PE Biosystems

Guidelines for Sequence Design of PNA oligomers

General Rules:

- PNAs can form duplexes in either orientation, but the **ANTI PARALLEL orientation** is strongly preferred. This will be the orientation for all antisense and DNA probe type applications. The N-terminal of the PNA oligomer is equivalent to the 5'-end of an oligonucleotide and is often referred to as "the 5'-end of the PNA".

- Because the PNA strand is uncharged a PNA•DNA-duplex will have a higher Tm than the corresponding DNA•DNA-duplex. Typically there will be an increase in Tm of about 1°C per basepair at 100 mM NaCl depending on the sequence. At lower salt the Tm differences are going to be even more dramatic. A 10-mer PNA will typically have a Tm of about 50°C, and a 15-mer typically a Tm of 70° with the anti parallel complementary oligonucleotide (DNA or RNA).

- Due to this higher affinity it is NOT necessary to prepare long PNA-oligomers, as opposed to the 25-40 units, which is the typical length for an oligonucleotide probe. Bear in mind that the shorter a probe the more specific it is. The impact of a mismatch is greater the shorter the sequence is. The primary concern regarding length of the PNA-probe should be the warranted uniqueness, i.e. what length is unique for the particular application. For most applications an oligomer length of 12-15 is optimal, and in many cases even shorter probes will work well. Longer PNA oligomers, depending on the sequence, tend to aggregate and are difficult to purify and characterize.

- Purine rich PNA oligomers tend to aggregate, with G-rich oligomers being the worst. As a rule never have more than 7 purines in any stretch of 10 units. Observing this rule will dramatically reduce the likelihood that your PNA oligomer will aggregate. The shorter the sequence the less attention needs to be paid to the sequence.

- As PNA/PNA interactions are even stronger than PNA/DNA interactions try to avoid self-complementary sequences.

- There are more restrictions on the design of PNA sequences than for the design of DNA sequences. It should, however, not generate problems, as it is easier to accommodate the requirements for the PNA oligomer. This is because you only need a probe length of 12-15 bases compared to a length between 25 and 40 which is the typical length for an oligonucleotide probe.

Specific Design Rules:

- **Length:** We will not synthesize any sequences with more than 18 bases, not including linkers, amino acids and labels.

- **Purine Content:** Purine rich PNA oligomers tend to aggregate and have low solubility.

  To avoid aggregation – Please follow these specific guidelines:

  1. Of any stretch of 10 bases in the sequence do not have more than 6 purines
  2. NO more than 4-5 purines in a row, specifically no more than 3 G's in a row
  3. Consider probing the other strand if you cannot follow the rules above

  Examples:

  GAT TAG CAG TCT ACG Good – No more than 6 of 10 are purines)

  ATT AGG GGC ATC TAC Bad – 4 G's in a row

  CTA GAT AGC AGG TTC Bad – 8 of 10 bases are purines)

  Notice that the complement, i.e. the probe for the "other strand" is good:

  GAA CCT GCT ATC TAG

- **Avoid self-complementary sequences such as inverse repeats, hairpin forming and palindromic sequences** as PNA/PNA interactions are even stronger than PNA/DNA interactions. For example would AATT be OK but not CCGG or ATTAAT. There is no problem with the synthesis but they are difficult to characterize and purify. If you still want a PNA oligomer containing a self-complementary sequence we cannot guaranty yield, purity or proper characterization.