General rules for PNA oligomers

1. PNA has higher affinity and specificity. Tm of PNA is about 1 °C per basepair higher than DNA. There is about 15 °C Tm difference for single base mismatch for PNA while it is about 10 °C for DNA.
2. Purine rich PNA oligomers have reduced solubility, and tend to aggregate. It is recommended that purine content of oligomer is less than 50%, and no more than 6 stretches of purine (specially G base) in one oligomer for PNA clamps as water solubility is quite important. Two Lysines can be added to improve solubility for long PNA or PNA with high amount of purine.
3. Please refer PNA Tool in our web site for design guidelines and Tm calculation. http://www.pnabio.com/support/PNA_Tool.htm

PNA Storage and Handling

1. PNA is very stable as a lyophilized powder (over 5 years) or as a solution in water (at least one year) if stored – 20 °C or lower. For long term storage, -70 °C is preferred.
2. When ready to use, spin down the tube and dissolve 50 nmole of PNA in 1 ml sterile water to make 50 uM stock. Vortex and mix well for complete dissolution.
3. Aliquot and store at – 20 °C or – 70 °C. Working stock can be diluted 10 times further (5 uM, 10x stock) and stored at 4 degree for a few weeks.
4. When thawing PNA, heat at 55 °C for 5 minutes for complete dissolution.

PNA Clamping for PCR Reaction

*Below is an example. The actual condition can vary depending on the sequences and needs to be determined empirically.

1. Prepare following PCR mix.
    a. 25 ul 2x PCR mix
    b. FW and RV PCR primers to final 0.2 uM each
    c. 5 ul PNA clamp (5 uM stock) to final 0.5 uM (range: 0.2~5 uM)
    d. DNA template: 20 ng (10~50 ng)
    e. Water to 50 ul
2. Run PCR program as below.
    a. Denaturation: 94 °C, 3 min
    b. Amplification (22~40 cycles depending on instrument)
        i. Denaturation: 94 °C 30 sec
        ii. PNA clamping: 65~75 °C for 20 sec (5~10 degree lower from Tm calculated from PNA Tool)
        iii. Primer annealing: 55~65 °C for 20 sec
        iv. Extension: 68 °C for 30 sec
    c. Extension: 72 °C, 10 min
    d. Keep at 4 °C
3. Take 5 ul of PCR product and run on the agarose gel.
4. Most common concentration of PNA in clamping reaction is 0.4~2 uM. In general, higher concentration of PNA gives better clamping if solubility is not compromised.

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