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 H_2O.
¡¡¡¡¡¡¡¡ MIND SECONDARY STRUCTURES!!!!!!!!!

QUANTITY (300ng)

YES: Spectrophotometric sample quantification (Nanodrop).

PCR PRODUCT

QUALITY

NO: Nucleotides, *primer*s, 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 Tm should be over 50 ^{0}C.

$$Tm = 2 \times (A+T) + 4 \times (G+C)$$

$$Ta = Tm - 5°C$$