

# 核酸定序核心-作業流程

第一共研電子報

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第四期

## DNA template preparation

the duty of a sample submitter

1. Prepare DNA template from plasmid, DNA fragment, BAC or YAC. Or prepare DNA template by PCR.
2. Clean up templates.
3. Examine DNA quality.
4. Determine DNA quantity.

★★★★★★★★★★

**Primer is added  
by the submitter or  
by our staff using  
primer we provide.**

★★★★★★★★★★

## Cycle sequencing

the duty of our staff

1. Select the sequencing chemistry.
2. Prepare cycle sequencing reactions.
3. Perform sequencing reactions in a thermal cycler.

Applied Biosystems Cycle Sequencing Kits:

- BigDye Terminator v3.1 kits
- dGTP BigDye Terminator v3.0

Perform an initial denaturation.

- a. Rapid thermal ramp to 96 °C
- b. 96 °C for 1 min

Repeat the following for 25 cycles:

- Rapid thermal ramp to 96 °C
- 96 °C for 10 sec
- Rapid thermal ramp to 50 °C
- 50 °C for 5 sec
- Rapid thermal ramp to 60 °C
- 60 °C for 4 min

介紹運作流程

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1. 各單位實驗室送來純化好的 DNA template 並且加入適當比例的 PRIMER，當中的 DNA template 與 PRIMER 的質和量，我們都有詳細的建議規範。
2. 核心實驗室進行 Cycle sequencing 反應實驗，使用 BigDye® Terminator v3.1 Cycle Sequencing Kits，有結構問題的 DNA 使用 dGTP BigDye Terminator v3.0 kits
3. 酒精沉澱合成產物。
4. 上機，毛細管電泳分析。
5. 軟體分析結果。

以上流程需要 1-2 工作天

## Automated DNA Sequencing Workflow

- DNA Template Preparation ( **the duty of a sample submitter** )
- Cycle Sequencing
- Extension Product Purification (Ethanol precipitation)
- Capillary Electrophoresis (Applied Biosystems instruments: 3730x/ analyzer)
- Data Analysis(Applied Biosystems software: Sequencing Analysis Software v5.4)