Guidelines for PNA oligomers

Handling and storage of PNA oligomers

1. Spin down the tube and dissolve in water to make a stock of 20~100 uM stock. For example, if you dissolve 500 ul water for 50 nmole PNA, the stock will be 100 uM. PNA is most stable in water.
2. For purine rich (>60%) or long PNA oligomers (>21mer), solubility might be reduced in water. If PNA is not completely dissolved in water, add organic solvent such as DMSO or DMF to up to 10% final concentration.
3. Store in aliquots at 4 ^{o}C for short term and -20 or -70 ^{o}C for long term.
4. For fluorescently labeled probes, protect from light.
5. When taking out the frozen stock, heat up the solution at 55 ^{o}C for 5 min and vortex well before using it.
6. PNA is very stable molecule and should be intact >1 year if properly stored.

General rules for PNA oligomers

1. PNA can form duplex with target nucleic acid in either orientation but antiparallel is strongly preferred.
2. PNA has higher affinity and specificity. Tm of PNA is about 1 ^{o}C per basepair higher than DNA. There is about 15 ^{o}C Tm difference for single base mismatch for PNA while it is about 10 ^{o}C for DNA.
3. Purine rich PNA oligomers have reduced solubility, and tend to aggregate. It is recommended that purine content of oligomer is less than 60%, and no more than 6 stretches of purine (specially G base) in one oligomer.
4. Due to the neutral backbone of PNA, the binding of PNA to nucleic acid is less dependent on ionic strength and pH. PNA binding to DNA or RNA is not affected significantly from 10mM to 1M of NaCl concentration. However when salt concentration is lower than 10mM, non specific binding can occur.
5. Please refer PNA Tool in our web site for design guidelines and Tm calculation. http://www.pnabio.com/support/PNA_Tool.htm

PNA Quantification

1. Calculate the molar extinction coefficient (ε260) of a PNA oligomer.

   ε260 (PNA oligomer) = $\Sigma\ \varepsilon_i \times n_i$

   (i = A, G, C and T, ε_i = molar extinction coefficient of base, n_i = number of base)

   ε260 (A) = 13.7 ml/(µmole x cm), ε260 (C) = 6.6 ml/(µmole x cm)

   ε260 (G) = 11.7 ml/(µmole x cm), ε260 (T) = 8.8 ml/(µmole x cm)
2. Make stock solution by dissolving the PNA oligomer in the appropriate volume (V_A) of water/or buffer.
3. Take appropriate volume (V_B) of the PNA solution and dilute it as needed.
4. Measure absorbance of the diluted PNA solution at 260 nm using spectrophotometer.
5. Determine the nanomolar amount of the total PNA oligomer by following formula.

   PNA concentration (mM) = (A260 / ε260) / path length (cm) X dilution factor (V_A / V_B)

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