

Modified Nucleoside Triphosphate Applications

An Overview of the SELEX Process

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TriLink's wide selection of modified nucleoside triphosphates offers researchers many novel ways to explore existing technologies typically used in drug discovery. One example is the SELEX (Systematic Evolution of Ligands by Exponential Enrichment) process of developing oligonucleotide aptamers. The SELEX process allows for the simultaneous screening of 1×10^{15} different oligonucleotides against a target of interest, such as a protein. The main goal of SELEX is to identify a small subset of aptamers from the original library that bind to the target (1). Three separate laboratories were simultaneously working on the development of this technology: Larry Gold and Craig Tuerk at the University of Colorado Boulder, Jack Szostak and Andy Ellington at Massachusetts General Hospital, and Gerald Joyce at the Scripps Institute in La Jolla, CA (2). The Colorado group was granted a patent in 1993.

The word aptamer comes from the Greek word "aptus", meaning "to fit" (3). An aptamer is an oligonucleotide ligand, generally 15-60 bases in length. It binds to the target of interest through conformational recognition, as opposed to the standard recognition mechanism for nucleic acids: hydrogen bonding of the bases to another nucleic acid strand. Oligonucleotides are often represented as linear sequences, but in reality they fold into specific conformations. It is these shapes that allow the lock-and-key fit between an aptamer and its target (4). As mentioned above, the ultimate goal of SELEX is to find an aptamer that binds to the active site of the target molecule.

Advantages of Aptamers

- Straightforward synthesis
- Easily modified to increase resistance to endonucleases
- Favorable toxicity profiles
- Greater stability than monoclonal antibodies
- Useful as therapeutic agents
- Highly specific; can discriminate between closely related proteins (5)

The SELEX Process

1. Define target molecule. (The target molecule can be a protein, small molecule, or a supramolecular structure.)
2. Create a "library" of random oligonucleotides ($\sim 1 \times 10^{15}$ oligonucleotides) The random pool of DNA generally has primer binding sites at the end of each oligonucleotide and wobble bases in between (6). This provides an efficient way to find and PCR amplify oligonucleotides that bind to the target molecule.
3. Expose the oligonucleotide "library" to the target molecule. A few of these oligonucleotides in the library will bind to the target and are then considered aptamers.
4. Non-binding oligonucleotides are separated from the binding oligonucleotides. Those that bind are amplified and then put through several additional selection cycles.
5. The number of high affinity binding molecules is reduced from trillions, to a small number through this process.
6. Individual aptamers are isolated, sequenced and refined using modified nucleoside triphosphates. For example, 2'-Fluoro-dCTP (TriLink Catalog # N-1008) and 2'-Fluoro-dUTP (TriLink Catalog # N-1010) are commonly used to modify aptamers. These types of modifications increase the stability of the aptamer and make it more resistant to endonuclease degradation (7).

Gilead currently holds the patent rights to the SELEX technology and has licensed it to Archemix, who is using the technology to develop pharmaceutical applications for aptamers. Archemix has sublicensed this technology to NOXXON, who is using it for the development of their Spiegelmer® technology (<http://www.noxxon.com>).

SomaLogic (<http://www.somallogic.com>), a company founded by one of the original inventors, Larry Gold, has developed their own technology, called PhotoSELEX. This technology involves photoaptamers, which use a 5-Bromo-2'-deoxyuridine-5'-Triphosphate (Br-dU) (TriLink Catalog # N-2008) residue instead of a standard Thymidine. In addition to the conformational binding, there is also covalent bonding between the Br-dU residue of the aptamer and a tyrosine amino acid on the target. The Br-dU oligonucleotides are exposed to radiation and those that are in the correct conformation will covalently crosslink to specific sites on the target molecule. Those that do not crosslink are washed away, while those that remain are amplified by PCR. These photoaptamers are put into arrays so that large numbers of proteins can be tested at the same time (8).

SELEX is just one of many applications that modified nucleoside triphosphates can be used in. TriLink offers a wide variety of modified nucleoside triphosphates that can be used for research purposes.

References

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3. Jayasena, S.D. Aptamers: An Emerging Class of Molecules that Rival Antibodies in Diagnostics (1999) *Clinical Chemistry* 45:9 1628-1650.
4. Somalogic <http://www.somallogic.com> (accessed December 2002)
5. Archemix <http://www.archemix.com> (accessed December 2002)
6. Burke, Donald, In vitro selections: Evolution In a Tube <http://bl-chem-ernie.chem.indiana.edu/~dhburke/SELEX.htm>
7. Archemix <http://www.archemix.com> (accessed December 2002)
8. Somalogic <http://www.somallogic.com> (accessed December 2002)

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PhotoSELEX is patented by SomaLogic, Inc.

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