Synonyms: SELEX

Related Topics: aptamers, random library, ribozyme

Definition:

The SELEX-method was invented by Larry Gold in 1990 and isochronally by Andrew E. Ellington. Both attempts are based on the principle of selective identification of target molecules via nucleic acid single strands. Gold worked with ssDNA, whereas Ellington used RNA.

The principle:
Single strand nucleic acid sequences are synthesized with random base pair sequence - only a small part is fixed, where specific primers will be bound while PCR. These nucleic acid strands form, because of intramolecular interaction, a threedimensional structure which is specific for every molecule. The most important interactions are electrostatic interactions, hydrogen bonds and base stackings. By this fixed structure target molecules such as proteins, hormones, bacterial toxins, amino acids, antibiotics, virus particles, increase factors, and even small organic molecules can be recognized. To obtain greater affinity bivalent cations which interact for example with the phosphate-part and thus amplify the tertiary structure, can be admitted to the nucleic acid library.

When a target has been linked to a nuclein acid sequence, this strand is refered to as aptamer (from the Latin aptus: fitting).

The process:
To construct nucleic acid libraries, the initial template is obtained via chemical synthesis of DNA fragments, each of them consisting of a random sequence of 30-60 nucleotide. These libraries mostly consist of about $10^{15}$ different nucleic acid single strands. For RNA-SELEX a DNA template is transcribed into an RNA which poses a nucleic acid single strand.

To select aptamers the combinatorial library is incubated with the target molecule. The separation, the key step of SELEX, can be effectuated by different methods such as affinity chromatography, sorption on nitrocellulose filters, or gel electrophoresis. All these selections take advantage of specific physical (or functional) proterties of the aptamer-target complex. To enrich the selected DNA-aptamers asymmetrical PCR is carried out. With the selection of RNA-aptamers on the other side cDNA must be synthesized by a reverse transcriptase firstly and then cDNA is amplified by PCR to an amout sufficient for the next round. Aptamers which specifically and efficiently bind with the target molecule can be yielded after about ten rounds. Dissoziation constants of 100 pM can be achieved.

One topical use is to generate a ribozym (from ribonucleic acid enzyme, also called RNA enzyme or catalytic RNA) that catalyzes a chemical reaction. Other important flieds of application of aptamers are medical diagnostics, therapeutic agents and environmental analysis.
Structures:

Fig. 2. Molecular recognition of the basic amino acids (A) arginine (left) and citrulline (right) by nucleic acid aptamers. The ligand-binding pockets are shown for arginine in complex with two different DNA aptamers (B and C) (18, 20) and RNA-aptamer complexes (19) of arginine (D), and citrulline (E). In all four complexes, the positively charged amino acid side chain (orange) penetrates deeply into the nucleic acid fold where intermolecular hydrogen bonds are formed exclusively with bases (cyan). The ligand-binding pockets are lined by clusters of bases (green) excluding both the negatively charged phosphate backbone and solvent water. Polar nitrogen (blue) and oxygen (red) atoms participating in hydrogen bonds are marked.

Fig. 3. Relative cell binding of U2OS-embedded SLEX pools. SLEX pools from round 21, round 12, and the starting round were assayed for binding to U2OS cells.

Publications:

Web-Links:
http://de.wikipedia.org/wiki/Aptamer
http://en.wikipedia.org/wiki/Ribozyme