PCR Blocker Protocol

PNA Storage and Handling

1. PNA is very stable as a lyophilized powder (over 5 years) or as a solution in water (at least two years) if stored – 20 °C or lower. For long term storage, store at – 70 °C.
2. When ready to use, dissolve 50 nmole of PNA in 1 ml sterile water each 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, 20x stock) and stored at 4 degree for a week.
4. When taking from freezer, heat at 55 °C for 5 minutes for complete dissolution.

PNA Clamping for PCR Reaction

1. For PCR in 50 ul, prepare following PCR mix.
    a. 25 ul 2x PCR mix
    b. 2 ul PCR FW primer (5 uM stock) to final 0.2 uM
    c. 2 ul PCR RV primer (5 uM stock) to final 0.2 uM
    d. 2.5 ul mPNA blocker (5 uM stock) to final 0.25 uM
    e. 2.5 ul pPNA blocker (5 uM stock) to final 0.25 uM
    f. DNA template: 20~200 ng
    g. Water to 50 ul
2. Run PCR program as below.
    a. Denaturation: 94 °C, 3 min
    b. Amplification (30~35 cycles)
        i. Denaturation: 94 °C 15 sec
        ii. PNA clamping: 75 °C for 10 sec
        iii. Primer annealing: 55~60 °C for 10 sec
        iv. Extension: 72 °C for 15~60 sec
    c. Extension: 72 °C, 10 min
    d. Stored at 4 °C
3. Take 5 ul of PCR product and run on the agarose gel.
4. The efficiency of PNA clamping (blocking PCR reaction) can vary depending on the amount of target and other conditions. In general, the more PNA oligo is added, the better the clamping effect is. To improve PNA blocking, mPNA and pPNA can be added to 1 uM final concentration.

PNA Bio
107 N. Reino Rd. #242, Thousand Oaks, CA 91320, USA
TEL 1-800-764-0478 / 805-504-2220 FAX 1-805-277-0629
Email info@pnabio.com Web *www.pnabio.com*