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- Optimize the condition of folding DNA origami – the mole ratio between scaffold and staple.

Mole ratio(scaffold:staple)	scaffold only	staple only	1:5	1:7	1:10
scafold(100nM)	52	0	2	2	2
staple(250nM)	0	8	4	5.6	8
10X TE	2	2	2	2	2
MgCl ₂ (200mM)	2	2	2	2	2
ddH ₂ O	14	8	10	8.4	6
total	20	820	20	20	20

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- Run the gel electrophoresis
 - Run two gels for each of the three structure. Six gels in total.
 - Box: 1-264(**A**)
 - bottom:265-348(**B**)
 - Top : 349-434(**C**)
 - 7 wells on one gel with the first one and the last one being the ladder.
Load 20 micro l in each well.
 - Choose the best ratio based on the gel result and conduct the PCR under different Mg²⁺ ion concentration accordingly.

Side notes:

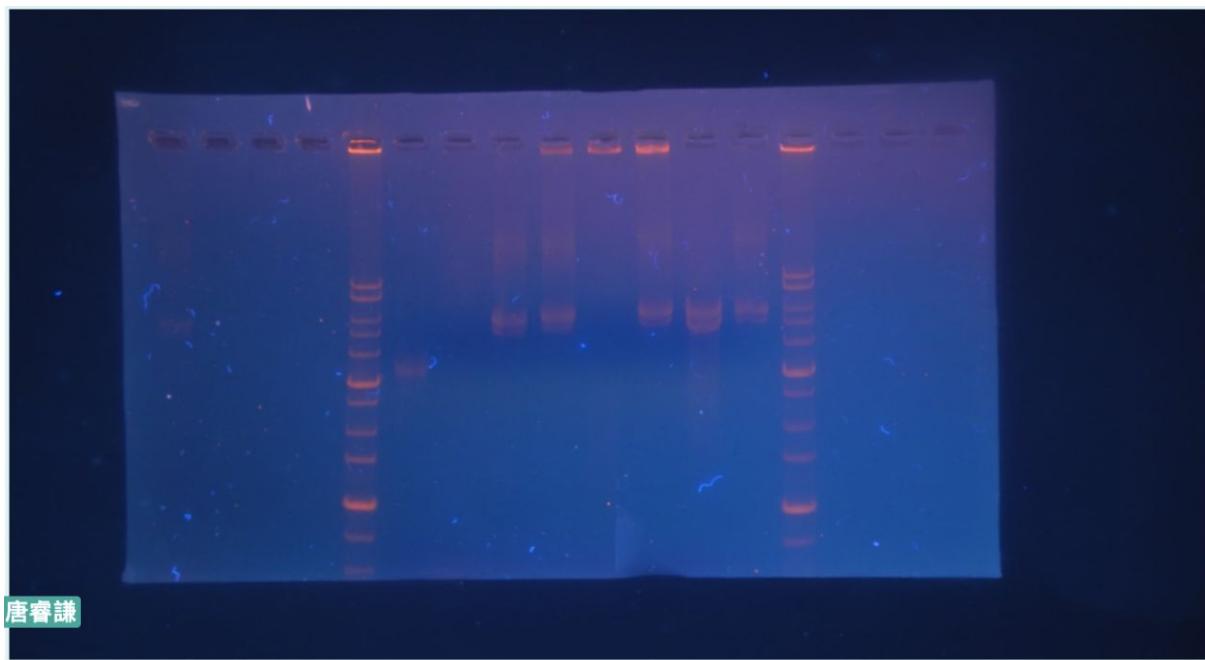
- PCR notation: f for scaffold, p for staple, Box: 1-264 are noted as **A**, bottom:265-348 are noted as (**B**)
- scaffold, staple (note 1, 256, 349 respectively),200mMMgCl₂, are all put in the -20 degree celcius fridge.

- Steps for running gel electrophoresis**

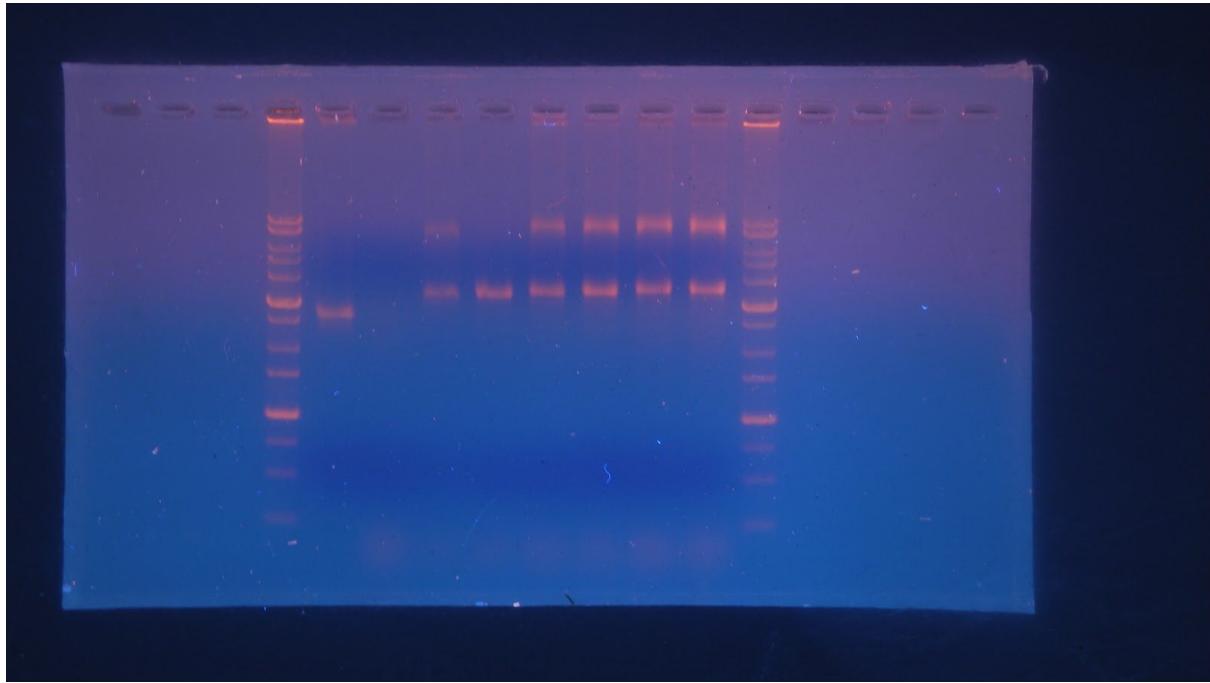
1. Mix 1xTAE buffer 150mL and agarose gel powder 1.5g, and heat up the mixture with microwave till the powder is completely dissolved and the solution becomes transparent.
2. Wait till the solution cools down a bit. Pour the mixture into the glue plate and put on the suitable tooth comb; leave it for 50 minutes for solidification.
3. Put the solid agar gel with the plastic case into the electrophoresis groove.
4. Mix 20 μ L of sample solution and 2.2 μ L of 10X loading dye, and load each sample to wells. (the first well and the last well is loaded with DNA markers.)
5. Add 3 μ L of (10^-5M) EtBr solution to the electrophoresis groove evenly.
6. Put on the lid and open the switch to run the electrophoresis with 110V half-voltage for an hour. (cover with tin foil to prevent EtBr from decomposing)
7. After the electrophoresis, put the agar gel in the UV box for observation.

- **determining scaffold/staple ratio**

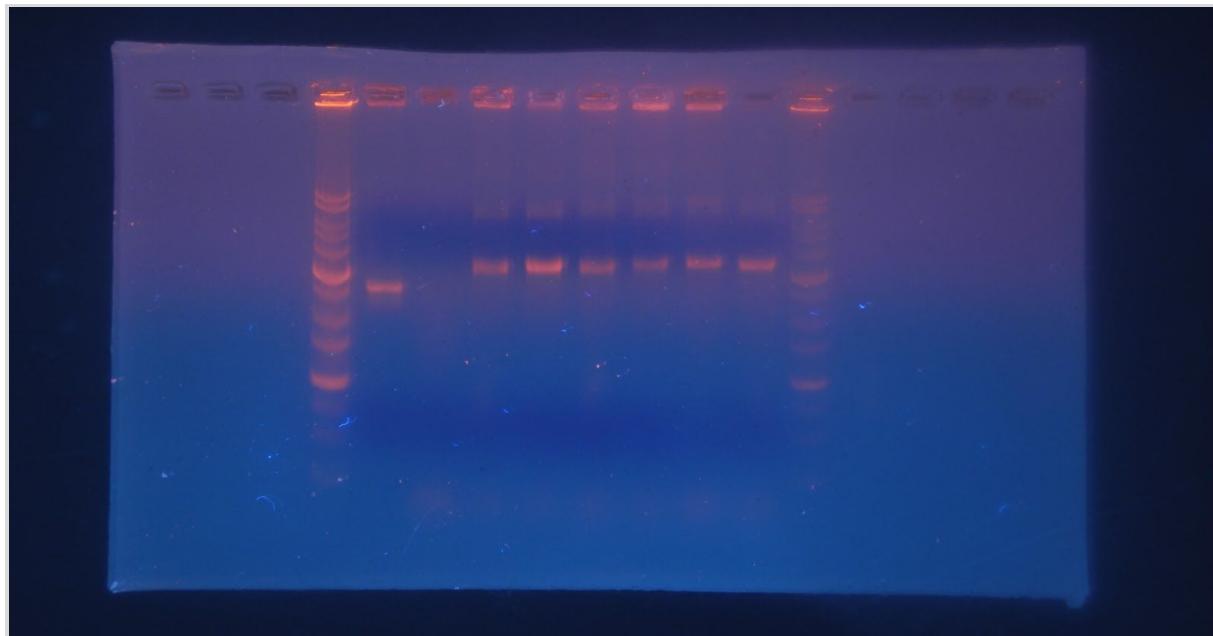
- box result (marker/scaffold/staple/1:5/1:5/1:7/1:7/1:10/1:10/marker)
 - We can see that all lanes have folded products, but we are not sure which position is the correct product. It seems that 1:5 and 1:10 are the two most productive ratios.



- bottom lid result
(marker/scaffold/staple/1:5/1:5/1:7/1:7/1:10/1:10/marker)



- top lid result (marker/scaffold/staple/1:5/1:5/1:7/1:7/1:10/1:10/marker)



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- Test 5 different Mg²⁺ ion concentrations. (10,15,20,25,30 microM)
 - $5 \times 2 + 1 + 1 + 2 = 14$ wells in total. 3 gels in total.
 - Since the heat released from the gel electrophoresis will disentangle the structure, we put the TAE buffer used during the experiment under 4°C for 30 min.

f&p formula. 20 micro l in total for each.

The image shows a handwritten lab notebook page. At the top, it says "30 min 10% ac". Below that is a sketch of a gel electrophoresis tank with two lanes labeled "f" and "p". To the left of the gel, there is a table for preparing "f" and "p" formulas. The table has two columns: "f" and "p". Under "f", there are four rows with values: 2, 2, 0, and 0. Under "p", there are four rows with values: 2, 3, 0, and 4. To the left of the table, there are handwritten notes: "10X TE 2", "MgCl₂ 30", "f 2", "p 4", and "H₂O 9".

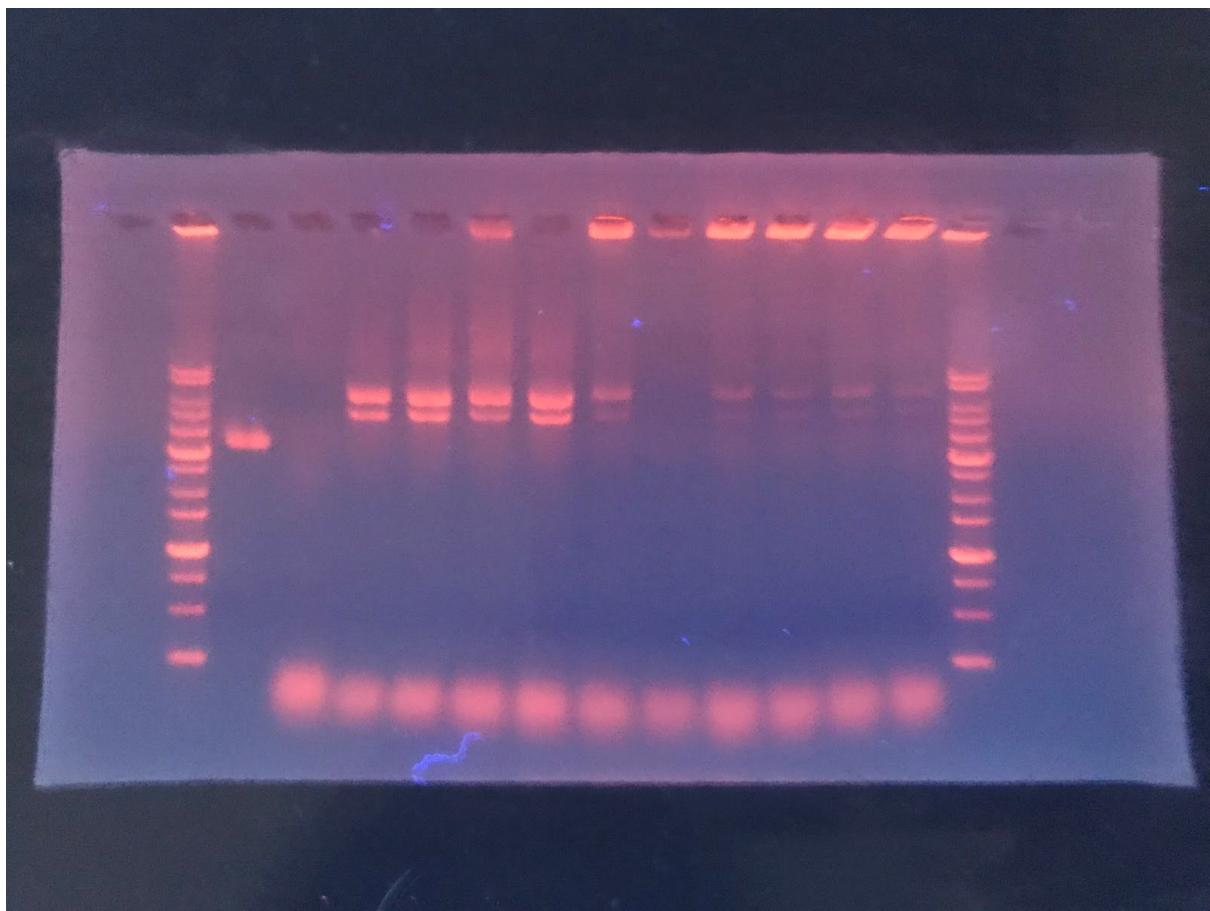
	f	p
	2	2
	3	3
	0	0
	0	4
H ₂ O	9	11

remember to run PCR for f & p as well.

Side notes :

1. The red dots on the staple are used.
2. Marker needs to be put in the -20 degree celsius firdge.
3. gel electrophoresis takes about 30 min. Wait till the deep blue dye reaches the third to last line.

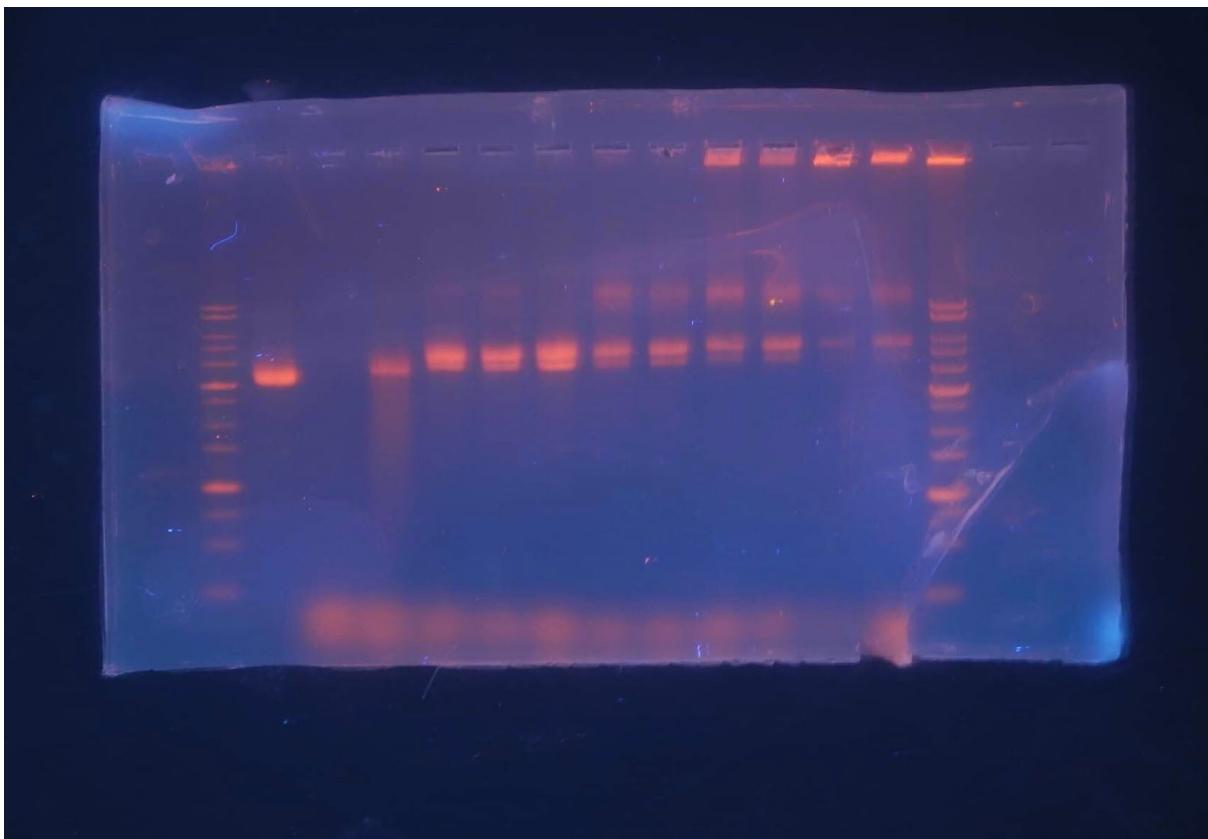
Result :



Box - choose 10mM
(marker/scaffold/staple/10mM/10mM/15mM/15mM/20mM/20mM/25mM/25mM/30mM
/30mM/marker)

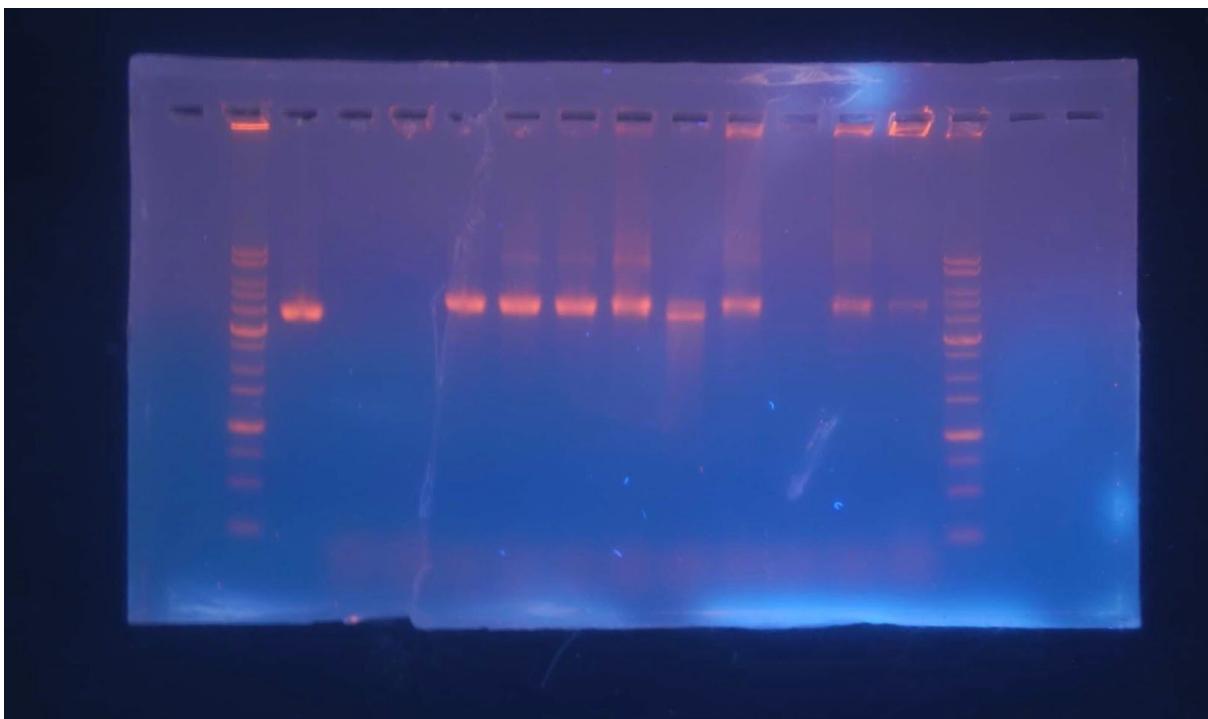
8/1

- Run the gel once again. The same as yesterday.



bottom lid - choose 15mM

(marker/scaffold/staple/10mM/10mM/15mM/15mM/20mM/20mM/25mM/25mM/30mM
/30mM/marker)



top lid - choose 15mM

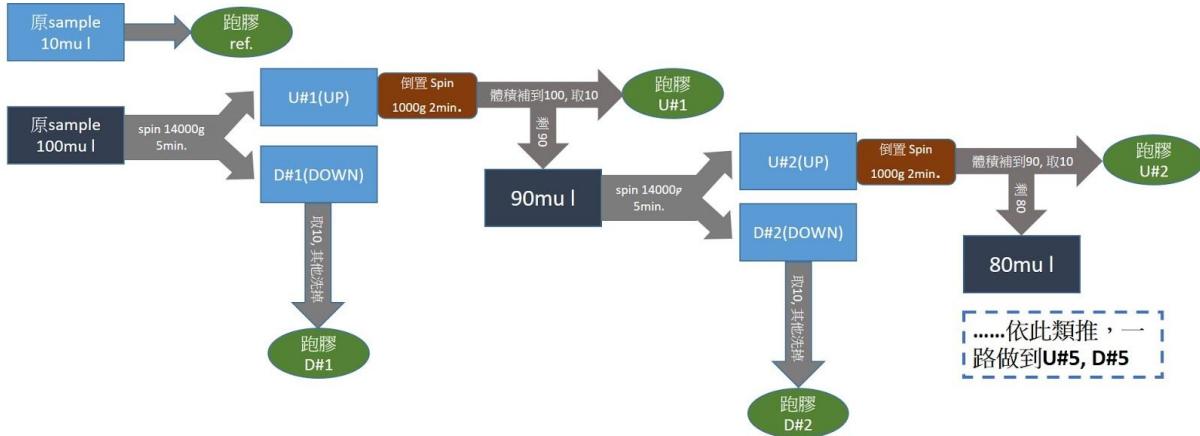
(marker/scaffold/staple/10mM/10mM/15mM/15mM/20mM/20mM/25mM/25mM/30mM /30mM/marker)

- box(10mM MgCl₂), bottom top(15mM MgCl₂) 50 μl for 10 tubes in total.
Run the PCR **using NanoMuscle protocol.**

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- **DNA origami Purification**
***wash before purify**
 1. assemble the filter and centrifuge tube, load 200μl 1xTEbuffer with 10mM Mg²⁺(box).
 2. 200μl 1xTEbuffer with 15mM Mg²⁺(lid)
 3. spin down at 14000g for 3 min
 - a. **7000g is suggested since little unbalance could lead to more serious result if the rotation is as quick as 14000g.**
 4. Turn the filter upside. (no need to cap). Spin the device at **1000 g for 2 min.**

***Purification Procedure**



這是原計畫。(一樣的步驟同時處理box, bottom, top lid 的材料。原材料各共有500 mu l · 也就是說處理一次如此純化要用兩管迷你eppendorf, 共計100mu l。)

註1: BOX 的 TOP 記做 X T; BOTTOM 的 DOWN 記做 B D. 另外，所有要跑膠的現在都在迷你eppendorf中(ref, U#1, D#1...)，標記皆在蓋子上。

註2: 之所以要補體積是為了要讓跑膠的每個well濃度皆相同，因為純化的目的就是要讓底部的渣渣逐漸消失，當濃度相同時，亮度相同，這樣比較時較有說服力。

※wash filter

1. wash with ddH2O*2
 - a. rotate in the 'up position' at 7000g for 3min.
 - b. rotate upside at 100g for 2 min.
2. sonication in ddH2O for 30~60min
3. keep in 20%EtOH

※plan for 8/4

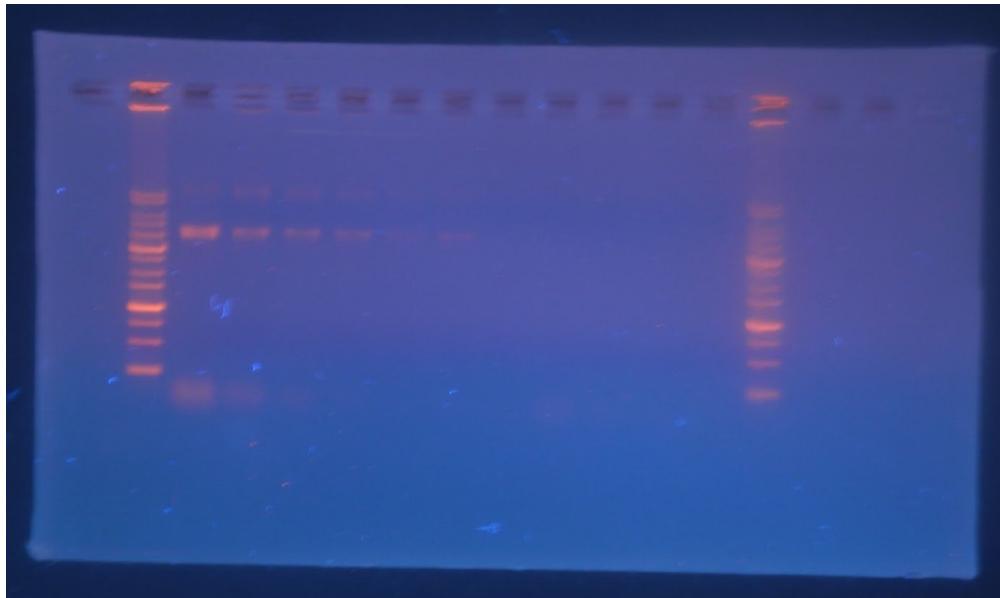
1. run three gels, add 2micro l loading dye of 6x conc. 10 micro l for each well.
2. original sample/U1/D1/U2/D2/U3/D3/U4/D4/U5/D5(x and t look quite similar, please pay extra attention to them.)

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- Run the gel after purification

bottom lid (B) :

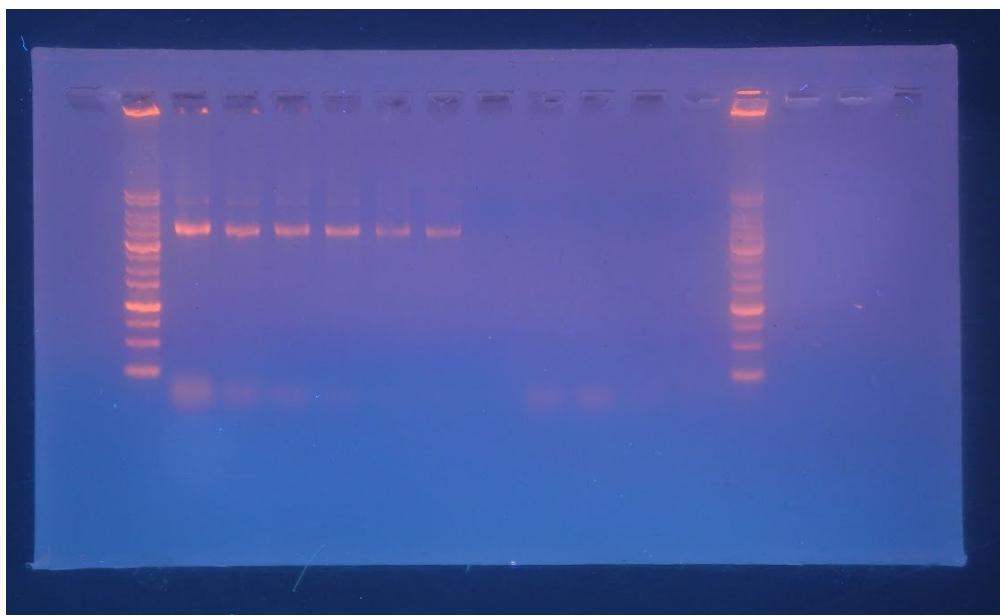
[from left to right] ladder/ref./U1/U2/U3/U4/U5/D1/D2/D3/D4/D5/ladder



- D is a bit weird. Nothing is seen.
- Purification is pretty good (U is apparently less in the third round). Maybe purified for three times is proper.

top lid (t):

[from left to right] ladder/ref./U1/U2/U3/U4/U5/D1/D2/D3/D4/D5/ladder



- Purification is pretty good. Purify 4 times is proper.

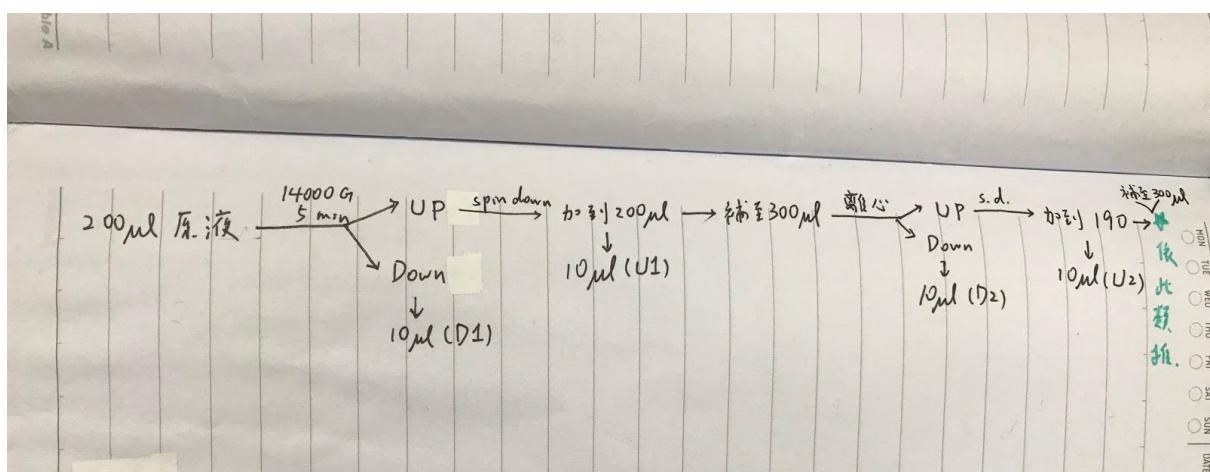
box (X) 由左至右依序為 : ladder/ref./U1/U2/U3/U4/U5/D1/D2/D3/D4/D5/ladder

純化效果不佳，U仍持續有staple，可能要再多純化幾次或擴大純化時的體積。（如
100microl->300microl）

U的上面（較近的亮帶，為摺疊產物）應該要維持一樣亮度，但這三張圖都顯示越來越淡，代表我們的摺疊產物在純化的過程中有所流失。而U和D的下方（遠端）是Staple等小分子，隨著純化進行應越來越淡。

8/5下午 佳儀、采蘩

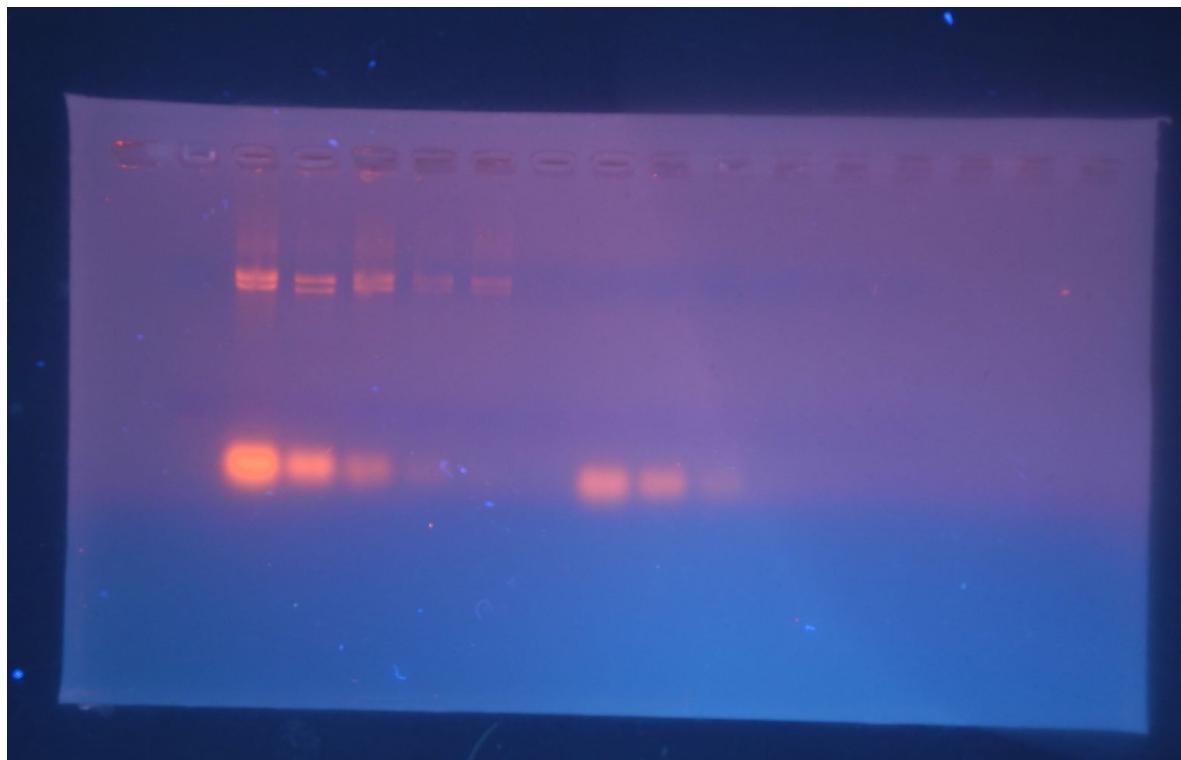
重新純化Box（共純化5次），流程圖如下：



Top/Bottom lid完成TEM要用的純化，top純化5次，bottom純化3次，皆按照之前8/2的流程

註1：第一次補體積補成水而非buffer，之後都有修正回buffer
 註2：Top lid 在第二次補體積時不小心補成100μl了（應為90μl）

8/5 晚上 采樣



新的Box 純化，由左至右依序為：

ladder/ref./U1/U2/U3/U4/U5/D1/D2/D3/D4/D5/ladder

討論決定採用第四次純化？

8/6

早上 采樣、佳儀

按照昨天流程完成Box純化，共純化4次

下午 采樣、佳儀、苡寧、泉浩 @生科大樓4樓 [生物影像平台]

TEM樣本前處理（未wash）。

生科院方提到如果產物都糾結在一起，可以用超音波震盪先處裡樣本。（據說上一屆花了很多時間2小時在震）

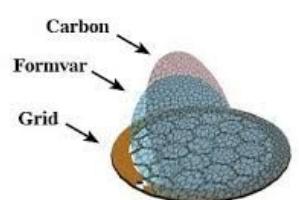
補充說明TEM銅網：把樣品load 7microl(形成完美包覆的液滴的最小需求體積)至**有膜的那面 (grid外圍的顏色較深的那面)**，用反夾鑷子夾取邊緣使用，不要碰到銅網。

負染UA用針筒裝，放在冰箱裡。由於是重金屬且用針釋液，故使用時要特別小心不要戳到手。

因樣品殘留MgCl₂，故潤洗load好樣品的銅網有助於去除鹽類，讓TEM的影像更美。

加完sample和UA的銅網存放在裝有濾紙的plate上，放入烘箱20分鐘。

關於TEM影像拍攝操作，可參考之前貼在群組的補充資料。



本次的protocol遵循上上屆：

1. Pick up a TEM copper grid (small) with negative tweezer.
2. Transfer 7 uL sample solution onto the TEM copper grid.
3. Wait for 35 seconds.
4. Remove sample solution from the side of the droplet with the edge of the filter paper.
5. Transfer a droplet of 2% UA (negative stain solution) onto the TEM copper grid.
6. Wait for 30 seconds.
7. Remove negative stain solution from the side of the droplet with the edge of the filter paper.
8. Place TEM copper grid under a lamp for 20 minutes.
9. Put TEM copper grid into TEM. (assisted by technician)
10. Observe with TEM and save the coordinates you intend to take photo of.
11. Take pictures and save files. (assisted by technician)

8/7 上午 苑寧、鈺璇、清璽、采蓁、重旬、睿謙

TEM操作

銅片的載入、卸載及照片拍攝都由老師操作，我們僅負責操作控制板，尋找欲留存的影像。

今天拍照的結果，有許多糊糊黑黑的團塊，且影像解析度不太好，看不到想要的結果。（尤以Box嚴重）

預期成果是透明，周圍有黑黑的UA染劑。

因此考慮將樣品稀釋／超音波震盪／加MgCl₂(學姊建議?)，但一次只操作一個變因，目前以重跑新的PCR（Nano Trap——加長annealing的時間）改變摺疊方式為目的，以期看到較為清晰的Box。

8/7 (重旬睿謙)



	A	B	C	D	E	F	
7	ddH2O		14	8	10	8.4	6
8	total		20	20	20	20	20
9							
10							
11	MgCl ₂ conc.(mM)	scaffold only	staple only		10	15	20
12	10X TE		2	2	2	2	2
13	MgCl ₂ (200mM)		1	1	1	1.5	2
14	scafold(100nM)		2	0	2	2	2
15	staple(250nM)		0	4	4	4	4
16	ddH2O		15	13	11	10.5	10
17	total		20	20	20	20	20
18							
19	10X TE composition(for 1 liter stock solution)可以依比例減少。				volume(ml)	final conc.(mM)	
20							

工作表1 ▾

工作表2

X 配 MgCl₂ 10mM / B, T 配 MgCl₂ 15mM 50 up 各六管

PCR protocol 改新的

	A	B
1		trap
2	denature	65oC for 15 min
3		to 45 @ 0.1oC / 7 min
4		Hold at 20oC



8/8

(A)睿謙 重旬 鈺璇 茲寧：

討論TEM無法找到產物的原因。歸咎2點：1.產物離心後沒有震盪，導致可能吸上的產物過少 2.沈降(settle)時間太少，僅有30秒，導致產物尚未附著於銅網，就被WASH掉

解套：將產物settle時間提升至5分鐘，染劑時間可能可以調整？

(B)睿謙 重旬 鈺璇 泉浩：

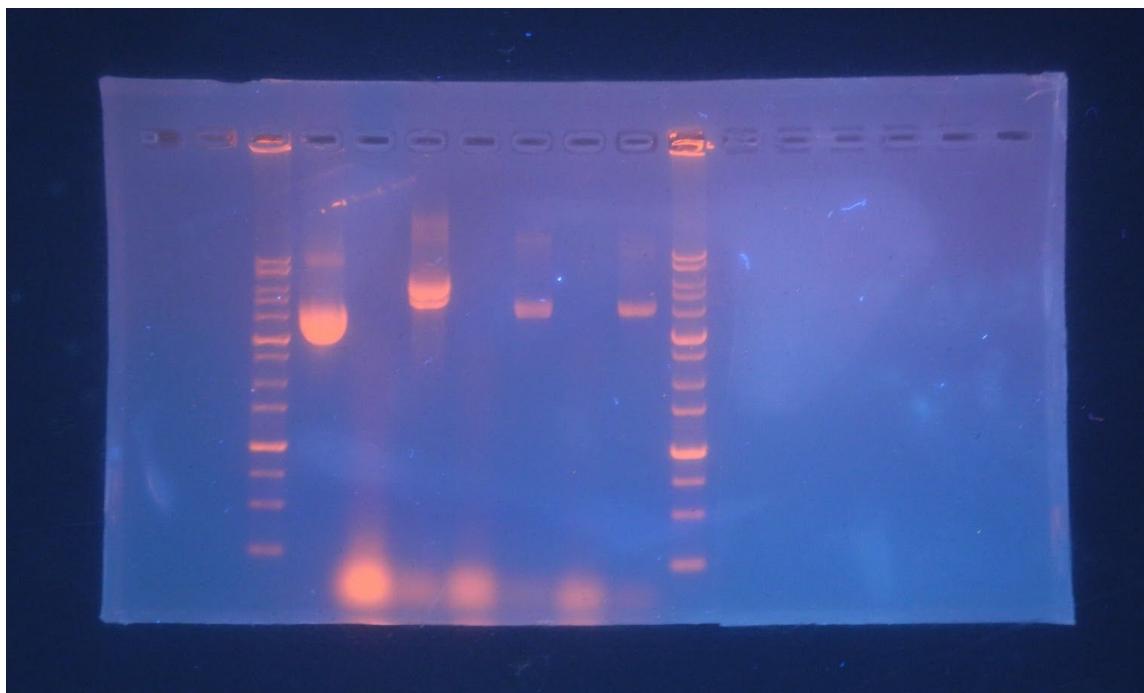
進行上步 pcr 後產物的電泳，炷膠時沒有放置tray，故使用罐子切割下來

跑電泳時，儀器短路導致DNA沒有分離，呈現隨機擴散狀態。

8/10 睿謙 重旬 泉浩

重新跑電泳(新的protocol, pcr跑24小時之成果，未純化)，以下是結果呈現。

各well分別是：marker/ scaffold / X staple / X / B p / B / T p / T / marker



解析：

BOX部分：於3、4處各有一條band，與第一次未純化結果大致相符。然而，3處的band和第一次的照片有所差異(第一次是在2~3處，此次是在3~4處)。另外，無法和純化後結果做比較。

BOTTOM的部分：於4~5有一條band，與第一次未純化結果相比，少了5~6的band；與純化後結果比較，相符，都是4~5。

TOP部分：結果與BOTTOM類似，於4~5處有band。少了第一次未純化的2處band，與純化後相符，只有一條4~5的band。

小結：

- 1.仍無法推測BOX的產物是哪一個亮帶，但應該是4處最有可能
- 2.bottom and top 的產物帶應該位於4~5處，因為和純化後結果相符，也同時代表我們新的protocol折疊效果更佳。

8/11 采葉、睿謙、泉浩

新PCR之純化+電泳

Tips: 擴大純化體積會延長純化所需時間，進而增加staple被濾出的機率。

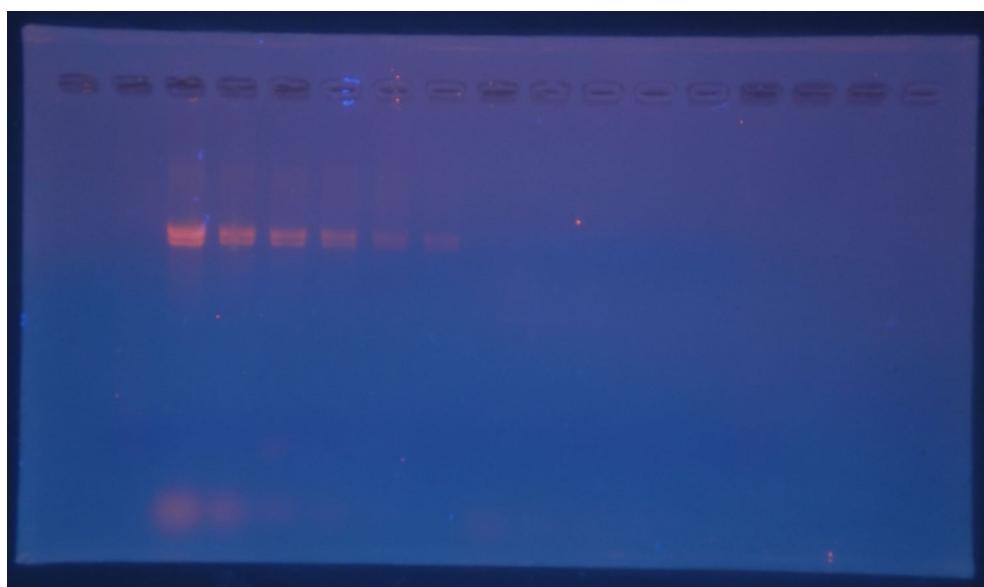
box—由左至右依序為（左二始）：

RNA ladder (加錯了所以看不到ladder) /未純化

/U1/U2/U3/U4/U5/D1/D2/D3/D4/D5/RNA ladder (加錯了所以看不到ladder)

由Up, 可以看到staple大約在純化第3次後大量減少, 因此**新protocol的box應純化3次**

至於down都沒有東西, 推測因為這次的純化將體積擴大到300microl, 導致Down (濾出液) 太稀而看不到東西。



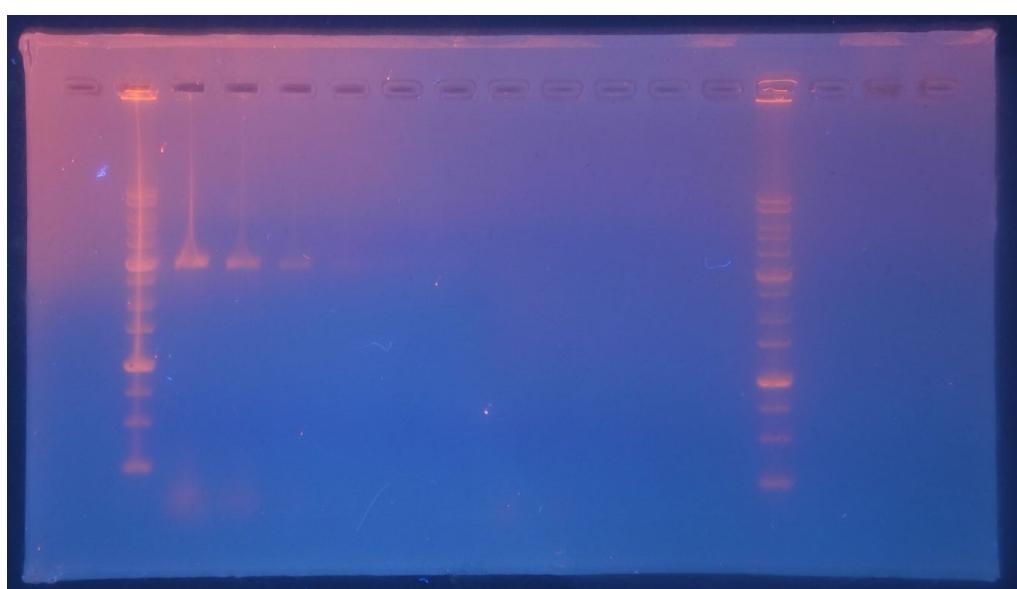
top lid—由左至右依序為：

DNA ladder/未純化/U1/U2/U3/U4/U5/D1/D2/D3/D4/D5/DNA ladder

down都沒有東西, 推測因為這次的純化將體積擴大到300microl, 導致Down (濾出液) 太稀而看不到東西。正常而言top lid & bottom lid的純化只需要把體積補回100microl即可。

前三排各有一條奇怪的垂直線, 原因未知, 可能是炷膠的問題。

和舊protocol的top lid (5~6)相比, 新protocol的產物在第7行。



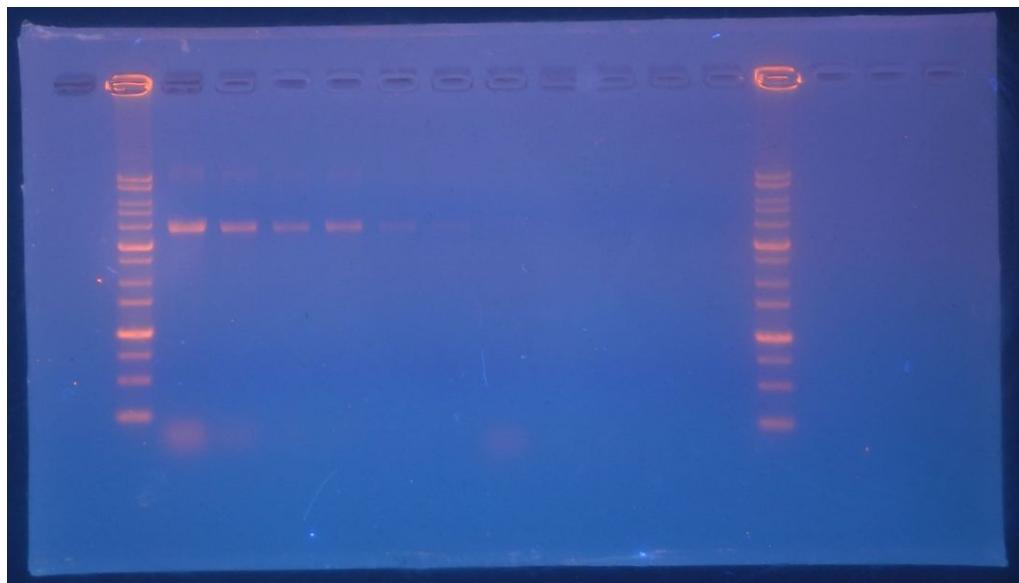
bottom lid—由左至右依序為：

DNA ladder/未純化/U1/U2/U3/U4/U5/D1/D2/D3/D4/D5/DNA ladder

down都沒有東西，推測因為這次的純化將體積擴大到300microl，導致Down（濾出液）太稀而看不到東西。正常而言toplid & bottom lid的純化只需要把體積補回100microl即可。

純化第三次似乎比第二次亮，推測是濃度／不均的原因。

和舊protocol的bottom lid相同，在第6行。



結論：box純化3次（要把體積補到300microl），top lid 3or4 次，bottom lid 3次。

注意：使用離心機務必注意轉速是*100G而非*100rpm，轉速過高會讓eppendorf斷頭很可怕！

8/12

早上：采葉、清靈

已純化5次的產物取30microl（還剩110microl）作超音波震盪2小時十下新protocol的pcr 各300microl

最後一管體積有點偏差，放在右二的位置。

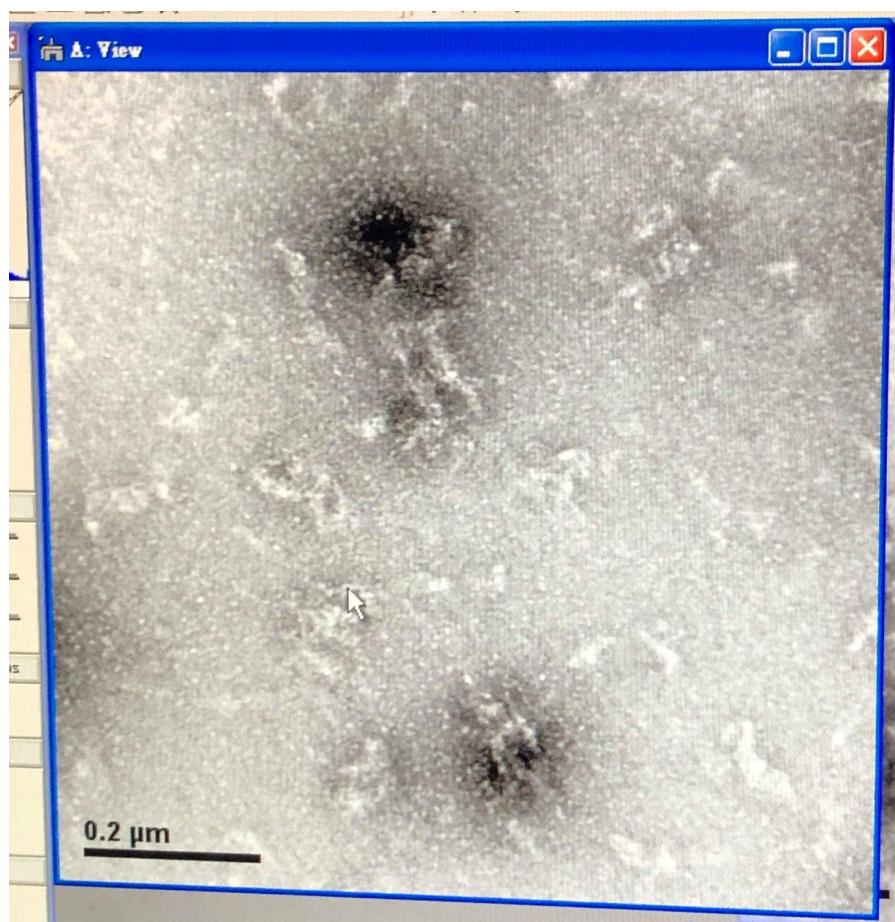
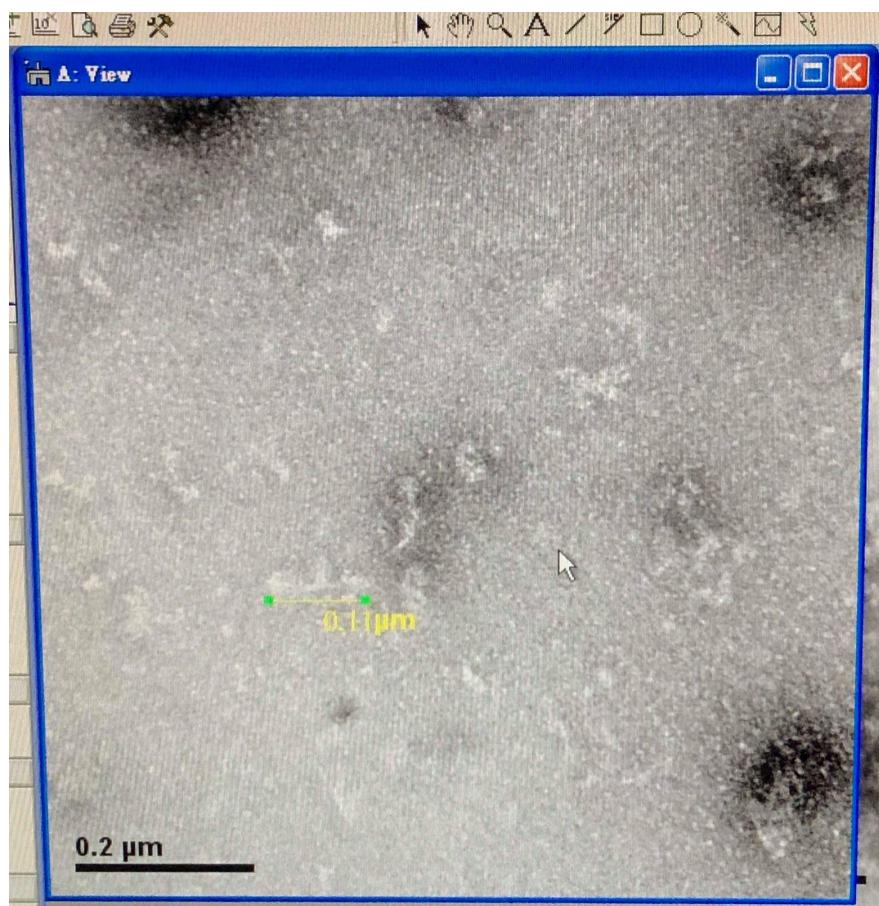
右一有點紅點，是額外配的50microl，因為當初配完250 microl才發現忘了預留保險空間。

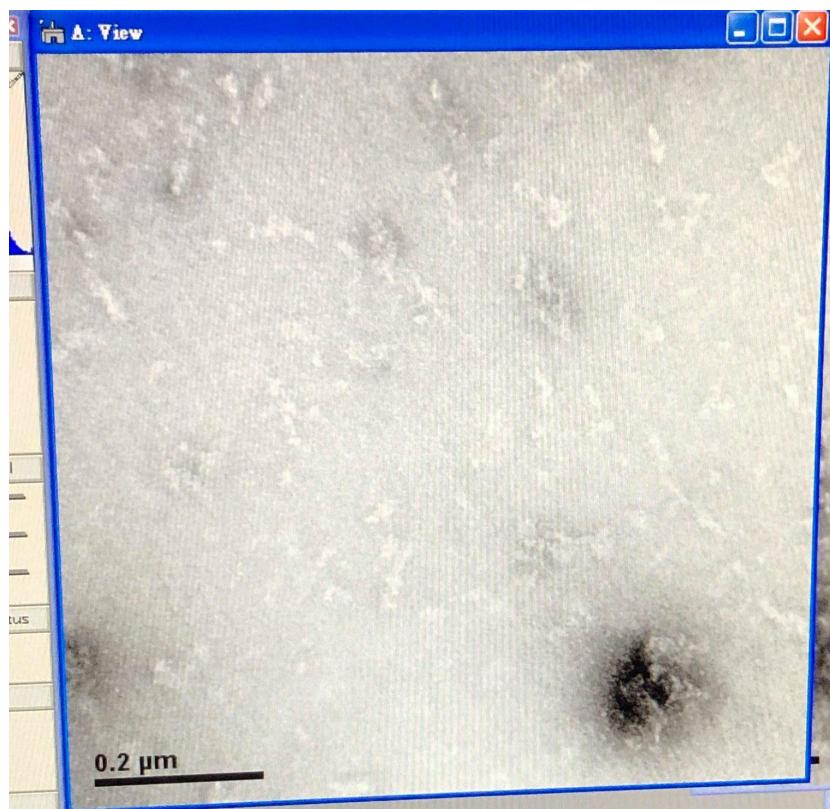
B和X的標示有塗改，敬請留意。

下午：重旬、泉浩、睿謙、采葉

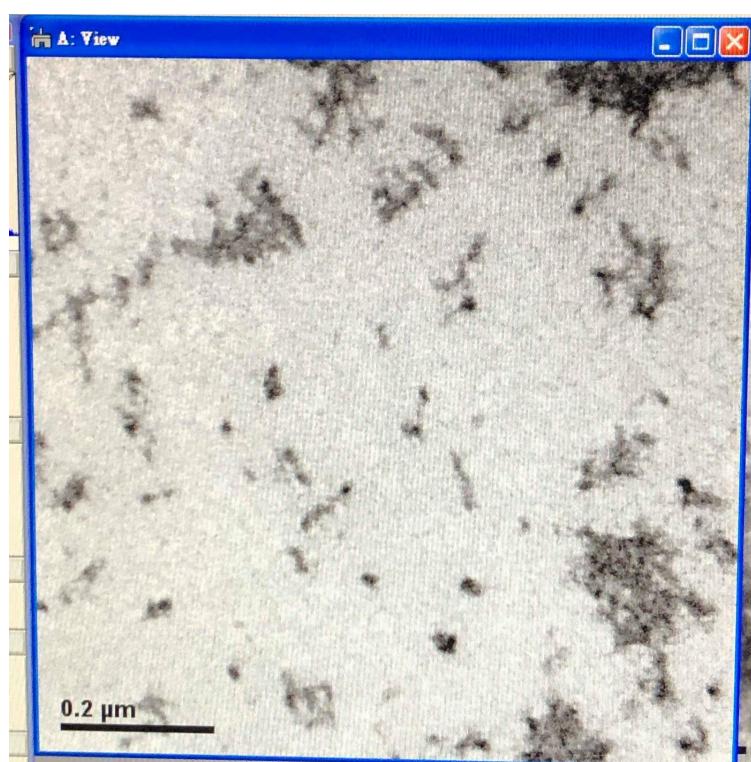
照Box（有&無超音波震盪）的TEM

已震盪2小時的box (settle 5 min, UA 35 sec.) 一可以看到重複性的條狀白色物，也許因為超音波震碎的關係，些許呈現破碎貌，也有一些糾纏在一起。





未震盪的box (settle 5 min, UA 35 sec.) —什麼東西都看不到的平原／黑黑的擠成一坨



結論：樣品要超音波震盪2小時以下（以防太碎）

8/13

早上：佳儀、清璽

box,top,bottom各取200μl原液純化三次，box有將體積補到300μl

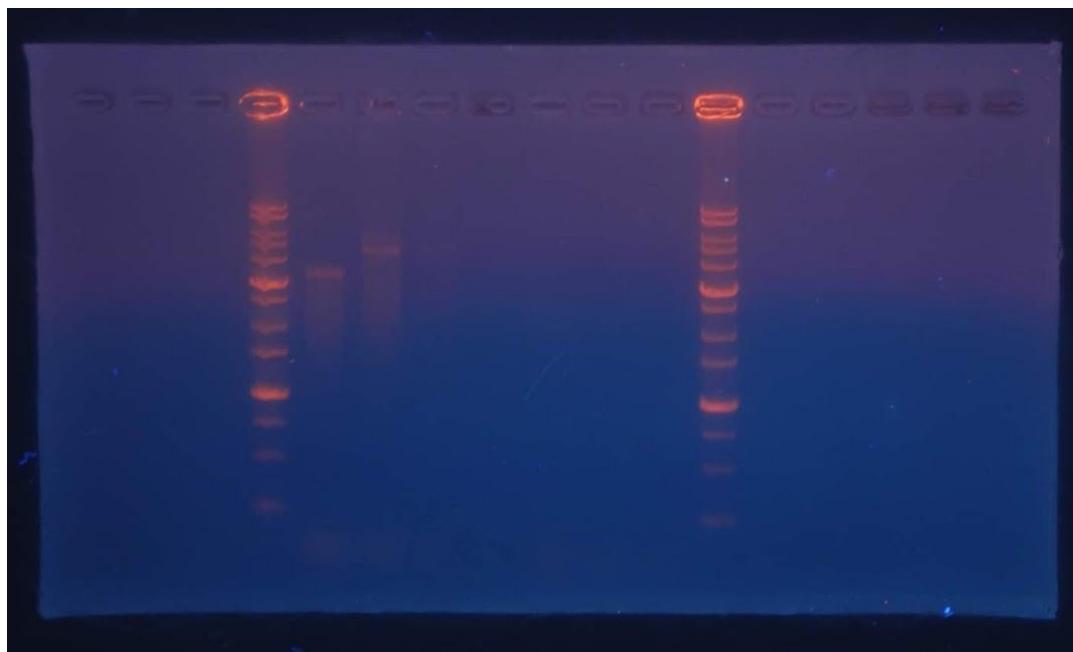
下午：佳儀、采蘋

將上午的結果跑膠

box

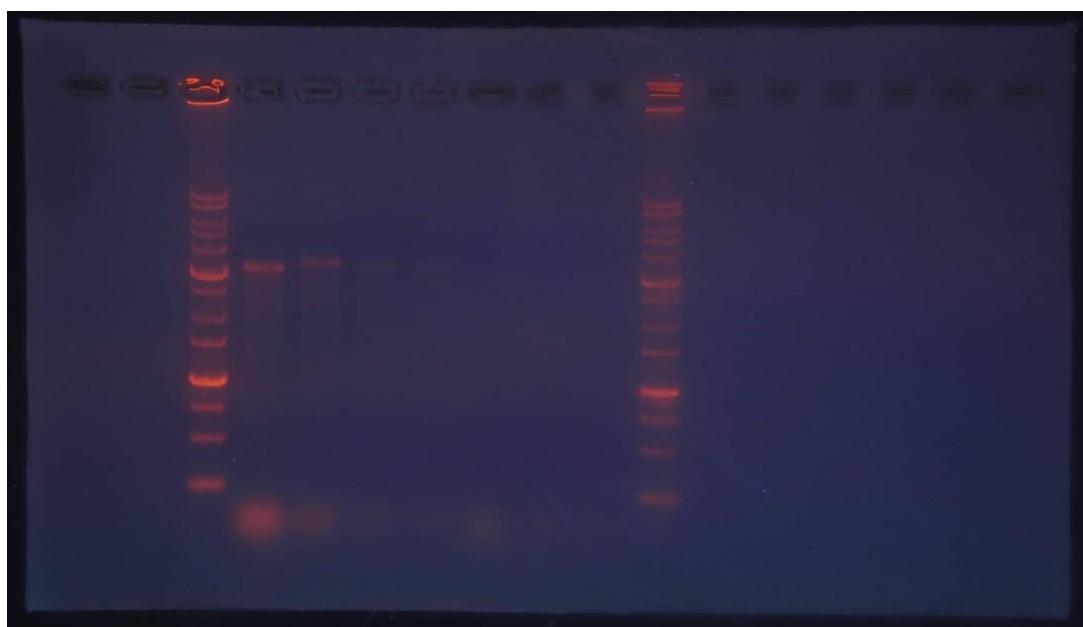
DNA ladder/未純化/U1/U2/U3/D1/D2/D3/DNA ladder

未純化和純化跑出的band不一樣，有些詭異，原因不明



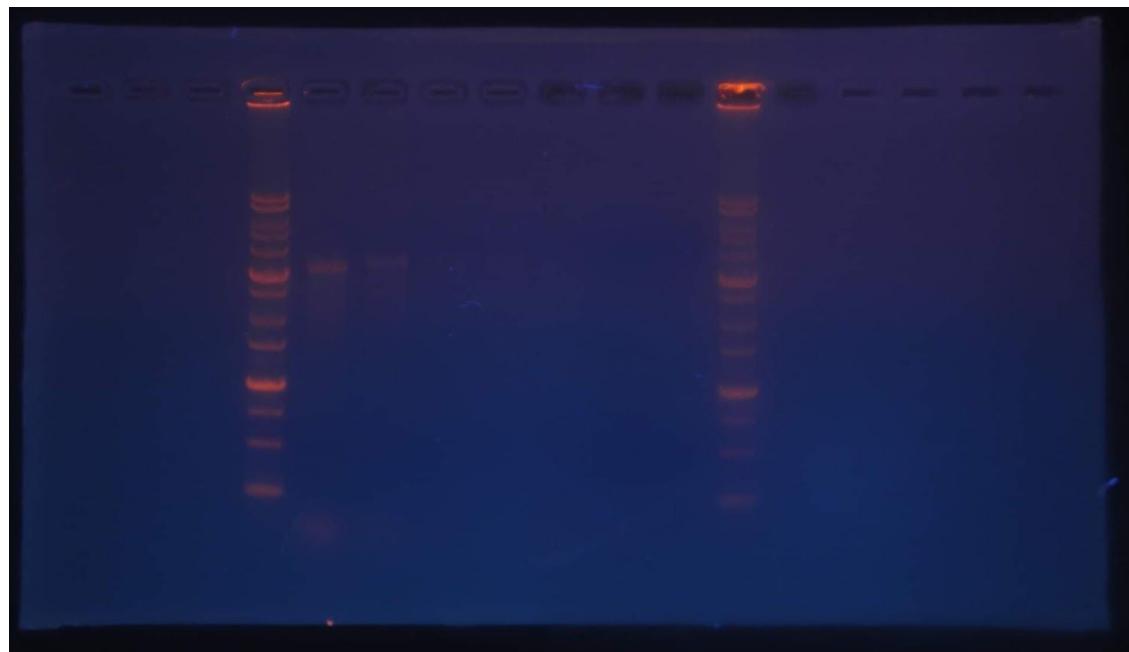
bottom lid

DNA ladder/未純化/U1/U2/U3/D1/D2/D3/DNA ladder



top lid

DNA ladder/未純化/U1/U2/U3/D1/D2/D3/DNA ladder



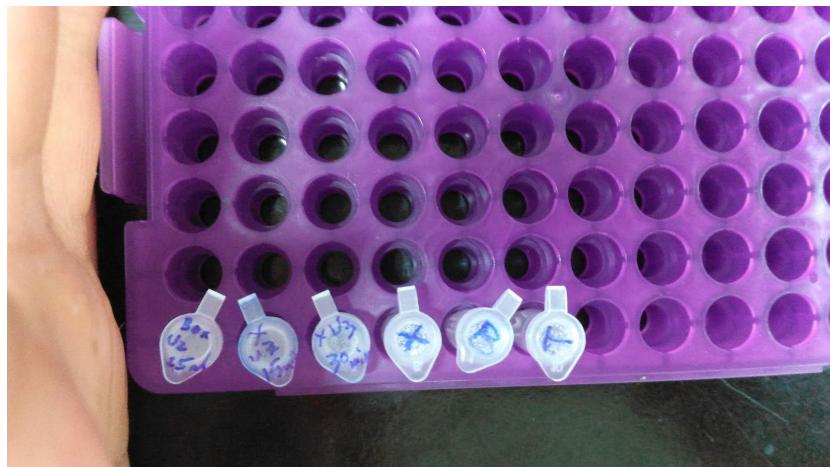
8/15 韋謙 重甸 鈺璇

- Box,lid純化三次(Box補到300mu l,lid補到100mu l)
- BoxU3--震15min ; BoxU3--震30min(有discharge)已處理好(settle5min,染50sec), 放在照電顯一進去, 左手邊櫃子從下面數來第二個, 有個圓形培養皿, 明早看TEM
- BoxU3--震15min ; BoxU3--震30min(不用discharge)還沒放在銅片處理, 可明早處理
- 震盪方式:15min是(連續震5min→停止震盪1min)*3
30min是(連續震5min→停止震盪1min)*6

注意震盪一段時間要換水, 避免水過熱(水會越來越熱, 要注意)



右上三管是Box和lid的U3, 體積剩55mu l



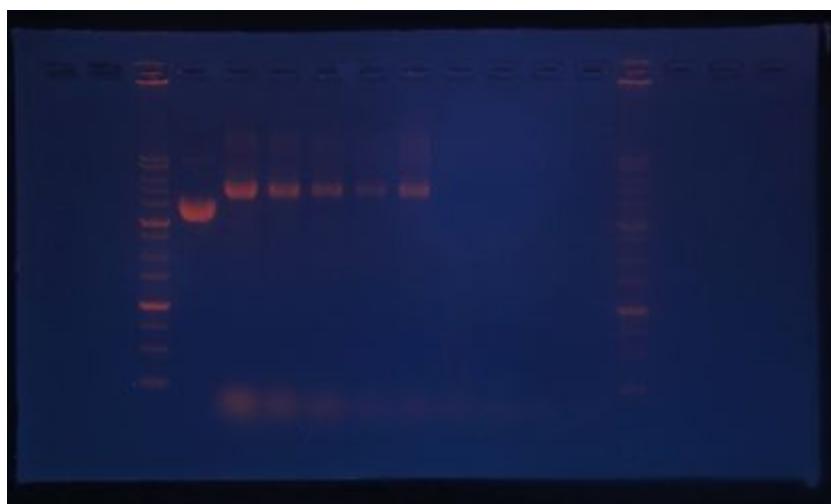
由左至右為BoxU2/BoxU3--震15min/BoxU3--震30min/XBT用24hour protocol合成的原液(8/14下的)

BoxU3--震15min/BoxU3--震30min明天可拿去照TEM, 做為沒有discharge的對照組

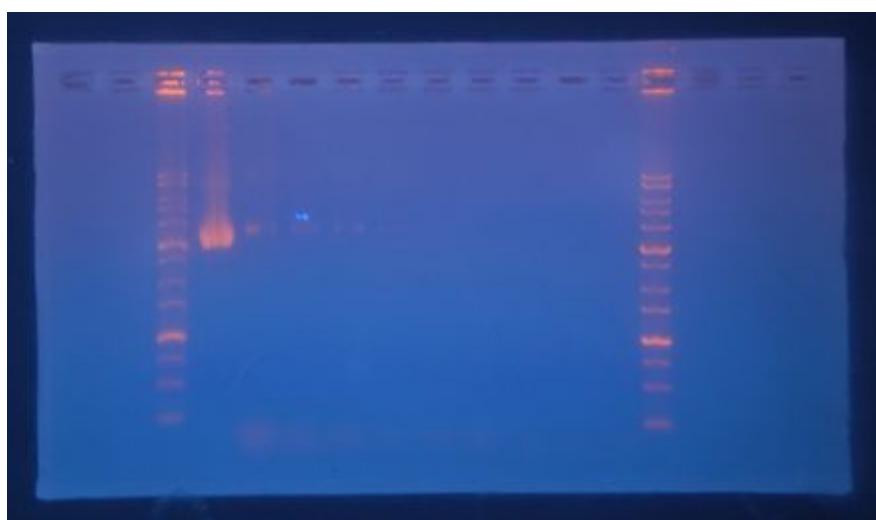
scaffold/原液/U1/U2/U3/U3(震15min)/U3(30min)/D1/D2/D3

Box:取200mu l純化, 體積捕到300mu l

震15min較30min好



Bottom:取100mu l純化，體積捕到100mu l



top:取100mu l純化，體積捕到100mu l



lid震盪後都沒band，可考慮震更短

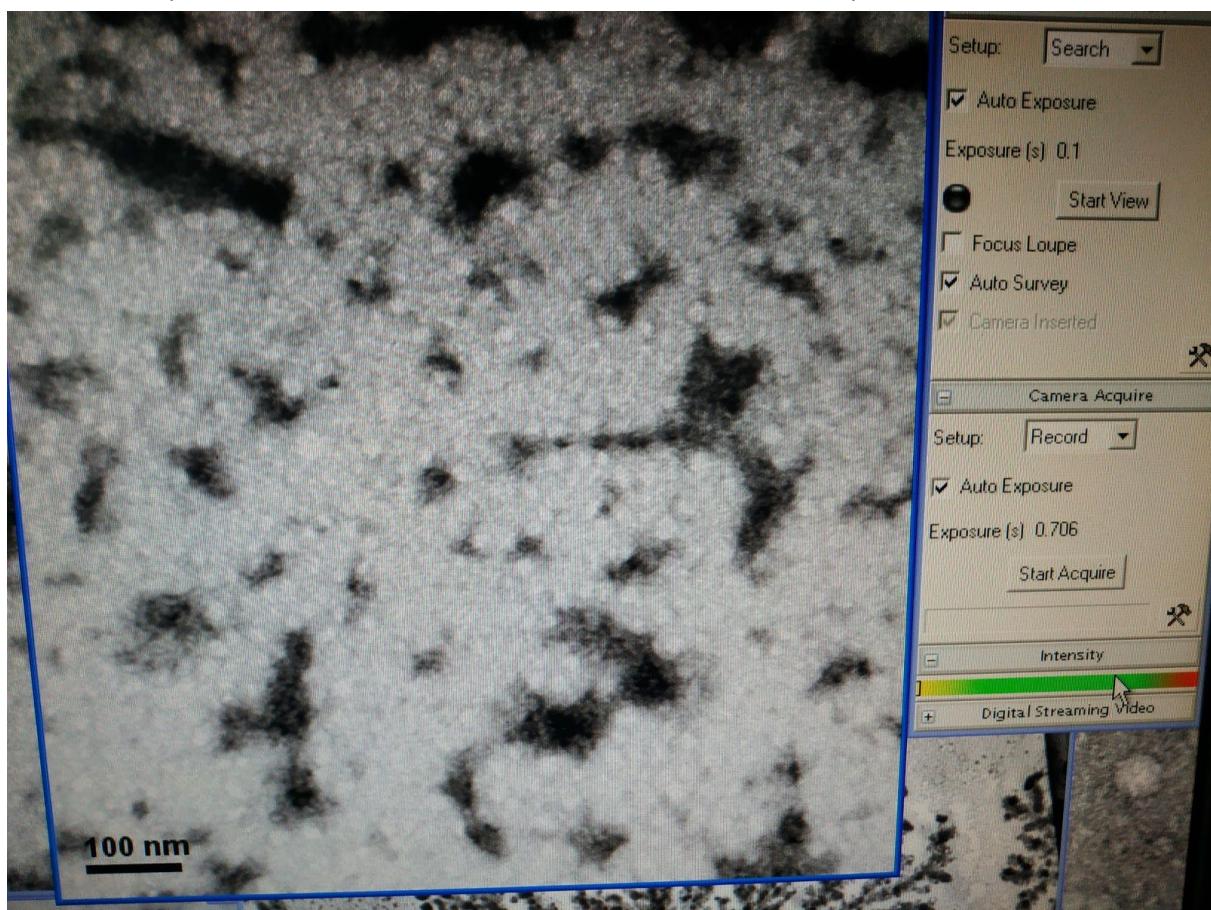
lid可能純到U2即可？

bottom這次band幾乎看不到(連原液都很淺)

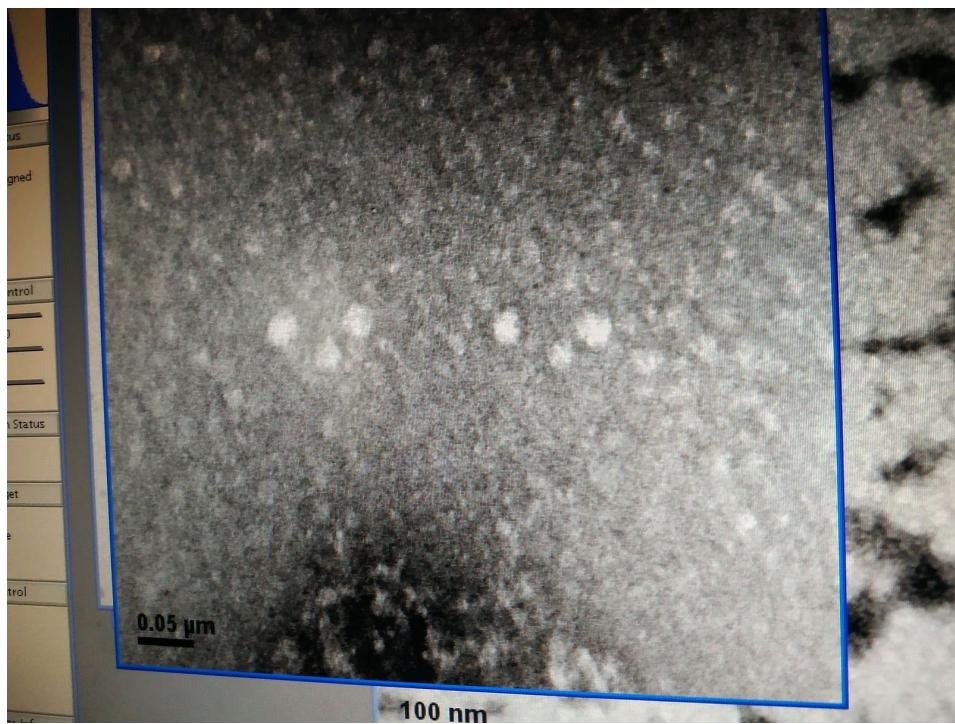
8/16 照TEM(早上：苡寧、采蘩、清璽)

(下午：苡寧、清璽)

- 1.不要做discharge 樣品的附著效果變差 且銅網破損多
- 2.震盪完後若閒置較久 在製作樣本前可再震盪1-2min才不會讓先前的震盪沒有效果
- 3.今天新製作的樣本有找到大小一致重複出現的白色小方格(推測就是我們的box) 但對比不明顯難以觀察 下次做染色時染色的時間應該可以加長
- 4.檢查廠商製作DNA時曾用過什麼enzyme(找廠商的報告) 確認沒有類似大小的蛋白質會影響觀察(DNA照出來的結果應該會和蛋白質差不多的顏色)

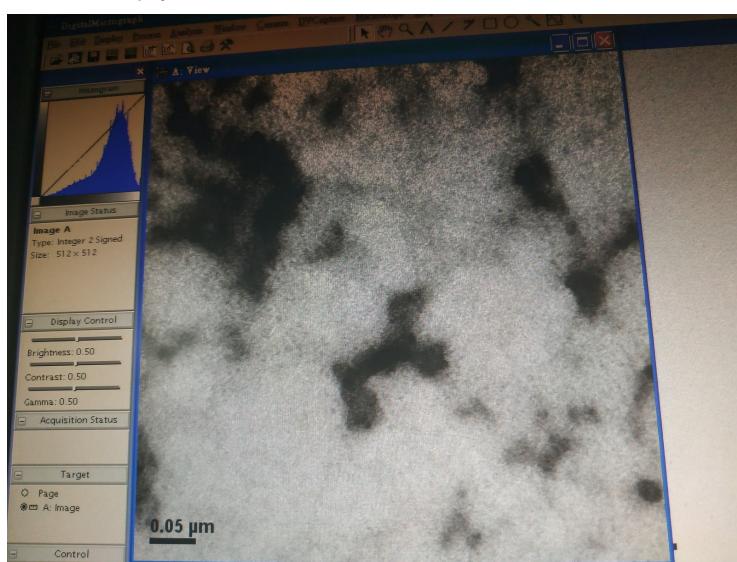


settle5min,stain1min,震15min



settle5min,stain1min,震30min, new pro.

Bonus:貴賓狗！！！



8/17 開會紀錄(睿謙 重旬 鈺璇 滕靈 泉浩)

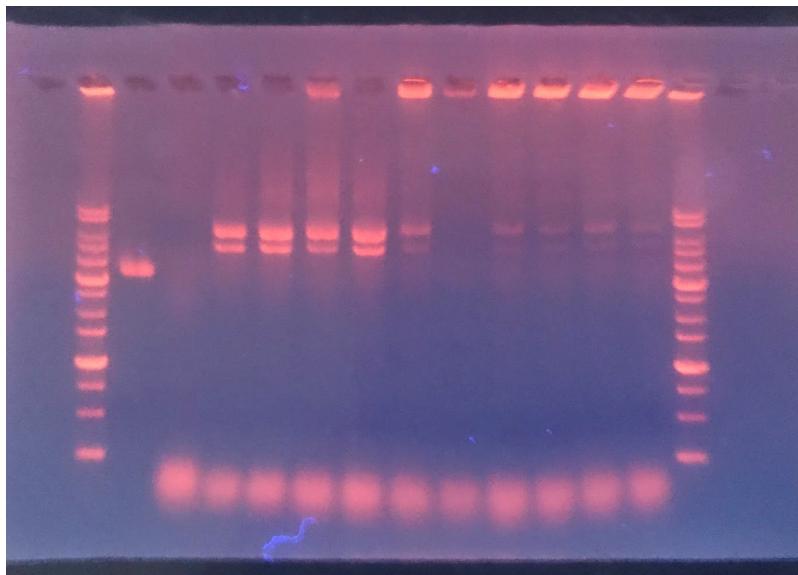
若使用目前材料繼續折疊，所需改變之變因。

- 1.震盪時間可以試試1分鐘、5分鐘、10分鐘，休息時間、頻率加長。
- 2.使用共同儀器中心(系館5樓)之震盪儀降低功率。 (現在440W/
- 3.溫度降低，以燒杯裝取冰塊，置放飄浮板。
- 4.染劑時間加長
5. $MgCl_2$ 濃度上升
- 6.agarose 濃度下降

8/18采葉，鈺璇

BOX 13小時protocol

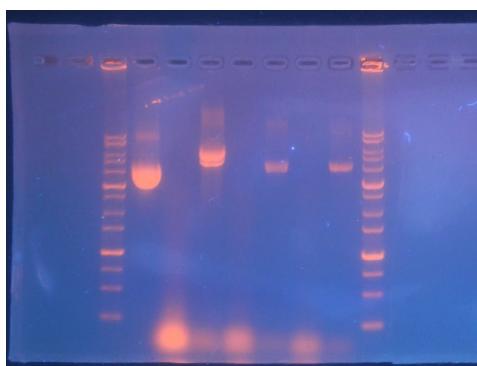
(marker/scaffold/staple/10mM/10mM/15mM/15mM/20mM/20mM/25mM/25mM/30mM
/30mM/marker)



舊protocol濃度15:看3和4的band是什麼？之前沒看到可能只是沒震
舊protocol濃度25:看卡在well裡的是什麼？

8/10 pcr跑24小時之成果

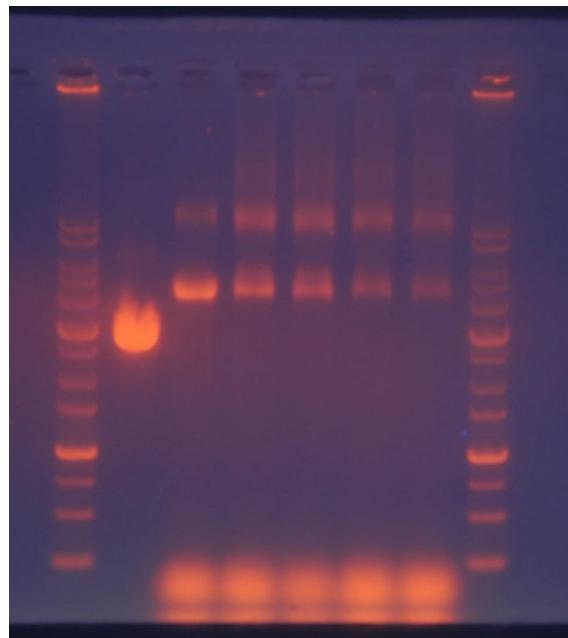
各well分別是：marker/ scaffold / X staple / X / B p / B / T p / T / marker



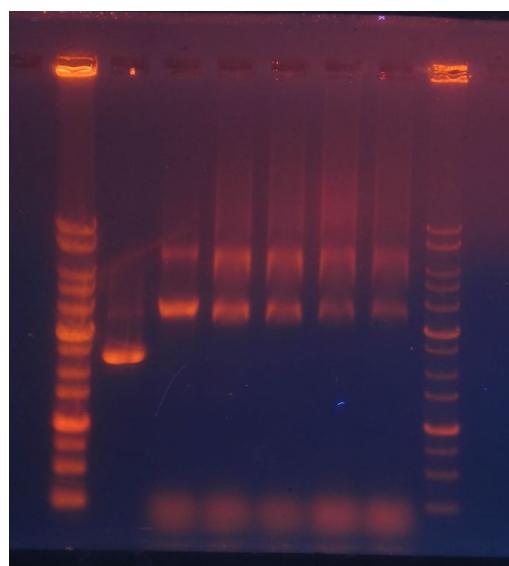
8/18

Box(24protocol): scaffold/10/15/20/25/30

0.8%膠



0.5%膠



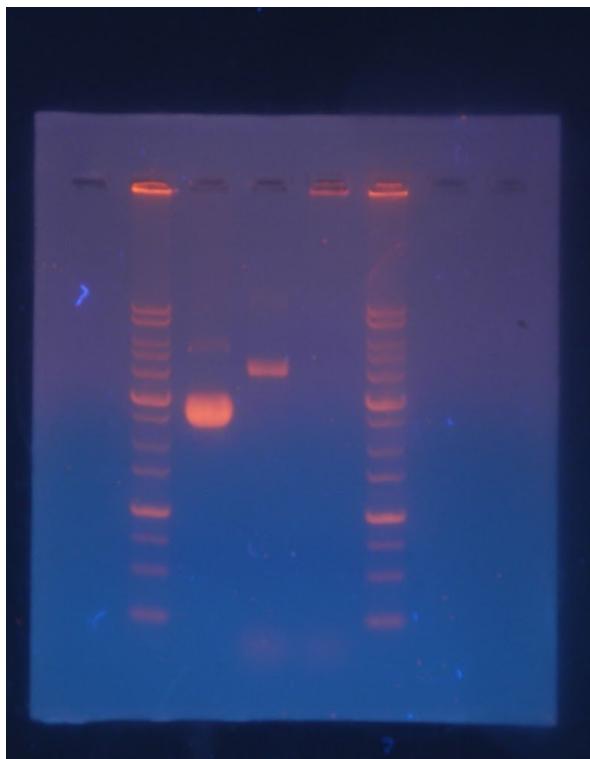
新protocol鎂濃度15,20:看上面新跑出的band是什麼

結論:跑舊protocol鎂15,25 新protocol鎂15,20 純化照TEM

8/20 賽謙 重甸

(舊protocol / 0.8% GEL/ 純化一次產物(U1))

ladder / scaffold / 10(應該是15??)mM / 25mM / ladder



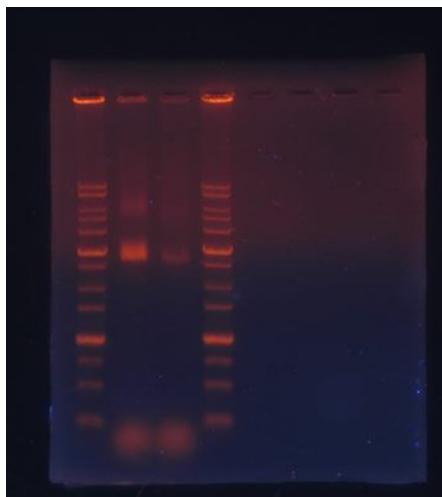
15mM沒有看到新奇的band 25mM 有卡住的跡象 可以試試0.5%GEL並且觀察不純化的產物

跑舊protocol的PCR 15mM/ 25mM

8/21采葉、清蠻，鈺璇

(舊protocol /0.5% GEL/未純化)

ladder / 15mM / 25mM / ladder



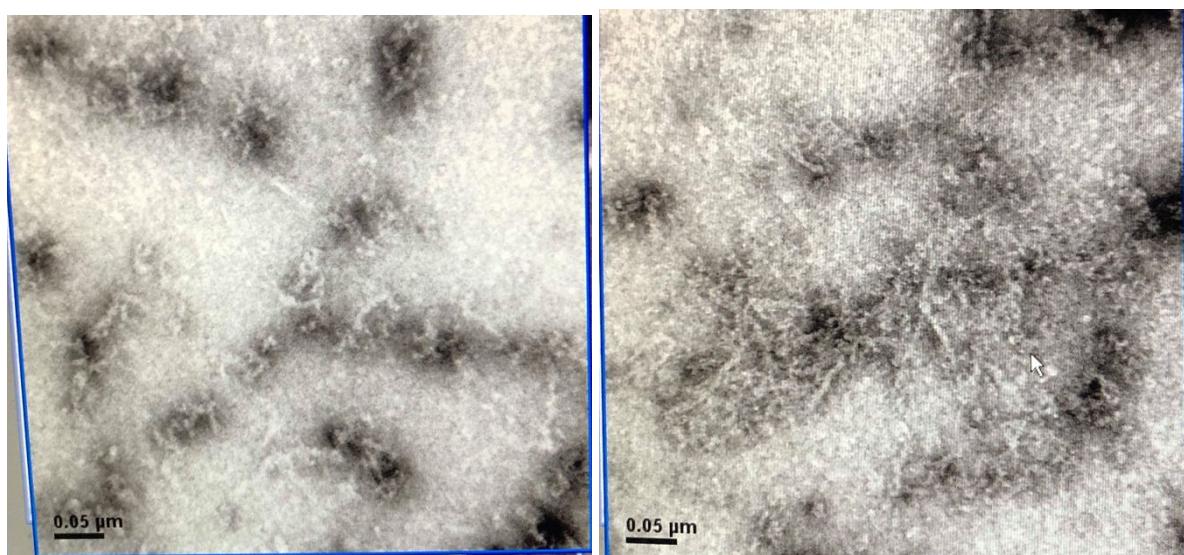
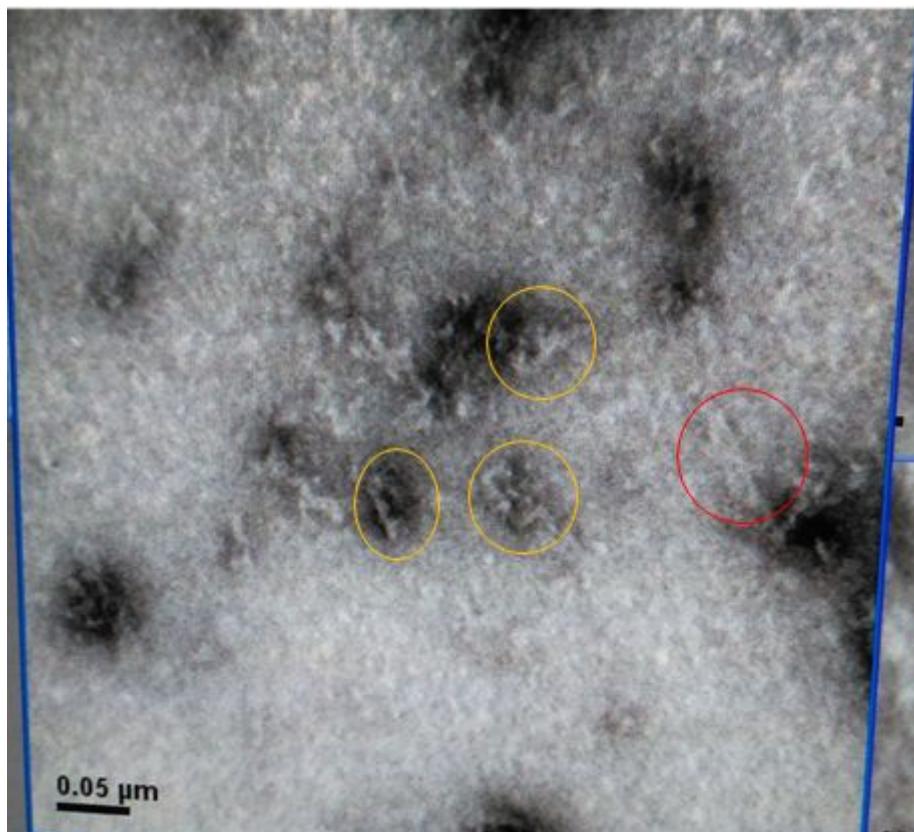
上面都有一個很糊很不明顯的band, 可能不是一制的結構

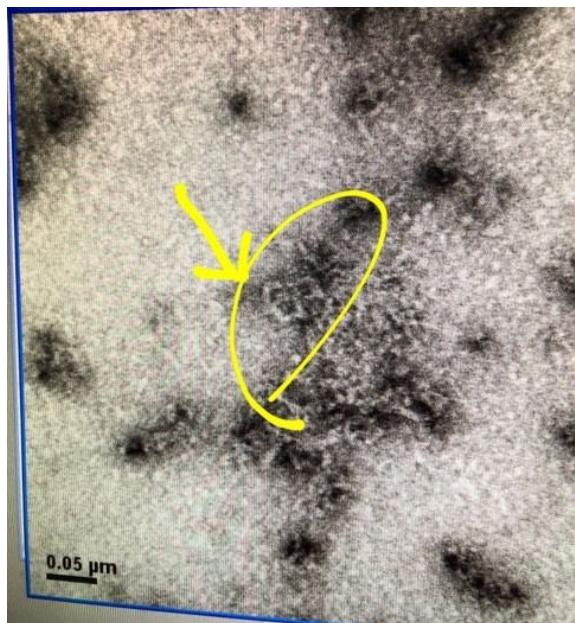
8/22早:清璽、鈺璇 下午:睿謙, 鈺璇

舊protocol未純化Box/震15min(90W)/Mg:15mM/

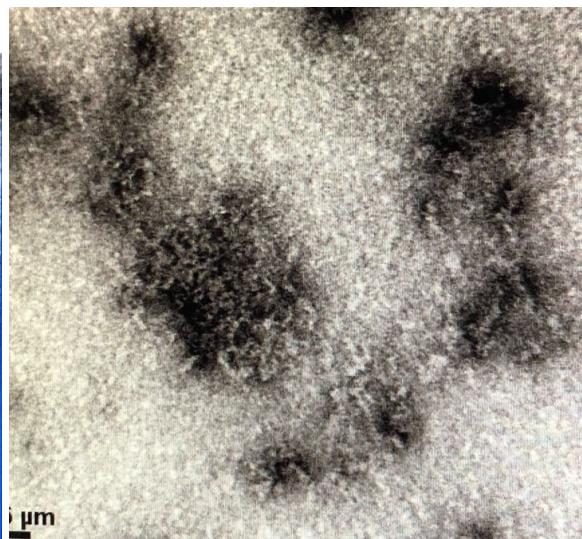
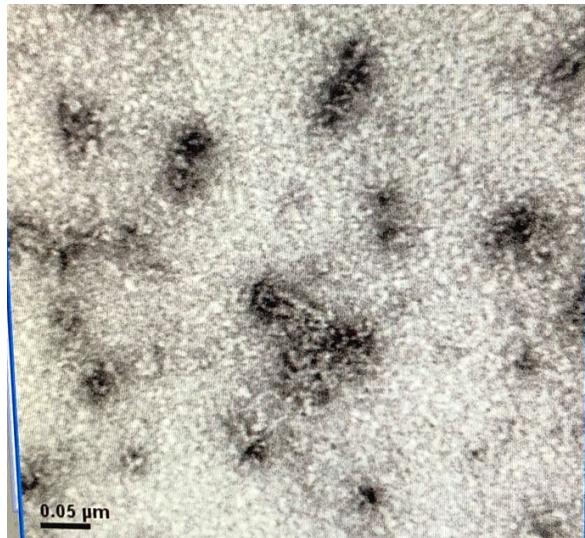
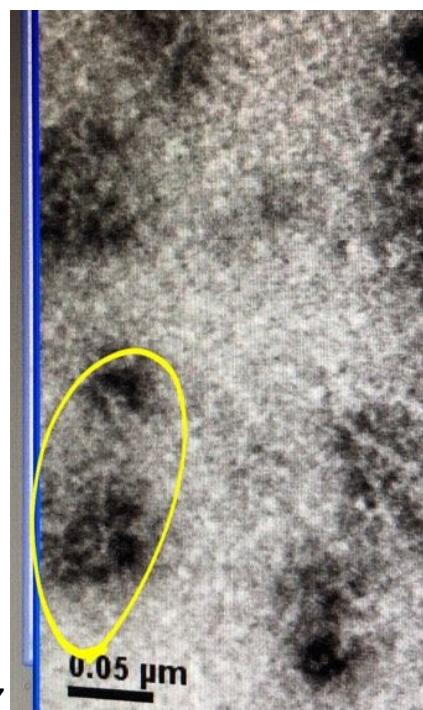
黃圈內的E很像側面的Box, 紅圈內似乎有底面的Box

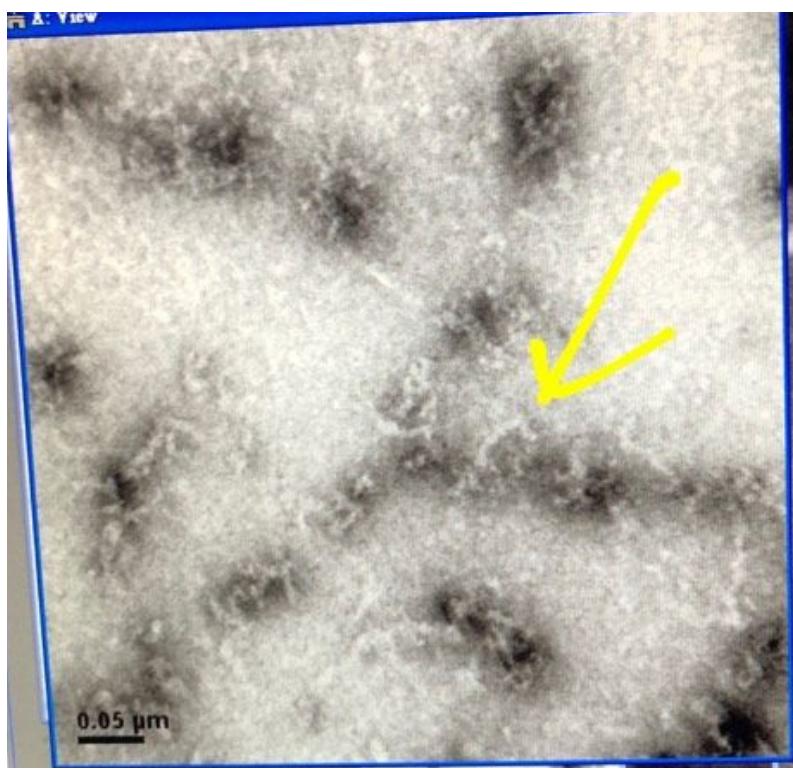
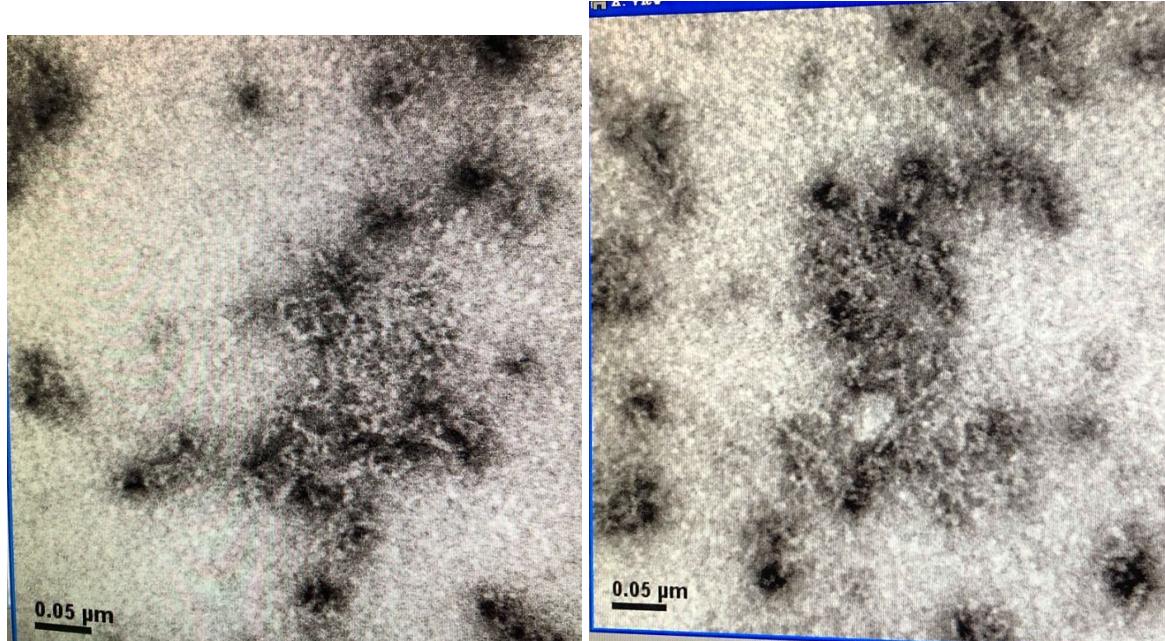
幾乎都看到側面的, 沒有從上或下的角度(長方形)可能厚度太薄?沒染出來?





877





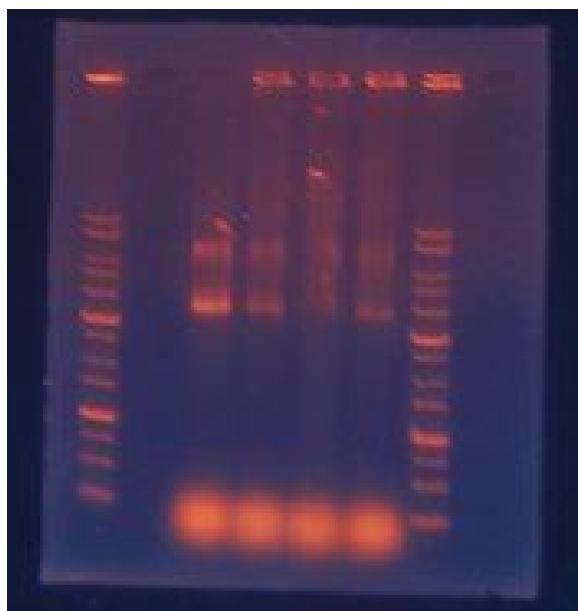
8/23 賈謙 重甸

以新的protocol 做 scaffold 20nM ; staple 100nM MgCl₂ 15mM

8/24 泉浩 鈺璇

0.5%GEL 新 protocol 的 box (sca 20nm, sta 100nm, MgCl₂ 15mM.)

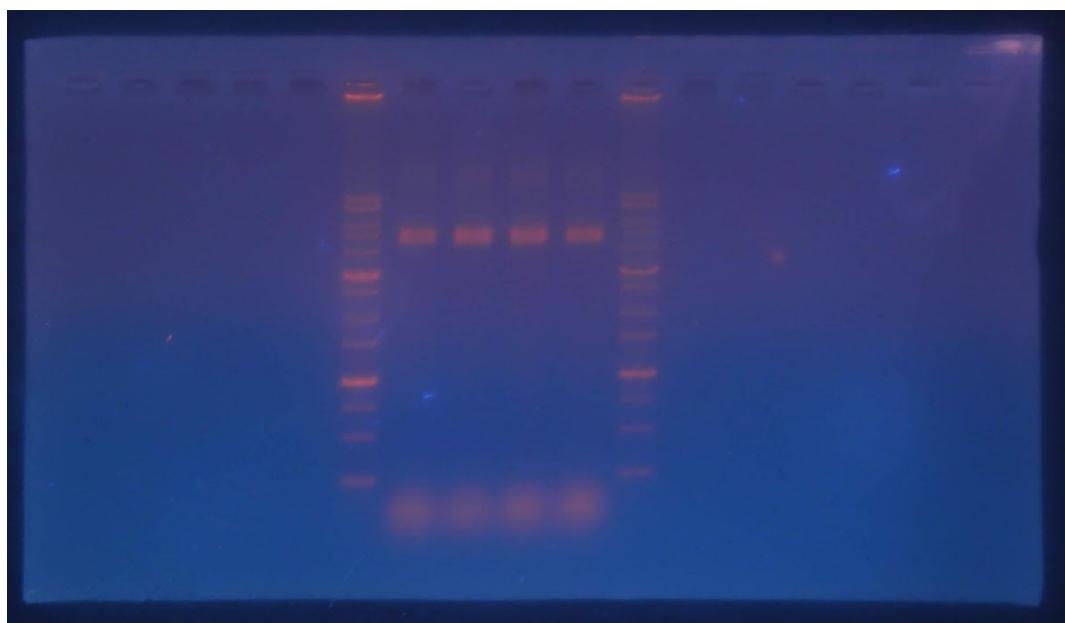
control / 沒震 / 震 5min / 震 10 / 震 15



震完後多出了一個卡well的巨獸

8/25 重旬 睿謙

舊protocol 15 MgCl 1%GEL



由左至右是震盪 1、5、10、15分

四位小朋友看起來一模一樣，且都沒有卡，推測在15min內結構還算穩定

8/27 滅靈 鈺璇

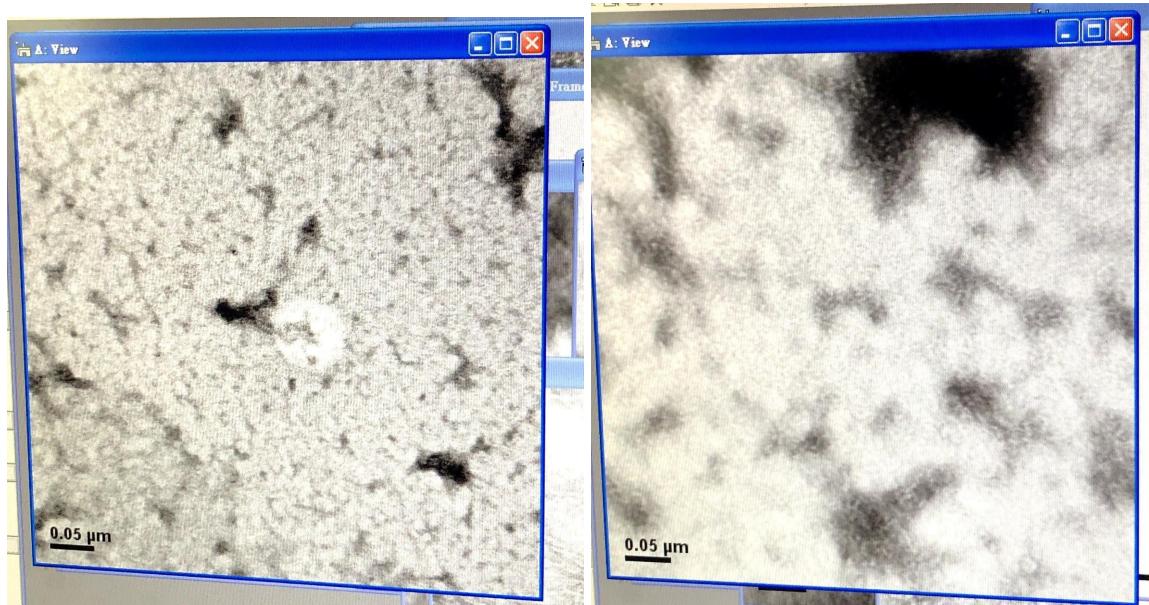
早上處理樣品

- 目的: 測試染2min.效果。
 - 舊pro 15mM 15min.改染2min. & control(only buffer) 染2min. 相互比較。
 - 新舊pro一起在五樓震15min.先不染新pro.
 - 下午照電顯依據染色效果決定染色時間。若舊pro染2min效果不好，新pro可以染1min和豪棒棒(舊pro)比較。
-

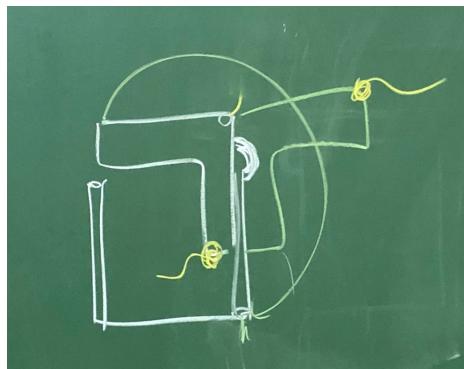
8/28 肅謙 鈺璇

下午觀察新protocol之震盪15min 和不震盪之TEM

結論：沒有發現產物



B計畫：重訂staple之結構



- 1.改成半個盒子
- 2.旋轉270度，做到隱藏/露出的效果

動機應注意問題：

不能同時把不相干的藥物加入盒子裡

8/29 上午 清靈、采蘿—跑膠、震盪、製不純,震15 min片

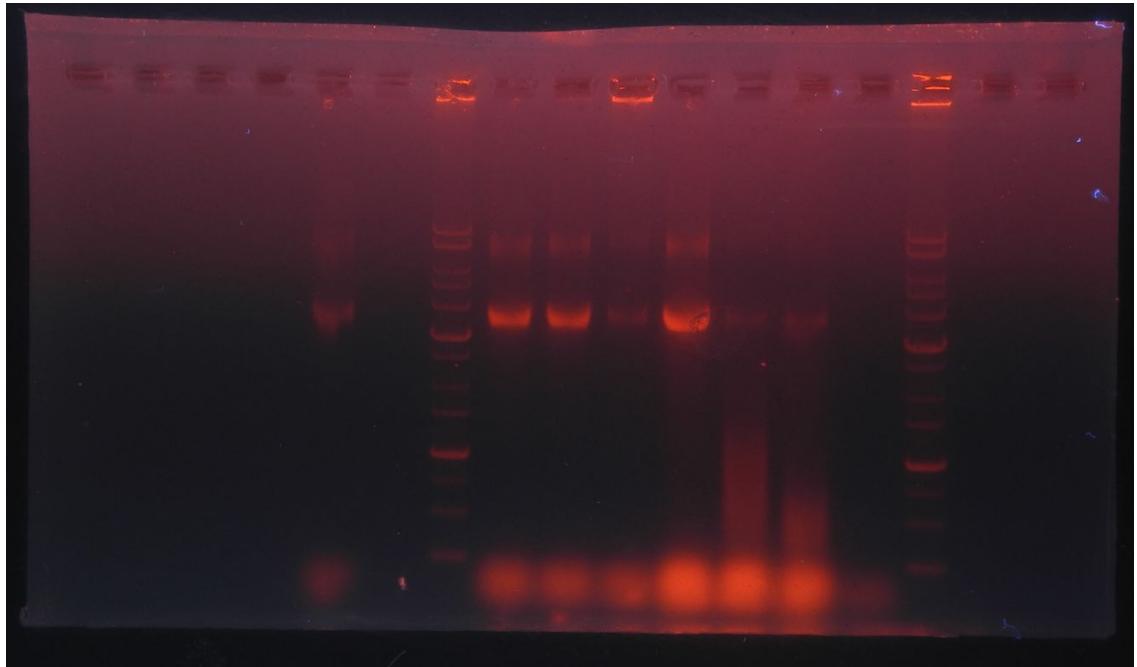
由左至右依序為：

Ladder／

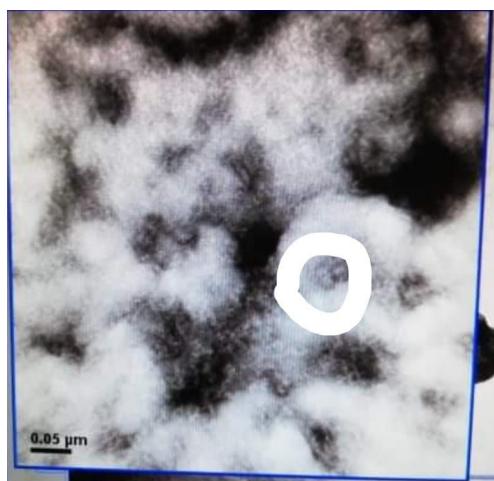
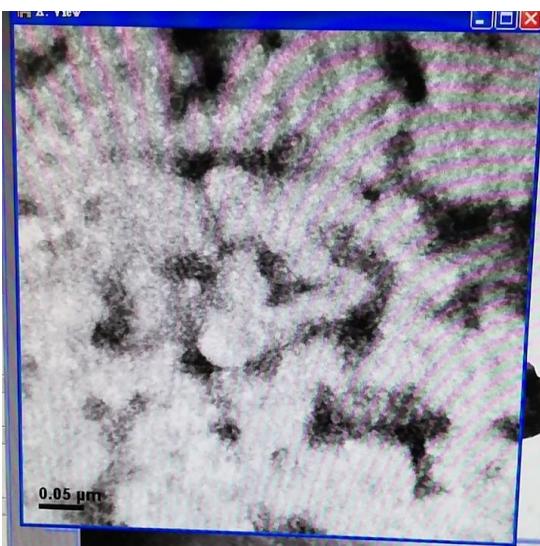
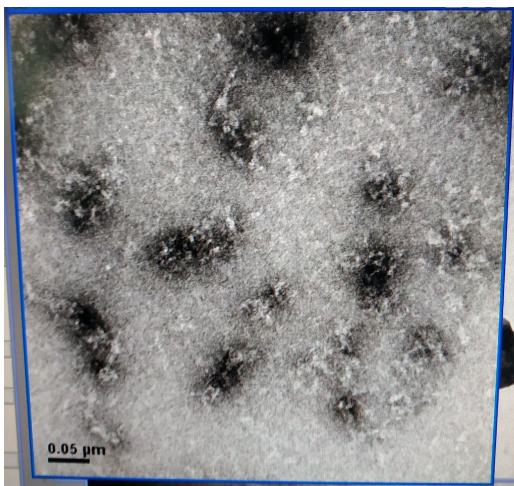
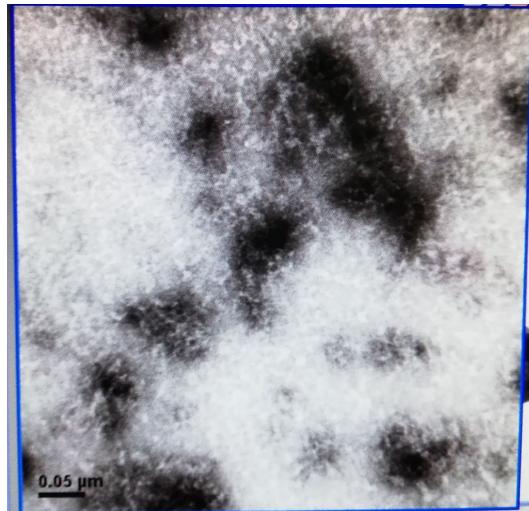
不純，不震／不純，震5 min／不純，震15 min／

純一次，不震／純一次，震5 min／純一次，震15 min／（純化）down／

Ladder



8/29 下午 采鑑、苡寧—TEM看不純,震15 min片 (好棒棒2.0)



學姊建議換ratio(1:7 or 1:10)跑舊protocol

9/2 鈺璇 睿謙 滕靈

靈：早上樣本處理 - 舊20:100 15mM 不純不震 染前小震1min. settle5 stain1。

上午：20:100不純不震 舊pro 15mM, 血球抹片。



下午：彥榮meeting。建議用簡單的定點翻面機制作動機，例如：

旋轉門、變裝娃娃、東南西北、電影院椅子。健妤建議用1:10或1:7跑跑看

晚上：下PCR for 10:50以及10:100 scaf:staple (Mg 15mM/ 舊protocol)

預計做：

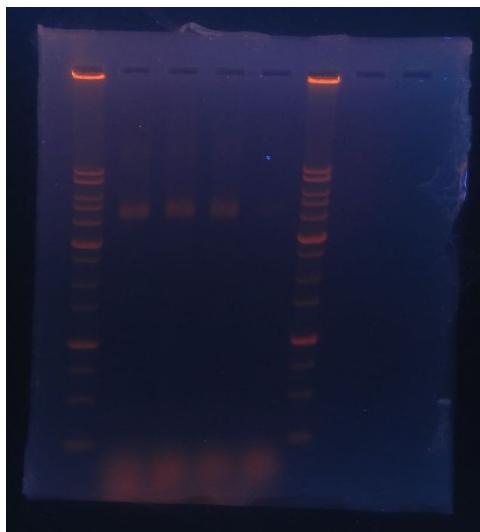
10:50 震15min 15mM diute2X 染2min

10:100 震 5/10 min X dilute/no dilute

9/3早上鈺璇 滴蠶

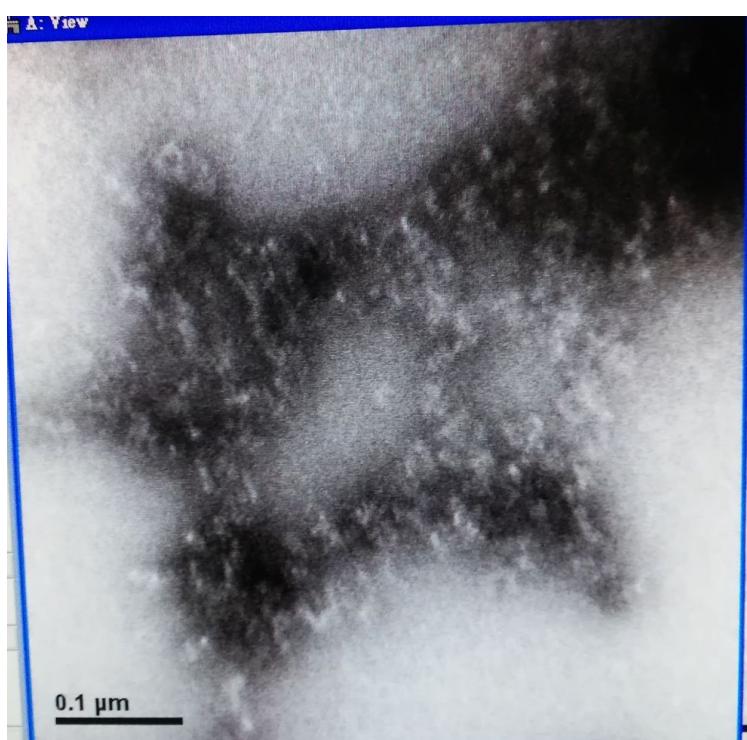
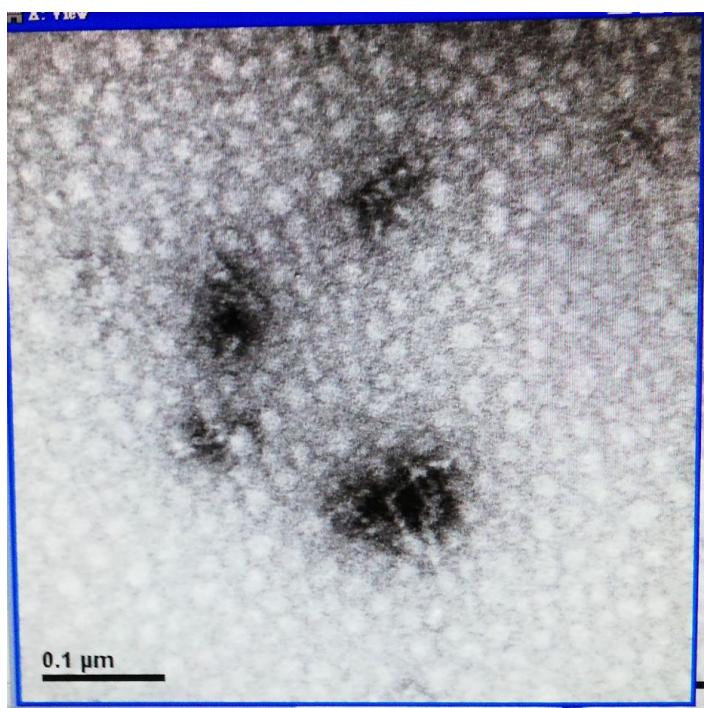
Box舊pro,sca:sta10:100/Mg15mM

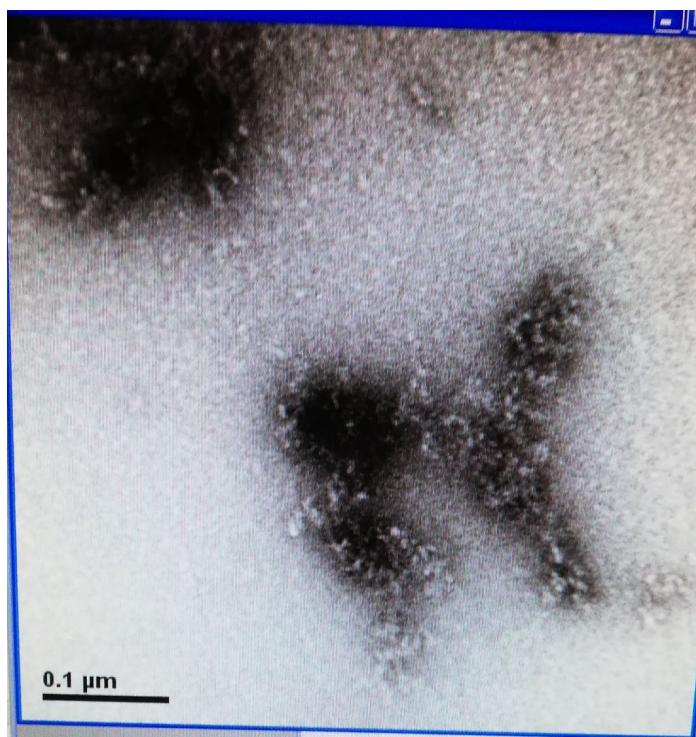
沒震/震5min/震10min/震15min



下午采蠶 重甸 鈺璇

box舊pro/10:50/dilute2X/Mg15mM/震15min/stain for 2min

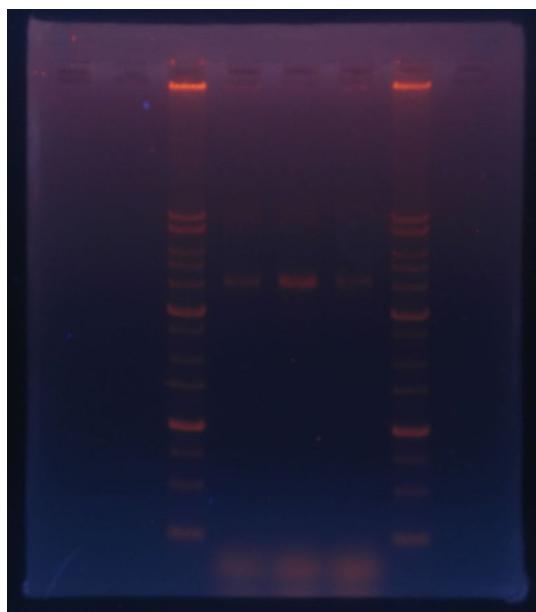




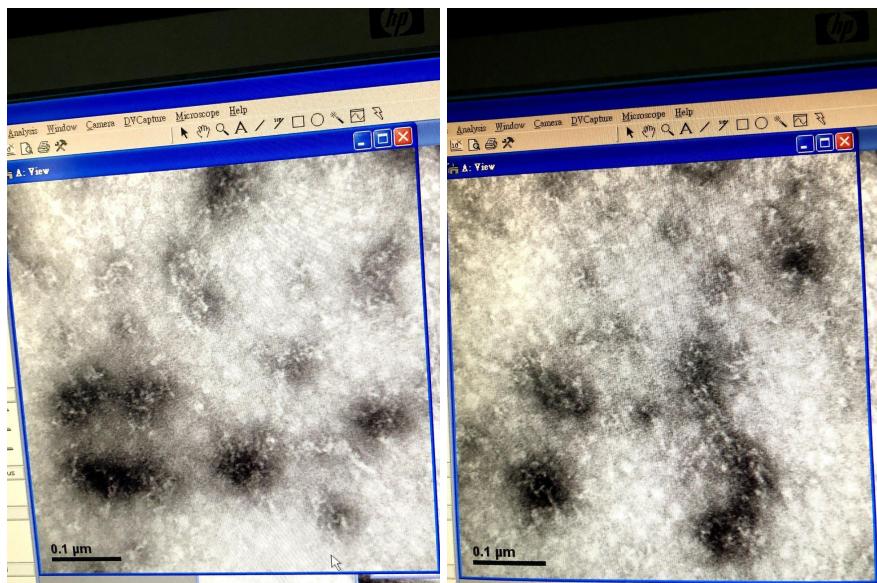
box舊pro/10:100/dilute2X/Mg15mM/

9/4 早上 采繫 睿謙 泉浩

跑膠 : MgCl₂ =15mM, 測三種FP比的box—由左至右依序為
ladder/1:5/1:7/1:10/ladder, 感覺1:7的效果最好。



觀察舊protocol 10:100/震5min/染2min 不dilute



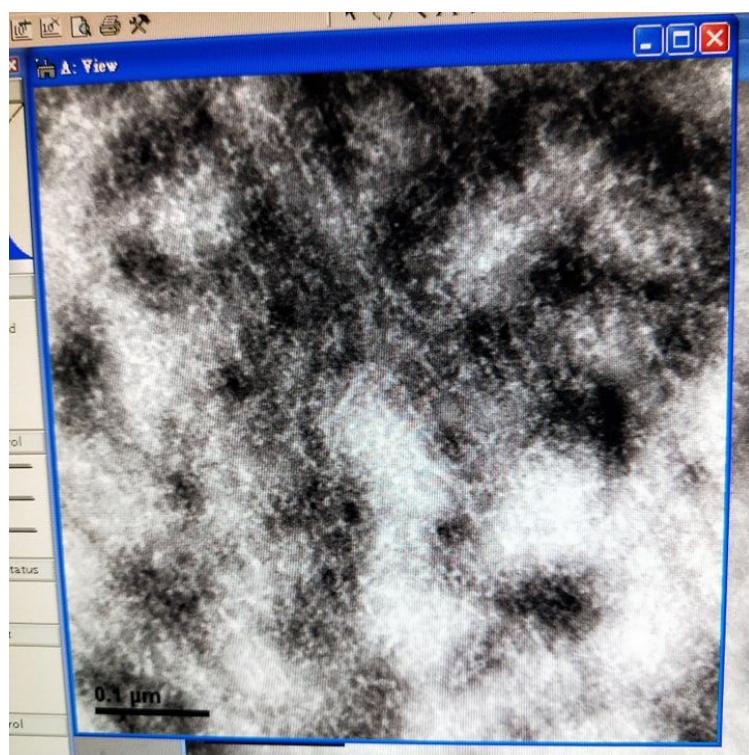
9/5 滅靈 重旬 泉浩

- 待觀察
 - 20 : 100 舊pro 震5min 染2min
 - 20 : 100 舊pro 震10min 染1min(已補一分鐘，共染2min).
 - 10 : 100 舊pro 震10min 染2min (今天做)
 - 10 : 70 舊pro 震10min 染2min (今天做)
- tem
 - 重看舊pro 震5min. 不純。(紅圈上面的那個銅網)
 - 太髒，破太多。沒結果。
 - 舊pro 震10min. 1:7 不純。
 - 未找到成品。
 - 舊pro 震10min. 1:10 不純



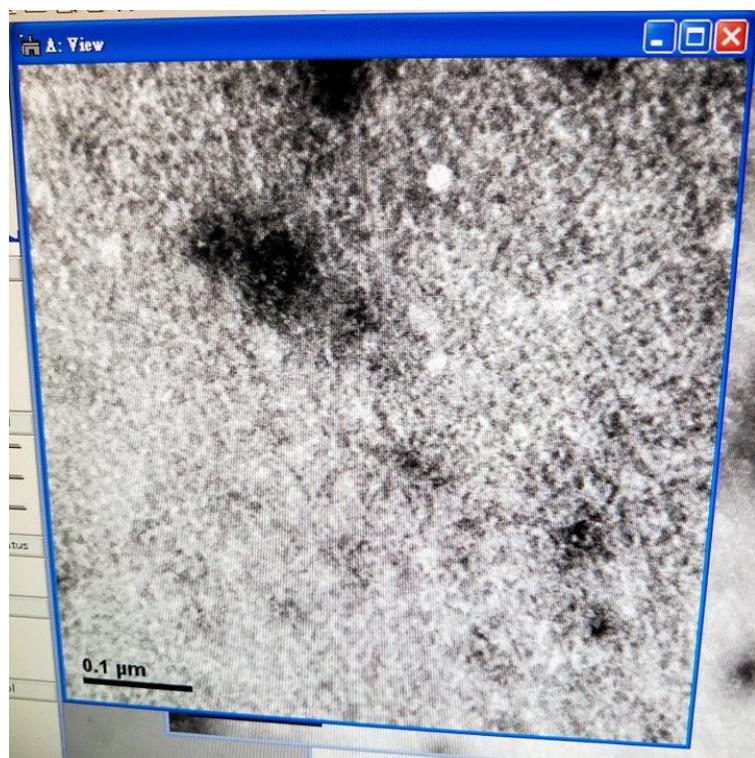


(可能是短邊，大約25nm左右，姐姐說可能稍微縮水，所以不會太精準。)



1:7 震10min 染2min 無dilute

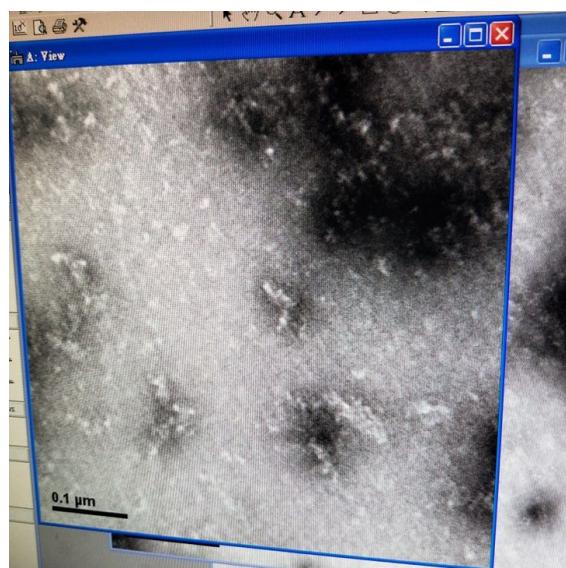
有大量的DNA，但是摺疊不完整，有直角特性的重複性不高



1:7 震10min 染2min 無dilute

有大量的DNA，但是摺疊不完整，有直角特性的重複性不高

並且出現許多朝著同一方向突起的DNA毛毛

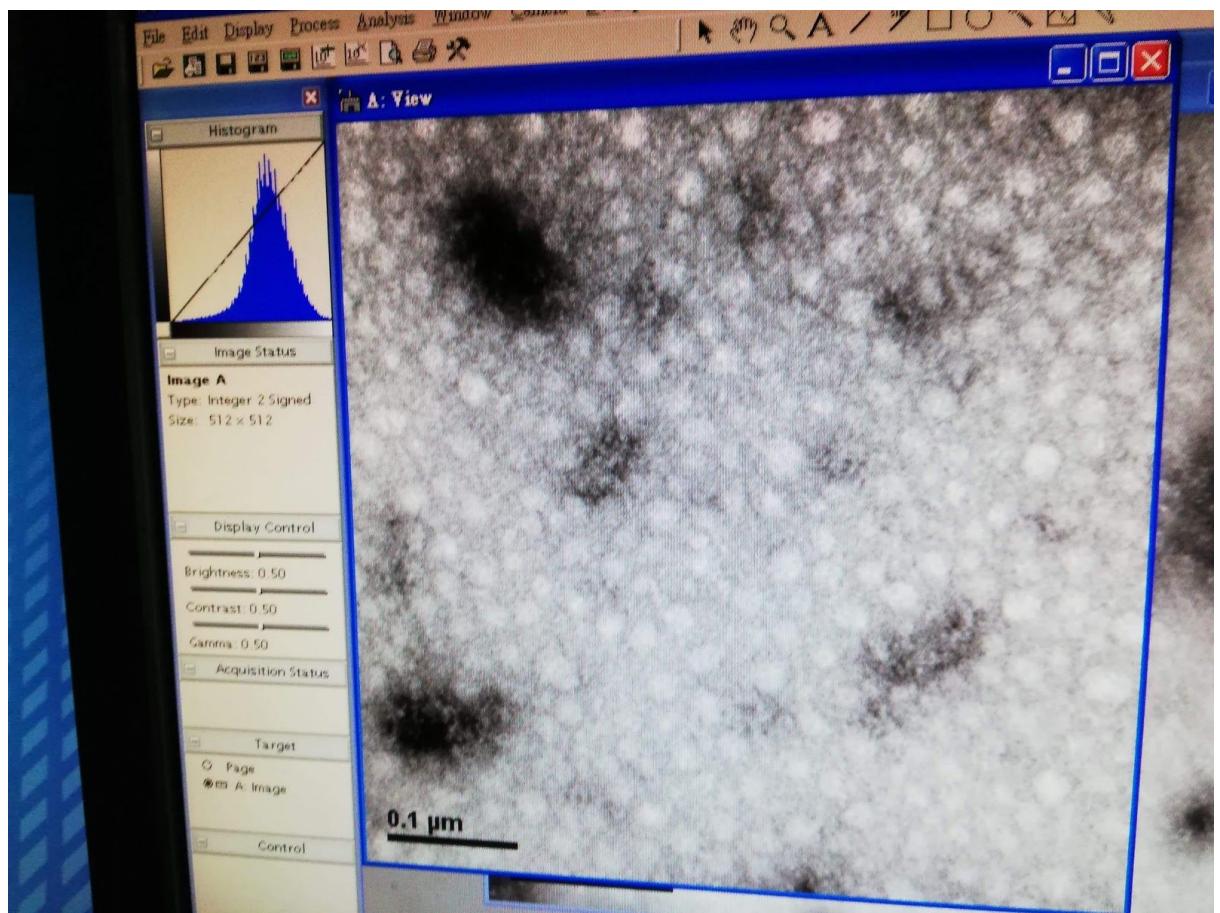


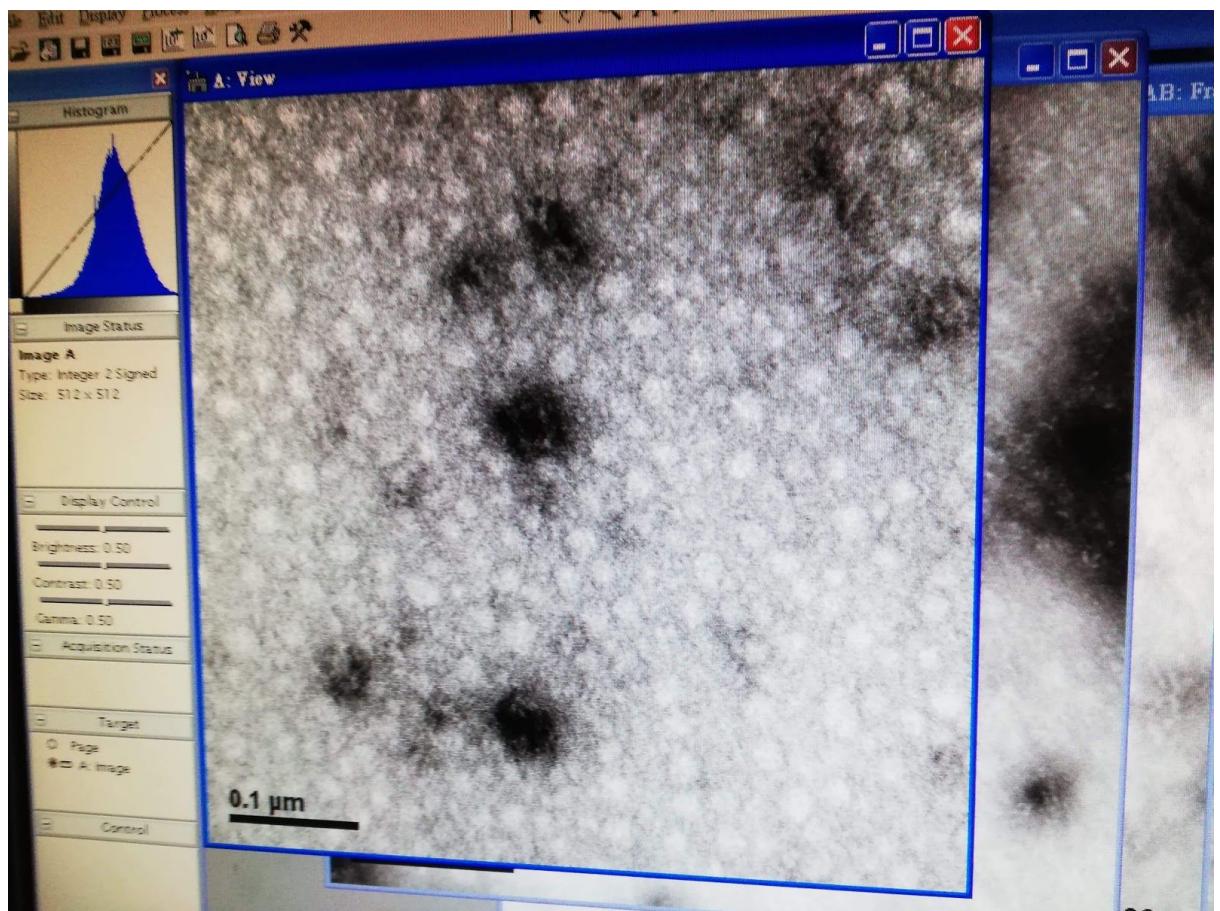
1:10 震10min 染2min 不dilute

銅網大多破掉，但仍可看到DNA產物，可是形狀再現性不高

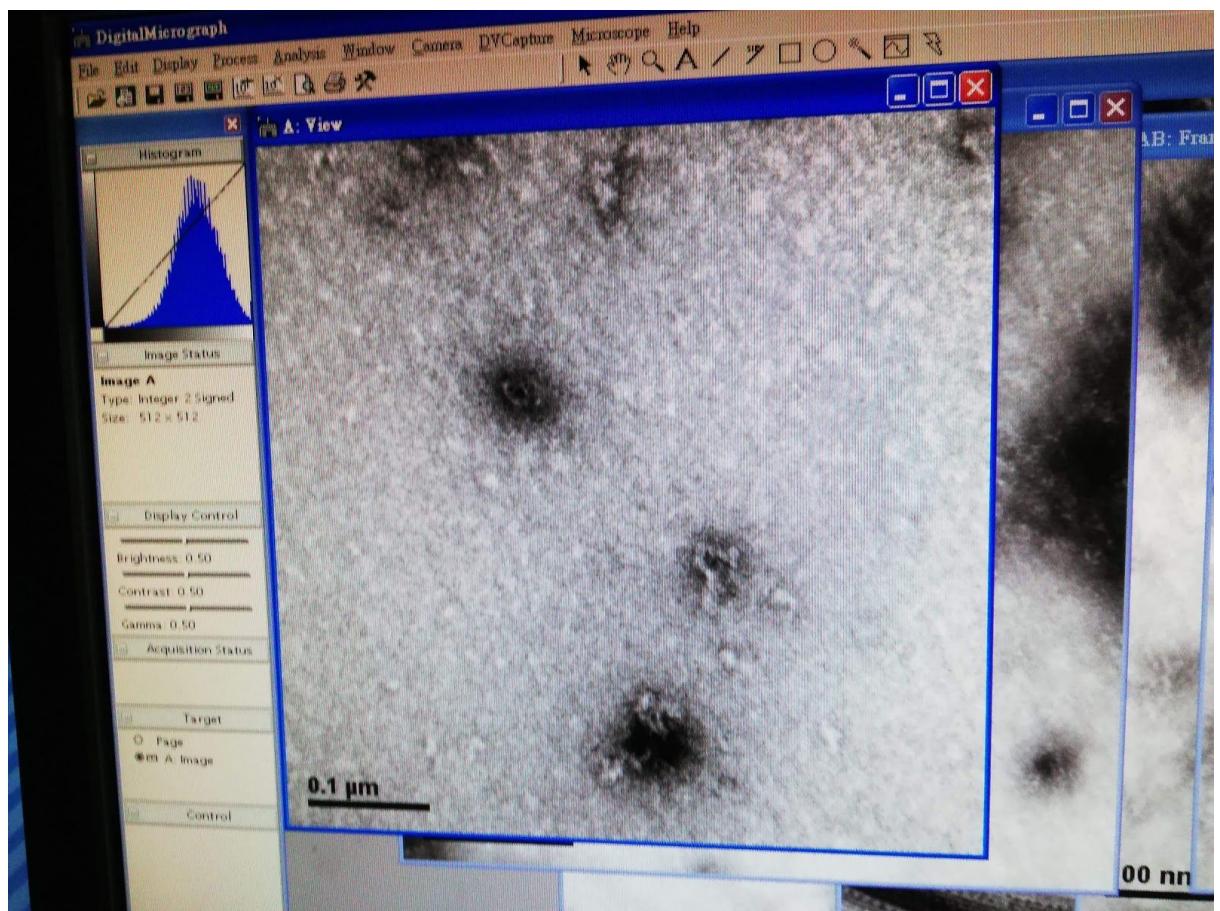
9/6 采蘩 滅靈 睿謙 泉浩

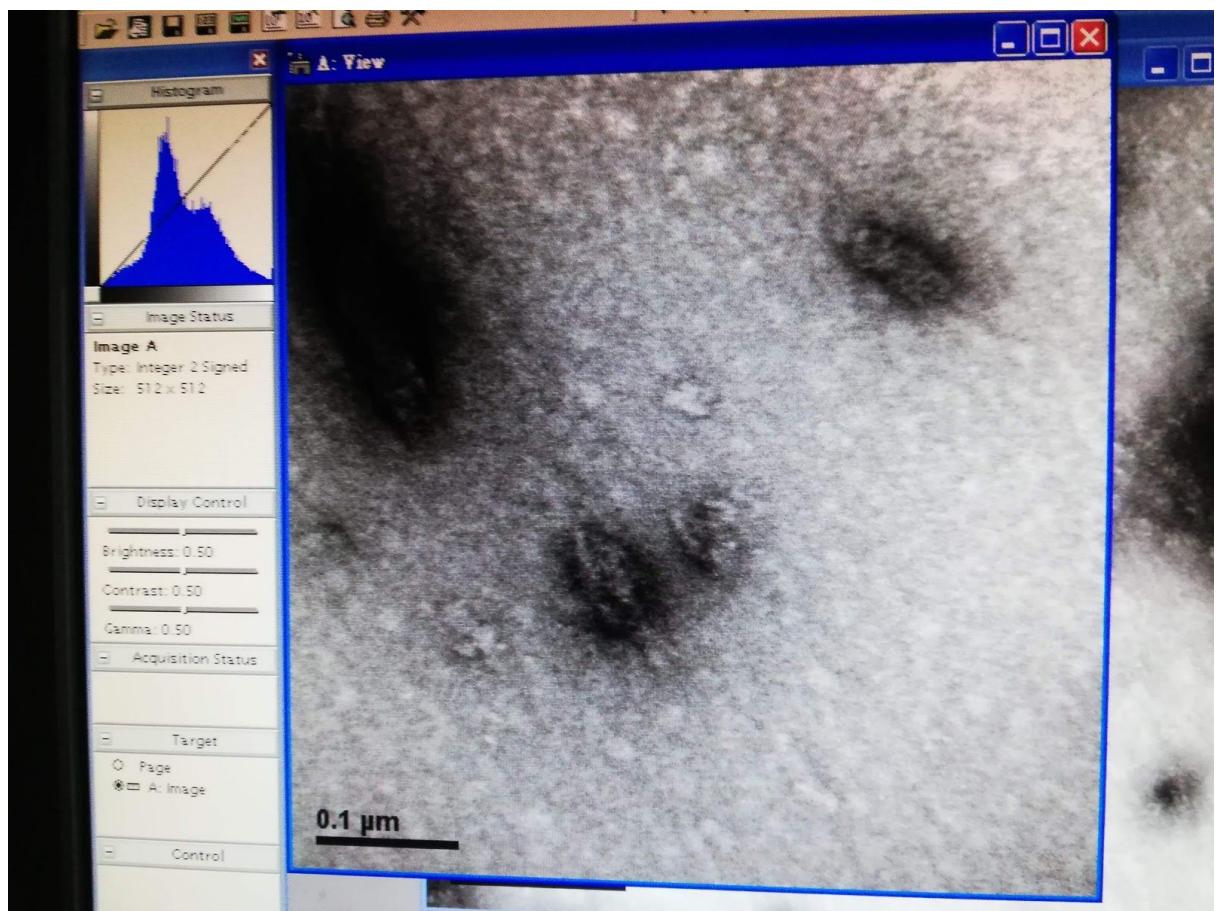
- 1:7 震10min. 15mM box stain 2min.

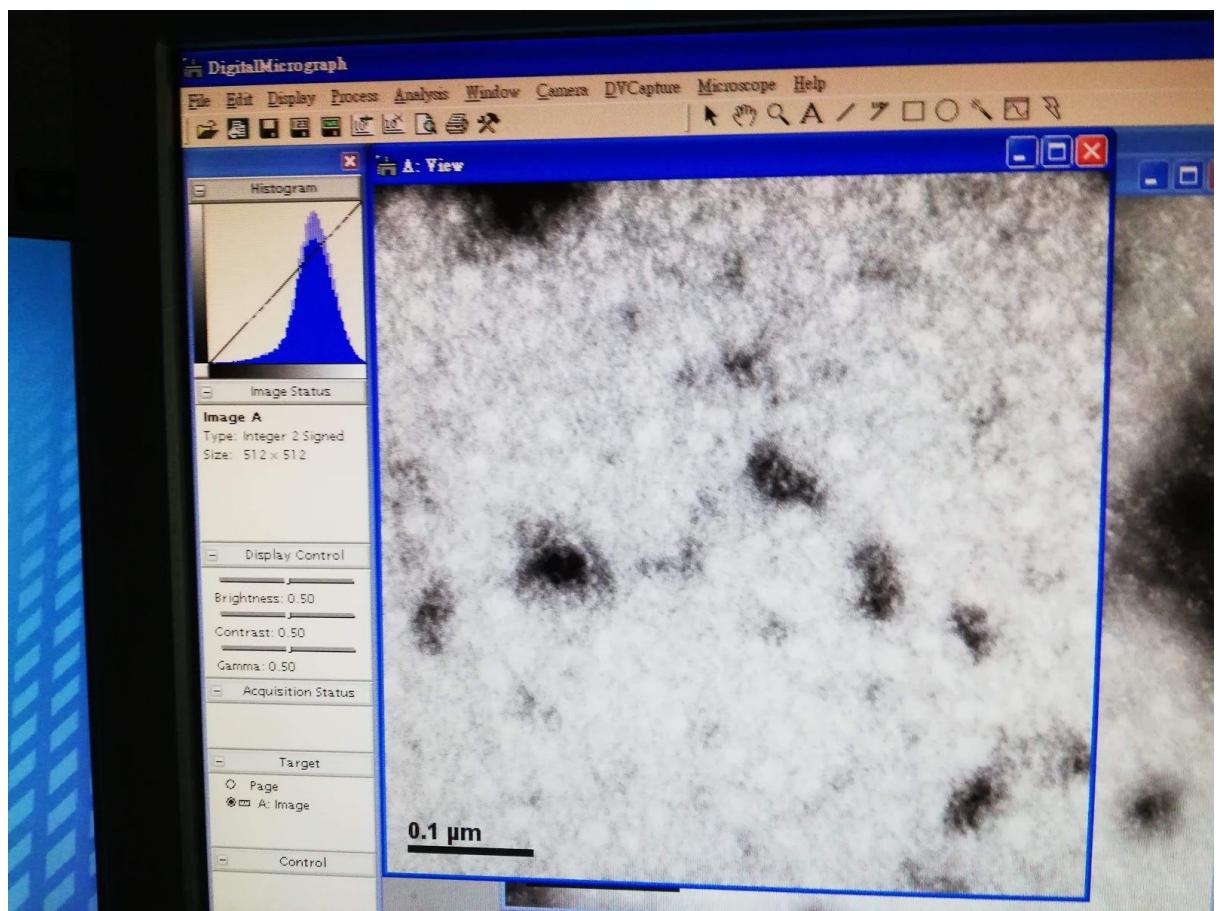


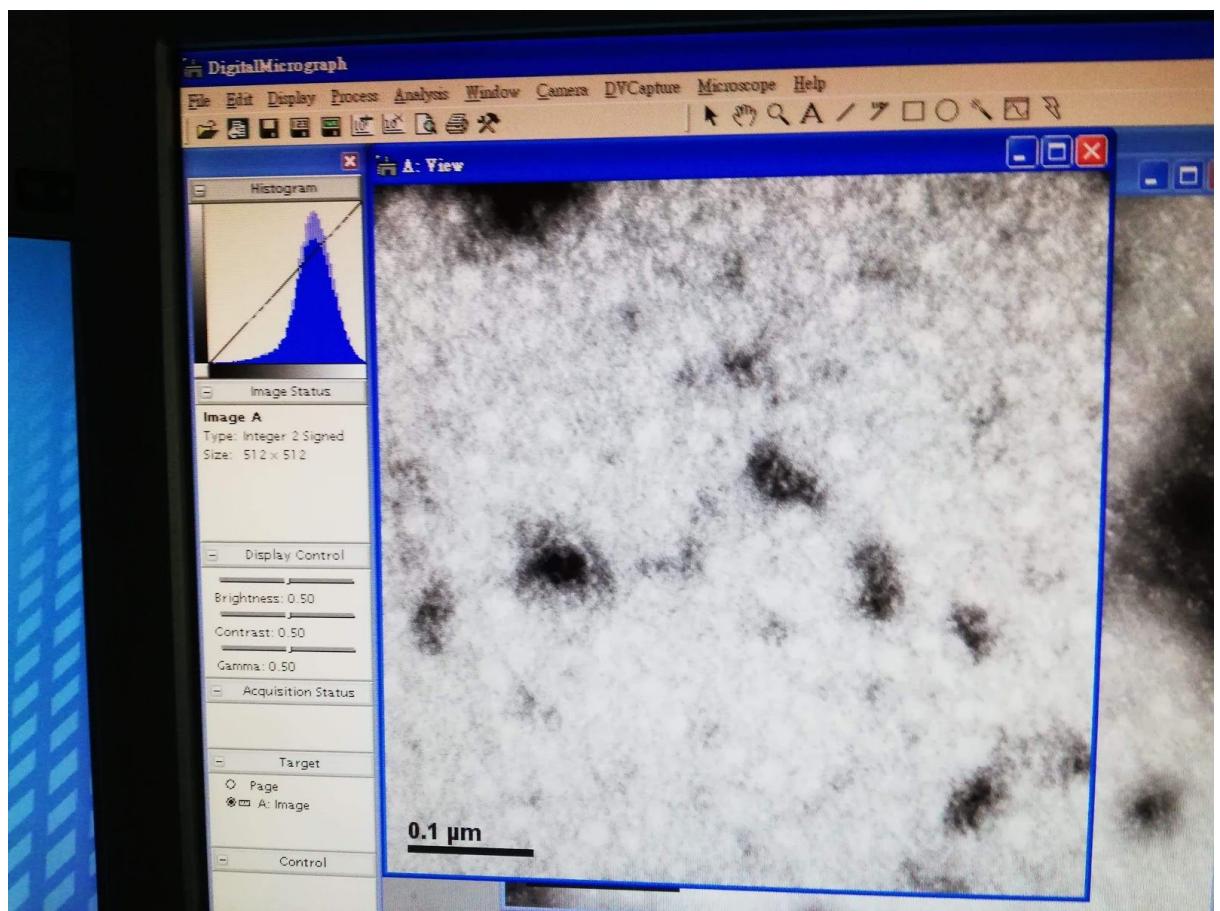


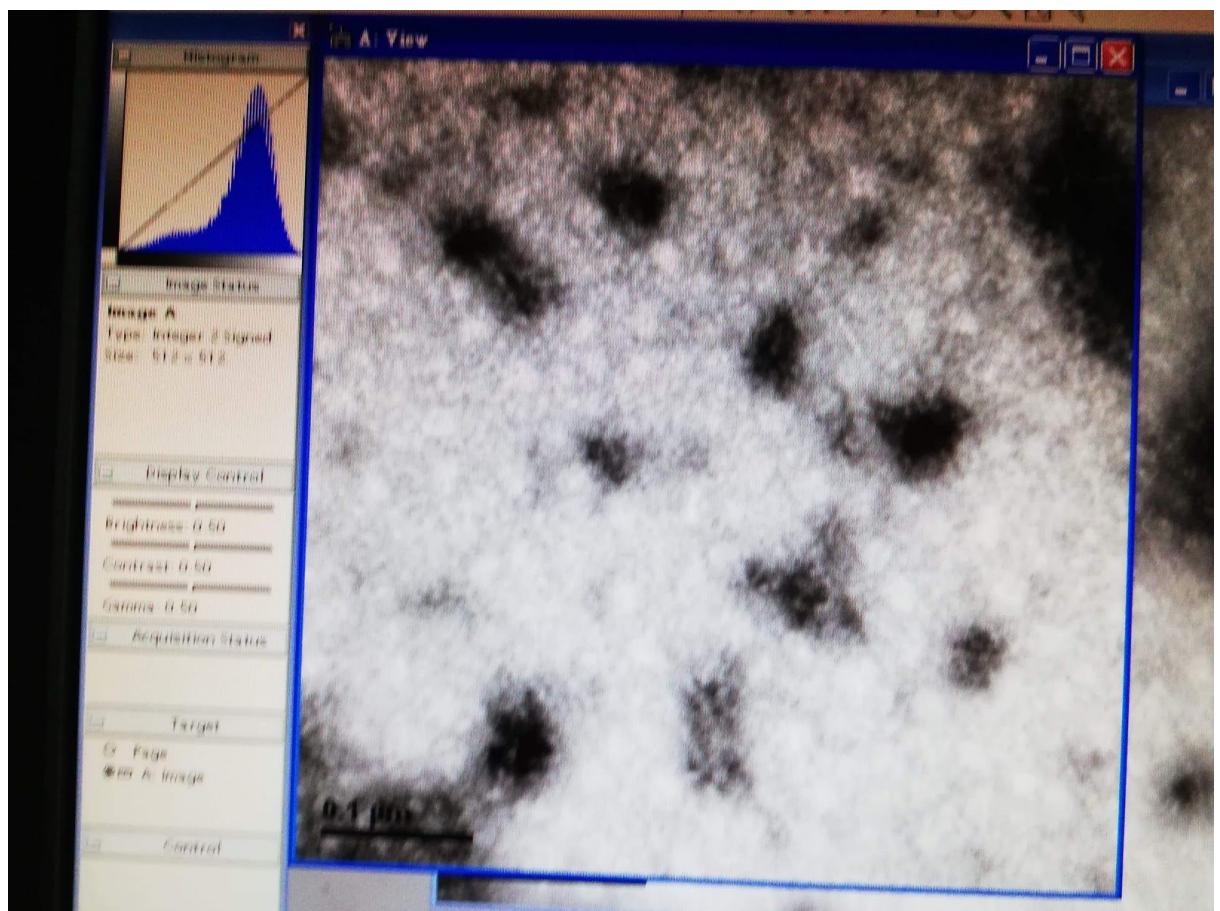


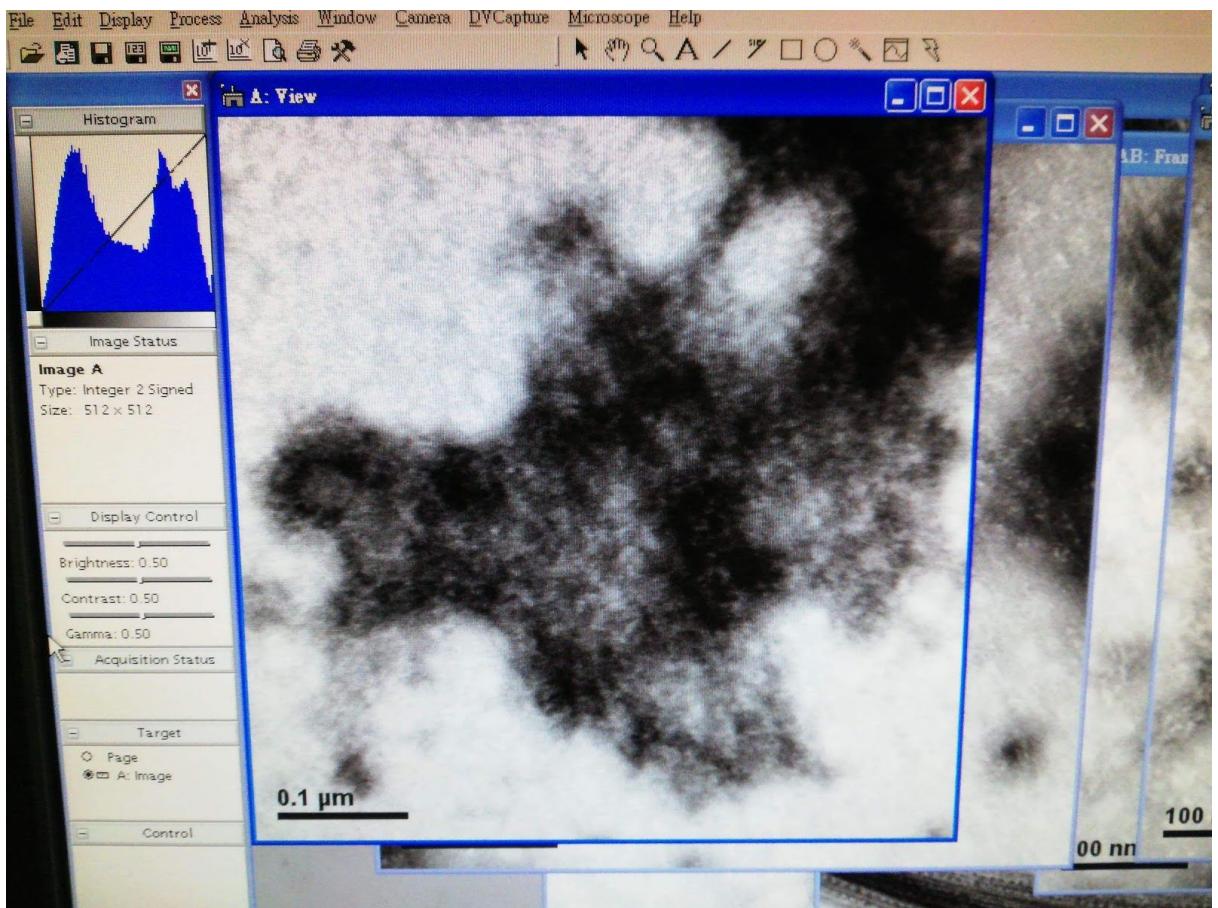




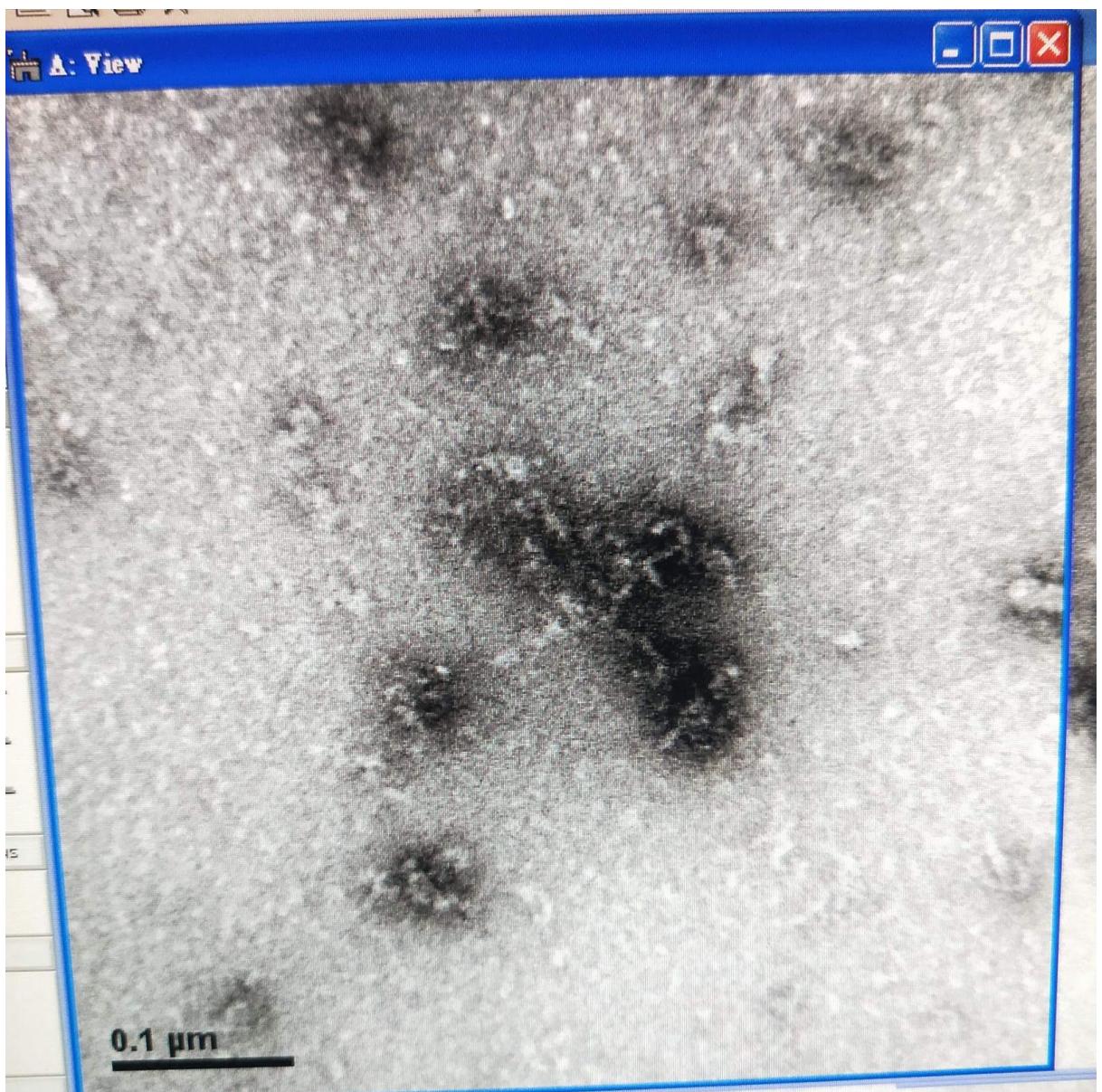


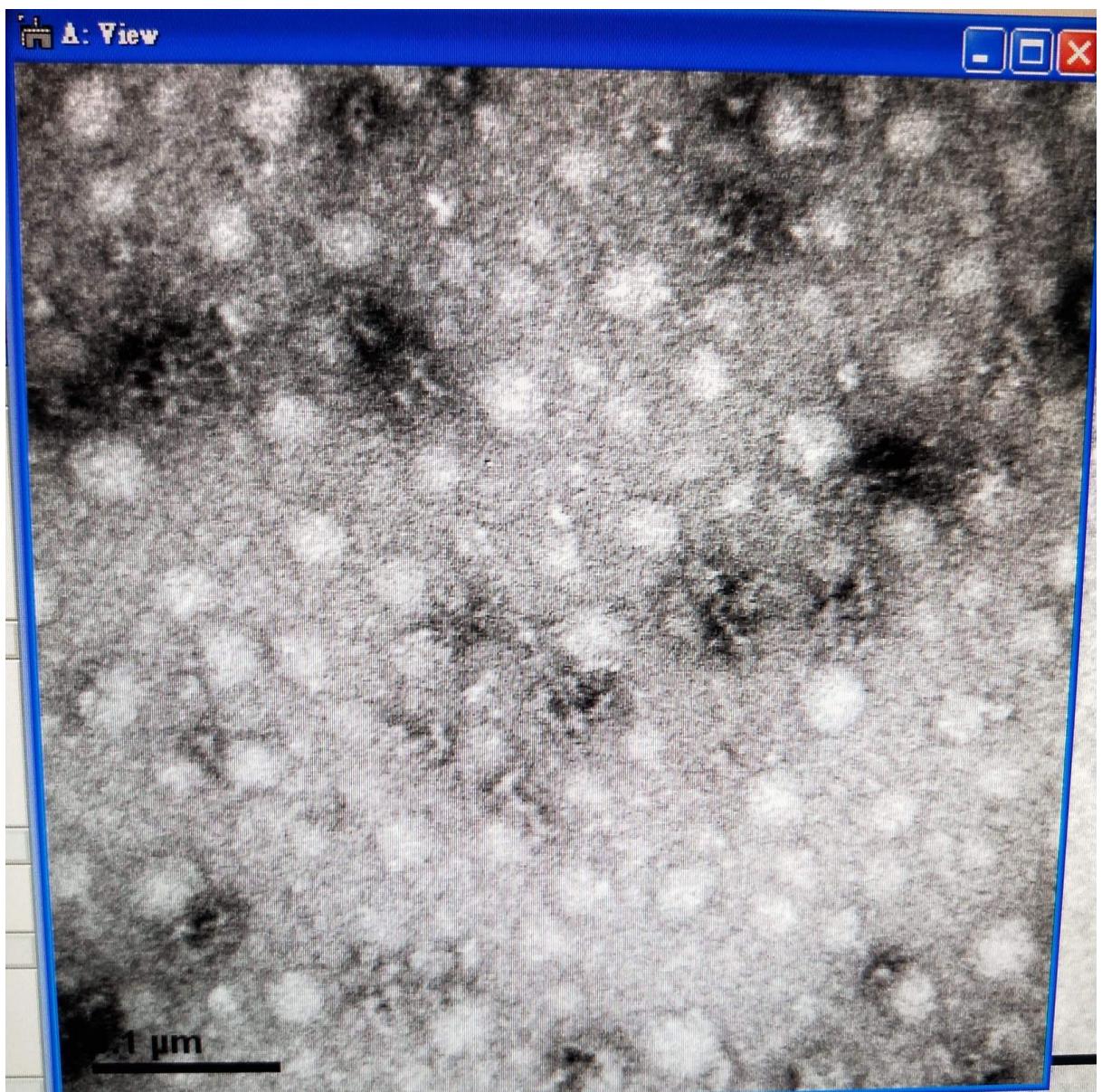




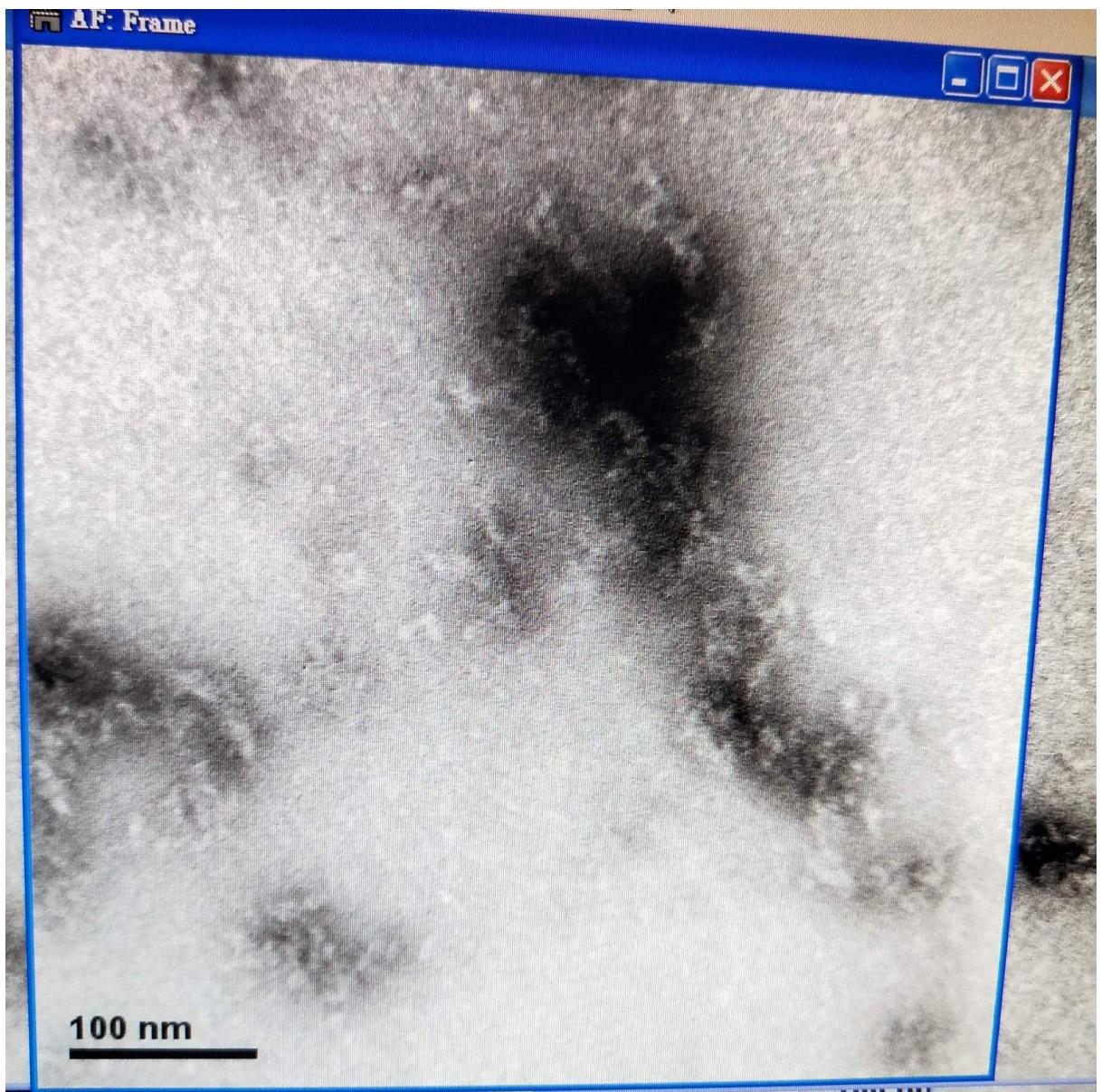


- 結論：東西不少，對比也夠，可惜沒有出現明顯產物形貌，多零碎狀。
- 染 1:7 bottom 不震 15mM / 1:7 bottom 震5min. 15mM / 1:10 bottom 不震 15mM
- 1:7 bottom 不震 15mM stain2 settle5 舊pro





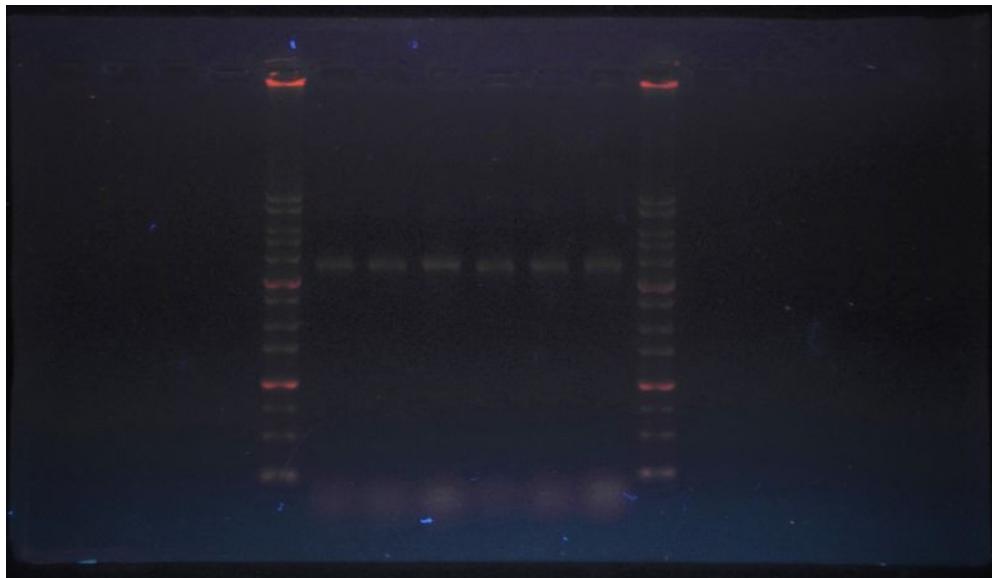
- 1:7 bottom 震5 15mM stain2 settle5 舊pro



- 結論：感覺震5跟不震似乎沒有太大區別。東西頗多，對比夠，但因為很小，所以必須到電腦上才可清楚分辨出L型。
- 待觀察銅網(已做好，在陪養皿中): 1:10 15mM 不震 stain2 settle5 舊pro
- 晚上
 - 采葉下了1:5/1:7/1:10 bottom, top lid 的pcr(故一共6管，每管體積40mul)。預計明天約9.可以收。
 - 收完明天就可以跑膠。算是對今天lid跑膠的補做。

9/7 睿謙 重甸

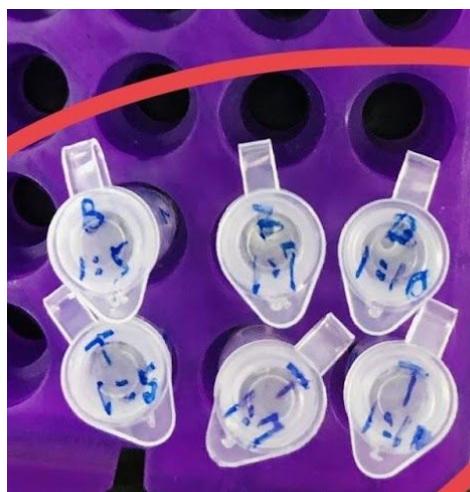
跑膠結果：



0.8% ladder/ B 1:10/ B 1:7/ B 1:5/ T 1:10/ T 1:7/ T 1:5/ ladder

感覺比例沒有太大影響

再做一次1:5/1:7/1:10 bottom, top lid 的pcr



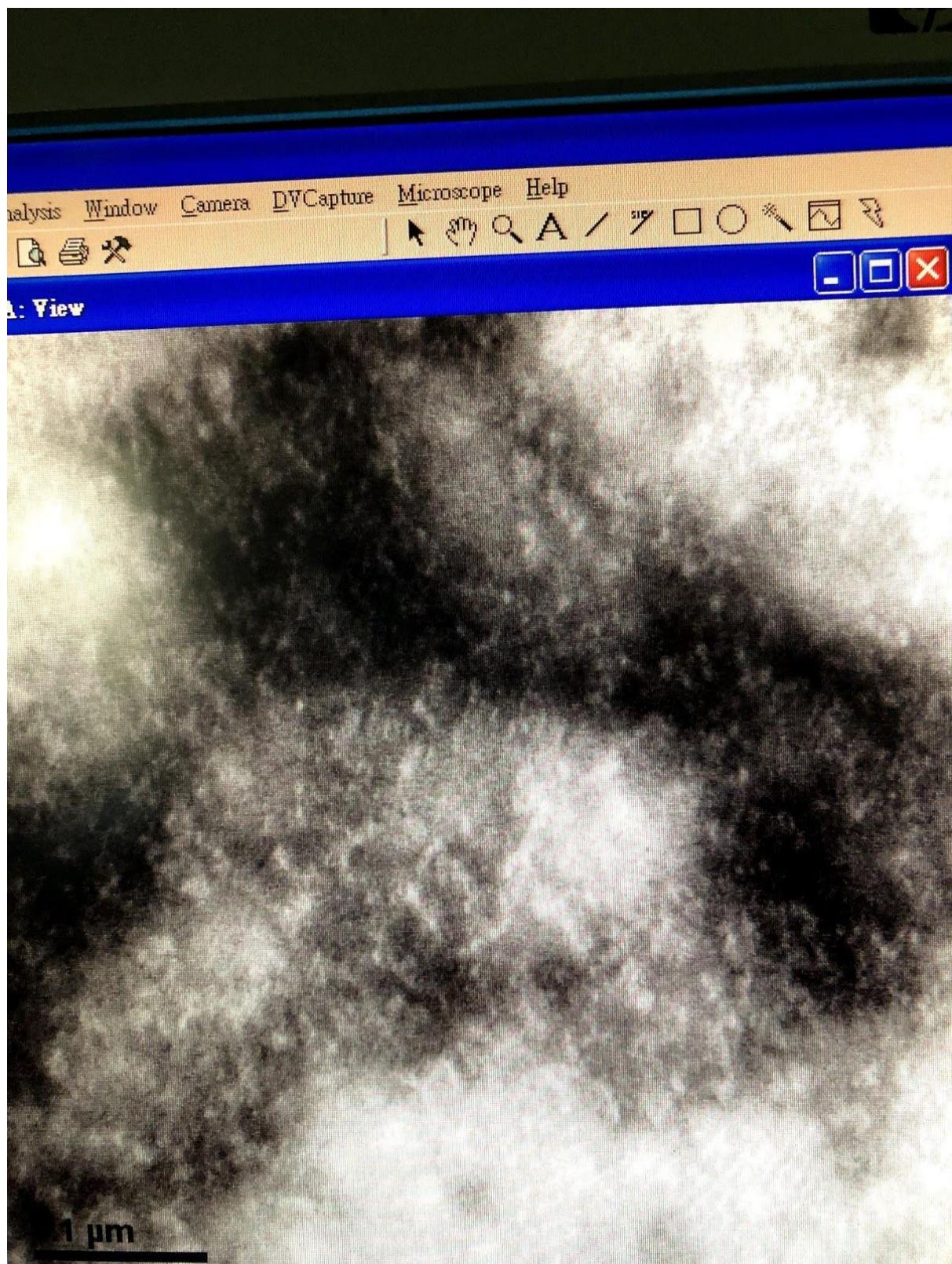
9/8 睿謙

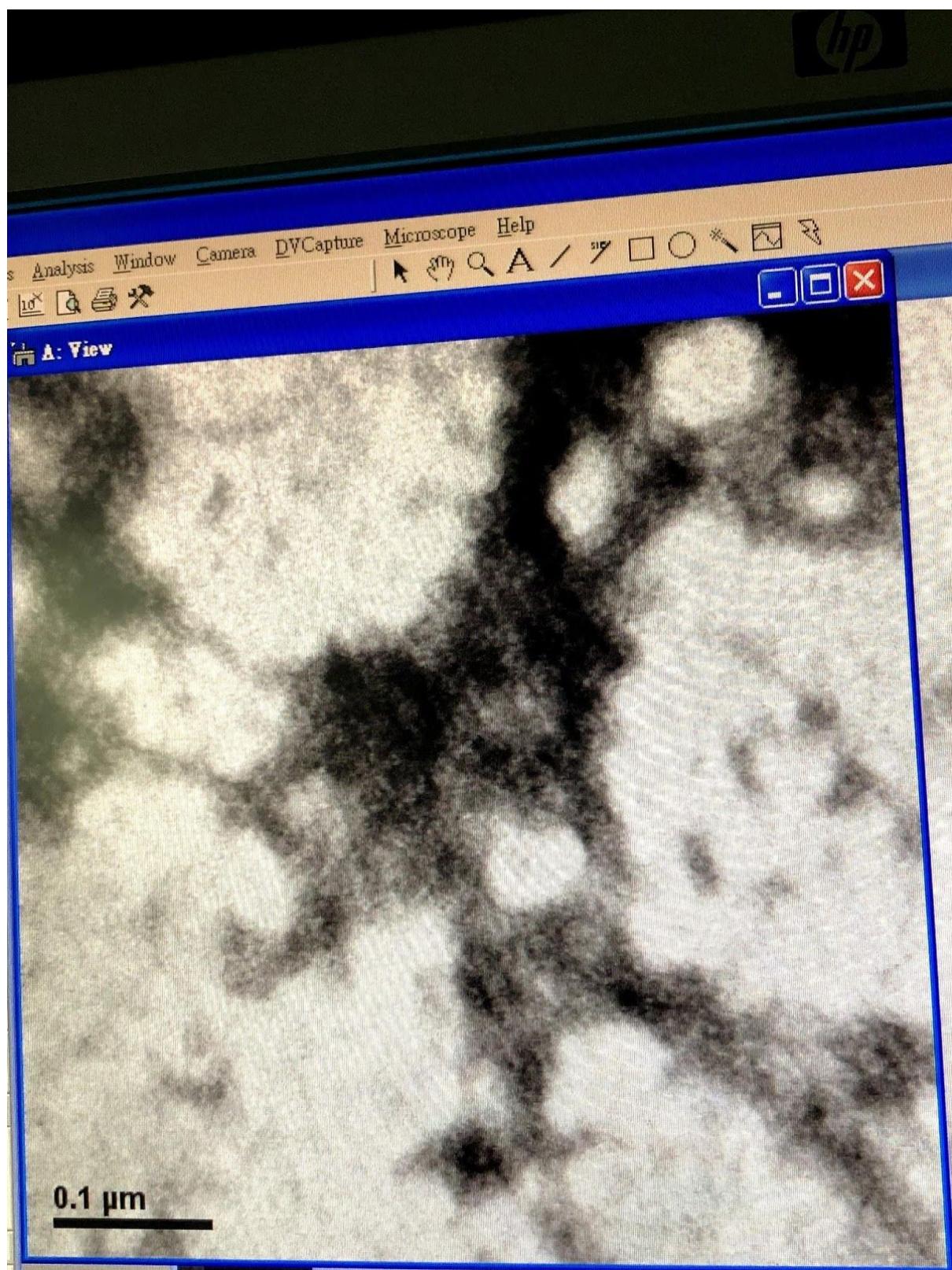


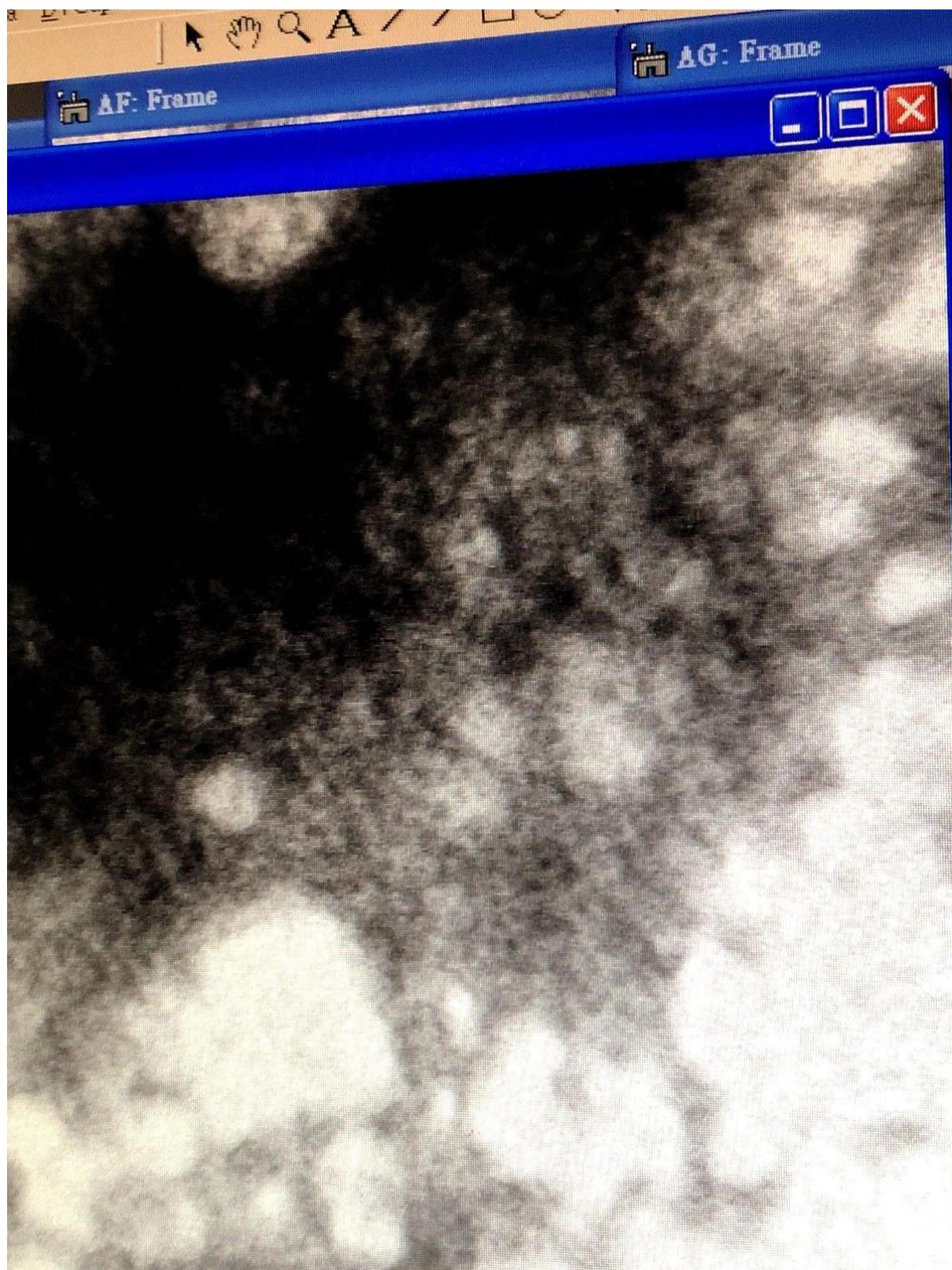
做 box 1:10 / top 1:5/ bottom 1:5 各50 mu l (15mM MgCl₂)

9/9 聰謙 戲寧 原豪(重旬 淸靈 鈺璇 泉浩 采葉 全員到齊

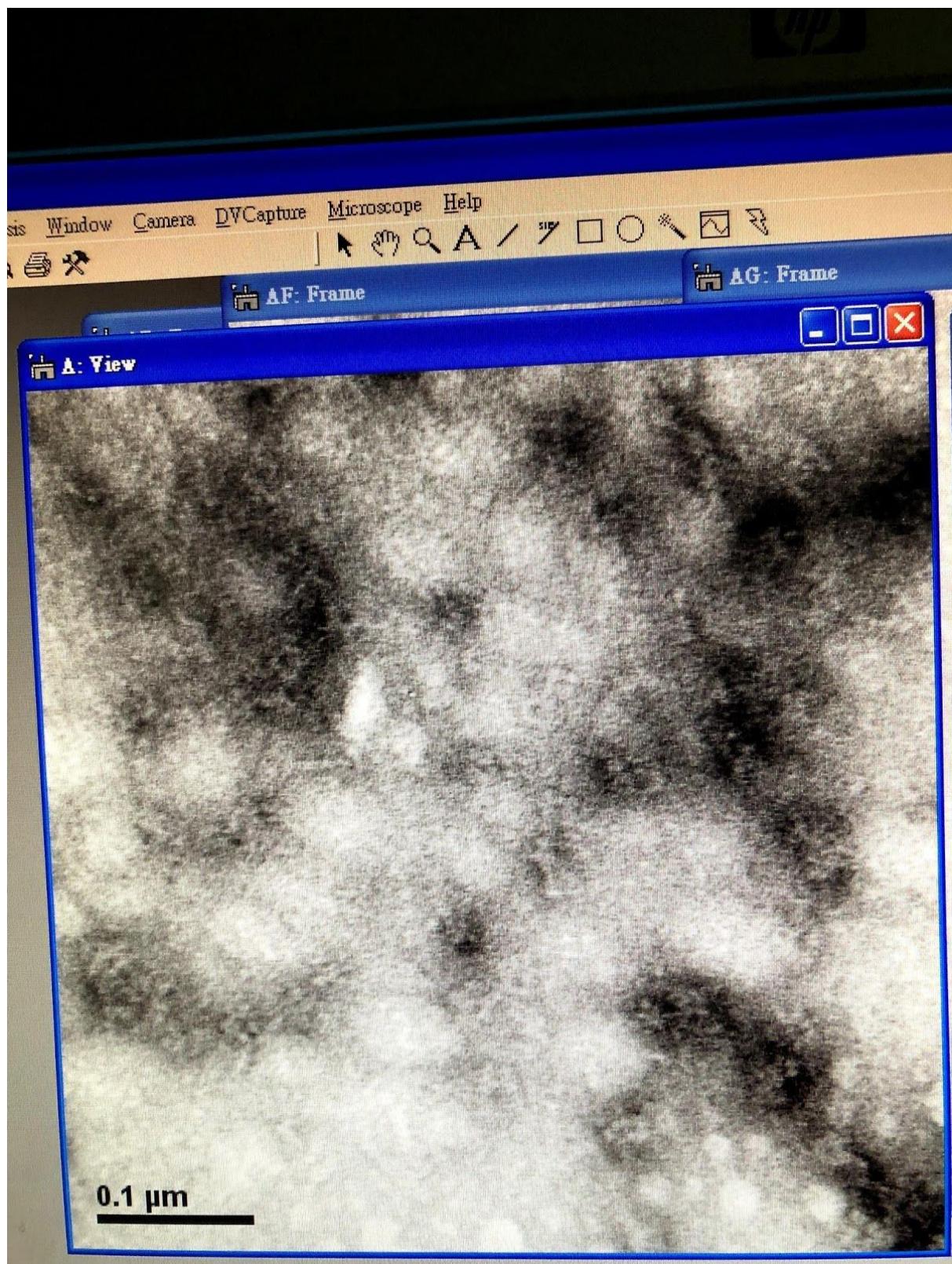
照 1:10 bottom 不震 15mM 2min 不純化

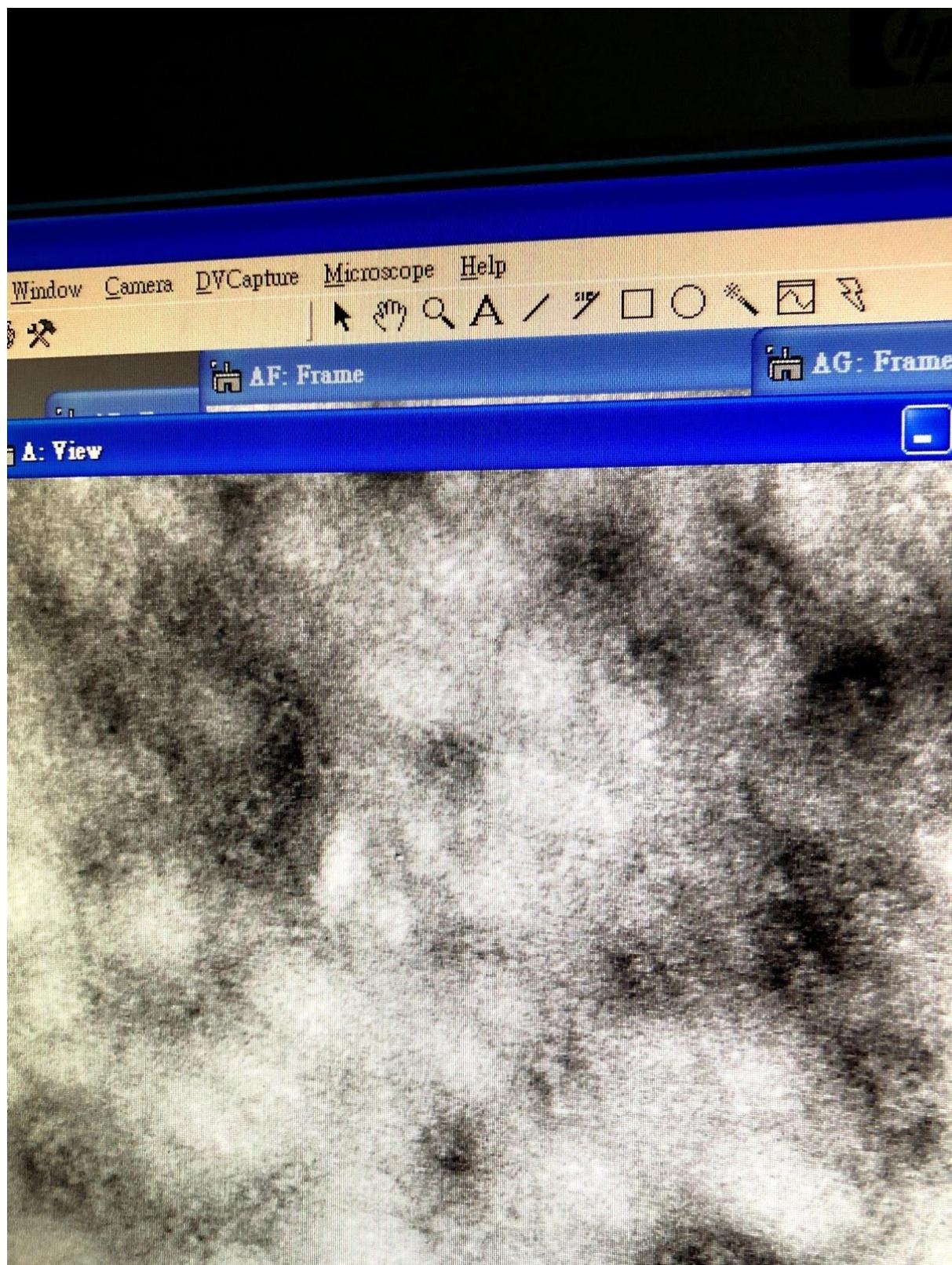


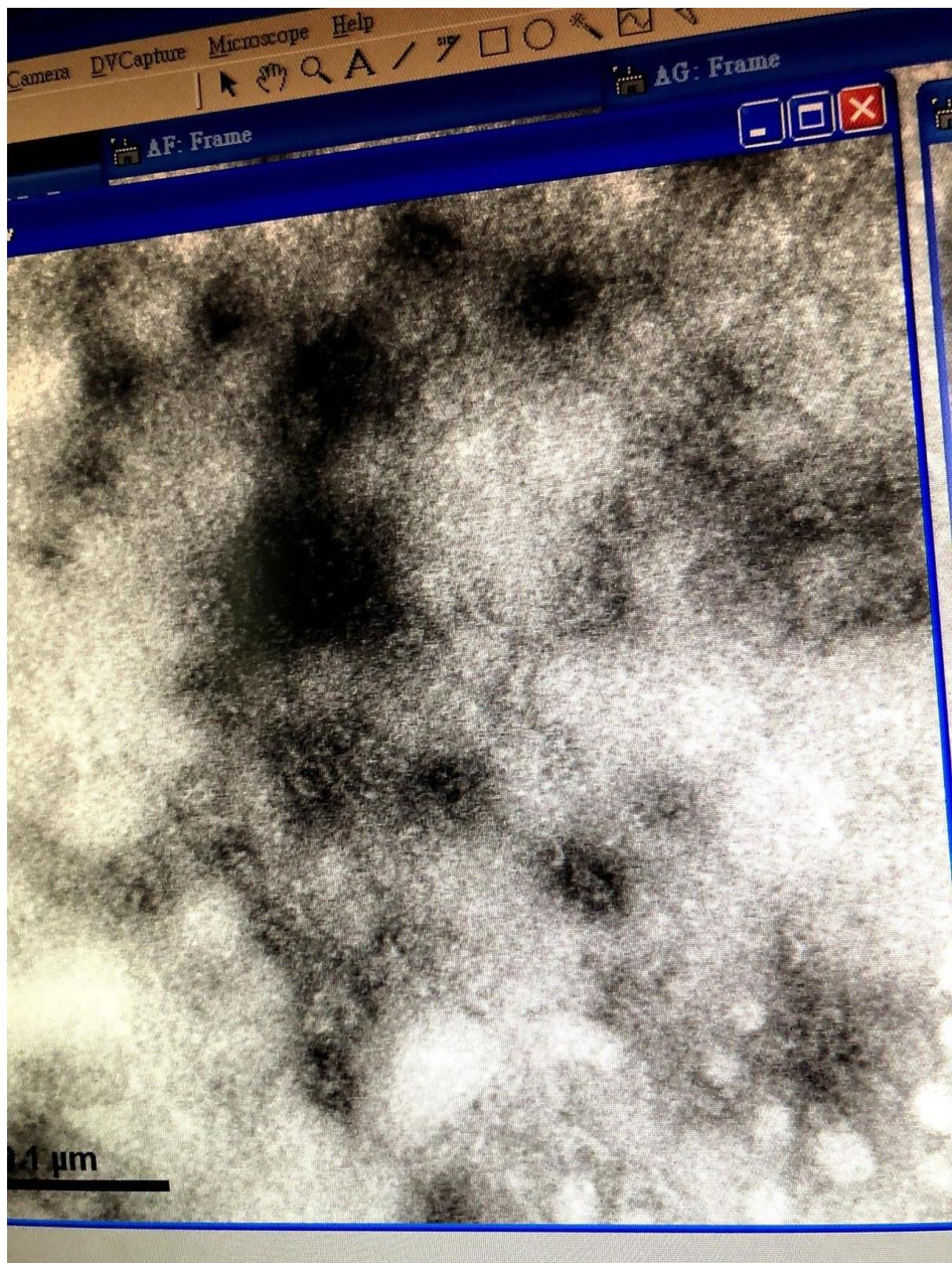


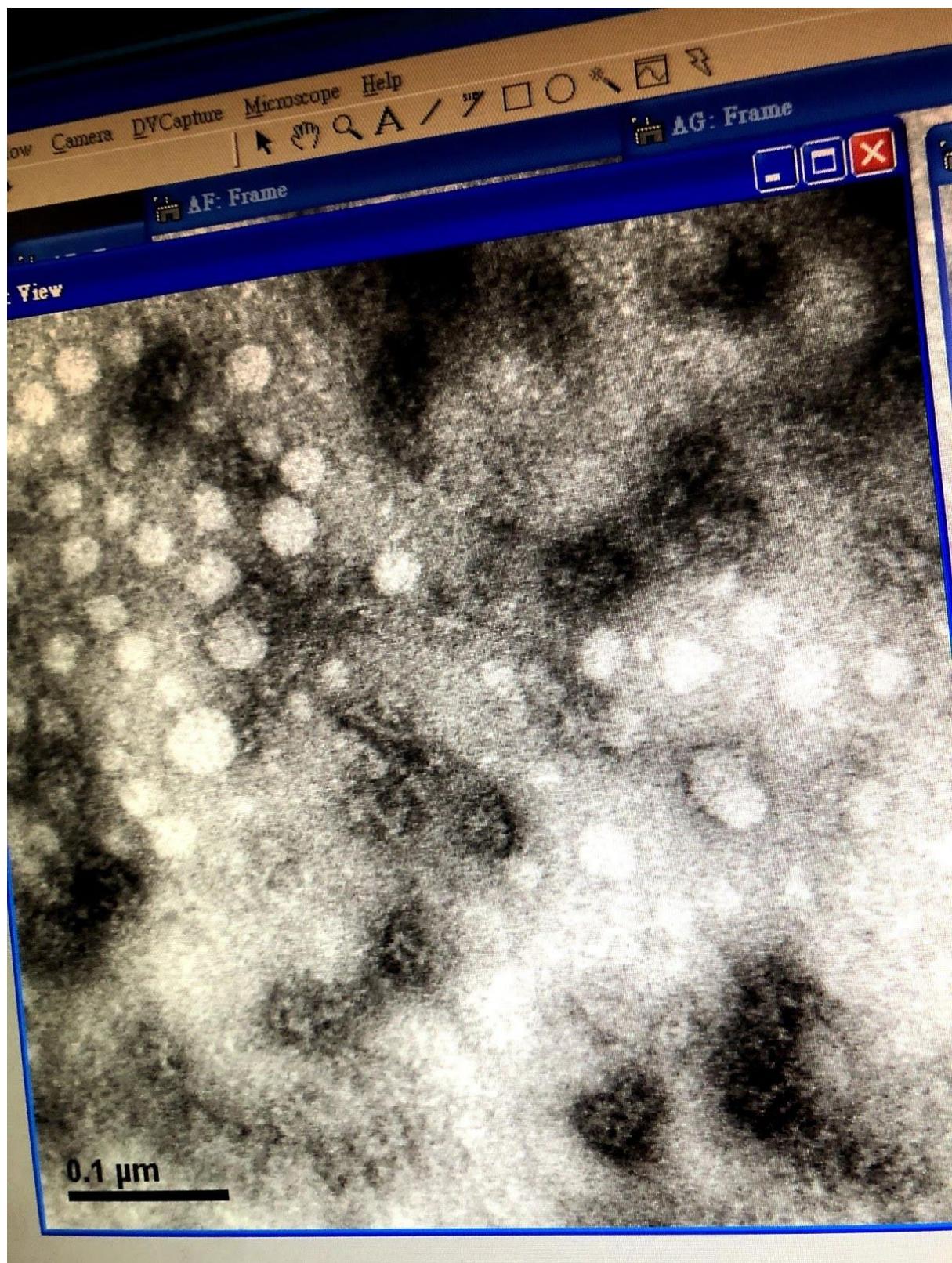


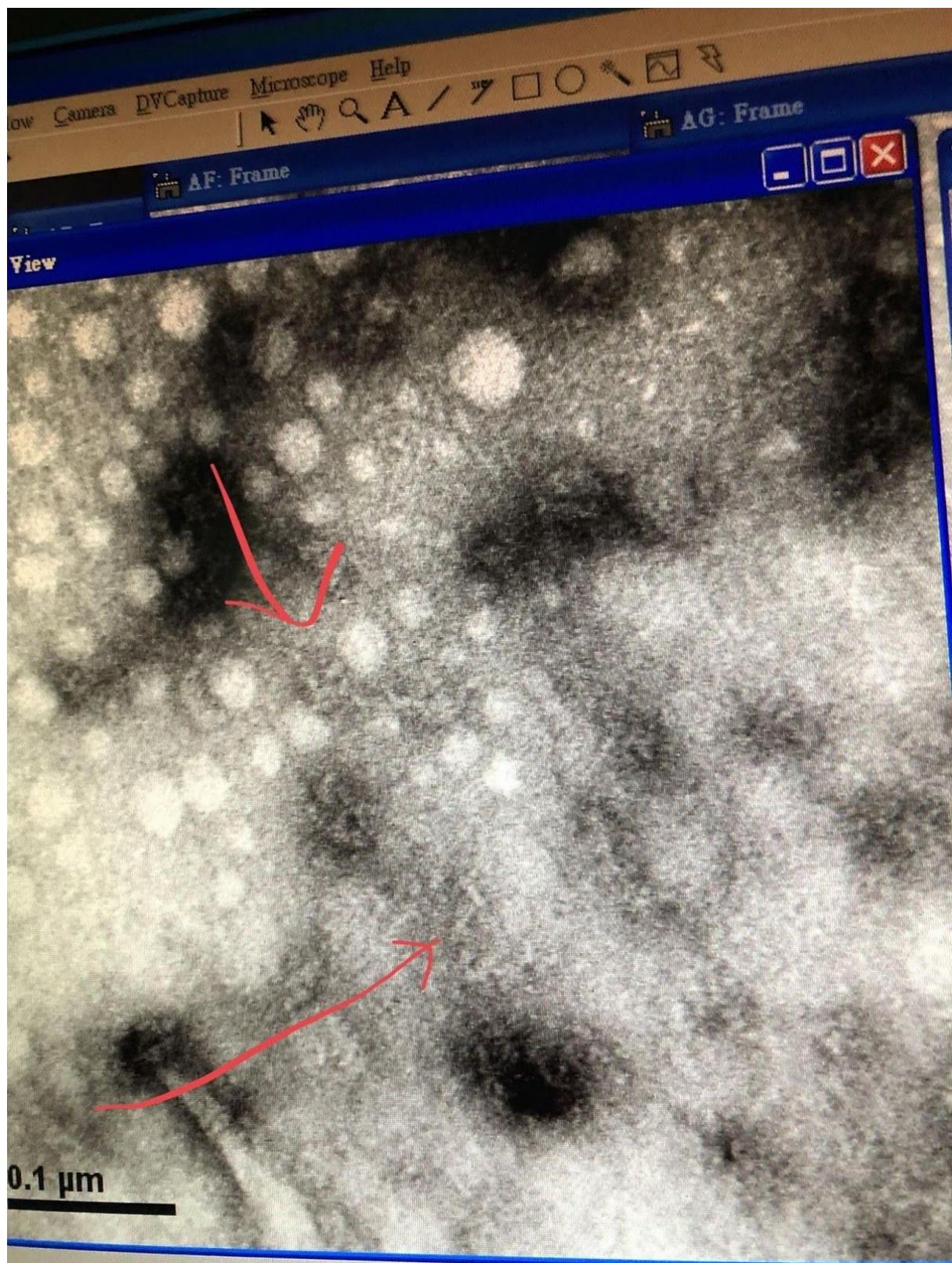
純化一次 1:5 bottom 不震 15mM 2min



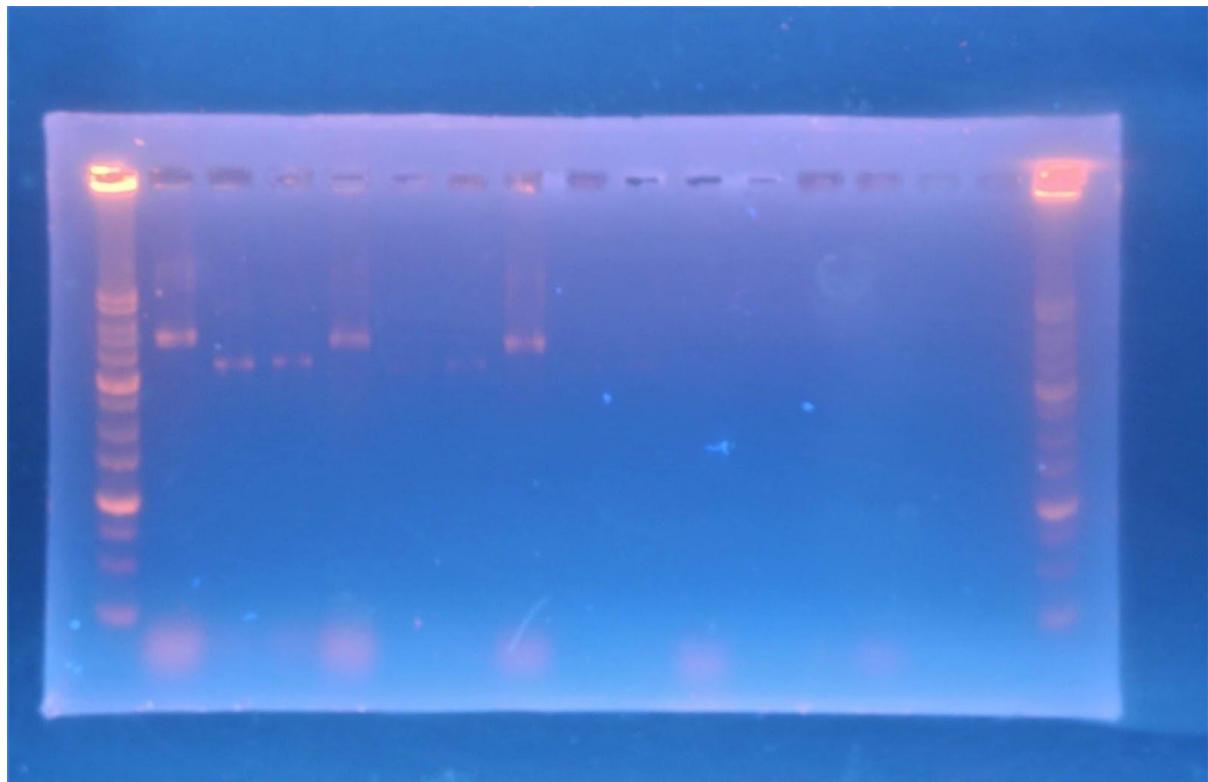








9/9睿謙 重甸

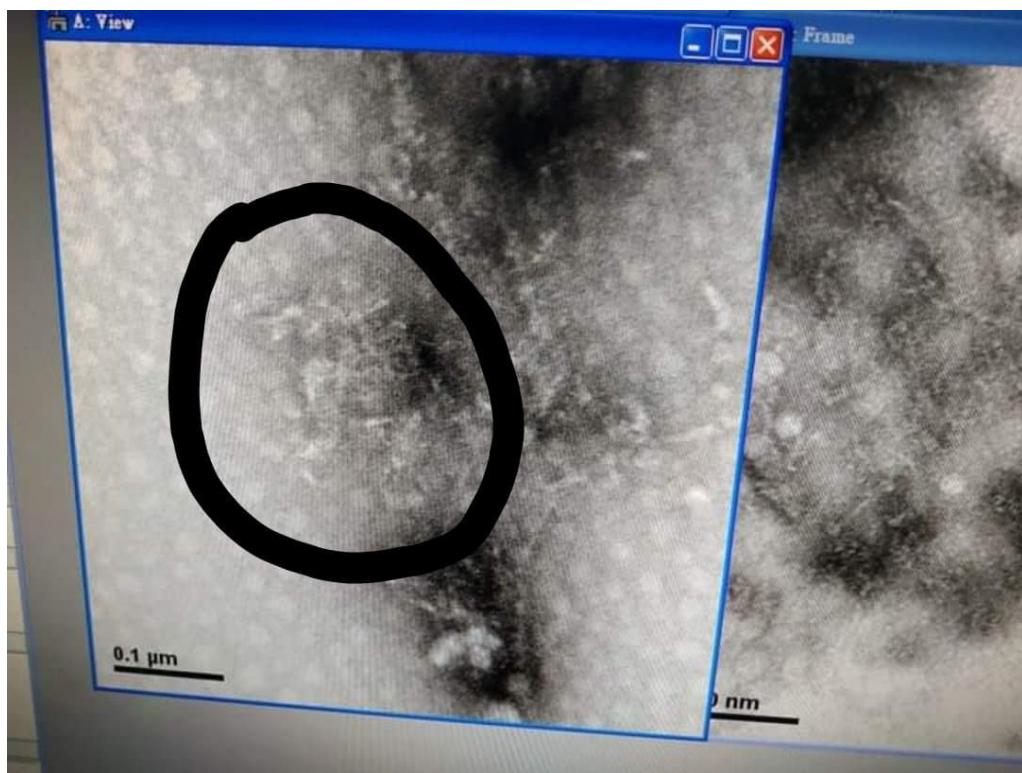
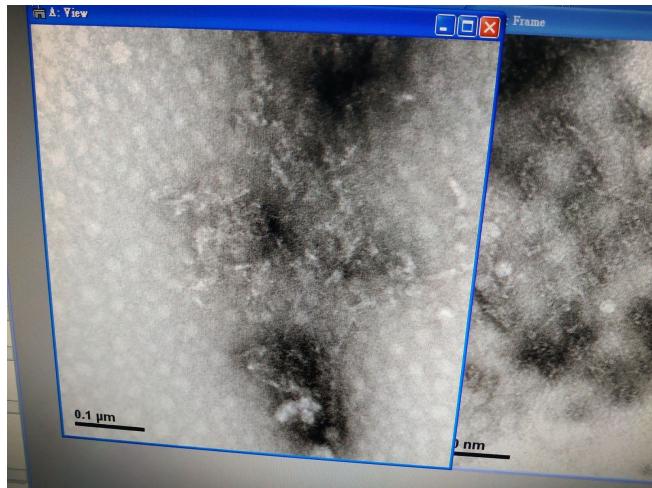


ladder/ X u1/ T u1/ B u1/ X u2/ T u2/ B u2/ X u3/ T u3/ B u3/ X d1/ T d1/ B d1/ X d2/
T d2/ B d2/ X d3/ T d3/ B d3/ladder 曝光60sec 可以看出蓋子純化三次後影響不大

9/10TEM采繫鈺璇苡寧

boxU1不震:堆在一起, 太黑

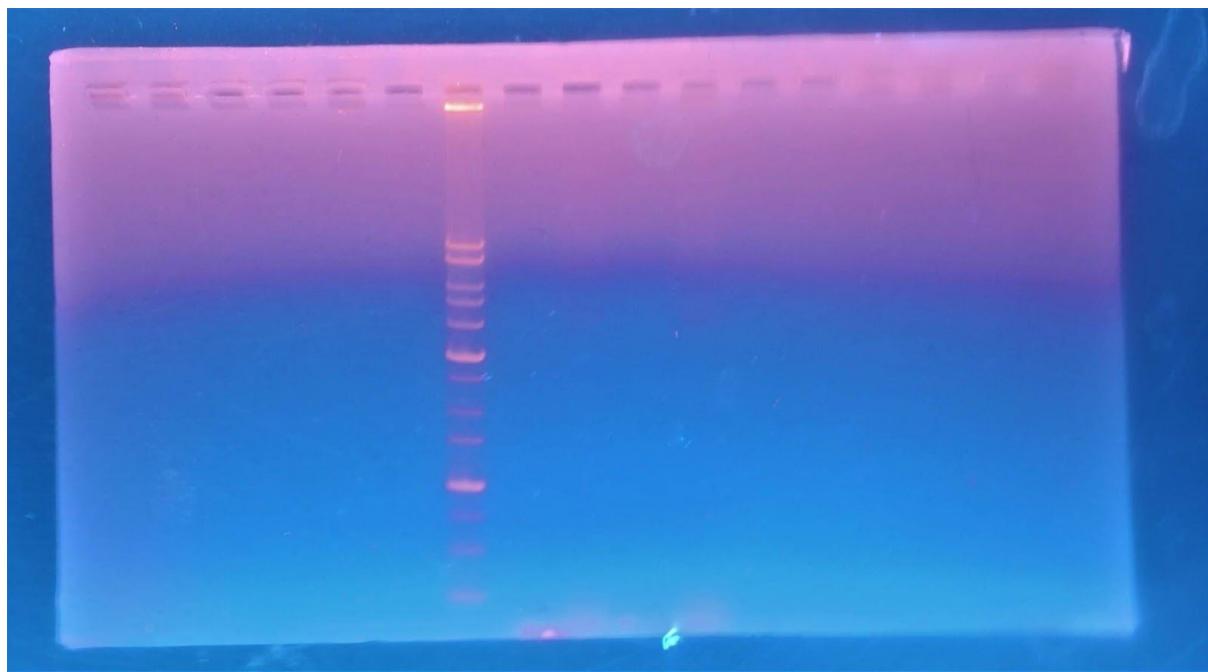
U5震5min:仍然有E, 但整片銅網中間很多格都很空, 只有周圍有點東西



TU1不震

樣本製作BT-U1-震5

X-U4和U5-震五



ladder/X D4/ X U4/ X D5/ X U5 曝光60sec 之後box純到第三次即可

組裝(protocol from nanomuscle):

method1(muscle)

1加initial blocker於box

(nanomuscle的big monomer的blocker濃度是scaffold的5倍)

(nanofolding有做unit:linker不同比例的跑膠測試)

2,Put the mixture in Mini-Shaking-Hybridisation Oven, and shake at 30 °C overnight

3,mix the blocked box and purified lid

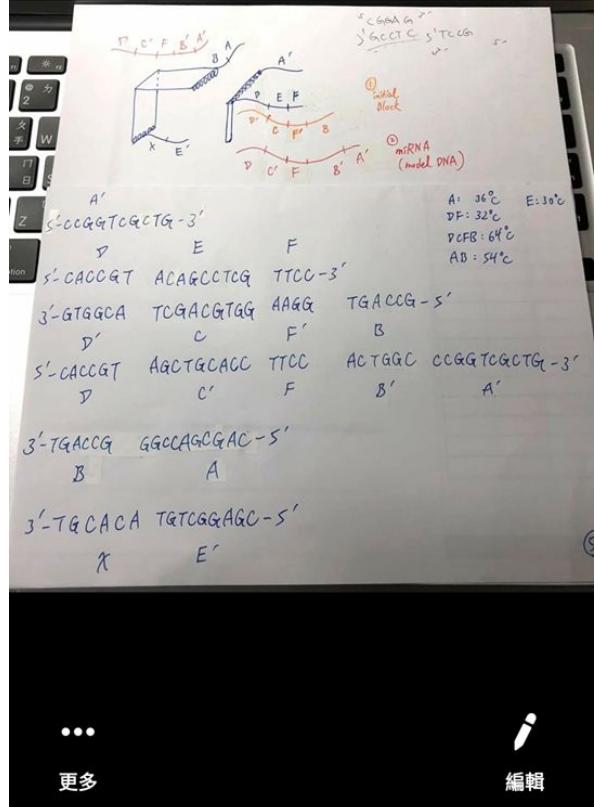
4,Put the mixture in Mini-Shaking-Hybridisation Oven, and shake at 30 °C for 3 day

bug:1沒純化，多餘的staple會干擾(和block接在一起)

2如果跑膠確實有疑似組起的，無法確定是A相接(有block好)，或E相接(沒block好)



BIOMOD BRAINSTORM!!!

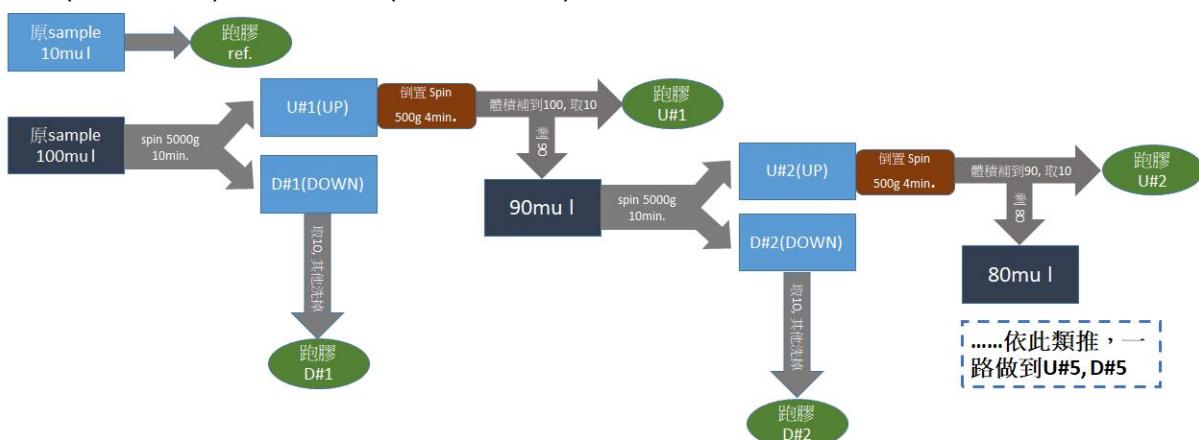


method2(folder)

incubated for 30 mins, in 25 °C

annealing

40度(1min hold), 降至25度(-0.1度/1min)



這是原計畫。(一樣的步驟同時處理box, bottom, top lid 的材料。原材料各共有500 mul，也就是說處理一次如此純化要用兩管迷你eppendorf, 共計100mul。)

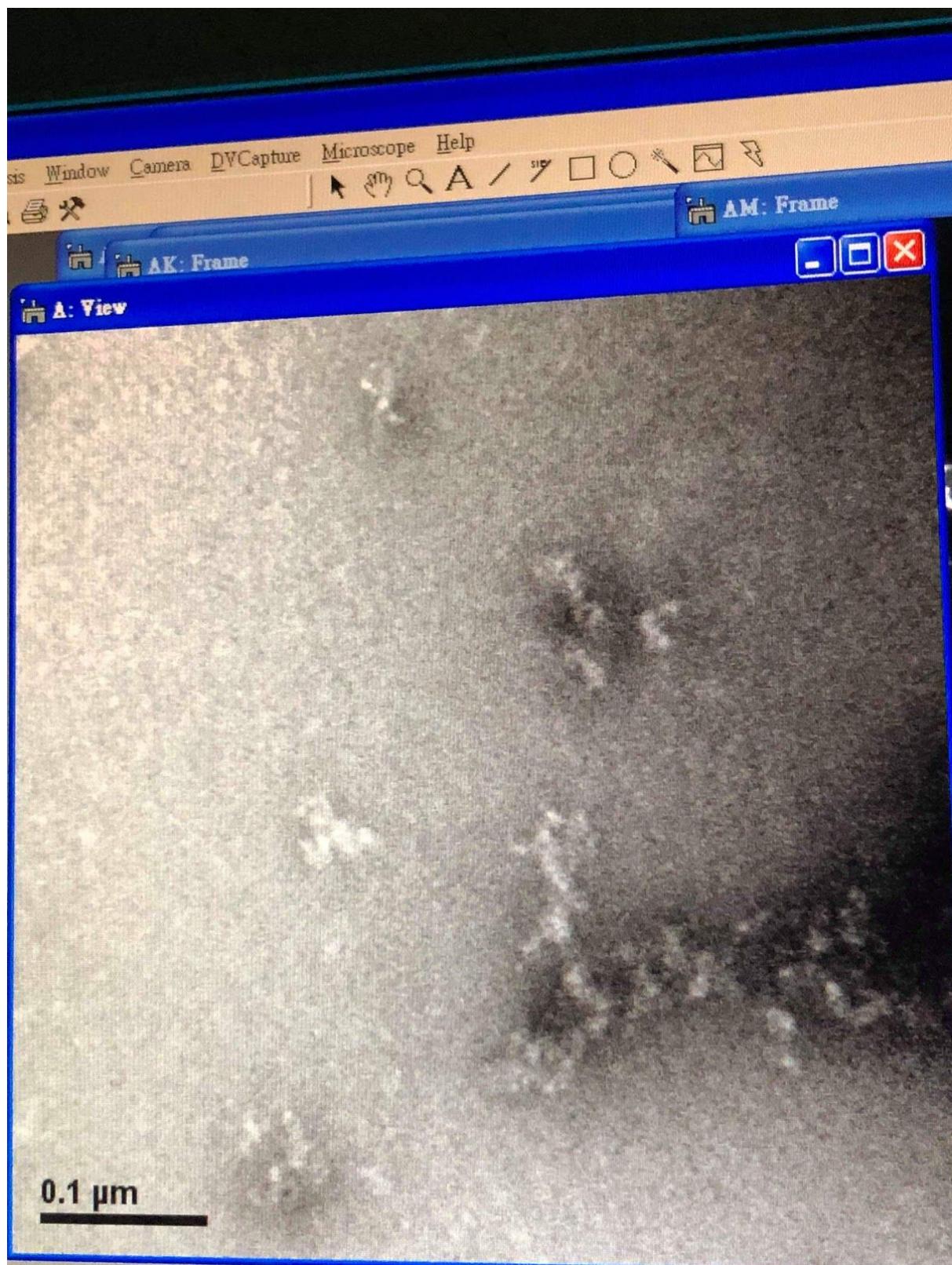
註1: BOX的TOP 記做X T; BOTTOM的DOWN 記做B D. 另外，所有要跑膠的現在都在迷你eppendorf中(ref, U#1, D#1...)，標記皆在蓋子上。

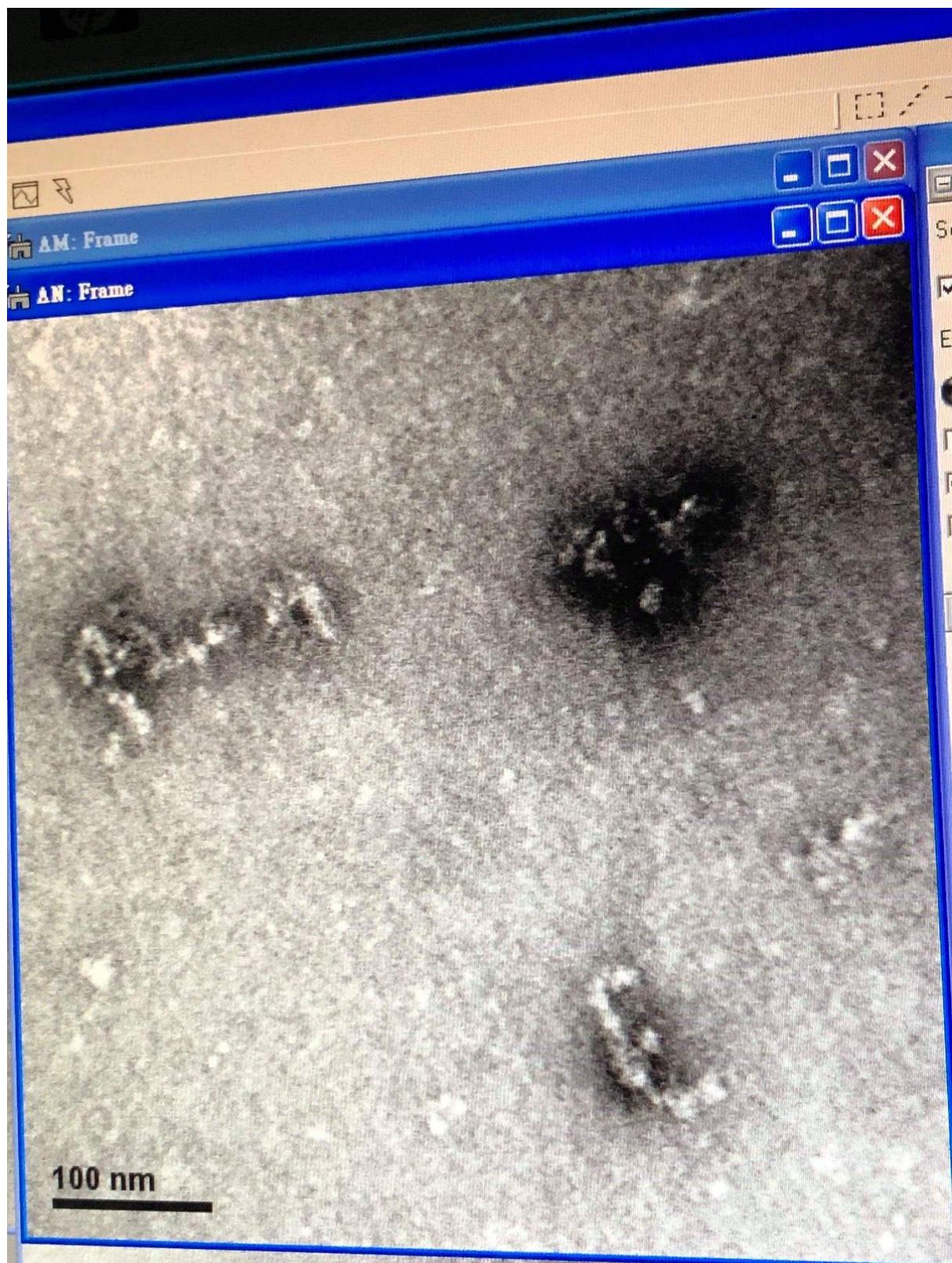
註2: 之所以要補體積是為了要讓跑膠的每個well濃度皆相同，因為純化的目的就是要讓底部的濁漿逐漸消失，當濃度相同時，亮度相同，這樣比較時較有說服力。

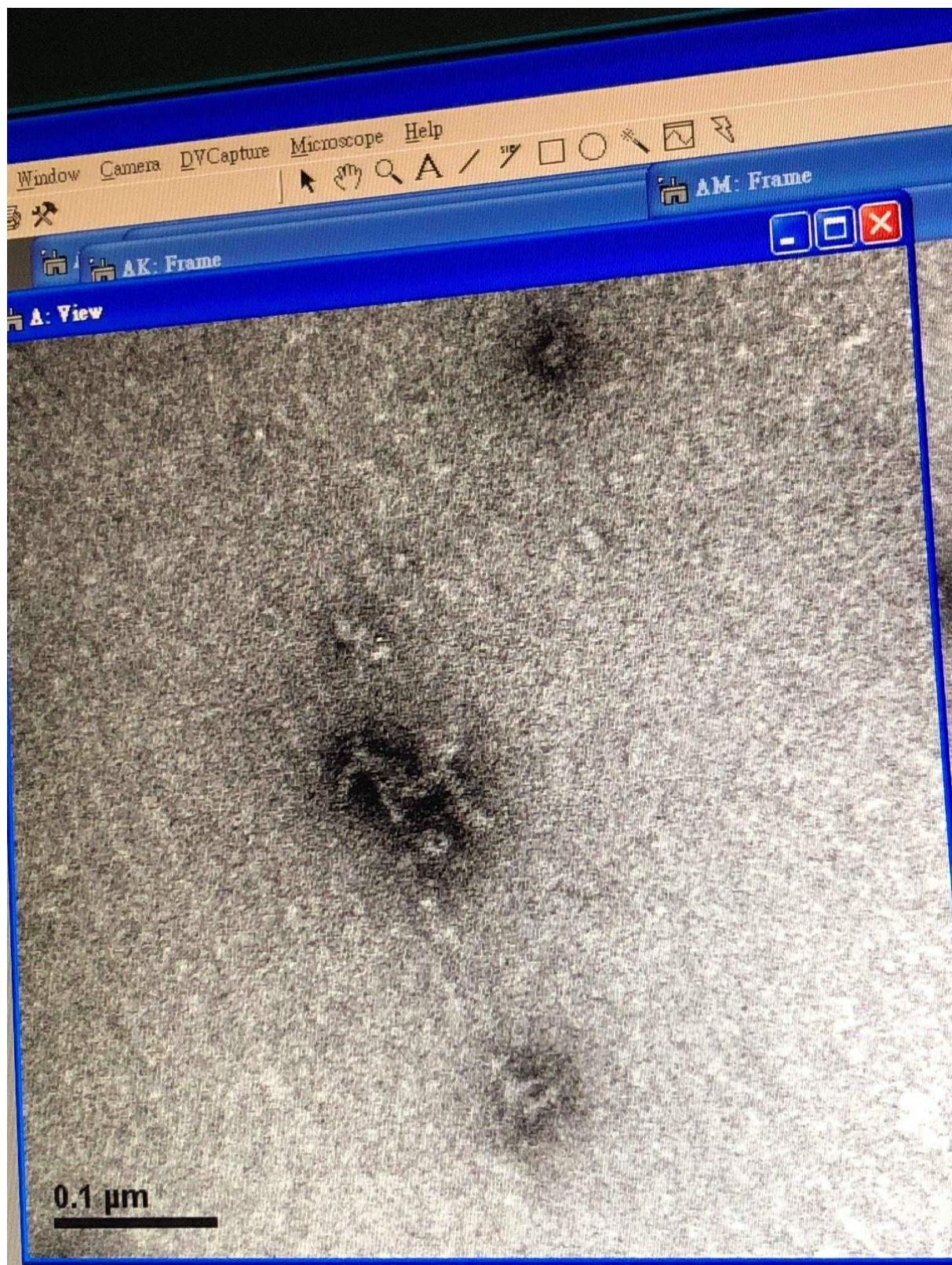
9/11 泉浩 采蘩 滴蠶 睿謙

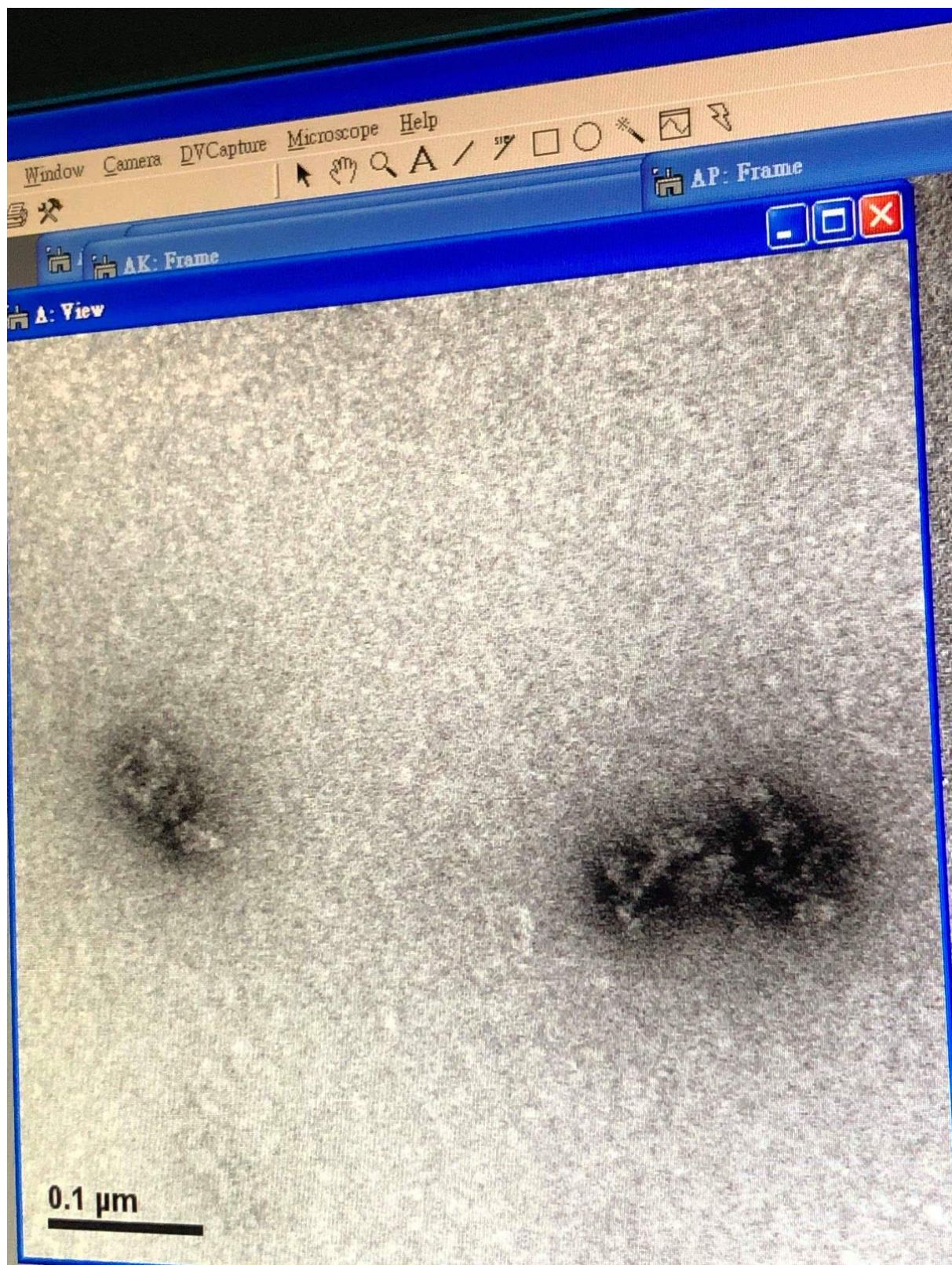
- lid 純1 震5min. 1:5 舊pro
 - 發現lid 震5min.效果很不錯，今天拍了5張照片。

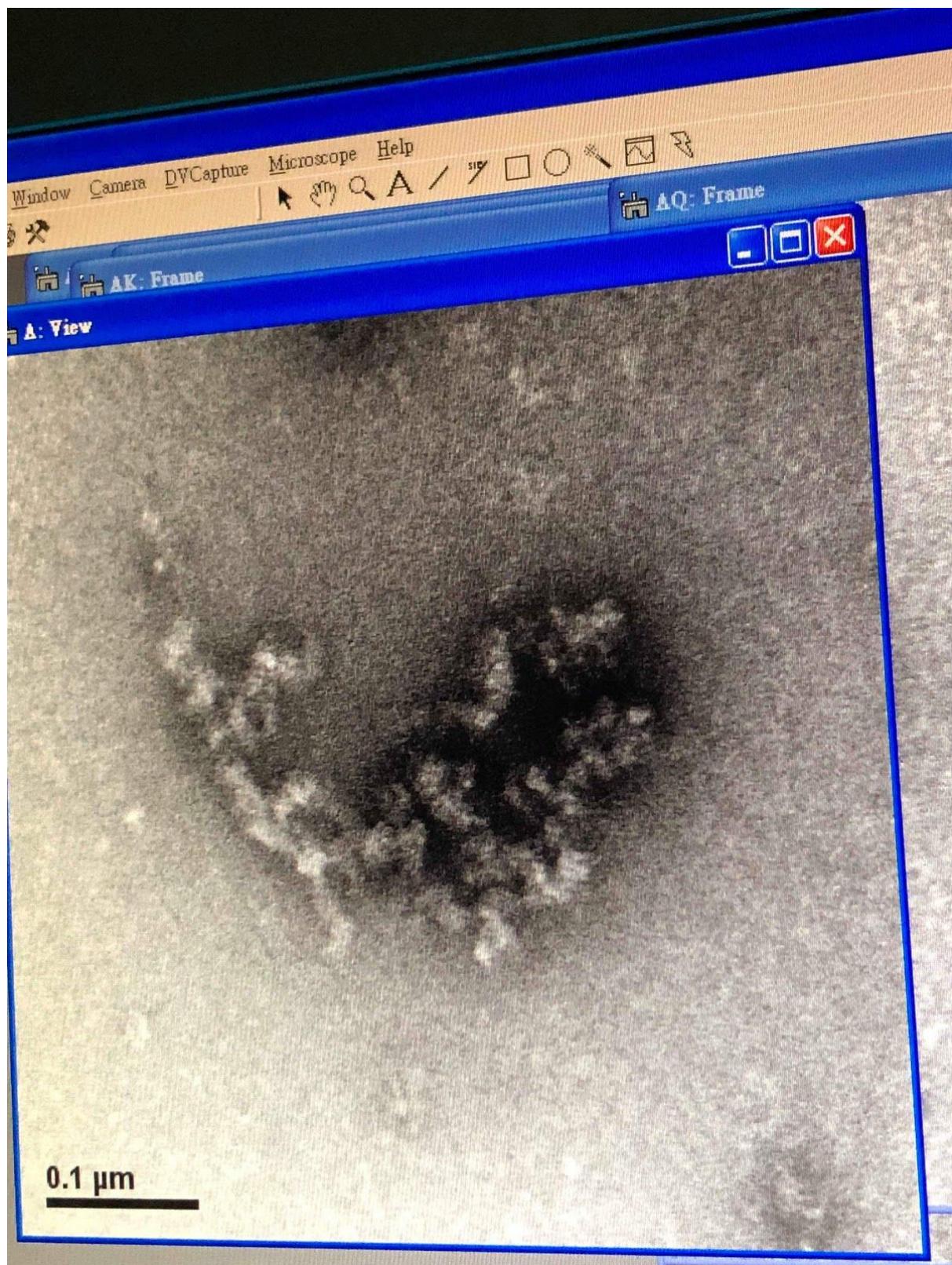




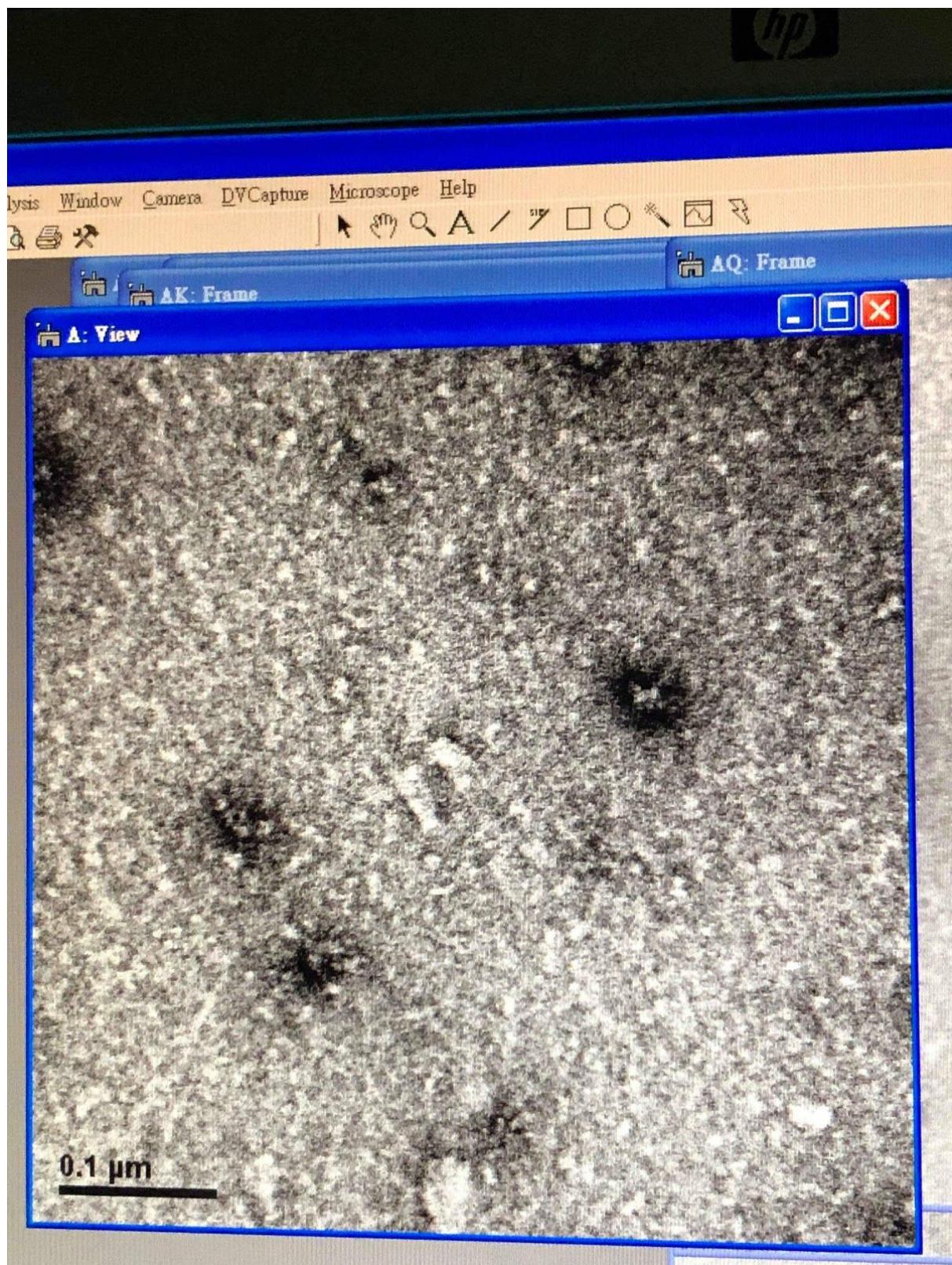




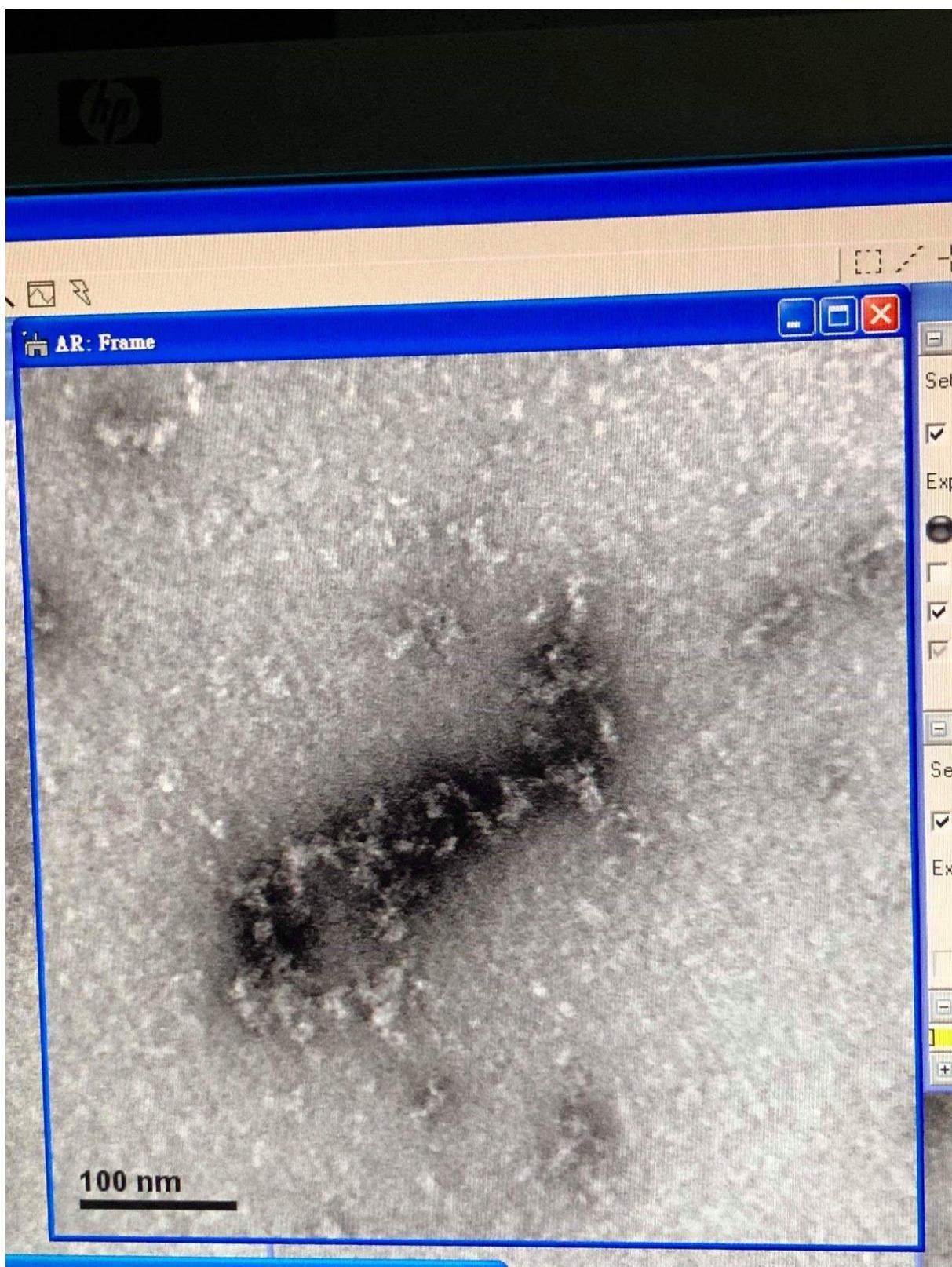


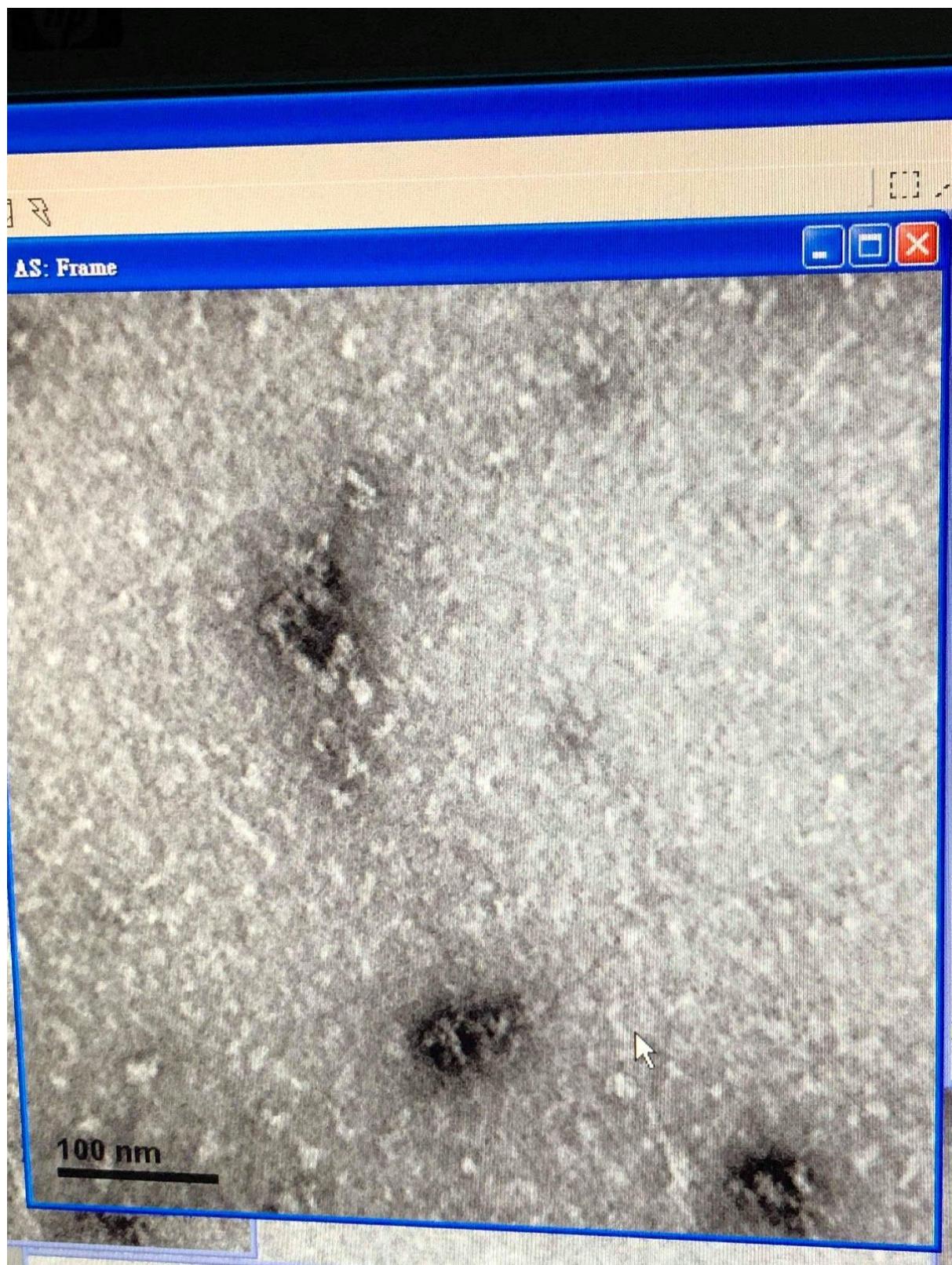


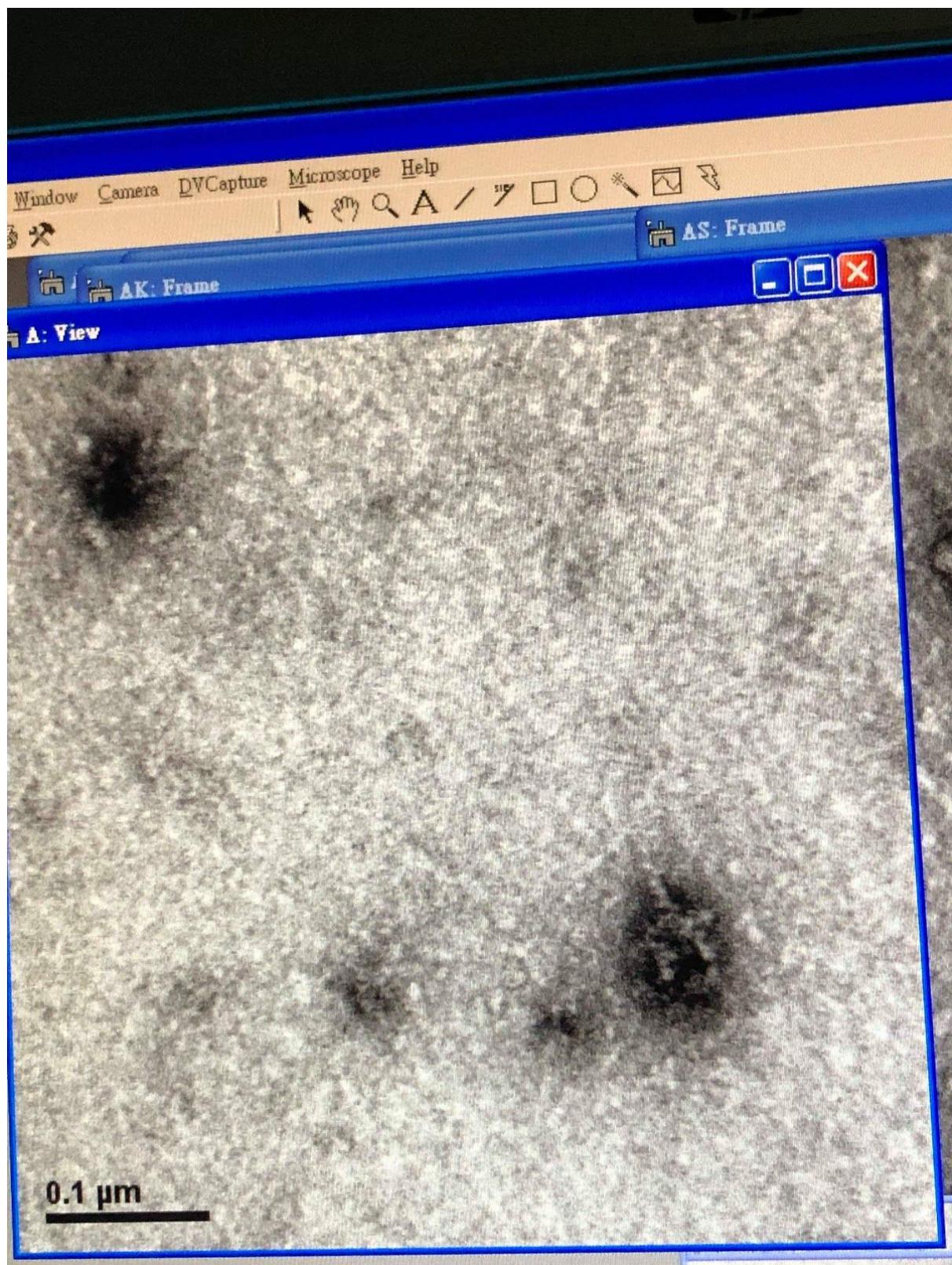
- box 1:10 純4 震5min.



N







9/13 采采 滕璽 泉浩

- 早上

- 我跟采采一起完成box純2 / BT純1。 分裝結果如下。



- 下午

- 開始進行零件組裝， 詳細實驗紀錄請見最上方pro的藍色字體。

protocol

PCR1→over night	→PCR2(9/14)	簡稱PP
PCR1→over night	→shake1天(9/15)	PS1
PCR1→over night	→shake2天(9/16)	PS2
PCR1→over night	→shake3天(9/17)	PS3
shake→PCR2(9/14)	SP	
shake→shake1天(9/15)	SS1	
shake→shake2天(9/16)	SS2	
shake→shake3天(9/17)	SS3	

9/13

※藍字為今天下午進度

1,U2box分300mul共兩管，每管加12mul稀釋20倍的blocker(435和437)

blocker皆取1.4mul, 加入 $1.4 \times 19 = 26.6$ ddH₂O

2,U1lid分200mul共兩管，每管加2mul稀釋20倍

hinge(T:439,440/B:441,442)

hinge 取1mul, 稀釋成20mul, 再取2mul. (剩餘保留)

翻面:BTX 取30 mul+3mul的Signal (稀釋10倍)

3,一管X和1管BT放進shaker(調30度, 不要搖太劇烈)

搖過夜, 預計9/14早上收。搖晃時直接擺在儀器底部即可, 跟上面的架子無關。

一管X和1管BT放進PCR1 40度(1min hold), 降至25度(-0.1度/1min)

PCR1需時150min.

4,跑完PCR1的box再純一次

清璽純化完已裝入寫有X 純化3的eppendorf.



5,PCR1完且又再純完一次的box和PCR完的lid,照下表配4組(共 $3 \times 4 = 12$ 管)

	x+B(50)	x+T(50)	x+B+T(75)
X	25	25	25
B	25		25
T		25	25

6,其中一組再跑PCR2(初溫代訂), 跑完今天跑膠

7,其餘三組放shaker

9/14

8,搖完一天的box純一次, 配四組(配法同step5), 三組拿去繼續搖, 一組跑PCR2

9,跑PS1和SP的膠

9/14 齋謙 重旬 鈺璇

將組裝後、加入blocker(hinge)做完pcr1跟shake後，再做組裝。

進行跑膠

加 Blocker or Hinge 時 shake overnight	加 Blocker or Hinge 時 PCR1(150 min)	組合時 PCR2 (120min)	組合時 shake 1 night	組合時 shake 2 night
DS1	DPI	APII	AS1	AS2

D:add block or hinge

S:shake

PI:PCR1 (150 min)

A:assembly (兩兩組裝)

PII:PCR2 (120 min)

數字：隔夜數

DS(18hour) / APII: Box+Top(30mul)完成; Box+Bottom(30)完成;

Box+Top+Bottom(55)

DS(18hour) / AS(9/14 15:30開始 預計一天、二天) 今日做 尚未收

DS(18hour) / AS(9/14 15:30開始 預計三天) : BTX(45 ul); BX(30 ul); TX(0 ul)

DPI(9/13 21:17收)/APII: Box+Top完成(30); Box+Bottom(30)完成;

Box+Top+Bottom(55)

DPI(9/13 21:17收)/AS1(9/14 16:00開始):

DPI(9/13 21:17收)/AS2(9/14 16:00開始):

DPI(9/13 21:17收)/AS3(9/14 16:00開始): BTX(75 ul); BX(20ul); TX(30ul)

(橘色字那六管在紫色rank上有標9/14made)

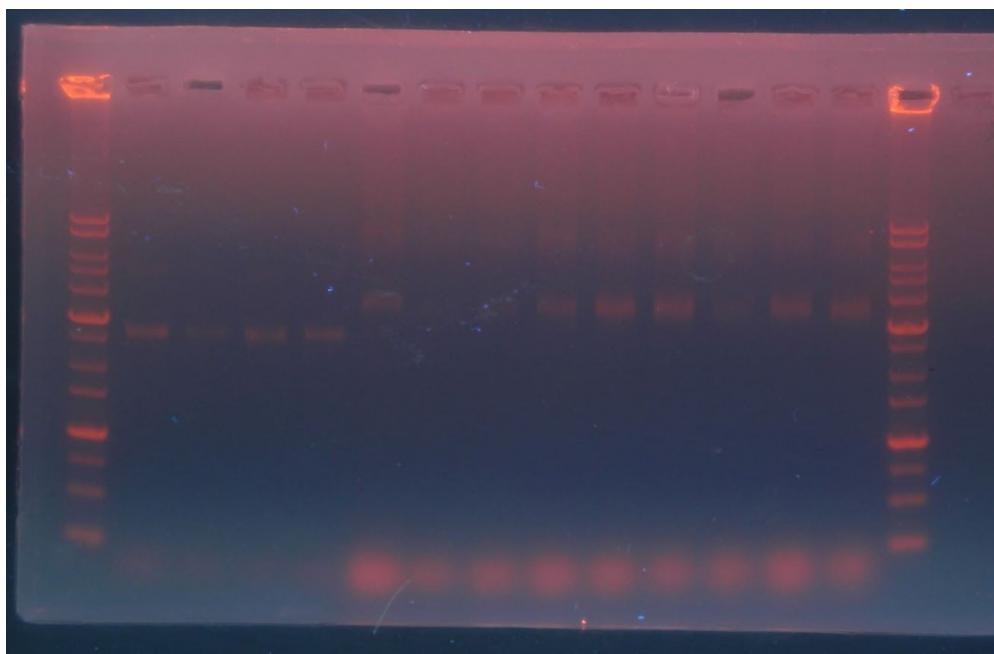
shaker內PS1和SS1的標示上都有塗改痕跡，明天收的人注意一下

9/14晚 鈺璇 泉浩

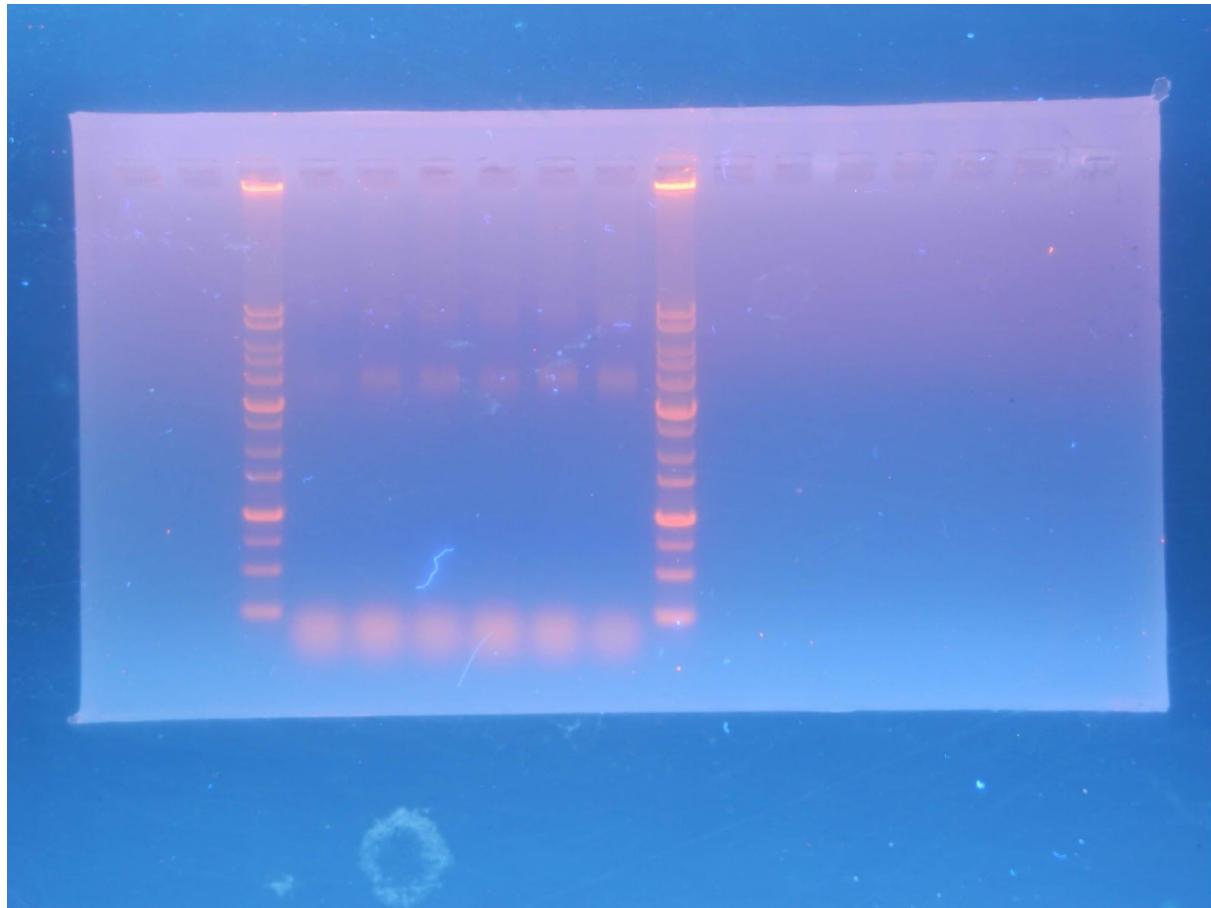
跑膠:

load10mul: DBS/DBP1/DTS/DTP1/DXS-U2/DXS-U3/DXP-U3

load20mul: SPII-BX/SPII-TX/SPII-BTX/P1PII-BX/P1PII-TX/P1PII-BTX



9/15 睿謙 重旬



Ladder/ PSI BX/PSI TX/PSI BTX/SSI BX/SSI TX/SSI BTX/LADDER

綜合兩張測試照片，SS 跟 SP 表現較佳

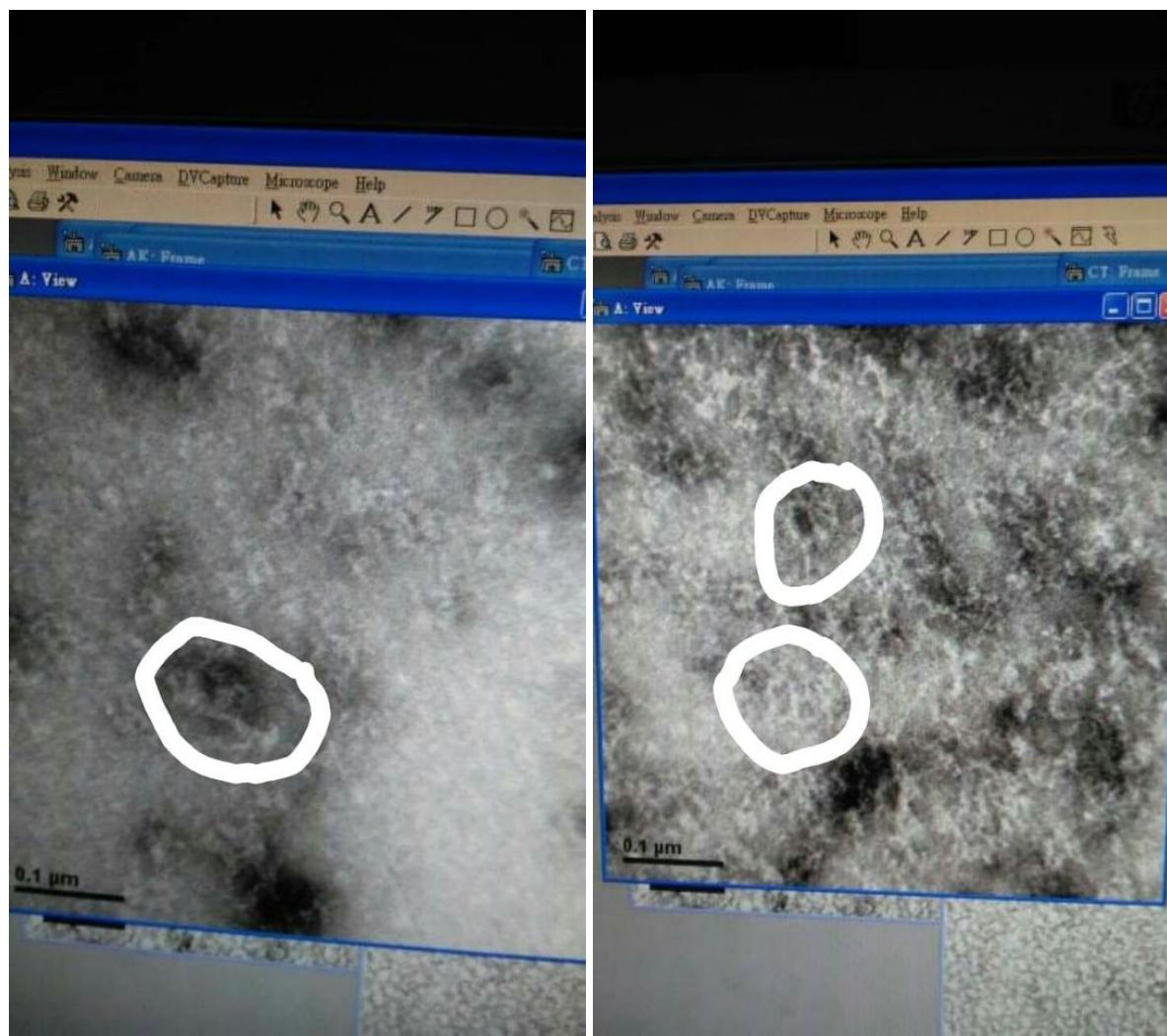
9/16 泉浩 清靈

下午2.多tem 热機中

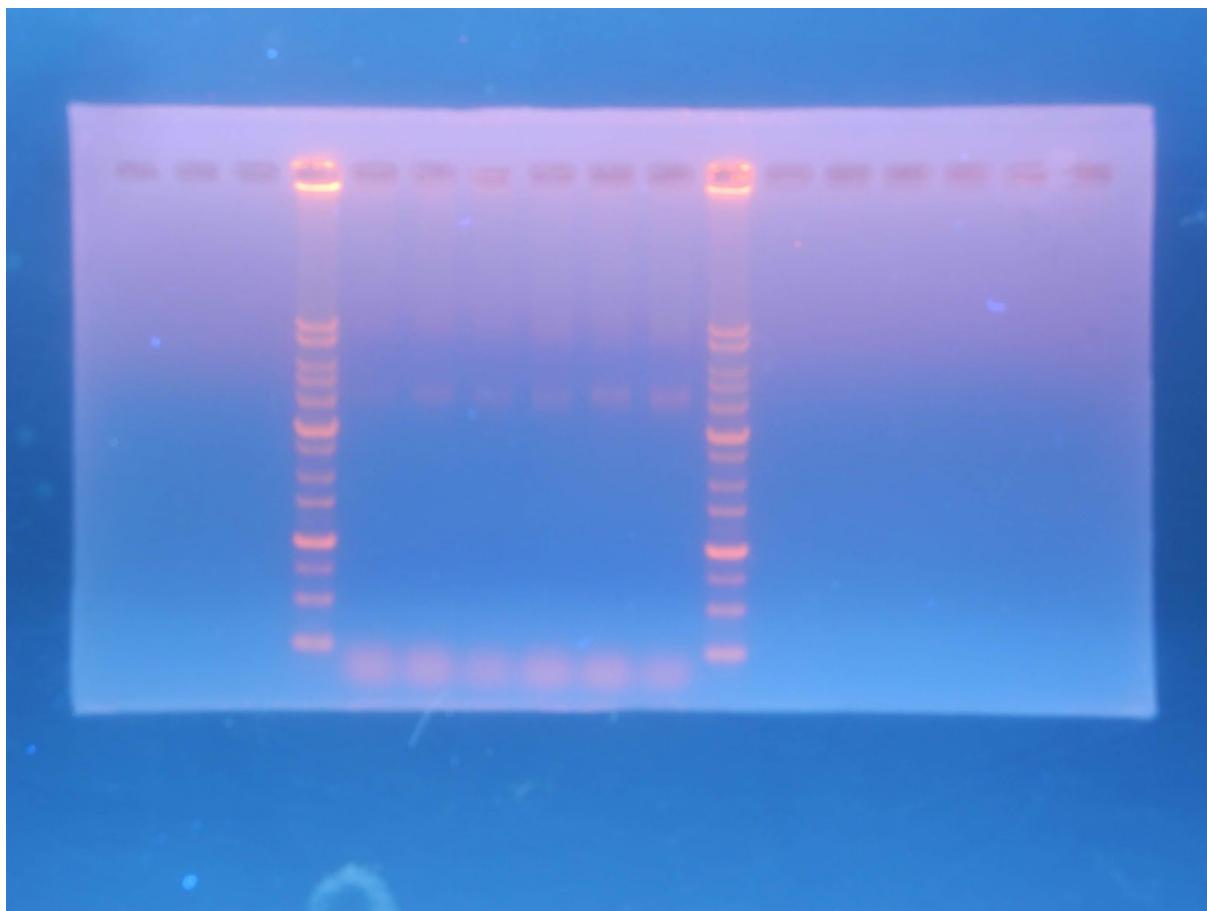
- settle5, 染色條件都是2min. 濃度15mM (box 1:10; lid1:5), 染前震1min.
- 染TX SS1很乾淨，推測應該是因為沒震導致全有全無。或是因為剛剛學姐的樣品有跟我們一起震，可能不小心卡到我們的東西，所以那1min.沒有震開。
 - 因為沒有東西所以我們會再重染，預計在BTX 後看。
- 染BTX

鈺璇 原豪

SS1-BTX 染前震1min



睿謙



LADDER/PS2 BX/PS2 TX/PS2 BTX/ SS2 BX/SS2 TX/SS2 BTX/LADDER

似乎喜歡SS protocol, 之後要用單獨的box、 lid、 scaffold做對照組

9/17 晚上:采 積

scaffold/box/lid +SS3

待補

9/18 采采 清靈 泉浩 睿謙 重甸

- PS1-BTX(沒震)可以繼續看，週一幾乎沒有看到。(采繫先看)
 - 染色、分散狀況很好，並未發現結塊。
 - 只是結構不完整，僅有看見一兩個E。未看見日字型。
- SS1-BTX、PS1-BTX皆震10min。之後染前小震1min.，一樣settle5, stain2。
- SS1-BTX (震10min.)
 - 背景有很多白泡。

9/19 睿謙 鈺璇 清璽

- 晚上 (清璽 鈺璇)
- 用完的離心機最後要將白色塑膠架取出，內部黑色轉子也要取出。故若是第一個要用，需要安裝的人務必要將螺絲鎖緊。
 - lid們留20mul跑膠(小eppendorf上標有20不純)，剩下80mul純1. 純完體積補回80mul.
 - box跟lid們混合，拿去shake 過夜(助教建議)
 - 注意shake要記得開fan才會為設定溫度，否則會是室溫。
 - 後續處理: amicon清洗完浸泡在新amicon酒精 / buffer*2, hinge, blocker, 200nM Mg已冰回大冰箱的muscle 盒。
-

9/20 清璽 重甸

跑膠

sca/x/T/B/X+blocker(XD)/TH不純/BH不純/TH-U1/BH-U1/BTX不純/BTX-U1

- 震overnight BTX 小管60mul 取10震5min., 10min. 做成TEM sample.

9/23 鈺璇 滕璽 原豪 睿謙 泉浩

- BTX純1 震5min. / 震 10min.
- 酮網製作
 - BTX 純1 震 5min dilute 2X
 - 沒東西。
 - BTX 純1 震5min. 沒有稀釋做稀釋效果比較
 - 沒雜質， 沒有破。
 - BTX 純1 震10min. 沒有稀釋做震盪時間比較
- 晚上
 - 純化 T+hinge (因為僅40mul所以純化前先補到100,之後純化完再補回40mul)一次。
 - 因為體積僅有40mul所以將體積補到100,根據以往經驗純化後應該為30幾，預計補回40.但發現竟然體積還有52mul，故目前濃度約為80%.
 - 將TX加在一起，shake overnight.

9/28采葉 鈺璇

XD/TH/BH shake for 1hour(30度)

TH/BH純一次(剩80mul放4度冰箱)

BTX(480mul)/TX(100mul)/BX(100mul)----4:30進shaker(30度)

預:

9/29

BTX,TX,BX先取20, 再純一次後取20(跑膠)

BTX:80mul(4°C)

signal:blocker	BTX	signalT	signalB
10:1	60	12	
	60		12
	60	12	12
7:1	30	4.2	
	30		4.2
	30	4.2	4.2
5:1	30	3	
	30		3
	30	3	3

9/30

1,可先做BTX+signal10倍的樣品、 BX和TX樣品

2,跑膠

(1)組裝:scaffold/x/B/T/XD/BH-U1/TH-U1/BX-U1/TX-U1/BTX-U1

(放在4度冰箱)

(x,B,T留20, 可以兩片膠各load10)

(BH,TH,BX,TX,BTX未純也有留20, 看要不要跑)

(2),翻面:跑不同ratio signal 的膠

scaffold/x/B/T/BTX/BTX-FT(5x,7x,10x)/BTX-FB(5x,7x,10x)/BTX-FBT(5x,7x,10x)

(在shaker, 預計9/30下午4:00取出,20跑膠,其餘冰4度)

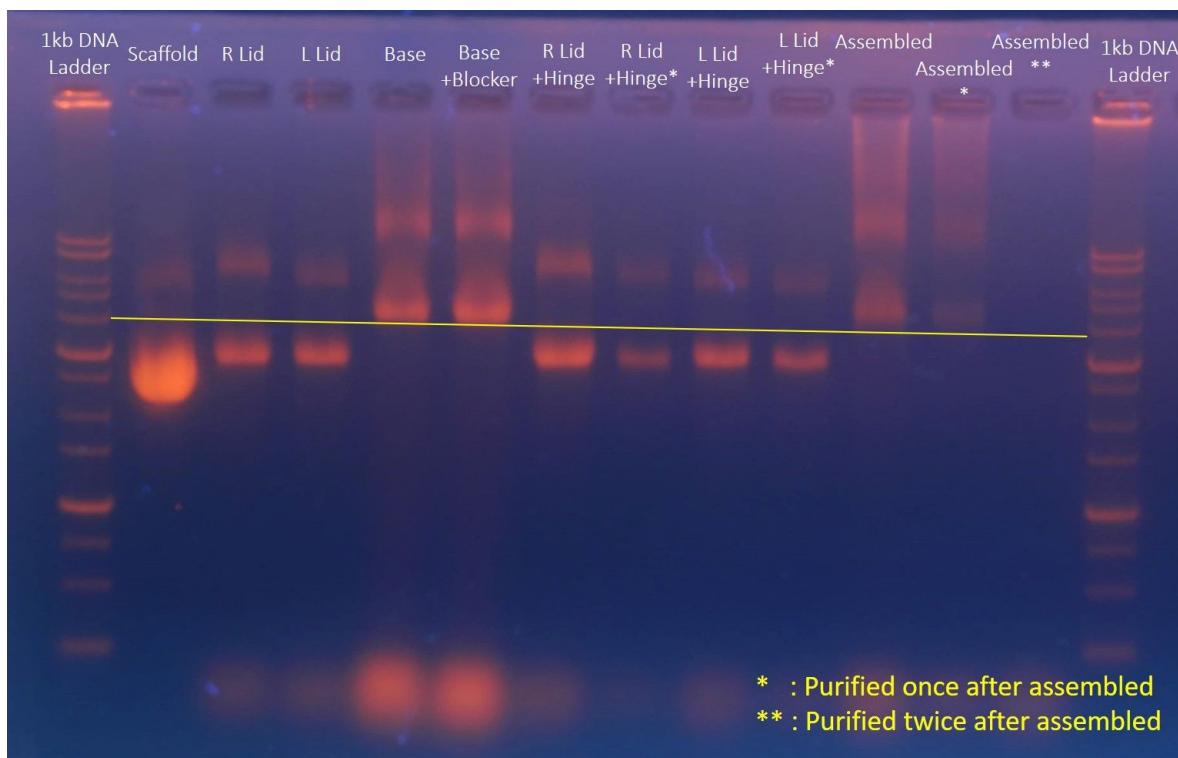
10/1

1. Add blocking staples solution to base solution. The mole of blocking staples is twenty times as the scaffold.
2. Put the mixture of base and blocker in Mini-Shaking-Hybridisation Oven, and shake at 25 °C for one hour.
1. Add hinge staples solution to lid solution. The mole of hinge staples is five times as the scaffold.
2. Put the mixture of lid and hinge in Mini-Shaking-Hybridisation Oven, and shake at 25 °C for one hour.
3. Purify the solution of lid with hinge to remove excess staples.

10/2

1. Mix the blocked base and purified left lid with hinge and purified right lid with hinge
2. Put the mixture in Mini-Shaking-Hybridisation Oven, and shake at 30 °C overnight.
3. Purify the solution of assemblies to remove excess staples.

10/3



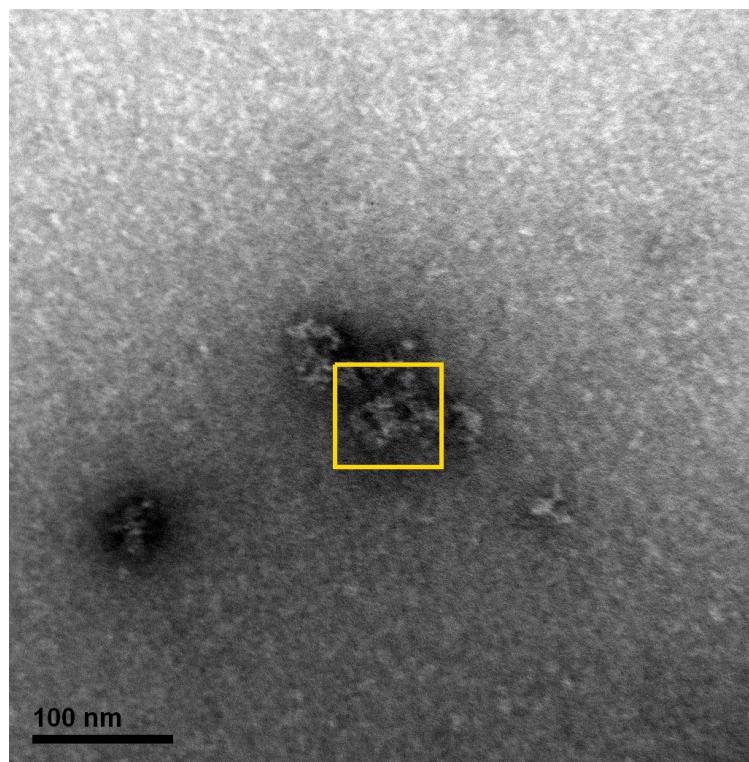
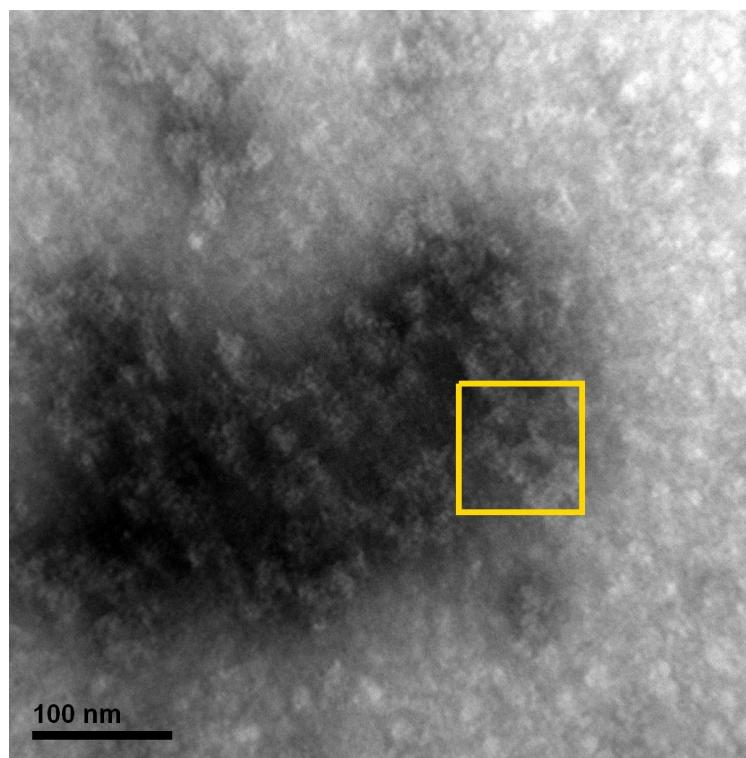
sample assembly and purification

Corresponding to the previous results, both Right and Left Lids exist between 4kb and 5kb, and the base appeared near 5kb. Notably, the components weren't purified in these three lanes. Since blockers and hinges are all short DNA fragments, we expect that no obvious band shifts will happen upon adding them, which matches the experiment results. In Figure 10 we could also observe that for Left and Right Lids, the bands of excess DNA slightly faded more than the band of Left/Right Lids + Hinges after the purifying process, so we decided to purify both Right/Left Lid + Hinge before assembling them with the base. Finally, the products are barely seen in the lane of Assembled**, so we decided to purify the Assembled components once only.

10/4

sonicate for ten minutes

BTX



10/5

1. To flip left lid: Add signal L to the mixture of Base and Both Lid
- To flip right lid: Add signal R to the mixture of Base and Both Lid
- To flip Both lids: Add signal L and signal R to the mixture of Base and Both Lid
(add signal according to the fold below)

signal:blocker	BTX	signalL	signalR
10:1	60	12	
	60		12
	60	12	12
7:1	30	4.2	
	30		4.2
	30	4.2	4.2
5:1	30	3	
	30		3
	30	3	3

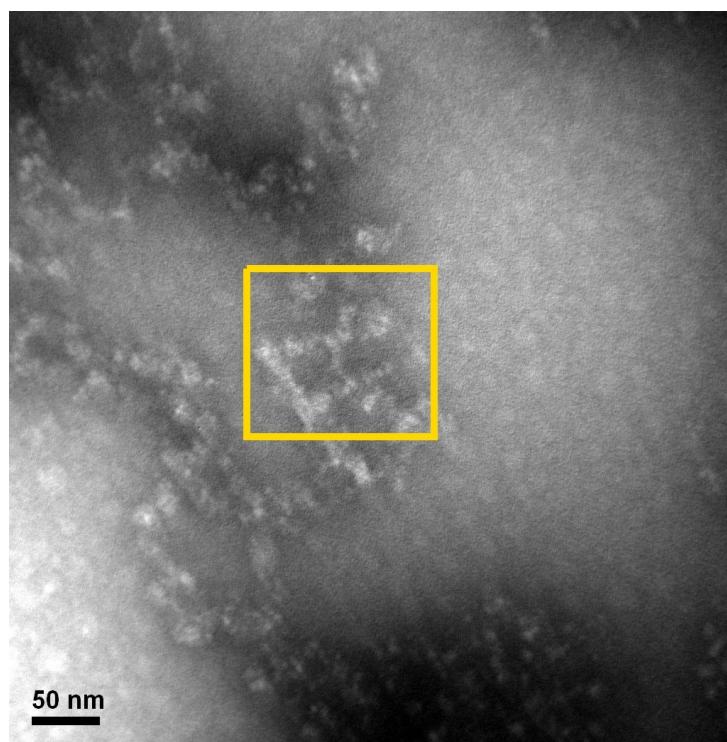
2. Put the mixture in Mini-Shaking-Hybridisation Oven, and shake at 30 °C overnight.

10/8

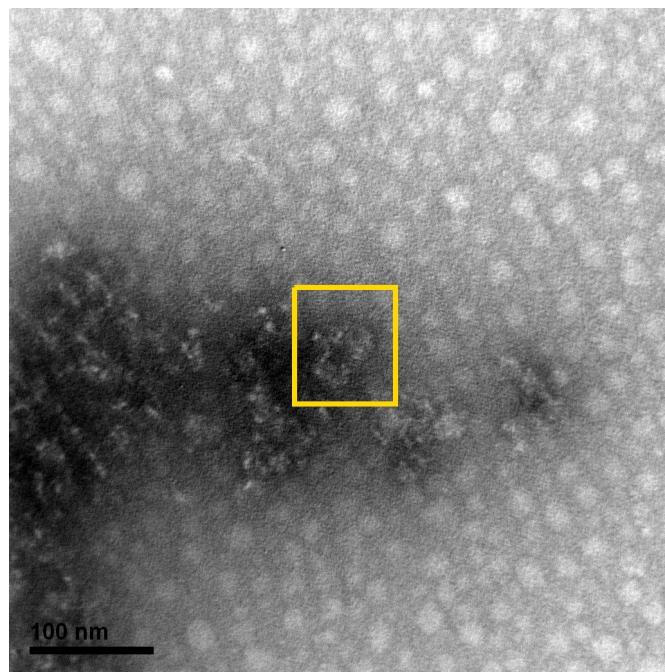
flip the left lid(BTX-FL)

signal:blocker=10:1

sonicate for 10min



the shape is similar with FL, but the size is two time bigger than our structure



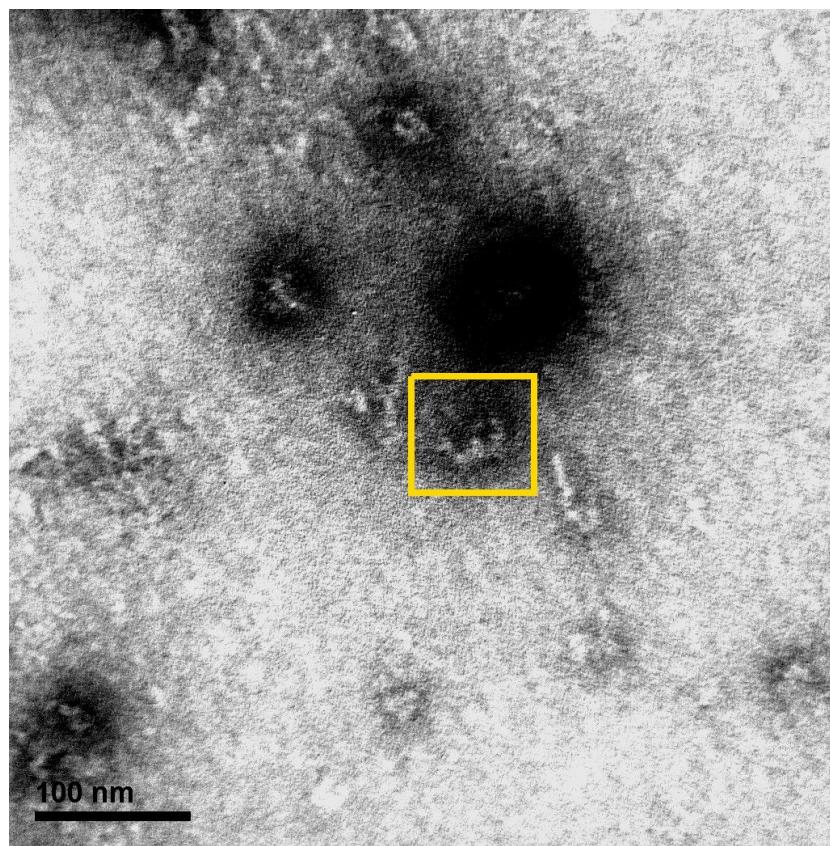
the shape and size is similar to flip the left lid(BTX-FL)

10/9

flip the right lid(BTX-FR)

signal:blocker=10:1

sonicate for 10min



10/14

flip both lids

the lids didn't attach to the base properly

