DNA Preparation and Purification Protocol for Enzymatically Synthesized Sequences

The following protocol describes the necessary pre-purification and purification steps for enzymatically synthesized DNA.

I. Poly(A) Tailing

1. Place the synthesized DNA tube on a magnetic rack and remove the supernatant.
2. To the tube of beads from the previous step (with the liquid removed), add the following components to create a 50 µl reaction mixture:
    - a. 5 µl of 10X TdT buffer
    - b. 5 µl of 2.5 mM $CoCl_2$
    - c. 0.5 µl of 10 mM dATP
    - d. 0.5 µl of TdT enzyme
    - e. 39 µl of nuclease-free water
3. Incubate the reaction for 30 minutes at 37°C, then stop the reaction by incubating at 65°C for 10 minutes. Agitation (e.g., mixing or vortexing) may improve reaction efficiency.
4. After the Poly(A) tailing reaction, wash the beads with 200 µl of water to remove the reaction solution. The specific steps are as follows:
    - a. Add 200 µl of water and mix well by pipetting.
    - b. Place the tube on the magnetic rack.
    - c. Let it stand for 2 minutes.
    - d. Aspirate and discard the supernatant.
    - e. Remove the tube from the magnetic rack.
5. Repeat the washing steps (a-e) two more times for a total of three washes.

II. Amplification

1. To the tube containing the tailed beads, prepare the following 50 µl reaction mixture:
    - a. 25 µl of 2x Phanta Max Master Mix
    - b. 2 µl of 10 µM PolyT primer
    - c. 23 µl of nuclease-free water
2. Perform the first PCR with the following program: 95°C for 3 minutes, followed by 10 cycles of (95°C for 15s, 65°C for 15s, 72°C for 30s), a final extension at 72°C for 5 minutes, and hold at 4°C.
3. Place the tube on a magnetic rack for 2 minutes. Collect the supernatant and prepare the following 50 µl reaction mixture:
    - a. 20 µl of supernatant
    - b. 2 µl of 10 µM P5 primer
    - c. 2 µl of 10 µM P7-i23 primer
    - d. 26 µl of nuclease-free water
4. Perform the second PCR with the following program: 95°C for 3 minutes, followed by 30 cycles of (95°C for 15s, 65°C for 15s, 72°C for 30s), a final extension at 72°C for 5 minutes, and hold at 4°C.
5. Place the tube on a magnetic rack for 2 minutes and transfer the entire supernatant to a new tube.

Primer Sequences:

- **PolyT Primer:** GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTTTTTTTTTTTTTTTTTTTT

- **P5 Primer:** AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATC*T
- **P7-i23 Primer:** CAAGCAGAAGACGGCATACGAGATATCCACTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T

III. Purification

1. To the supernatant from the final PCR step, add 110 µl of Vazyme VAHTS DNA Clean Beads and mix thoroughly by pipetting 30 times.
2. Incubate at room temperature for 10 minutes to allow the DNA to bind to the beads.
3. Place the sample on a magnetic rack. Wait for the solution to clear (approximately 5 minutes), then carefully remove and discard the supernatant.
4. Keeping the sample on the magnetic rack, add 200 µl of freshly prepared 80% ethanol to wash the beads. Incubate at room temperature for 30 seconds, then carefully remove and discard the supernatant.
5. Repeat step 4 once for a total of two ethanol washes.
6. Keeping the sample on the magnetic rack, air-dry the beads for 7 minutes at room temperature.
7. Remove the tube from the magnetic rack. Add an appropriate volume of nuclease-free water. Resuspend the beads thoroughly by vortexing or pipetting, and let stand for 2 minutes at room temperature. Place the tube back on the magnetic rack for 5 minutes until the solution is clear. Carefully transfer the supernatant (containing the purified DNA) to a new nuclease-free tube.

In conclusion, this protocol provides a comprehensive approach to purifying enzymatically synthesized DNA sequences.