Cross-Linkers Introduction

The ability to utilize UV light to cross-link bases in double-stranded and triple-stranded DNA, or cross-link bases in oligonucleotides with other types of molecules (such as proteins), is often necessary for successful performance of structural studies, for example the probing of nucleic acid secondary structure, or the structure of protein-nucleic acid complexes. Cross-linker modifications generally fall into two categories, nucleic acid intercalators (for example, psoralen) and halogenated bases (for example, 5-Br-dC). Incorporation of a nucleic acid intercalator into an oligo permits site-specific targeting of the cross-link into double-stranded and triple-stranded DNA. Incorporation of a modified base, capable of forming cross-links, into an oligonucleotide is often the method of choice when an intra-strand cross-link is needed, or a direct cross-link to a protein or other molecule is desired.

<table>
<thead>
<tr>
<th>Modification</th>
<th>Catalog Number</th>
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<tbody>
<tr>
<td>5-bromo deoxycytosine (Br dC)</td>
<td>26-6411</td>
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<tr>
<td>5-bromo deoxyuridine (Br-dU)</td>
<td>26-6412</td>
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<td>5-iodo deoxycytosine dC</td>
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<tr>
<td>5-iodo deoxyuridine dU</td>
<td>26-6415</td>
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<tr>
<td>Convertible dG (2-Fluoro deoxy inosine)</td>
<td>26-6671</td>
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<tr>
<td>Psoralen C6</td>
<td>26-6686</td>
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Cross-Linkers Design Protocols

Oligonucleotides Incorporating Cross-Linkers--Design Considerations

Oligonucleotides containing photo-crosslinker modifications can be valuable research tools for probing the structure of DNA-protein complexes. In particular, it is those amino acids and bases in contact with each other that can be cross-linked, and thus identified as the specific units involved in binding DNA to protein. Cross-linking can be generated either by steady-state UV irradiation or pulsed lasers. For such studies, 5-halogenated uracils/cytosines are commonly used as cross-linker modifications in the DNA template. (7,8), and several excellent protocols are publicly available (9). Positioning of the halogenated bases within the DNA can be done systematically by progressive substitution along the DNA until cross-linking with an amino acid is achieved, or other structural information can be used to guide the choice of where to place the modified base(s).

When inter- or intra-strand cross-linking between duplex or triplex DNA at an thymidine position is desired, the cross-linking intercalator psoralen is typically chosen. The amount of cross-linking achieved can be tightly controlled by varying the dose of 360 nm UV light applied. Although the use of psoralen-modified oligos is primarily considered with respect to their ability to cross-link duplex or triplex DNA, cross-linking to mRNA is also possible. A 5'-psoralen-modified DNA oligo containing puromycin can be cross-linked to the 3'-end of a long mRNA template. The resulting photo-crosslinked product efficiently forms mRNA-protein fusion products (10).
Cross-Linkers Applications

One of the most commonly used intercalator cross-linkers is psoralen, which is used to probe nucleic acid secondary structure at specific points in both duplex and triplex DNA. Specifically, psoralen forms cross-links with thymidine. In duplex DNA, after intercalation, psoralen can form either monoadducts with one adjacent thymidine, or diadducts with two thymidines adjacent to it, depending on the particular UV wavelength it’s exposed to (1). These adducts can occur on the same or complementary strands. For triplex DNA, psoralen C6-modified homopyrimidine oligos are used to bind to a complementary homopurine-homopyrimidine duplex, thereby forming a triplex that can be cross-linked together at the triplex-duplex junction point (2). Demonstration of the existence of triple-helix-directed gene modification and the involvement of nucleotide excision repair mechanism in DNA interstrand cross-link repair are two examples of the use of psoralen-modified oligos as research tools (3-4). Halogenated bases are a second class of UV cross-linker used for probing biomolecular structure, particularly the structure of protein-DNA complexes. 5-Br-dC and 5-Br-dG have been incorporated into dG-dC oligos capable of easily changing into the Z-conformation. This property allowed such oligos to function as probes for detecting and studying Z-DNA binding proteins (5). Substituting 5-Br-dU at several thymine positions of oligos allowed them to be used to characterize the binding of Nuclear Factor BA1 with DNA (6). See the relevant tech sheets of the different halogenated bases offered by Gene Link for additional examples.
References