Buffers

TAE buffer series

40 mM trisaminomethane (Tris), 20 mM acetic acid, 1 mM ethylenedia-minetetraacetic acid (EDTA) disodium salt (pH ~8). We typically add 12.5 mM magnesium acetate in the solution for application related to DNA (termed TAEM buffer).

1. 1 L 50× TA buffer (we have > 0.9 L left in the lab in April 2022). Weight 242 g of Tris and 57.1 mL of acetic acid and dissolve in 0.7 L of DI water. Add water to final volume of 1 L
2. 50 mL 10× TAE buffer. Weight 0.186 g EDTA disodium salt and add to 10 mL 50× TA buffer. Add DI water to final volume of 50 mL. Notes:
    a. EDTA dissolves really slow. When preparing, planning ahead is very important
    b. Sometimes we need 2× of regular EDTA concentration in the final regular buffer. In such a case, weight 0.372 g EDTA disodium salt and the rest are the same
    c. Normally, the concentration is marked as '10× TAE buffer. For 1×, 40 20 1 or 40 20 2'. The numbers are concentration of 3 components in mM
    d. When we need '10× TAE buffer. For 1×, 40 20 1' with '10× TAE buffer. For 1×, 40 20 2' and '10× TAE buffer. For 1×, 40 20 0' available, we can mix these 2 under 1:1 volume ratio and create '10× TAE buffer. For 1×, 40 20 1'
3. 10 mL 1× TAEM buffer. Add 5 – 8 mL DI water first, then add 1 mL 10× TAE buffer and 125 µL of 1 M magnesium acetate. Add water to final volume of 10 mL. Notes:
    a. Sometimes we need Mg^{2+} concentration to be 6 mM instead of 12.5 mM. Therefore, 60 µL of 1 M magnesium acetate should be added
    b. Similar to Note c in step 2, we can mix TAEM buffer with 6 and 12.5 mM Mg^{2+} to create TAEM buffer with Mg^{2+} between 6 and 12.5 mM with a suitable volume ratio
4. 7.5 mL 4/3× TAEM buffer. Add 5 – 6 mL DI water first, then add 1 mL 10× TAE buffer and 125 µL of 1 M magnesium acetate. Add water to final volume of 7.5 mL. The same notes in step 3 apply here
5. 1.2 mL fixing buffer (TAEM buffer with 2 mM nickel chloride). Add 900 µL of 4/3× TAEM buffer and 96 µL of 25 mM nickel chloride solution (we have quite some in stock)
    a. We can start with different concentrated TAEM buffer and nickel chloride solution. Just make sure the final concentration is desired
    b. We may have different combination of EDTA, Mg^{2+} and Ni^{2+} concentration. The most important aspect is the correct and clear marking. Without it, the buffers are useless

MES buffer

50 mM 2-(N-morpholino)ethanesulfonic acid (MES), 5 mM magnesium chloride, and 200 mM sodium chloride (pH ~6.5)

1. 50 mL 0.5 M MES sodium salt solution. Weight 5.43 g MES sodium salt and dissolve in 40 mL of DI water. Add water to final volume of 50 mL
2. 10 mL 2 M sodium chloride solution. Weight 1.169 g sodium chloride and dissolve in 7 mL of DI water. Add water to final volume of 10 mL

3. 50 mL 2 M magnesium chloride solution. Weight 20.33 g magnesium chloride hexahydrate. Add DI water to final volume of 50 mL. Notes:
    a. Magnesium chloride hexahydrate can get really messy due to the water in the salt. Weighting has to be quick otherwise the crystals will dry
    b. If magnesium chloride is not available, magnesium acetate can be used in place of it. Don't dilute 1 M magnesium acetate unless in the buffer. For example, don't make 50 mM magnesium acetate in DI water alone. Rather, make 10× MES buffer where there are 50 mM magnesium acetate
4. 50 mL 1× MES buffer. Add 35 mL DI water first, then add 5 mL of 0.5 M MES sodium salt, 125 µL of 2 M magnesium chloride, and 5 mL of 2 M sodium chloride. Add water to final volume of 50 mL
5. Sometimes we need 1× MES buffer with nickel chloride. Similar to step 5 in making TAEM buffer, adding nickel chloride to concentrated MES buffer followed by dilution will be all we need. Typical final Ni^{2+} concentration is 2 – 3 mM

TBE buffer series

89 mM trisaminomethane (Tris), 89 mM boric acid, 2 mM ethylenedia-minetetraacetic acid (EDTA) disodium salt (pH ~8.3). Normally purchased directly from Thermo Fisher in 10× concentration. There is a problem with precipitation due to the high concentration. Currently we just use the 10× buffer with precipitations at the bottom and pretend the precipitations don't exist. Dr Chengde Mao's student, Dake Mao, suggested dilution into 5× concentration. We typically use 0.5× TBE buffer with 11 mM magnesium chloride for application related to agarose gel of DNA.

1. 0.5 L 0.5× TBE with 11 mM magnesium. Add 0.4 L DI water first, then add 25 mL 10× TBE buffer and 2.75 mL of 2 M magnesium chloride. Add water to final volume of 0.5 L.

DNA origami

Scaffold DNA

1. M13mp18 (7249-nt) is supplied by Bayou Biolabs (1.0 µg/µL, total 500 µg). When covering the concentration into molar, the molar mass for ss- version needs to be used. After the conversion, it should be 442 nM to be exact or 450 nM for a bulk part number
2. M13 variant (P8064, 8064-nt) is supplied by tilibit nanosystems (100 nM, 0.5 mL)

DNA oligomers

All short strands (including staples, linkers, and releasers) are obtained from Integrated DNA Technologies, and are stored at −20 °C.

If they are in DNA plate form, the concentration is 70 – 75 µM. The actual value depends on the options available at IDT. Our strands before 2020 are mostly 75 µM if in DNA plates. Before 2017, the total volume is large, which can be more than needed (> 50 µL). Then the volume went down and so did the concentration. Therefore, we have to plan carefully, or the strands can run out. Starting from 2021, the price went up and the ordering got harder. We may move away from IDT.

Storing at −20 °C is very important, which keeps the strands from degradation. Even at low temperature, the ice can sublimation and thus the total amount in each well may seems less than before. Before using the DNA plates, thawing and centrifuging are required. Before making sure all the liquid are at the bottom of the wells, the rubber sealings shouldn't be open. Otherwise, the DNA strands will contaminate each other.

During using, all the strands should be transfer into a container. Normally, the short strands are subject to be mixed so putting each strand in a separate container is not needed. The container can first be a PCR tube strip and then collect all the liquid into on 0.2 mL PCR tube or a 1.5 mL tube depending on the quantity. The 0.2 mL tube has better sealing against drying.

If they are in DNA tube form, the concentration is by our dilution. The tube arrives in dried DNA form. It's recommended to add DI water for 100 μM concentration. If they are used with the DNA planes, 70 – 75 μM might be a better idea so that the mixture can have the same volume ratio for the same final concentration. Sometime due to volume limitation, it's not feasible. The operator has to make a decision. Regardless, the final concentration should be marked on the tube for future reference.

DNA Origami Assembly

The related staples, linkers, and releasers should be pre-mixed together, respectively. Generally speaking, each origami has 150 staples. The final concentration of each staple should be 500 nM. Scaffold concentration is 5 or 10 nM. The final volume is 55 μL. Molar ratio of scaffold and staple is 1:4. Thus, staple to be added is 2-5 μL. After the annealing or incubation, store the mixture in the refrigerator at 4 °C for at least 5 min before imaging. Here are some typical origami structures

1. Rectangular tile. Add 41.25 μL 4/3× TAEM buffer in 0.2 mL PCR tube. (Alternatively, 27.5 μL 2× TAEM buffer and then 13.75 DI water). The add 1.22 μL M13mp18, 5 μL staple mixture. Finally, add water to final volume of 55 μL. If the difference before and after adding water is very small, skipping the final step is fine. The scaffold concentration is 10 nM and the scaffold: staple is ~1:4. Thermally anneal the mixture on a thermal cycler from 75 to 4 °C at −1 °C/min
2. Ribbons: Mix 10 nM rectangular tile with 10× linkers (~2 μL) and incubate the mixture at 37 °C for 30 min. If the yield is too low, rise the temperature to 40 °C and extend the time to 1 h. Sometimes adding more buffer can work. If nothing can be seen in the AFM, it could be dilution problem during deposition. Try a higher deposition concentration may help. If the polymerisation is too much, ribbons can hardly be seen. Therefore, lowering linker concentration, incubation temperature and time would be good
3. Special ribbon with controlled length. There are rectangular tile type A and B. The definition in Haorong's ACS Nano 2016 paper is different from the labels on the DNA plates and tubes if purchased before 2016. Double check the sequence before using can be the best practice. The ribbon with type A and B can have the length controlled, which can go up to 8 units. Please refer to Figure S1 of the paper and understand the overall scheme. Important points

a. As long as the steps are followed, the final result will be ABBABAAB
b. Each time of adding the linkers and incubation, for example, LAB, there is a following step of adding the respective quenchers and incubation. The is the key of controlling the size
c. After each time of the linker-quencher cycle, either purification or adding more linkers in the next round is needed. For example, the 1st round takes 1 μL of linker and 1.5 μL of quencher. Without purification, the 2nd round should take 2 μL of linker and 3 μL of quencher. This can add up really fast if any interference between the cycles is detected. Sometimes considering purification is easier. There are protentional issues with it, too. When purify, centrifuge is normally used. If the ribbon is too long or the spinning speed is too high, the ribbon can be easily damaged. The threshold for 'too long' or 'too high' is case sensitive and might need more experiments to figure out

4. Fight ring or similar wireframe structures. Add 41.25 μL 4/3× TAEM (6 mM Mg^{2+}) buffer in 0.2 mL PCR tube (or 2× TAEM buffer with water). The add 0.61 μL M13mp18 (final concentration 5 nM). The volume of staples depends on the staple concentration in the staple mixture. Targeting for 1:4 molar ratio is the general way. The volume should be ~ 3 μL. Then, add water to final volume of 55 μL. There are two possible annealing method.
    a. '9545' in the thermal cycler. Developed by Haorong Chen. First, the mixture was annealed from 95 °C to 65 °C at -1 °C per 2 min; then from 65 °C to 60 °C at -1 °C per 25 min; after that, from 60 °C to 50 °C at -1 °C per 60 min; later on, from 50 °C to 45 °C at 1 °C per 25 min; finally, from 45 °C to 25 °C at 1 °C per 2 min.
    b. 'HaoYan' in the thermal cycler. Acquired from Hao Yan's work in 2015 Nature Nanotech. 90 °C to 85 °C at -4 °C per 5 min; then from 85 °C to 70 °C at -1 °C per 5 min; after that, from 70 °C to 40 °C at -1 °C per 15 min; finally, from 40 °C to 25 °C at -1 °C per 10 min.
    c. The methods are subjected to be changed. To make the structures better, some temperature ranges should be slower whereas others should be faster. It is not always the slower the better.

DNA Origami Reconfiguration

Different origami structures are capable of different reconfigurations. Origami tile can be cyclised along the helical direction or perpendicular to that direction. They can also be released back to flat state. The wireframe origami can have different states. By adjusting the jack edge length, different states can be switched from each other

1. Cylinder from rectangular tile (normally perpendicular to the helical direction). This can be assembled from rectangular tile (2-step assembly) or from stretch (1-pot).
    a. 2-step: Mix 10× linkers with 10 nM DNA origami tiles (~2 μL linkers for 55 μL origami). Then incubate the mixture at 40 °C for 1 h. For the best result, dilute DNA origami tiles to 1.3 nM with TAEM buffer and then mix with 154× linkers. The total volume is approximately 30 μL. After that, incubate the mixture at 50 °C for 2 h

    b. 1-pot: Similar as the preparation for rectangular tile. Beside adding the scaffold and staples, also add linkers as well. The volume of linkers mixture is the same as in step a. 1-pot gives better result than 2-step
2. Rectangular tile from cylinder. Add 20× releasers with elongated tubes assembled from origami cylinders (~3 μL linkers for 55 μL origami). Then incubate the mixture at 40 °C for 6 h
3. Wireframe structures method 1.
    a. Toehold-mediated strand displacement. Mix the origami with 30× releasers. Incubate at 44 °C for 12 h
    b. Purification. Remove all the chemical waste (including free short ssDNA and dsDNA strands by using the centrifugal filter (100 kDa) from Amicon (we might need a different provider). The process should be performed 3 times. In each time, mix ~60 μL DNA solution with TAEM buffer (make sure the Mg^{2+} concentration is the same) to reach 500 μL and put on a centrifugation rapidly at 5000 RPM for 3 min. Discard the solution which passes through the filter and collect the solution left in the filter (~60 μL) for the next filtration
    c. Reannealing. Mix 20× new jack staples with the purified structures and incubate at 40 °C for 18 h. Then, reduce the temperature from 40 °C to 20 °C at a rate of -1 °C per 1 min (for a total of 20 min for cooling)
    d. This method is slower, but the results are normally better than method 2
4. Wireframe structures method 2.
    a. Toehold-mediated strand displacement. Mix the origami with 10× releasers. Incubate at 55 or 60 °C for 1 h
    b. Purification is same as in method 1
    c. Reannealing. Mix 20× new jack staples with the purified structures and incubate at 45 °C for 1 h. Then, reduce the temperature from 45 °C to 20 °C at a rate of -1 °C per 1 min (for a total of 25 min for cooling)
    d. This method is faster, but the results is not as controllable as method 1. This is more for a quick and dirty try-out

DNA Intercalation

EtBr: Dilute the DNA origami ribbons to 2 nM with MES buffer and mix with concentrated EtBr solution to reach different final concentrations from 0 to 3.5 μM of EtBr. The final volume is approximately 10 μL. Incubate the mixtures at room temperature for 5 min

There are other intercalators, which may have different properties from EtBr. EtBr is the widest used intercalator, nonetheless