to cleave.
c. Wipe off any silicon dust with a Texwipe Cleanroom Paper.
d. Use the cleaver to break the silicon along the etched lines.
    i. Line up the etches on the wafer with the dip on the bottom of the machine.
    ii. Push down on the moving part of the machine until the silicon cleaves.

2. Cleaning Silicon Wafers

a. Fill a glass beaker with a 3:1:1 ratio of DI water : hydrogen peroxide : ammonium hydroxide. Cover the beaker with aluminum foil and poke holes in it with tweezers to allow the release of pressure.
b. Heat the solution on a hot plate at approximately 100°C for 3-5 minutes or until boiling. After the solution comes to a boil, further heat the solution for 20 minutes.

c. Pour DI water into the glass beaker to cool the solution, then dispose of all the liquid into the designated waste bottle. Triple rinse the silicon wafers and glass beaker with DI water.
d. Fill a plastic petri dish with 10 mL of DI water and 1 mL of hydrofluoric acid. Submerge silicon pieces into solution and allow them to float for 30 seconds.
e. Triple rinse with DI water and dry the wafers.

3. Spinning Polymethyl Methacrylate
   a. Spin silicon wafer on spincaster once to dry the water off of it.
   b. Spin silicon wafer on spincaster once with pure toluene to dissolve any leftover contaminants not washed off from silicon cleaning procedure. Apply toluene to silicon wafer using a glass pipette. Dispense toluene onto wafer until the liquid covers the entire silicon wafer's surface. Spin.
   c. Spin silicon wafer on spincaster once with PMMA solution, which was created by dissolving 15 mg of polymethyl methacrylate in 1 mL of toluene.
   d. Remove wafer from spincaster.
4. Annealing Samples
   a. Place samples carefully in the vacuum oven using plastic tweezers as not not scratch the silicon.
   b. Do not use without supervision and follow the instructions located next to the oven.
5. Making DNA Solution
   a. Remove concentrated Lambda DNA and SyBr Gold dye from freezer.
   b. Vortex the tube containing DNA for 10 seconds.
   c. Use appropriate micropipettes to put 2µL DNA, 3µL SyBr Gold, and 995 µL of NEBuffer 3.1 into a separate tube, changing pipette tips each time, to create a 1mL of 0.2% DNA solution.
   d. Vortex solution for 10 seconds.
   e. Place DNA solution in fridge when not in use.
6. Dipping PMMA-Coated Wafers
   a. Remove DNA solution from the fridge and incubate in 60 °C oven for 30 minutes.

b. Use micropipette to move 700μL of the DNA solution into a teflon well.
c. Using computer program and laboratory exclusive dipping machine, place the well underneath the sample and have machine dip the wafer into the DNA solution, pulling out after 2 minutes.
d. Remove sample from machine and store in petri dish until desorption.
e. Dispose of remaining DNA solution as directed by principal investigator.

7. Making 3% Agarose Gel
   a. Using a scale, measure out 0.9g of agarose powder on a weighing plate and pour it into a glass beaker.
   b. Fill the beaker with slightly more than 30mL of NEBuffer 3.1 as some will evaporate when it is being heated.
   c. Swirl the solution and place in the microwave for 30 seconds or until the solution is completely clear.
   d. Remove the beaker from the microwave using oven mitts as it can be hot.
   e. Pour the solution into petri dishes up to about 4 mm deep and allow the gel to cool.
   f. Use an X-ACTO® knife to cut solid gels into 1 cm x 1 cm squares so they can fully cover silicon wafer samples. Keep gels submerged in NEBuffer 3.1 before use.

8. DNA Desorption
   a. DNA-dipped samples will be assigned to several different treatments.
   b. Two samples will be placed in wells, one covered with a 3% agarose gel made with NEBuffer 3.1 and the other just in NEBuffer 3.1 solution, and both will incubate in a 60°C oven overnight.
   c. Several others will be placed in wells with NEBuffer 3.1 and an electric field parallel to the sample at field strengths for different lengths of time, with and without a gel.
   d. All samples will be re-dyed in diluted SYBR® Gold solutions, washed with DNase reaction buffer, and blown dry with nitrogen gas.

9. Confocal Microscopy
   a. All samples will be observed using a Leica TCS SP2 confocal microscope before and after desorption.

b. Samples can be seen on a computer through a camera connected to the microscope using a 63x water lens.
c. Pictures will be taken with the computer of many areas of each sample to get an average of the amount of DNA on each sample before and after desorption

F. **Risk and Safety**

1. Cleaving Silicon Wafers
   a. Use personal protective equipment (gloves, lab coat, safety goggles)
   b. Silicon slivers may scatter when cleaving – be careful of splinters
2. Cleaning Silicon Wafers
   a. Acids and bases can be extremely harmful if inhaled, ingested, or contacted with skin. Handle with care.
   b. Hydrofluoric acid must be handled with extreme care by an experienced professional. Calgonate (calcium gluconate) should be present in any lab in which hydrofluoric acid is used. HF must also be contained in plastic as it dissolves glass.
   c. Disposal of chemicals should occur within a fume hood. All hot solutions should be cooled before disposal. Chemicals must be disposed of into appropriately labeled waste containers and these containers should be stored in a safety cabinet that resists fire.
3. Spinning Polymethyl Methacrylate
   a. Toluene and PMMA solution (since the PMMA is dissolved in toluene) should be kept away from plastics as toluene dissolves plastic. All containers holding toluene should be made of glass.
4. Annealing Samples
   a. Parts of the oven may be hot when removing samples. Use proper protective equipment and only use with supervision.
5. Making DNA Solution
   a. All dyes used are mutagens and teratogens. Handle with extreme care and avoid contact with skin or ingestion of any of the chemicals used.
6. Dipping PMMA-Coated Wafers

a. Use mitts when removing the DNA solution from the oven as it can get hot.

7. Making 3% Agarose Gel
   a. The glass beaker and/or agarose gel solution may be hot right after it is removed from the microwave, so be careful and use oven mitts.
8. DNA Desorption
   a. Do not touch the exposed electrodes when the power supply is on to avoid getting shocked.
9. Confocal Microscopy
   a. Look closely at sample when changing magnification or focus to avoid crashing the lens.
   b. Clean the water lens after use to prevent drying spots

G. Data Analysis

A Leica TCS SP2 confocal microscope will be used to photograph several areas on each sample before and after DNA is desorbed and ImageJ will be used to quantify the percent change in DNA on the samples.

H. Bibliography

[1] Pareek, Chandra Shekhar, et al. “Sequencing Technologies and Genome Sequencing.” *Journal of Applied Genetics*, vol. 52, no. 4, 2011, pp. 413–435., doi:10.1007/s13353-011-0057-x.

[2] Goodwin, Sara, et al. “Coming of Age: Ten Years of next-Generation Sequencing Technologies.” *Nature Reviews Genetics*, vol. 17, no. 6, 2016, pp. 333–351., doi:10.1038/nrg.2016.49.

[3] Allemand, J.f., et al. “PH-Dependent Specific Binding and Combing of DNA.” *Biophysical Journal*, vol. 73, no. 4, Oct. 1997, pp. 2064–2070., doi:10.1016/s0006-3495(97)78236-5.

I. **Post Summary**

NO CHANGES EXIST