

DNA SANGER SEQUENCING

Volumen: 6µl
ADN: 250-300 ng
PCR: 10-20 ng/100 pb
Primer: 3,2 pmoles
Tubos: 0,2 ml

DNA AND PRIMERS REQUIREMENTS

PLASMID DNA

QUALITY

- NO: Phenol, chloroform, proteins, chromosomal DNA, RNA, ethanol, salts, detergents, PEG, EDTA (TE).
YES: Column purified plasmid DNA.
DNA in H₂O.
iiiiiii MIND SECONDARY STRUCTURES!!!!!!!!!!

QUANTITY (300ng)

- YES: Spectrophotometric sample quantification (Nanodrop).

PCR PRODUCT

QUALITY

- NO: Nucleotides, *primers*, dNTPs or PCR amplification enzymes.
YES: Column purified DNA, exo I y SAP treated samples or PCR product dilution.

QUANTITY 10-20 ng/100pb

- YES: Spectrophotometric sample quantification (Nanodrop).

PRIMERS

QUANTITY 3,2 pmoles

QUALITY

Avoid *primers* with 3 or 4 Gs ó Cs in a row.
Primers should be at least 18 bases long to avoid non specific hybridization. If possible anchor *primers* with GCs in 3'.

Avoid secondary structures in *primers* sequences, dimmers and mismatches.
Primers T_m should be over 50 °C.

$T_m = 2 \times (A+T) + 4 \times (G+C)$
$T_a = T_m - 5^{\circ}\text{C}$