Manual PNA Fmoc-synthesis

Synthesis Preparation

1. Prepare the following solutions:

   (A) 0.202 M HATU in NMP: Weigh out 0.768 g HATU and dissolve in NMP to give 10 mL.

   (B) 20% Piperidine in DMF: Mix 20 mL Piperidine and 80 mL DMF to give 100 mL total volume.

   (C) 5% DIEA in DCM: Mix 5 mL DIEA with 95 mL DCM. This reagent is also used for PNA synthesis so some of it may already be available.

   (D) 5% Ac_2O (acetic anyhydride)/6% 2,6-Lutidine in DMF: Mix 5 mL Ac_2O, 6 mL Lutidine and 89 mL DMF.

Swelling the resin. Soaking the resin in DCM causes the polymer to swell, increasing the accessibility of reagents to the functional groups on the support.

2. Weigh out 100 mg of resin and place in a reactor. We typically use PAL-PEG-PS, 0.21 mmol/g loading.

3. Add 10 mL DCM and agitate for 2 hours to overnight.

4. Drain the solvent, then wash the resin three to five times with DCM. Add enough DCM to disperse the resin, and mix the resin with a pipet prior to draining. After the last washing, open the reactor to vacuum only long enough to drain the solvent. (If the resin is completely dried, it will no longer be swelled).

Solutions for PNA synthesis

1. **Monomer Solutions**. Monomers are dissolved in NMP at a concentration of 0.215M.

A (725 g/mol)	G (741.8 g/mol)
T (506 g/mol)	C (701 g/mol)

- *Prepare the solutions in microcentrifuge tubes or small glass vials. Calculate how much of each monomer you will need based on the PNA sequence and weigh out only what you will need. Heating and vigorous mixing will be necessary to get the solids to completely dissolve.*

2. **20 % piperidine in DMF**. This solution is used to remove Fmoc-protecting groups from the PNA N-terminus. Store in an amber bottle in the hood.

3. **0.8 M base solution.** This solution provides the base that is necessary for activation on the PNA monomer with HATU prior to coupling.

- *Use Base Solution purchased from Applied Biosystems.*

4. **0.2M HATU in DMF**. This solution is used to generate the activated PNA monomer by esterifying the carboxyl group of the monomer with a hydroxybenzotriazolyl group. Displacement of this group by the N-terminus of the PNA is facile.

- *Use HATU Solution purchased from Applied Biosystems*

5. **Ac_2O/Lutidine/DMP (5:6:89).** This solution is used to cap any unreacted amino groups as acetamides, preventing elongation of failure sequences.

Coupling the PNA monomer to the resin. The carboxylic acid group of the protected monomer must be activated prior to coupling to the resin. The monomer is mixed with equal parts PNA activator and base solution (Applied Biosystems) for at least 2 minutes to fully activate the carboxylic acid and then added to the resin. The amount of monomer added to the resin is calculated as follows:

$$monomer\_added(mL) = \frac{resin\_amount(mg) * resin\_loading(mmol/g)}{monomer\_concentration(mM)} * stoichiometric\_excess$$

Typically, a 5x stoichiometric excess is used for all monomers.

1. Add the activated monomer solution to the resin.

2. Agitate the resin for 20 minutes to 1 hour.

3. Wash the resin thrice with DMF, once with 5% DIEA in DCM (agitate for 30 seconds during this wash) and twice with DCM.

4. Remove a small portion of the resin and add to a microcentrifuge tube.

5. Add one drop each of Kaiser solutions (A) and (B).

6. Heat microcentrifuge tube to 100 ºC for two minutes.

7. If the test is negative (beads are yellow), proceed with capping. If the test is positive (blue), repeat steps 1-3, perform Kaiser test again to ensure complete coupling.

Capping Unreacted Sites. It is critical to cap any unreacted sites on the resin, otherwise, PNAs will be synthesized that lack the C-terminal lysine residue. The capping reaction is done by adding acetic anhydride to the resin, resulting in conversion of all unreacted sites to the acetamides.

1. Add enough of the capping solution (D) to disperse the resin (at least 3 mL), then agitate for 5 minutes. Repeat once.

2. Wash the resin thrice with DCM.

3. Repeat above protocol for each additional PNA monomer until desired sequence is synthesized in stepwise fashion.

Kaiser Test. The "Kaiser Test" is a colorimetric test for the presence of amino groups; we use it to make sure that each coupling step in PNA synthesis goes to completion. It is based on the reaction of ninhydrin with amino groups to form a blue adduct. Therefore, an incomplete coupling cycle will lead to a positive Kaiser test, demonstrated by the development of a blue color, while coupling to completion will yield a negative (yellow) test.

Prepare the following solutions:

1. Dissolve 8 g phenol in 2 mL absolute ethanol. Warming of the solution will be required to completely dissolve the phenol. (Note: It is easiest to weigh out phenol when it is still cold. Still, the large crystals make it difficult to weigh out exactly 8 g. Try to get close and adjust the volume of ethanol accordingly.)

2. Dissolve 13 mg KCN in 20 mL water. (Transfer the DCN into a tared vial in the hood, then carry to the balance with the cap on!) Check in the hood before preparing this solution as there might already be some available.

3. Dilute 20 μL of aqueous KCN solution with 980 μL pyridine, then add to 100 μL of the phenol/ethanol solution. This is the Kaiser (A) solution.

4. Dissolve 1.0 g ninhydrin in 20 mL absolute ethanol. This is the Kaiser (B) solution

5. Store Kaiser (A) and (B) solutions in amber dripper bottles.

Cleavage of PNA oligomers from Resin.

This procedure uses a combination of trifluoroacetic acid (TFA) and m-cresol to cleave the PNA oligomers from its solid support. The procedure also removed the Boc protecting group from the side chains.

1. Prepare a solution of TFA:m-cresol (4:1) *(1600 μL TFA with 400 μL m-cresol)*

2. Add the solution to the resin and let stand for 2 hours with no mixing.

3. Elute liquid into conical glass centrifuge tube. Use pipet bulb to push liquid through frit.

4. Repeat steps 2-3, combining the eluted fractions.

5. Add at least a 5-fold excess of dry ether.

6. Mix and place on dry ice for 10 min.

7. Centrifuge in hood for 5 min.

8. Carefully pour off supernatant.

9. Add dry ether to 10 mL and mix to resuspend pellet.

10. Place on dry ice for 5 min.

11. Centrifuge in hood for 5 min.

12. Carefully pour off supernatant.

13. Repeat steps 9-12 four times with the following dry ice incubation times: 5 min, 2 min, 0 min, 0min.

14. Dry PNA with gentle stream of N_2.

15. Store dried PNA in refrigerator until ready for HPLC purification.