

Primer design concerns

Primer concerns:

1. GC>50%, Tm~66-72oC, both primers should have similar GC percentile and Tm.
2. deltaG (free energy) for hairpin and dimer close to Zero.

Subclone

miniprep, purify with 30 ul water. Take 6 ul, digest , purify with 30 ul water. For subcloning, add 1 ul vector