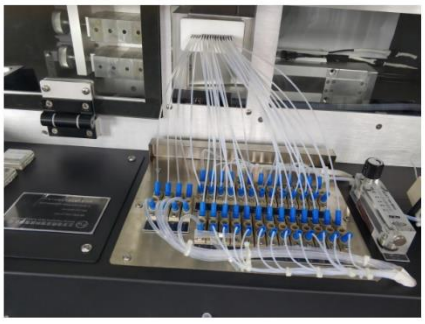
# OVERVIEW OF DNA PRIMER PRODUCTION LINE

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# DNA PRIMER PRODUCTION LINE

DNA primer is single stranded DNA used to prime the polymerase chain reaction. The single stranded DNA is produced synthetically using DNA synthesizer. Typical process used in synthetically creating DNA is the phosphoramidite method. This method is replicated in the 3'- to 5'- direction which is opposite to that in nature on a column containing solid supporting material. The typical solid support for DNA synthesis is Polysterene (PS) and Controlled Phore Glass (CPG). One amidite base is added per synthesis cycle on solid support. When the sequence is completed phosphoramidite, the DNA is separated from the solid support and the base protecting groups are removed. Further post synthesis process is required until the synthetic DNA can be used as PCR primer.

## A. THE COMPLETE CYCLE FOR DNA SYNTHESIS INCLUDE:

1. **Reagent Preparation**
   Dilution of Phosphoramidite Base Powder to the desired concentration suitable for DNA synthesis. Can be done with dissolver instrument to avoid Oxygen contamination.
2. **DNA synthesis**

**a. Detritylation**

Detritylation is the removal of protecting (blocking) group Dimethoxytrityl (DMT) from phosphoramidite base. DMT prevent the 5'-end from reacting with anything. Detritylation is typically done with **Trichloroacetic Acid (TCA) or Dichloroacetic Acid (DCA) in Dichloromethane**. DCA is preferable for longer DNA synthesis due to weaker acid, however the process is slower (60 sec). For faster DNA synthesis (usually shorter DNA), TCA is preferable (35 sec). Longer exposure to acid can cause depurination/mutation.

**b. Activation**

After DMT is removed, an **amidite base (A-C-G-T)** is dissolved in **Acetonitrile (ACN)** and mixed with activator solution **Tetrazole or Ethylthiotetrazole (ETT)**. The activator protonates the 3'-end to facilitate reaction with 5'-end of the oligo.

**c. Oligonucleoside coupling on solid support**

- **Universal solid support**: Solid support column with the linker only, without any nucleoside attached.
- **Standard support**: Solid support with the first nucleoside already attached

**d. Capping**

To prevent the extension of DNA sequence that did not react in the coupling step, the remaining 5'-hidroxyl group must be blocked with a mixture of two capping solution known as **CAP-A (Acetic Anhydride) and CAP-B (N-Methymidazole)**. This also allows for easier purification in post synthesis

**e. Oxidation**

The bond of the attached amidite base is strengthened trough iodine oxidation to prepare for the next step of detritylation. Backbone modifications be done as a replacement for the oxidation step.

**The Oxidizer:**

- Iodine and H2O
- Pyridine
- Tetrahydrofuran

**f. Washing**

After the oxidation step a wash of **Acetonitrile (ACN)** is run through the line before the next detritylation. Another wash step is also performed after detritylation.

## B. POST DNA SYNTHESIS

Once the DNA sequence is completed, the oligo must undergo post synthesis operation in order to become biologically active

**a. Cleavage**

The first step to post synthesis is to separate the finished oligo from the solid support. Cleavage is done with strong bases **Ammonium Hydroxide, Methylamine, or AMA** (a mixture of Ammonium Hydroxide and Methylamine)

**b. Deprotection**

The second step requires removing the amidite base protecting group from the oligo by raising temperature to 70 – 90 °C.

**c. Oligo quantification**

- Nanodrop
- Qubit Assays
- Epoch Microplate reader (for batch quantification)

Overview of Post synthesis operation: **Cleave + Elute -> Deprotection -> Oligo Quantification**

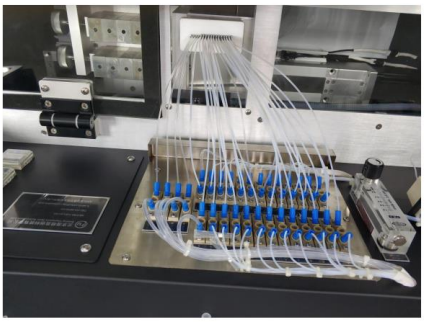
## D. PRICE LIST OF REAGENT AND CONSUMABLES FOR DNA SYNTHESIS

| No. | Reagents & Consumables | MOQ | Price (USD) |
|---|---|---|---|
| 1. | Trichloroacetic Acid (TCA) | 4 Liter | **35** |
| 2. | Phosphoramidite base - A/T/G/C | 5 grams | **22.5** |
| 3. | Acetonitrile | 4 Liter | **33** |
| 4. | Tetrazole or Ethylthiotetrazole | 4 Liter | **106** |
| 5. | CAP-A (Acetic Anhydride) | 4 Liter | **82** |
| 6. | CAP-B (N-Methymidazole) | 4 Liter | **92.5** |
| 7. | Iodine | 4 Liter | **86** |
| 8. | Ammonium Hydroxide | 1 Liter | **60** |
| 9. | Reverse phase silica column - C18 (price/bag) | 1 bag (100 pcs) | **160** |
| 10. | CPG column HJ-Z-12-100 100nmol (price/bag) | 1 bag (1000 pcs) | **160** |
| 11. | Filter | 100 | **160** |
| 12. | Molecular trap(10g/piece) | 100 | **950** |
| 13. | Molecular trap(5g/piece) | 100 | **785** |

## E. THE REQUIRED MACHINERY FOR PRODUCING DNA PRIMER:

1. Amidites Dissolver Instrument (Step 1)

2. 12 DNA synthesizer (Step 2 a,b,c,d,e, f)



### TECHNICAL SPECIFICATION

| No | Parameter | HJ 12-Channel Synthesizer |
|---|---|---|
| 1. | **Synthesis column** | 12 |
| 2. | **Number of amidite inlet** | 16 |
| 3. | **Number of reagent inlet** | 7 |
| 4. | **Synthesis range** | 25 nmol – 300 µmol |
| 5. | **Synthesis rate** | 4-6 Coupling/minutes |
| 6. | **Total time for 20-unit Poly-T synthesis** | 2 h 30 min |
| 7. | **Total solvent (acetonitryl) consumption per coupling** | 2.6 mL |
| 8. | **Coupling efficiency** | 99% (60 base, 50nmol) |
| 9. | **CPG feed** | CPG in Pre-packed column |
| 10. | **Availability of DMT monitoring *** | Yes |
| 11. | **Production scale (weekdays)** | 500-600 primer tube/month |

*DMT carbocation absorbs at 495 nm and thereby produces an orange color that can be used to monitor coupling efficiency.

3. Ammonolysis instrument (Step 3 a,b)

4. Nanodrop/Qubit/Epoch Microplate reader (Step 3 c)


5. Pipetting workstation (Optional)
   The Pipetting workstation with the function of Elution and purification, this workstation uses for different step in the synthesis cycle, because it has different function in one.

6. Tube Labeling Machine