OUC-iGEM Protocols

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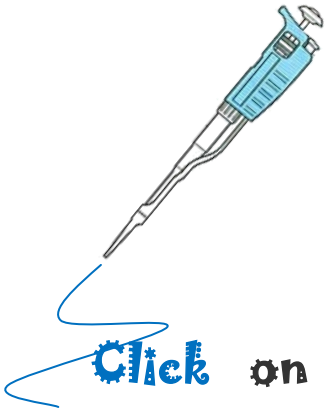
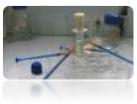




Trace stratified sampling device Manufacture

Polymerase Chain Reaction

1. Keep everything on ice. The PCR reaction mixture in the ice preparation, and then placed in a PCR reaction in the PCR reaction apparatus. This cold start (Cool Start Method) can enhance the specificity of PCR amplification to reduce non-specific reactions in the PCR process, can get good PCR results.
2. Make up a master mix of everything into PCR tubes.
   a. **25.0μL reaction system**

      18.3μL ddH2O
      2.5μL 10x Buffer (mg^{2+})
      2.0μL dNTPs
      0.5μL forward primer
      0.5μL reverse primer
      0.2μL DNA polymerase
      1.0μL template DNA
      --------**25.0μL** Total

   b. **50.0μL reaction system**

      37.75μL ddH2O
      5.0μL 10x Buffer (mg^{2+})
      4.0μL dNTPs
      1.0μL forward primer
      1.0μL reverse primer
      0.25μL DNA polymerase
      1.0μL template DNA
      --------**50.0μL** Total
3. Chose a suitable program, and adjust your annealing temperature and extention time as described below:

Annealing: 55°C for 0:30 min (different primers different annealing temperature)

Extention: 72°C for t min ("t" depends on the length of goal sequence, 1min per 1000bp)

Final extension: 72°C for 10:00 min

Colony PCR

1. Keep everything on ice. The PCR reaction mixture in the ice preparation, and then placed in a PCR reaction in the PCR reaction apparatus. This cold start (Cool Start Method) can enhance the specificity of PCR amplification to reduce non-specific reactions in the PCR process, can get good PCR results.
2. Make up a master mix of everything into one microcentrifuge tube.
3. Pipette up and down in the microcentrifuge tube, drain 25μL or 50.0μL solution to each PCR tube.
   a. **25.0μL reaction system**

      18.3μL ddH2O
      2.5μL 10x Buffer (mg^{2+})
      2.0μL dNTPs
      0.5μL forward primer
      0.5μL reverse primer
      0.2μL DNA polymerase
      Colony stab (template DNA)
      --------**25.0μL** Total

   b. **50.0μL reaction system**

      37.75μL ddH2O
      5.0μL 10x Buffer (mg^{2+})
      4.0μL dNTPs
      1.0μL forward primer
      1.0μL reverse primer
      0.25μL DNA polymerase
      Colony stab (template DNA)
      --------**50.0μL** Total
4. Pick colonies from plates, spot onto these PCR tubes.
5. Run the "Colony PCR" program, and adjust your extention time as described below.

   The "Colony PCR" program
   Initial denaturation: 95°C for 5:00min
   30 cycles of:
   94°C for 0:30 min

55°C for 0:30 min (different primers different annealing temperature)
72°C for t min ("t" depends on the length of goal sequence, 1min per 1000bp)
Final extension: 72°C for 10:00 min

Gel Electrophoresis

1. Prepare a 1% weight-to-volume agarose gel (400ml)
   a. Dilute stock of 50×TAE to 1×with ddH2O.
   b. Measure 400 ml of 1×TAE buffer.
   c. Transfer 1×TAE buffer to Duran bottle
   d. Weigh out enough agarose to make 1% gel. (1% of 400mL is 4.0 g)
   e. Transfer agarose to Duran bottle.
   f. Melt agarose in microwave, stirring ever 15-20 seconds until completely melted.
   g. Allow gel to cool until Duran bottle can be handled comfortably, pour agarose into gel tray, assemble gel pouring apparatus by inserting gate into slots
2. Allow agarose to cool, place the gel in the apparatus rig with the wells facing the negative end (black-colored)
3. Fill the rig with 1x TAE buffer
4. Load 2μL of DNA maker into lane
5. Mix 1μL of 6x loading buffer with 2μL DNA sample, load them into lane.
6. Run at 130V for 30 min.
7. Use Ethidium bromide dyeing gel ten minutes.(EB is dangerous to work with; Gloves must be worn at all times during the whole procedure)
8. Use Gel imaging system check gel.
9. Take picture for gel

Gel Extraction of DNA (Spin Column Extraction)

E.Z.N.A.™ Gel Extraction Kit

1. **Excise gel slice containing DNA fragment of interest.**
   a. Gel electrophoresis fractionates DNA fragments.
   b. The gel is exposed to UV to find the DNA fragments (stained by Ethidium bromide).
   c. The goal DNA band is identified.
   d. Physically remove the slice of gel contains the goal DNA with clean surgical blade.
2. **DNA Purification**

e. Determine the appropriate volume of the gel slice by weighing it in a clean 1.5 ml microcentrifuge tube.
f. Add an equal volume of Binding Buffer (XP2).
g. Incubate the mixture at 55°C-60°C for 7 min or until the gel has completely melted.
h. Mix by shaking or vortexing the tube in increments of 2-3 minutes. (IMPORTANT: Monitor the pH of the Gel/Binding Buffer mixture after the gel
has completely dissolved. DNA yields will significantly decrease when pH >
8.0. If the color of the mixture becomes orange or red, add 5 µl of 5M Sodium
Acetate, pH 5.2 to bring the pH down. After this adjustment, the color of the
Gel/Binding Buffer mixture should be light yellow.)
i. Place a HiBind® DNA column in a provided 2 ml collection tube.
j. Apply 700 µl of the DNA/agarose solution to the HiBind® DNA column, and centrifuge at 10, 000 x g for 1 min at room temperature.
k. Discard liquid and place the HiBind® DNA column back into the same collection tube. For volumes greater than 700 µl, load the column and centrifuge successively, 700 µl at a time. Each HiBind® DNA column has a total
capacity of 25 µg DNA. If the expected yield is larger, divide the sample into
the appropriate number of columns.
l. Add 300 µl of Binding Buffer (XP2) into the HiBind® DNA columnCentrifuge at 10,000 x g for 1 min at room temperature to wash the column.Discard the flow-through and re-use the collection tube.
m. Wash the HiBind ® DNA column by adding 700µl of SPW Wash Buffer diluted with absolute ethanol. Centrifuge at 10,000 x g for 1 min at room temp.
Note: SPW Wash Buffer Concentrate must be diluted with absolute ethanol before use. See label for directions. If refrigerated, SPW Wash Buffer must be brought to room temperature before use.
n. Repeat step 8 with another 700µl of SPW Wash Buffer diluted with absolute ethanol.
o. Discard liquid and centrifuge the empty HiBind® DNA column for 2 min at maximal speed ($ 13,000 x g) to dry the column matrix. Do not skip this step, it is critical for the removal of ethanol from the HiBind® DNA column.
p. Place a HiBind® DNA column into a clean 1.5 ml microcentrifuge tube. Add 30-50µl (depending on desired concentration of final product) of Elution Buffer (10 mM Tris-HCl, pH 8.5) directly onto the column matrix

and incubate at room temperature for 1 minute. Centrifuge for 1 min at maximal speed ($13,000 x g) to elute DNA. This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower co[illegible]

Restriction Enzyme Digestion

To check if the two selected restriction enzymes can perform effective catalysis in the same solution

1. Mix DNA solution with the suitable amount of the master mix.
   a. **25.0μL reaction system**

      20.0μL DNA solution
      0.25μL BSA
      2.5μL 10x NEB Buffer
      1.0μL of each restriction enzyme
      1.25μL ddH2O

      --------**25.0μL** Total

   b. **50.0μL reaction system**

      40.0μL DNA solution
      0. 5μL BSA
      5.0μL 10x NEB Buffer
      1.5μL of each restriction enzyme
      2.0μL ddH2O

      --------**50.0μL** Total
2. Pipette up and down in the EP tube.
3. Incubate: 37°C for 2 hours

Ligation

1. Check the concentration of DNA fragments and vector which are going to be ligated.
2. Calculate the amount of partA/partB and vector added, based on the fragment length. Note that a ligation using a molar ratio of 1:3-1:5 vector to inserts.
3. Add DNA/buffer and ligase together in the EP tube.
   20.0μL reaction system

A µL part A
B µL part B
VµL vector
2µL 10x T4 buffer
1.25µL ddH2O
1µL T4 ligase

--------20.0µL Total

4. Mix the reaction by pipetting up and down Gently and microfuge briefly.
5. Incubate at 16°C overnight (high concentration T4 DNA Ligase can be used in a 10 minute ligation)
6. Chill on ice and transform10-20 µl of the reaction into 50 µl competent cells.

Transformation of E.*coli*

1. Remove competent cells from freezer and allow to thaw on ice for 10 min
2. Take care not to disturb the competent E.*coli*: do not vortex them or pipette them up and down.
3. Add 50 µL of thawed competent cells and then 1 - 2 µL of the re-suspended DNA to the labeled tubes. Make sure to keep the competent cells on ice.
4. Incubate the cells on ice for 30 minutes.
5. Heating shock the cells by immersion in pre-heated water bath at 42°C for 60 seconds. A water bath improves heat transfer to the cells.
6. Incubate the cells on ice for 5 minutes.
7. Add 200µl of SOC broth (make sure that the broth does not contain antibiotics and is not contaminated)
8. Incubate the cells at 37°C for 2 hours while the tubes are rotating or shaking.
9. Prepare two dishes with LB agar and the appropriate antibiotic(s) with the part number, plasmid, and antibiotic resistance. Plate 20 µl and 200 µl of the transformation onto the dishes, and spread. This helps ensure that you will be able to pick out a single colony.

10. Incubate the plate at 37°C for 12-14 hours

11. Always keep agar plates upside down so that drips of condensation and falling debris do not contaminate them.

E. Coli Cell Culture

1. Media preparation: Add 10µl of 100mg/ml concentrated ampicillin for each 5 ml of liquid broth. Be careful to keep the medium sterile especially if not adding an antibiotic, to avoid contamination.

2. Label on the plates the colonies you wish to culture.

3. Pour 30 ml of the prepared medium into a falcon tube labelled to match the desired colony.

4. Using a sterile loop, transfer the colony to the liquid medium in the tube.

5. Incubate for 14-16h in a shaking incubator at 37 degrees.

MiniPrep

SanPrep Plasmid DNA Kit

1. Preparation
   a. Make sure that RnaseA has been added into BufferP1
   b. Make sure that ethno has been added into Wash Solution (stored at 4°C)
   c. Make sure that P2 and P3 don't have any sediment
2. Extract 1.5-5ml overnight suspension culture and centrifuge at 8000g for 2 minutes to recollect bacteria and discard culture.
3. Add 250µl BufferP1 and suspend bacteria
4. Add 250µl BufferP2, immediately overturn the tube for 5-10 times. Stay in room temperature for 2-4 minutes to split bacteria.
5. Add 350µl BufferP3. Large amount of flocks appear. Overturn the tube for 5-10 times. Be careful don't let the flocks disperse.
6. Centrifuge at 12,000g for 5-10 minutes. Move supernatant into a absorbing column and centrifuge 8000g for 30s. Discard liquid in collection tube.
7. (Optional) Add 500µl Buffer DW1 and centrifuge 30s at 9,000g. Discard liquid in collection tube.

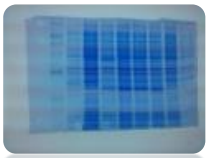
8. Add 500μl Wash Solution, centrifuge at 9,000g for 30s. Discard liquid in collection tube.
9. Repeat step8
10. Centrifuge empty tube at 9,000g for 1min

(Using a vacuum centrifuge enrichment machines concentration, using a vacuum centrifuge enrichment machines concentration of alcohol solvent model 45 degrees 3 minutes, you can effectively remove the residual alcohol, to ensure the quality of plasmid elution.)

Extraction of Genomic DNA from cyanobacteria

1. Add 1.5ml cyanobacteria culture media to 1.5ml eppendof tube. Centrifuge at 5000~6000rpm for 5min.
2. Remove the supernate.
3. Centrifuge at 12000rpm for 5min.
4. Remove the supernate.
5. Collect the precipitate and freeze in liquid nitrogen. Grind the cells using mortar and pestle in the presence of liquid nitrogen until finely ground. Transfer the powder to 1.5ml eppendof tube. Add 65 degree Celsius preheated CTAB and mercaptoethanol, then waterbath at the same temperature for 1h.
6. Add 600ul phenol-chloroform-isopropanol (24:25:1) mixture and mix by inversion to form an emulsion. Then centrifuge at 12000rpm for 15min. Collect the supernate.
7. Add chloroform-isopropanol (24:21) mixture and mix by inversion. Then centrifuge at 12000rpm for 15min.
8. Add 1ml -20 degree Celsius precooled anhydrous ethanol. Mix well. Place in refrigerator -20 degree Celsius for 3~4h or -80 degree Celsius for 30min.
9. Centrifuge at 12000rpm for 10min.
10. Remove supernate and wash with 75% v/v ethanol for 2~3 times. Centrifuge at 12000rpm for 5min each time. Vacuum-dry or dry in ultraclean area.
11. Add TE or ddH_2O and dissolve for use.

SDS Page Gel Electrophoresis



1. Preparation of the Gel

(1)Combine all reagents except the TEMED for the 15% separating gel. (10ml)

15% Separating Gel Components (4.195 mL)

2.3 mL deionized water
5.0mL 30% acrylamide/Bis
2.5 mL 1.5 M Tris, pH 8.8

0.1mL 10% SDS

0.1mL 10% ammonium persulfate

When ready to pour the gel, quickly add the TEMED

0.004mL TEMED, pH 8.9

(Warning: Acrylamide is a neurotoxin. Use gloves, do not ingest.) **--------10.0ml** Total

(2)Mix using a Pasteur pipette, and transfer the separating gel solution between the glass plates in the casting chamber to about 3/4 inch below the short plate.

(3) Add a small layer of absolute ethyl alcohol on top of the gel prior to polymerization to straighten the level of the gel and remove unwanted air bubbles that may be present. Once the gel has polymerized, the ethyl alcohol can be removed by absorption with Kimwipes or filter paper. (4) Dry ethanol at RT to pouring the stacking gel.

(5)Combine all reagents except the TEMED for the 5% stacking gel.

5.0% Stacking Gel (3.0 mL)

2.1 mL deionized water

5.0mL 30% acrylamide/Bis

0.38 mL 1.0 M Tris-HCl, pH 6.8

0.03mL 10% SDS

0.03mL 10% ammonium persulfate

When ready to pour the gel, quickly add the TEMED

0.003mL TEMED, pH 8.9

(Warning: Acrylamide is a neurotoxin. Use gloves, do not ingest.) **--------3.0ml** Total

(6)Mix using a Pasteur pipette, and transfer the stacking gel solution between the glass plates in the casting chamber.

(7)Insert the well forming comb into the opening between the glass plates.

(8)Both the separating and stacking gels should polymerize within six minutes.

(9)Once the stacking gel has polymerized, the comb can be gently removed. The polymerized gel between the short plate and spacer plate forms the "gel cassette".

2. Sample Preparation

(1)Place some water in a 600 mL beaker and leave on a hot plate to boil. (This can take 15 minutes or more.)

(2)Centrifuge bacterial suspension at 4 ° C for 3 minutes at 15000g.Discard liquid and use 500μL Buffer G (0.5M Glycerol,1mM Na_2HPO_4)Resuspend it.

(3)Repeat step (2)

(4)Centrifuge bacterial suspension at 4 ° C for 3 minutes at 15000g .Combine 500μL Buffer G resuspend it. Add 100μL lysis buffer n every microcentrifuge tubes mix by gently inverting.

(5) Centrifuge at 15000g for 2-3min.
(6) Mix 15μL Supernatant with 5μL 4x Laemmli sample buffer. Mix 500μL Precipitation with 125μL 4x Laemmli sample buffer.
(7)In separate tubes, aliquot 10 mL of MW marker. (MW markers are already prepared in Laemmli sample buffer.)
(8)Boil the samples for 10 minutes to fully denature the proteins. Leave the samples at room temperature until ready to load onto the gel.

3.Electrophoresis

(1)Remove the gel cassette from the casting stand and place it in the electrode assembly with the short plate on the inside.
(2)Slide the electrode assembly (with the gel cassette) into the clamping frame. Press down on the electrode assembly while clamping the frame to secure the electrode assembly. This step is important to minimize potential leakage during the electrophoresis experiment.
(3)Pour some 1X electrophoresis buffer into the opening of the casting frame between the gel cassettes. Add enough 1X electrophoresis buffer to fill the wells of the gel. Use a gel loading tip to pipette some buffer into each well to ensure cleanliness.
(4)When all wells are sufficiently cleaned, slowly pipette 20μL of denatured sample or MW marker into each well.
(5)When the gel has been loaded, lower the clamping frame into the electrophoresis tank.
Fill the region outside of the frame with 1X electrophoresis buffer.
(6)Cover the tank with the lid aligning the electrodes (black or red) appropriately.
(7)Connect the electrophoresis tank to the power supply.
(8)Allow the samples to run at 80V until the dye front reaches the bottom of the 5.0% Stacking Gel (3.0 mL). change the electrophoresis Voltage 120V, This can take as long as around 2 hour.
(9)When electrophoresis is complete, turn off the power supply and disassemble the apparatus.

Measure the fluoscence Induction curves of Comparator

1. Activate strain on a Medium plate.
2. Incubate 12 hours at 37°C

3. Pick colonies from plates, spot into 20mL LB Liquid medium .
4. Incubate 3-5 hours at 37°C when its OD Approaching to 0.05 and the broth is a little bit turbid.
5. Add inducer into 96X black hole plate in advance.
6. Pipet 100 uL broth into 96X black hole plate Shaking culture 5 hours at 37°C.
7. Measure the fluorescence in the plate reader under the excitation wavelength of 485/20nm, emission wave wavelength of 516/20nm.
8. Export data and Analysis it.

Measure the response curves of phosphate

1. Prepare 1X MOPS without K2HPO4 added

2.Certain concentration of K2HPO4 stocks(for example,40mM K2HPO4).when we decide to measure the response curves of engineered cells,particular volumes of the stock solutions of K2HP04 are added to each subpackaged EP tube with 1mL MOPS medium without Pi.Since the induced concentration of phosphate is extre mely low, accurately quantificaiton needs to be done.

3. Finish a series of phosphate concentration gradient 1X MOPS media.

4. Cultivate engineered cells in the same LB medium for 6-8 hours to micro mixed state.

5.

Subpackage 1ml liquids into each EP tubes.Concentrifuge at 9,000 xg for 30 min.

6. Discard the medium and resuspend with 1mL 1X MOPS medium without Pi.

7. Repeat step 6.

8.

Pipett 10uL cells in each sample(in ep tube) and transfer into each prepared grad ient 1X MOPS

in EP tube.

9.

The induction time varies from 6 h-18h.The gradient of induction time can be eas ily set every

1-2 hours.

10. Pipet 100 uL samples into 96X black hole plate and measure the fluorescence in the plate reader under the excitation wavelength of 488nm.

Attachment：

MOPS Minimal Medium(C.Neidhardt, F., Culture Medium for Enterobacteria. J Bacteriol, 1974. 119(3): p. 736.)

10X MOPS mixture	**100**

	ml	
0.132 M K2HPO4	10 ml	
milliQ H2O	880 ml	
1mg/ml thiamine	0.1 ml	(optional - we do not use thiamine because it does not affect the growth rate of E. coli K-12 MG1655 in this medium.)
TOTAL	990 ml	

1. Mix ingredients above and adjust the pH to 7.2 with approximately 300 microliters 10 M NaOH.
2. Filter sterilize. Can be stored at 4 degrees for up to 1 month.
3. Before use add 10 ml 100X carbon source (as appropriate - we typically use a final concentration of 0.1% glucose).

·10X MOPS Mixture

1. In a 1 L beaker with a stir bar, add the following to ~300 ml milliQ H2O:

Component	FW	grams
MOPS	209.3	83.72
Tricine	179.2	7.17

2. Add 10 M KOH to a final pH of 7.4 (10 to 20 ml)
3. Bring total volume to 440 ml
4. Make fresh FeSO4 solution and add it to the MOPS/Tricine solution:

Component	FW	grams	H2O vol (ml)	stock conc (M)
FeSO4•7H2O	278	0.028	10	0.01

5. Add the following solutions to the MOPS/tricine/FeSO4 solution (see below how to make each of these):

Mix in the order shown!

Component	Volume
1.9 M NH4Cl	50 ml
0.276 M K2SO4	10 ml
0.02 M CaCl2•2H2O	0.25 ml
2.5 M MgCl2	2.1 ml
5 M NaCl	100 ml
Micronutrient stock	0.2 ml
Autoclaved milliQ H2O	387 ml
TOTAL	1000 ml

6. Filter sterilize with 1 L capacity 0.2 micron filter
7. Aliquot into sterile 100 or 200 ml plastic bottles and freeze at -20°.

·Stocks used in 10X MOPS mixture

Make each separately, mixing the amount indicated into the specified volume.

Store at room temp.

Component	FW	stock conc (M)	grams	vol (ml)	note
NH4Cl	53.49	1.9	50.82	500	
K2SO4	174.3	0.276	4.8	100	
CaCl2•2H2O	147	0.02	0.294	100	
MgCl2	203.3	2.5	50.75	100	
NaCl	58.44	5	292.2	1000	

·Micronutrient stock (100 ml)

Mix everything together in 40 ml autoclaved milliQ H2O, bring up total volume to 50 ml. Store at room temp.

Component	Formula	FW	Grams for 50 ml
ammonium molybdate	(NH4)6Mo7O24•4H2O	1235.9	0.009
boric acid	H3BO3	61.83	0.062
cobalt chloride	CoCl2	237.9	0.018
cupric sulfate	CuSO4	249.7	0.006
manganese chloride	MnCl2	197.9	0.040
zinc sulfate	ZnSO4	287.5	0.007

·Potasium phosphate K2HPO4 Solution:

Can be stored at room temperature after autoclaving.

Component	FW	stock conc (M)	grams	Vol (ml)	note
K2HPO4	173.2	0.132	23.0	1000	autoclave

Error Prone PCR

R C Cadwell and G F Joyce,1994.Mutagenic PCR. Genome Res. 1994 3: S136-S ,140

1. Prepare a 10× mutagenic PCR buffer containing 70 mM MgC12, 500 mM KC1, 100 mM Tris (pH 8.3 at 25°C and 0.1% (wt/vol) gelatin.
2. Prepare a 10× dNTP mix containing 2 mM dGTP, 2 mM dATP, 10 mM dCTP, and 10 mM TTP.
3. Prepare a solution of 5 mM MnC12. DO NOT combine with the 10× PCRbuffer, which would result in formation of a precipitate that disrupts PCR amplification.
4. Combine 10 ul of 10×mutagenic PCR buffer, 10 ul of 10×dNTP mix,

30 pmoles of each primer, 20 fmoles of input DNA, and an amount of H2Othat brings the total volume to 88 ul. Mix well.

5. Add 10 ul of 5 mM MnCl2. Mix well and confirm that a precipitate has not formed.

6. Add 5 units (2u1) of Taq polymerase (Cetus or licensed supplier), bringingthe final volume to 100 u1. Mix gently. Cover with mineral oil or a waxbead, if desired.

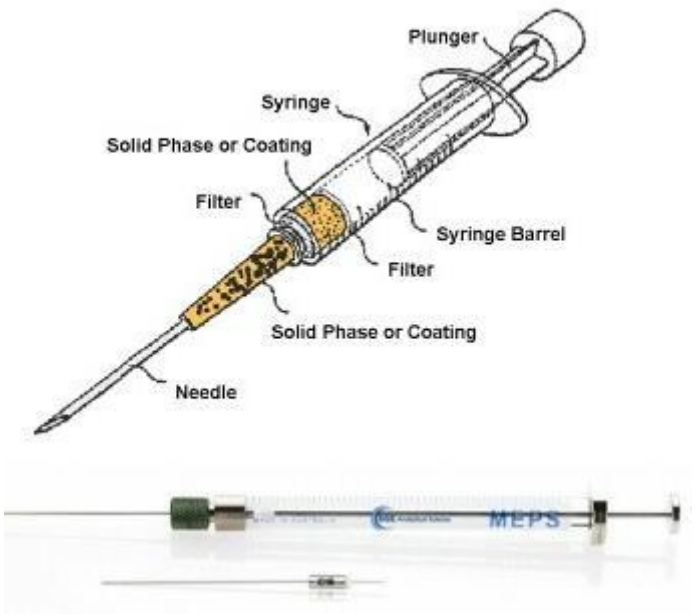
7. Incubate for 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1min. Do not employ a "hot start" procedure or a prolonged extension time at the end of the last cycle.

8. Purify the reaction products by extraction with chloroform/isoamyl alcohol[24:1 (vol/vol)] and subsequent ethanol precipitation.

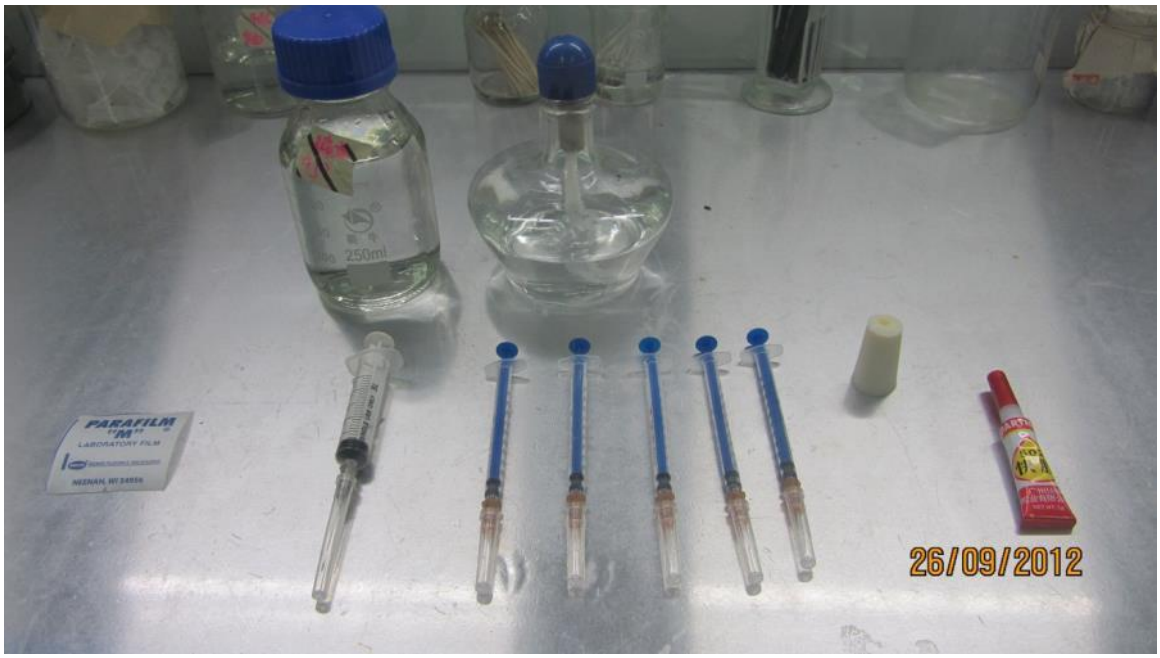
9. Run a small portion of the purified products on an agarose gel stained with ethidium bromide to confirm a satisfactory yield of full-length material.Mutagenic PCR should be carried out in parallel with standard PCR (omittingthe four changes listed above); the yields of full-length DNA should be comparable.

NEW APPROCH:

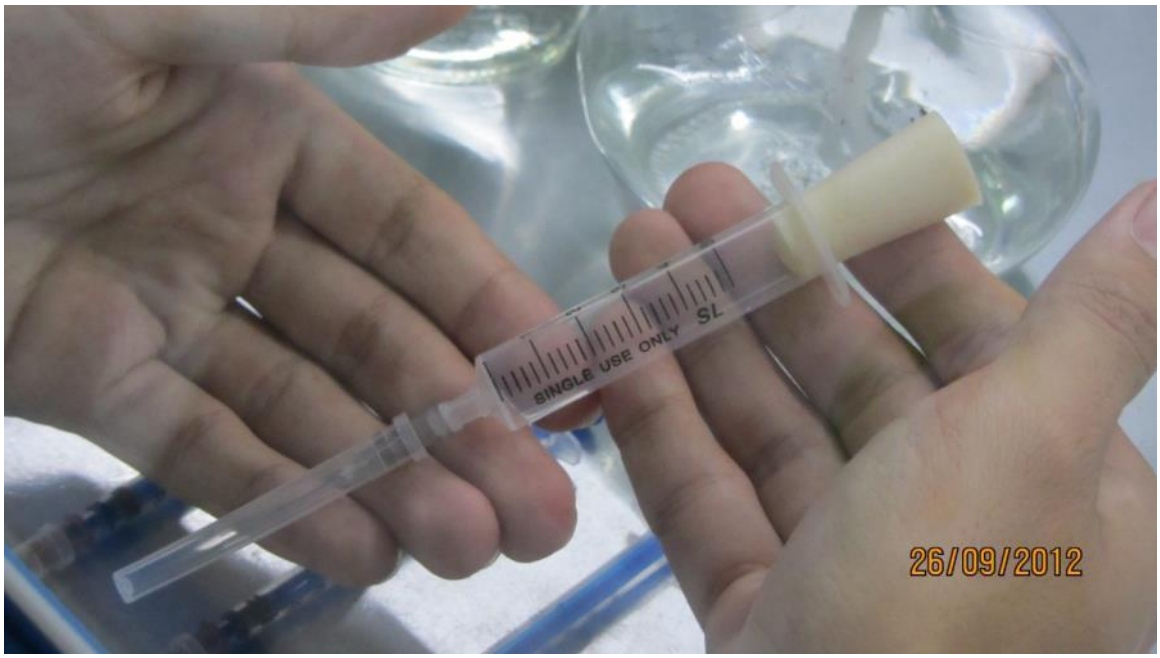
Trace stratified sampling device Manufacture process:



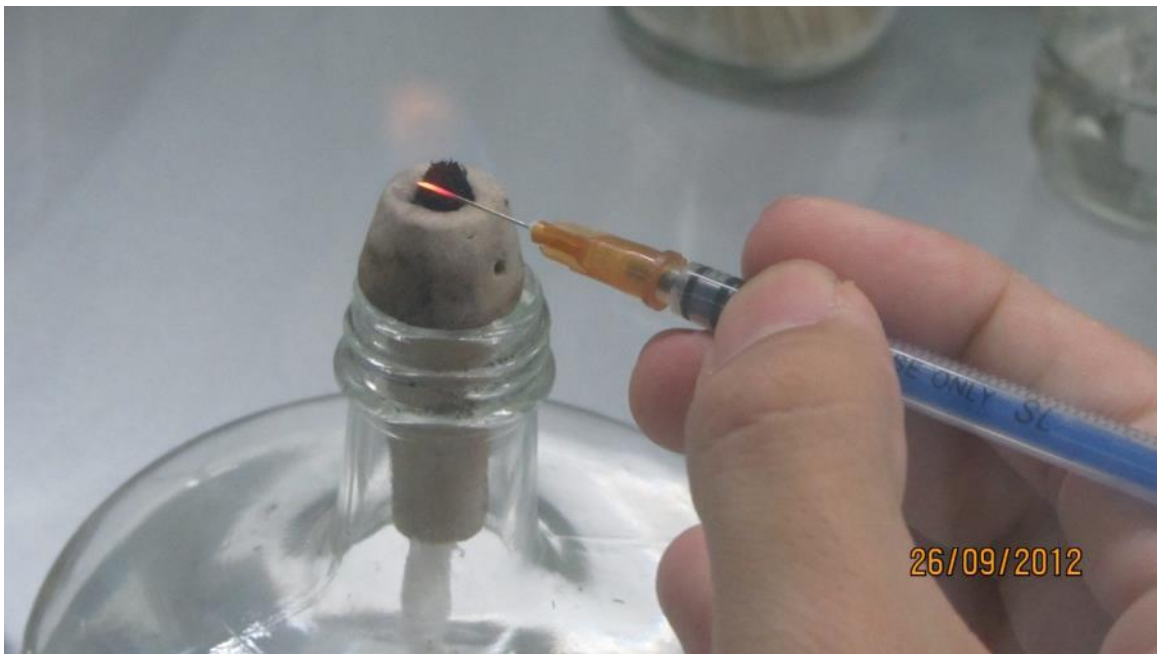
Structure of syringe



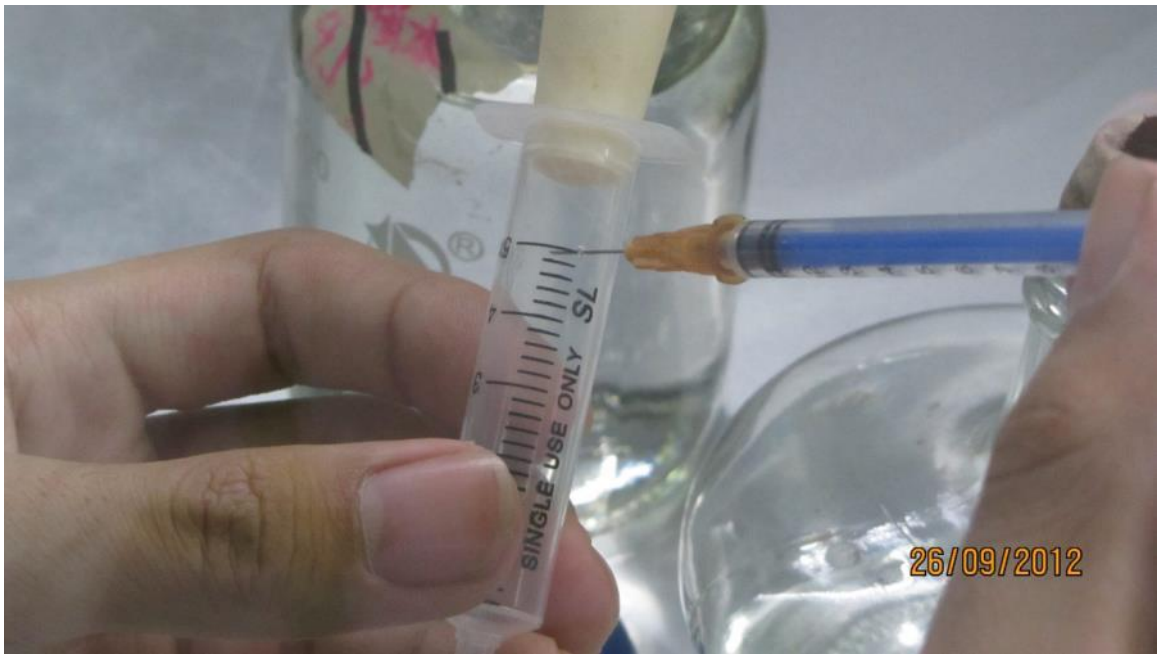
Step1：Before you make the trace stratified sampling device, you should prepare a 10 ml of aseptic syringe and five 1 ml of aseptic syringes one LaBoratoRy FILM, Silica gel plug, alcohol burner sterile water and seccotine.



Step2: Please pull out the plunger and needle. To cut the parafilm into small pieces posted on the Filter. Then fort silica gel plug and plug the needle again.

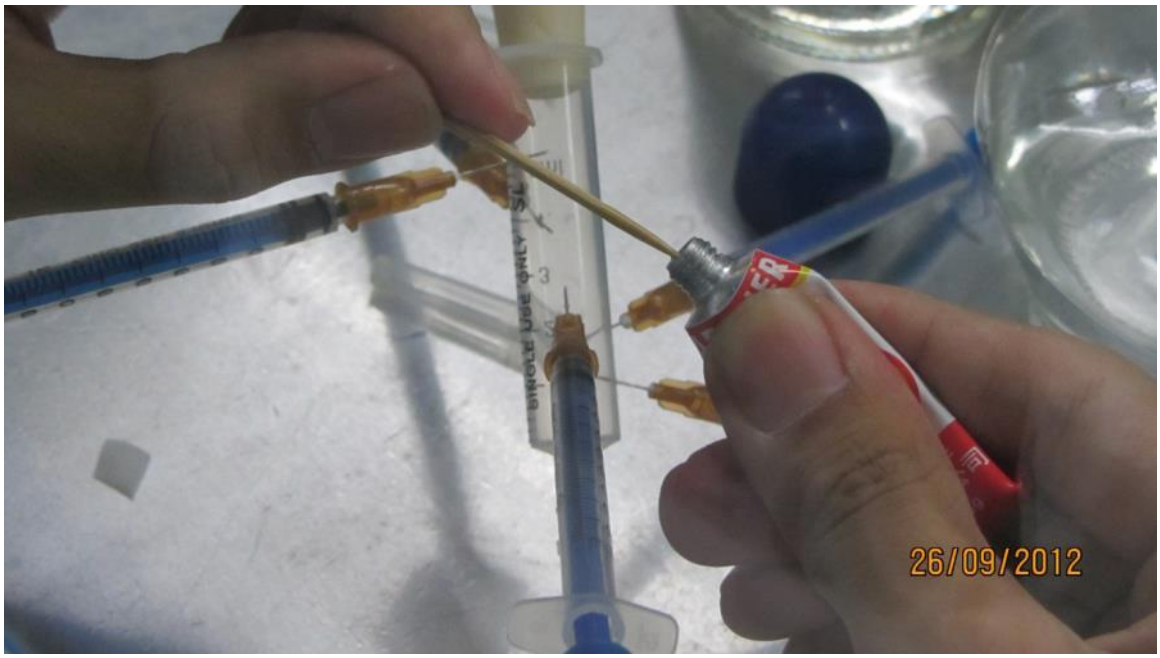


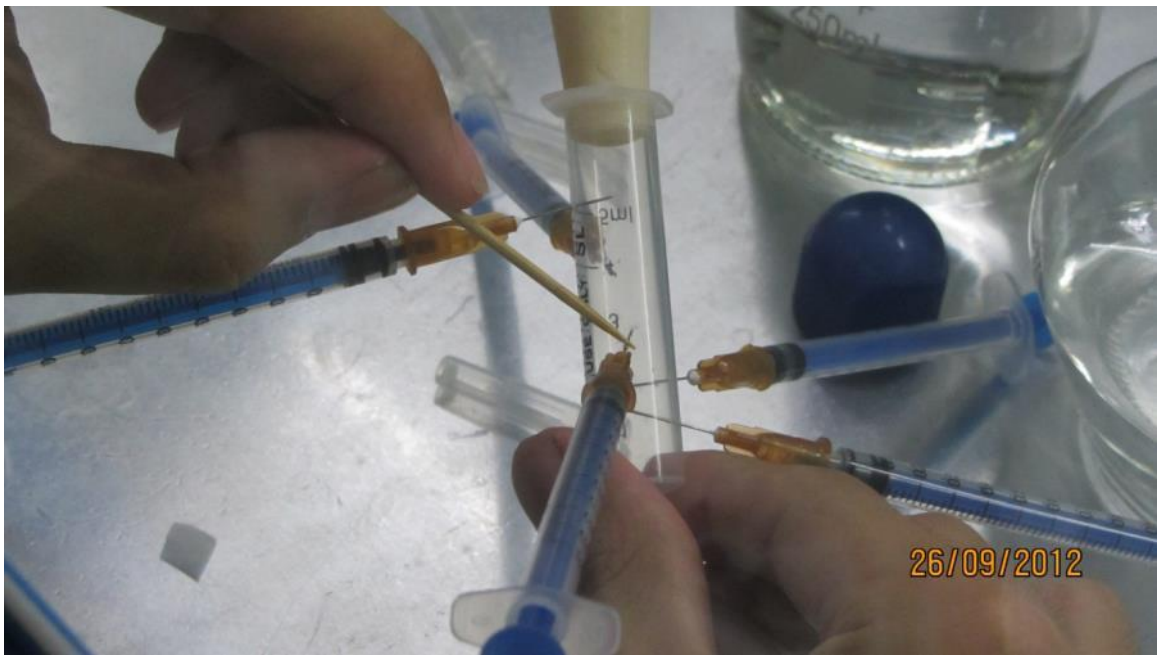
Step3: Put the 1 ml syringe needle in the alcohol burner flame on heating to red heat state.



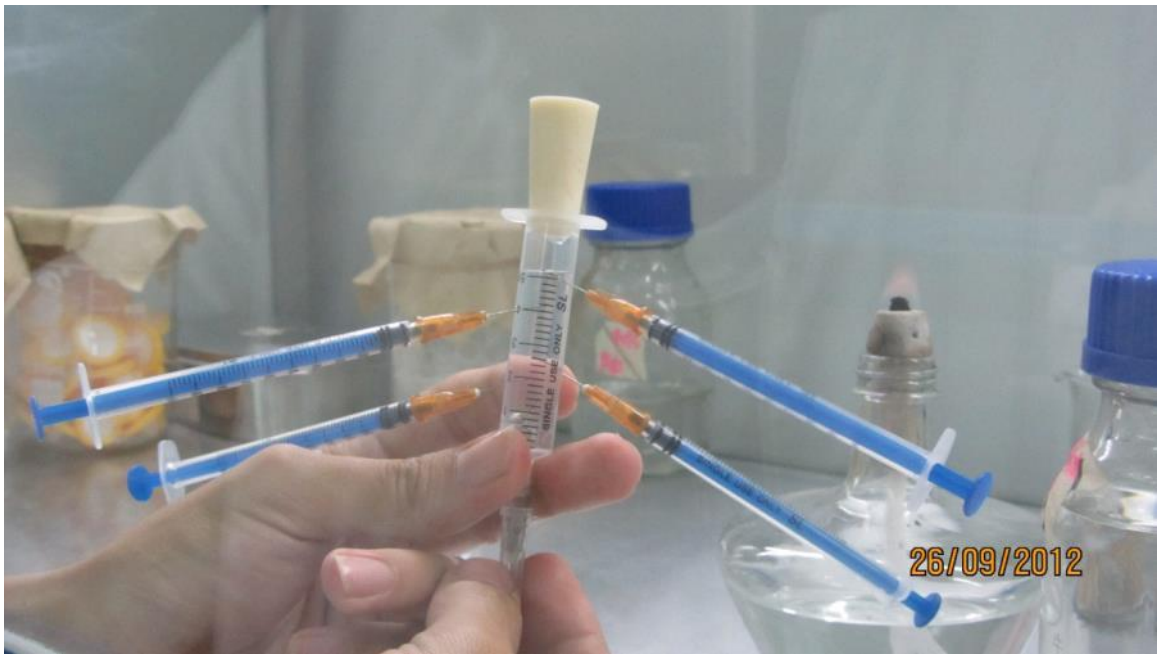
Step4: Find 10 ml syringe on corresponding position and forting the needle and putting off the air in the needle immediately at the same time to avoid melting plastic and block the pinhead.

Step5: Repeat the step 3 five times on the different corresponding position.

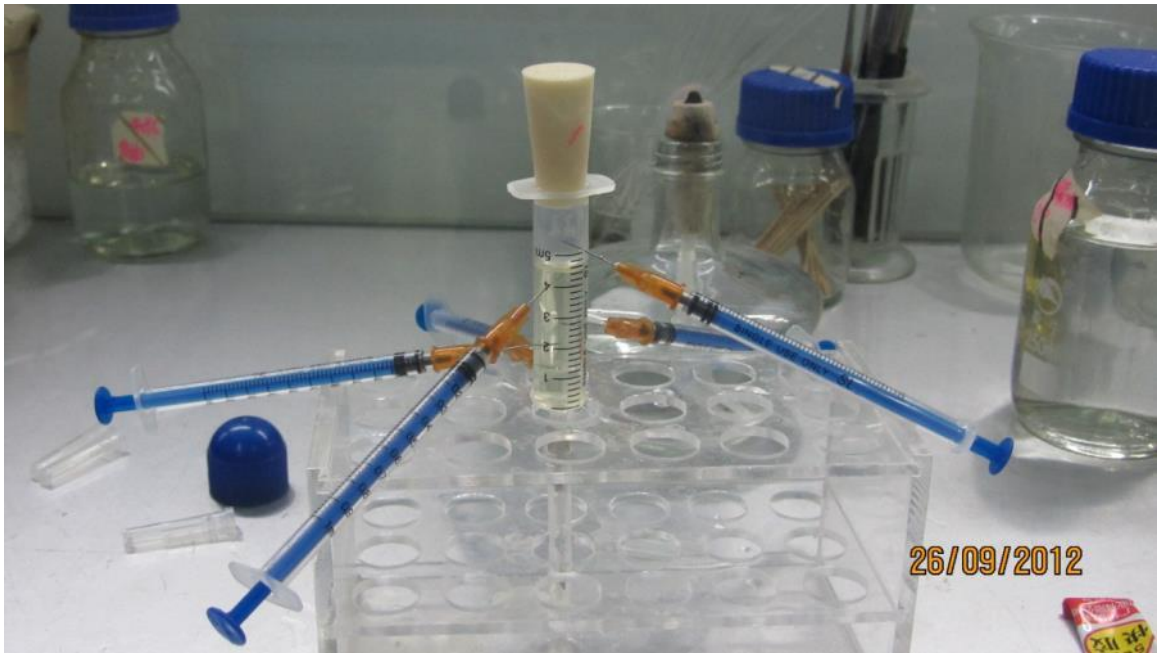




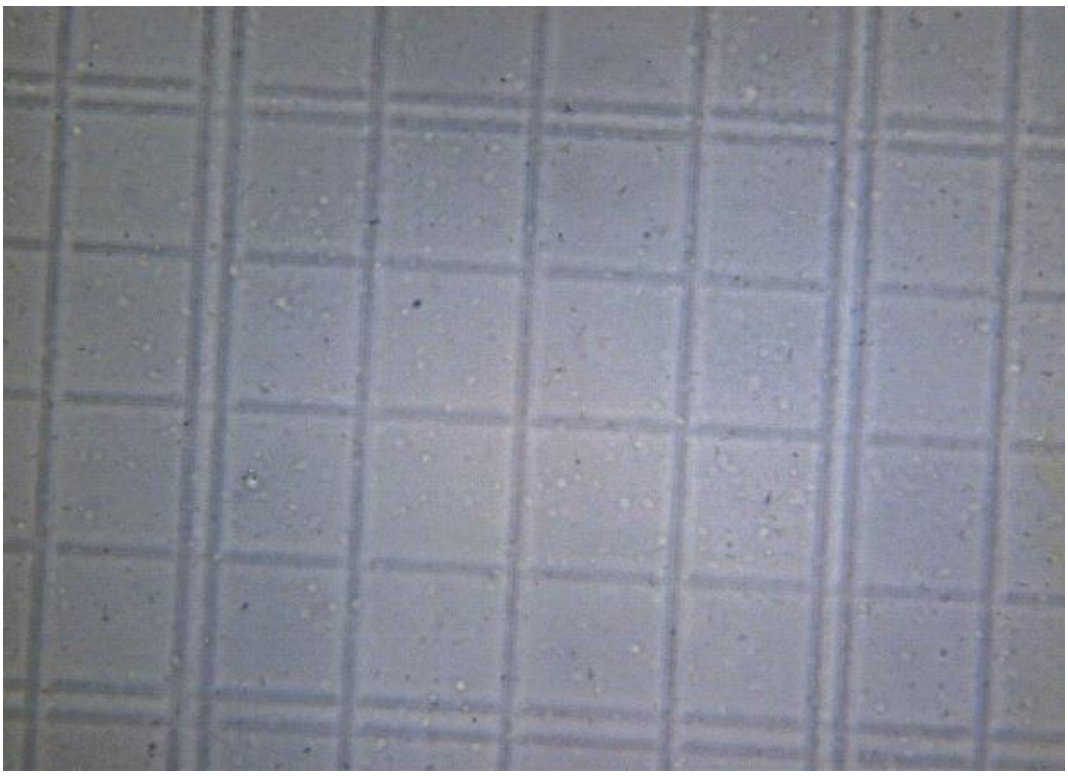
Step6: Use super glue to seal the gap when stab the needle.



Step7: After the Manufacture process, pour away the sterile water into syringe Barrel and Check whether leakage.



Step8: Pouring away the culture solution into syringe barrel and foster in standing.



Step9: Suck out bacteria to detect OD/600 and do blood counting.

Annotation: The whole process must be asepsis
The Angle between the 1ml syringes is 72 degrees