# Protocol


1. DNA origami ……3

1.1 Amplification of M13 bacteriophage ……3

1.11 Transform ……3

1.12 Get M13 bacteriophage ……3

1.2 Extract scaffold DNA—M13 DNA ……4

1.3 Verify scaffold DNA ……5

1.4 DNA Origami……5

2. Synthesis and modification of gold nanoparticles ……6

2.1 Synthesis of golden seeds ……6

2.2 Measuring the size and zeta potential of golden seed ……7

2.3 Synthesis of 30 nm gold nanoparticle ……7

2.4 Conjugation with short stand ……7

2.5 Purification ……8

2.6 TEM observation ……8

3. Modification of magnetic nanoparticles ……9

3.1 Fluorescence- biotin-avidin system (F-BAS) ……9

3.2 Conjugation with F-BAS ……9

3.3 Purification of magnetic nanoparticle……10

3.4 Measure the hydrate sphere size and zeta potential……10

4. Assemble of nano-robot ……11

4.1 Purification of DNA origami……11

4.2 Setting PCR program……11

4.3 Assemble ……12

4.4 TEM observation ……12

# DNA origami

## 1.1 Amplification of M13bacteriophage

In this stage, we need to transform the M13 bacteriophage RF dsDNA into JM109 Escherichia coli cells to get much M13 bacteriophage. And we finally get plaque by white-blue plaque selection. So at first we need to get X-Gal—IPTG LB agar plate. On each plate, we streak 40ul 20mg/mL X-Gal and 12ul 50mmol IPTG.

## 1.11 Transform

In the following experiment, we suppose that there's 100uL competent cells.

1. First incubate competent cells on ice until it melt.
2. Add target DNA into cell suspension. CAUTION the volume of DNA should less than suspension. Shake the centrifuge tubes slightly to mix the contents. Then incubate on ice for 30 minutes.
3. Put the centrifuge tube in 42℃ water for 60-90 seconds. Then quickly put it on ice for 2-3 minutes. BE CAREFUL not to shake the centrifuge tube.
4. Add 500uL LB medium into the centrifuge tube, then oscillating at 37 ℃/180 rpm for 1 hour to revive the cells and make related gene express.
5. Streak adequate amount transformed cells (about 100uL) on the LB plate, incubate the LB agar plate at 37 °C upside down for 12-16 hours.

## 1.12 Get M13 bacteriophage

**Then we need to get as much bacteriophage as possible:**

1. After overnight incubation, pick a single colony from the LB agar plate and use to inoculate a 50 mL LB culture. Incubate for 8 hours at 37 °C and ~250 rpm

2. Collect bacteria by centrifugation at 6,000 g for 20 minutes at 4°C.

3. Recover the supernatant (bacterial pellet can be discarded) and precipitate the bacteriophage that bears the 7249-base scaffold by adding polyethylene glycol (average MW=8,000) and NaCl to final concentrations of 4% and 0.5 M, respectively.

4. Mix with magnetic stir bar until all PEG8000 has dissolved. **CAUTION** At this point, the supernatant should be a cloudy suspension. If it is still clear, it is likely that there are little or no phage present.

5. Incubate on ice for 30 minutes, then collect the precipitated bacteriophage by centrifugation at 6,000 g for 20 minutes.

6. Re-suspend pelleted bacteriophage in 100 mL of 10 mM Tris (pH~8.5), 1 mM EDTA. This is "pre-inoculation" bacteriophage that will be used in following steps to scale up production of the M13mp18 scaffold strand.

<PAUSE POINT> The pre-inoculation bacteriophage can be stored at -20 °C for at least 12 months

7. To generate the "inoculation" phage, pre-warm an LB agar plate at 37 °C for ~30 minutes.

8. Streak JM109 Escherichia coli cells on the pre-warmed LB agar plate to generate single colonies.

9. Incubate the LB agar plate overnight at 37 °C.

10. After overnight incubation, pick a single colony from the LB agar plate and use to inoculate a 3 mL 2xYT culture. Incubate overnight at 37 °C and ~250 rpm.

11. Use all 3 mL of the overnight culture from the previous step to inoculate a 500ml conical flask containing 150 mL of 2xYT medium supplemented with 5 mM $MgCl_2$.

12. Shake at 280 rpm and 37 °C until OD650nm = 0.5. That need about 2~2.5 hours.

13. Add 50 mL of the pre-inoculation phage stock. Continue shaking at 37 °C for 4 hours at 280 rpm.

14. After above steps, we can extract scaffold DNA from bacterial suspension.

## 1.2 Extract scaffold DNA—M13 DNA

In this stage, we use ***E.Z.N.A.® M13 DNA Mini Kit*** to extract M13 DNA. Here is the experiment procedure (You can seek from the web http://omegabiotek.com/store/product/e-z-n-a-m13-dna-mini-kit/)

**Before Starting:**

1. Prepare SPW Wash Buffer according to the instructions on Page 4.
2. Heat the Elution Buffer to 65°C

## Procedure:

1. Prepare a 4 mL culture of infected M13.
2. Incubate at 37°C for 6-7 hours with vigorous shaking.
3. Centrifuge at 5,000 rpm for 15 minutes at room temperature.
4. Transfer 1.5 mL of the supernatant obtained containing the M13 bacteriophage, into a fresh reaction tube.

**Note:** Be careful not to disturb the bacterial pellet during the transfer. If the supernatant is not clear, repeat the centrifugation step.

5. Add 300 µL MPG Buffer to the M13 supernatant and mix by vortexing.
6. Let sit at room temperature for 10-15 minutes.
7. Add 700 µL sample to a HiBind® M13 DNA Mini Column inserted into a 2 mL Collection Tube.
8. Centrifuge at 10,000 rpm for 30 seconds. Discard the filtrate and reuse the collection tube.
9. Repeat Steps 7 and 8 until all of the sample has been passed through the HiBind® M13 DNA Mini Column.

10. Add 700 µL MPX Buffer.
11. Centrifuge for 30 seconds at 10,000 rpm.
12. Discard the filtrate and reuse the collection tube.
13. Add 700µL MPX Buffer.
14. Let sit for 1 minute at room temperature.
15. Centrifuge at 10,000 rpm for 30 seconds.
16. Discard the filtrate and reuse the collection tube.
17. Add 700 µL SPW Wash Buffer.

    **Note:** SPW Wash Buffer must be diluted with ethanol before use. Please see Page 4 for instructions.

18. Centrifuge at 10,000 rpm for 30 seconds.
19. Discard the filtrate and reuse the Collection Tube.
20. Repeat Steps 17-19 for a second SPW Wash Buffer wash step.
21. Centrifuge the empty column at maximum speed for 1 minute.
22. Insert the HiBind® M13 DNA Mini Column into a clean 1.5 mL microcentrifuge tube (not provided).
23. Add 30-50 µL Elution Buffer heated to 65°C.

**Note:** Make sure to add Elution Buffer directly onto the HiBind® M13 DNA Mini Column matrix.

24. Let sit at room temperature for 1 minutes.
25. Centrifuge at maximum speed for 1 minute.

Store eluted DNA at -20°C.

After we get M13 DNA, we measure the concentration by nucleic acid analyzer. We find that the concentration is not high enough. So we try to concentrate the bacteriophage suspension first. Using the same way in the first stage, we extract bacteriophage from bacterial suspension first. Then we use 1/10 volume of the suspension. And then we can get high concentration M13 DNA solution. So we finally made the concentration improve 10 times.

## 1.3 Verify scaffold DNA

In this stage, we need to verify that the DNA we extract from bacteriophage is what we need-M13 DNA. There're several ways to test: 1. Sequencing the DNA; 2.Transform the E. coli by single strand DNA; 3.PCR identification……In our experiment we choose the second way. Using the same way in the first stage to do a transforming, we finally get a blue spot which confirms that we have got scaffold DNA.

## 1.4 DNA Origami

Finally we've got enough scaffold DNA, we can start our DNA origami reaction eventually. After a series of gradient experiment, we optimize the reaction condition: For a 50uL reaction system, the final concentration of every reagent:

**scaffold(M13mp18)** **10nmol/L**

**staple(284)** **100nmol/L**

**$Mg^{2+}$** **8 mmol/L**

**1*Buffer (5mmol/L Tris , 1mmol/L EDTA(pH7.9 at 20℃)**

**Add dd$H_2O$ to 50uL**

PCR procedure:

Lid Control Mode: reheating at 98°C

Incubate at 95.0°C for 5 minutes

Decrease from 95°C to 65°C by 1.0°C/30s every cycle

Decrease from 65°C to 40°C by 1.0°C/5min every cycle

Decrease from 40°C to 25°C by 1.0°C/1min every cycle

stored at -20°C

## 2. Synthesis and modification of gold nanoparticle

## 2.1 Synthesis of golden seeds

1. Soak all the glassware and magneton with 50% aqua regia more than 5 minutes.
2. Recycle the aqua regia, suspended by dd$H_2O$, ultrasonic concussion.
   Wash the glassware more than twice.
3. Add 2ml 50mM chloroauric acid and 98 ml dd$H_2O$ into the flask, away from light.
4. Put the flask on the heating magnetic stirrer until the solution is boiling. See Fig 2.1
5. Add 1ml 1.75% sodium citrate, and heat for another 15 minutes.
6. Stop heating, and let it cool down slowly.

**Fig 2.1 Synthesis of golden seed**

## 2.2 Measuring the size and zeta potential of golden seed

1. Turn on the laser sphere analyzer, preheat more than 10minutes.
2. Wash the cuvettes more than three times with $ddH_2O$.
3. Mix 0.5ml solution got from the step 1.1 with 1.5 ml $ddH_2O$, incubate for a minute.
4. Add the mixture into cuvette, then place the cuvette in the groove correctly.
5. Start the program, set its parameter, measure the hydrate sphere size and zeta potential.

## 2.3 Synthesis of 30 nm gold nanoparticle

1. Add 4 ml golden seed and 320μL chloroauric acid into a flask.
2. Ultrasonic concussion meanwhile adding 960μL MSA.
3. Mix the solution above with 95 ml $ddH_2O$, place the flask in room temperature away from light for a night.
4. Measure the hydrate sphere size and zeta potential as the step 1.2.

## 2.4 Conjugation with short stand

1. Clean the glassware and magneton as the step 2.1 mentioned.
2. Mix 2ml 30nm collaurum with 4.2nmol sulfhydryl short ssDNA （*MOD14-SH* ） in a 5ml bottle.
3. Put the flask on the heating magnetic stirrer for 2 days. See Fig 2.2. Keep the magneton

rotating without heating.

4. Measure the hydrate sphere size and zeta potential as the previous step.
5. Store the modified collaurum at 4℃

**Fig 2.2. Link with sulfhydryl DNA**

## 2.5 Purification

1. Soak the 100KD Amicon® Ultra-4 Centrifugal Filter Units with 20% ethanol for 2 hours.
2. Add 1ml 30nm modified colloidal golden into the tube, mix with 3 ml $ddH_2O$.
3. Centrifuge in a fixed angle rotor at 1250g at room temperature for 5 minutes.
4. Discard filtrate, add $ddH_2O$ to the solution until the volume is 4ml.
5. Repeat the above steps 4-5 times.
6. Store the purified collaurum at 4 ℃.

## 2.6 TEM observation

1. Clean a petri dish and dry it.
2. Put grid on the petri dish, keep carbon membrane side up.
3. Pipette 2μL of collaurum sample and put it on grid surface.
4. Put 1-5 g calcium chloride beside the grids.
5. Cover the lip until the sample dried. See Fig 2.3

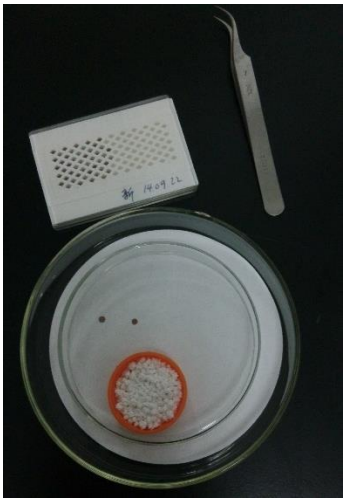

**Fig 2.3 Sample preparation**

## 3. Modification of magnetic nanoparticle

### 3.1 Fluorescence- biotin-avidin system (F-BAS)

1. Dissolve biotinylated short ssDNA with dd$H_2O$.
2. Dissolve 5mg streptavidin with 1 ml phosphate buffer(PH=7.2)
3. Pipette 56μL streptavidin solution into the 2ml centrifuge tube
4. Mix 14.4nmol biotinylated short ssDNA（each of four DNA R1 ,R2, L1, L2 are 3.6nmol）with4.8 nmol fluorescently-labeled biotin.
5. Add all the biotin and ssDNA into the centrifuge tube, blend.
6. Place the centrifuge tube in the Orbital Shaker Incubator, incubate at room temperature for 30min.
7. Store the at 4 ℃.

### 3.2 Conjugation with F-BAS

1. Ultrasonic concussion HOOC-PEG-COOH coated nano-magnetic sphere solution before using.
2. Dissolve 1.5mg N - butyl hydroxy diimide(NHS) and 1mg 1-ethyl-3-3(3-imethylaminopropyl) carbodiimide (EDC ) with 1ml phosphate buffer(PH=7.2).
3. Pipette 100μL magnetic sphere solution into a 2ml centrifuge tube, mix with solution above.
4. Place tube in the Orbital Shaker Incubator, incubate for 30 minutes, and activate

carboxyl on the surface.

5. Add conjugated streptavidin produced in the step 3.1 into the tube.
6. Incubate at room temperature for more than 4 hours.
7. Storage at 4 ℃. Don't freeze!

## 3.3 Purification of magnetic nanoparticle

1. Soak the 100KD Amicon® Ultra-4 Centrifugal Filter Units with 20% ethanol for 2 hours.
2. Add 0.5ml magnetic sphere solution into the tube, mix with another 1 ml $ddH_2O$.
3. Centrifuge in a fixed angle rotor at 850g at room temperature for 4 minutes.
4. Blow slowly with a pipette if there is sediment.
5. Gather all MP solution in a centrifuge tube, ultrasonic concussion for 1 minute.
6. Add the MP solution into the tube again.
7. Add $ddH_2O$ until the bulk volume is 1.5ml.
8. Repeat the above steps for 5-6 times.

Store the purified magnetic sphere at 4 ℃. See Fig 3.1

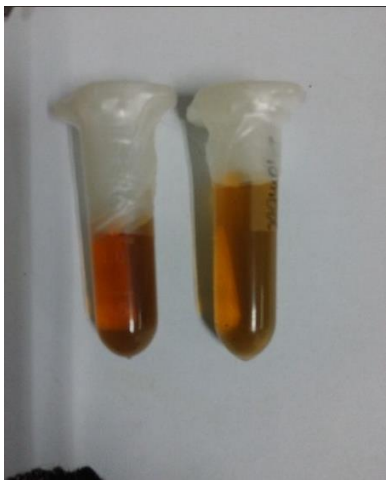

**Fig 3.1 Magnetic sphere**

## 3.4 Measure the hydrate sphere size and zeta potential

This step is the same as the step 2.2.

# 4. Assemble of Nano-robot

## 4.1 Purification of DNA origami

1. Prepare a 100KD Amicon® Ultra-4 Centrifugal Filter Units. See Fig 2.5
2. Add 0.25ml DNA origami into the tube, mix with another 1.25 ml $ddH_2O$.
3. Blow it slowly with pipette.
4. Centrifuge in a fixed angle rotor at 1250g at room temperature for 5 minutes.
5. Discard filtrate, Add $ddH_2O$ until the solution volume is 1.5ml.
6. Repeat for another 5-6 times $ddH_2O$
7. Store at -20℃.


**Fig 4.1 Prepared Centrifugal Filter Units**

## 4.2 Setting PCR program

Program as shown blew:

| step | temperature /℃ | time/min | step | temperature /℃ | time/min |
|---|---|---|---|---|---|
| **1** | 37 | 5 | 14 | 30.5 | 5 |
| **2** | 36.5 | 5 | 15 | 30 | 5 |
| **3** | 36 | 5 | 16 | 29.5 | 5 |
| **4** | 35.5 | 5 | 17 | 29 | 5 |
| **5** | 35 | 5 | 18 | 28.5 | 5 |
| **6** | 34.5 | 5 | 19 | 28 | 5 |
| **7** | 34 | 5 | 20 | 27.5 | 5 |
| **8** | 33.5 | 5 | 21 | 27 | 5 |
| **9** | 33 | 5 | 22 | 26.5 | 5 |
| **10** | 32.5 | 5 | 23 | 26 | 5 |
| **11** | 32 | 5 | 24 | 25.5 | 5 |
| **12** | 31.5 | 5 | 25 | 25 | 5 |
| **13** | 31 | 5 | **Three Cycles** | | |

## 4.3 Assemble

| | DNA origami | magnetic particle | modified collaurum | 10 X origami buffer | 100mM Mg2+ |
|---|---|---|---|---|---|
| volume / μ L | 30 | 100 | 30 | 19.5 | 15.6 |

1. Add all the composition as the table above into a PCR tube.
2. Ultrasonic concussion before incubating.
3. Incubate in the PCR instruments.
4. Store product at 4℃.

## 4.4 TEM observation

1. Pipette 2μL of modified Nano-robot sample on copper grid surface and wait for 5minutes.
2. Blot excess liquid with filter paper
3. For each sample, place 2 droplets of uranyl acetate (7μL each) on dishes.
4. Briefly submerge grid into the first droplet and blot excess liquid immediately with filter paper immediately.
5. Submerge grid into the second droplet for 15s and blot excess liquid with filter paper
6. Put 1-5 g calcium chloride beside the grids.
7. Cover the lip, protecting from dust in the air.
8. Wait for at least 30 minutes until copper grid is totally dry