定序成功的關鍵

送件要點

- 影響定序成功與否，主要是 DNA 的質與量。
- 量過多或過少都不佳，請依照建議範圍。
- 務必將 DNA template 純化，確保無其他干擾物影響 cycle sequencing 反應，且雜質也會導致毛細管受損，若發生毛細管因而損壞，送件單位須照價賠償。
- 請將 DNA template 與 primer 溶於 ddH2O 中，勿溶於任何 buffer。
- 送件前確認 DNA template 為

  1. single band （ except uncut plasmid DNA）

  2. A260/280≧1.7
- 每個樣品包含單一 DNA template＋1 端 primer (非 1 對)放在 200ul 微量離心管 ，補 ddH2O 至最後體積 6μl。

樣品種類	含量(ng)	**PCR product** 長度(bps)	含量(ng)
ssDNA	50~90	100~200	2~5
含 2 級解構	150~200	200~500	5~15
dsDNA plasmid	300~500	500~1000	15~30
含 2 級結構	600~800	1~2k	30~60
BAC, Cosmid	1500~2000	≧ 2k	60~100

Primer quantity→ 6 pmol （6μM*1μl）

送件樣品DNA template 含量的建議範圍

DNA Template Quantity

The amount of DNA template used in a sequencing reaction can affect the quality of the data.

Too much template makes data appear top heavy, with strong peaks at the beginning of the run that fade rapidly. Too little template or primer reduces the signal strength and peak height. In the worst case, the noise level increases so that bases cannot be called. DNA template quantitation is critical for successful sequencing reactions. The most common way to determine DNA quantity is to measure the absorbance (optical density or O.D.) of a sample at 260 nm in a spectrophotometer

DNA Template Quality

Poor template quality is the most common cause of sequencing problems. Results characteristic of using poor quality templates:

- Noisy data or peaks under peaks
- No or low signal
- Early loss or termination of extension

Contaminants in cycle sequencing reactions negatively affect polymerase binding and amplification or extension. Resulting sequences produce poor quality data with low signal or high noise.

Potential ontaminants:

- Proteins
- RNA
- Chromosomal DNA
- Excess PCR primers, dNTPs, enzyme, and buffer components (from a PCR amplification used to generate the sequencing template)
- Residual salts
- Residual organic chemicals, such as phenol, chloroform, and ethanol
- Residual detergents
- Agarose gel, if DNA was extracted from a gel