Biotechnology Advances 35 (2017) 275–301

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

ABCs of DNA aptamer and related assay development

Tarun Kumar Sharma [a,b,*], John G. Bruno [c,**], Abhijeet Dhiman [d,e]

[a] Center for Biodesign and Diagnostics, Translational Health Science and Technology Institute, Faridabad, Haryana 121001, India
[b] AptaBharat Innovation Private Limited, Translational Health Science and Technology Institute Incubator, Haryana 121001, India
[c] Operational Technologies Corporation, 4100 NW Loop 410, Suite, 230, San Antonio, TX 78229, USA.
[d] Department of Biotechnology, All India Institute of Medical Sciences, New Delhi 110029, India.
[e] Faculty of Pharmacy, Uttarakhand Technical University, Dehradun 248007, Uttarakhand, India

ARTICLE INFO

Article history:
Received 23 August 2016
Received in revised form 19 November 2016
Accepted 17 January 2017
Available online 18 January 2017

Keywords:
Aptamers
SELEX
Aptamer library
Colorimetric assay
Aptamer Linked Immunosorbent Assay (ALISA)
FRET
ssDNA generation
Post-SELEX optimization
Translation of aptamers

ABSTRACT

This review is intended to guide the novice in aptamer research and development to understand virtually all of the aptamer development options and currently available assay modalities. Aptamer development topics range from discussions of basic and advanced versions of Systematic Evolution of Ligands by EXponential Enrichment (SELEX) and SELEX variations involving incorporation of exotic unnatural nucleotides to expand library diversity for even greater aptamer affinity and specificity to improved next generation methods of DNA sequencing, screening and tracking aptamer development throughout the SELEX process and characterization of lead aptamer candidates. Aptamer assay development topics include descriptions of various colorimetric and fluorescent assays in microplates or on membranes including homogeneous beacon and multiplexed Fluorescence Resonance Energy Transfer (FRET) assays. Finally, a discussion of the potential for marketing successful aptamer-based assays or test kits is included.

Contents

* Correspondence to: T. K. Sharma, Centre for Biodesign and Diagnostics, Translational Health Science and Technology Institute, Faridabad, Haryana 121001, India.
** Correspondence to: J. G. Bruno, Operational Technologies Corporation, 4100 NW Loop 410, Suite230, San Antonio, TX 78229, USA.
E-mail addresses: tarun@thsti.res.in (T.K. Sharma), john.bruno@otcorp.com (J.G. Bruno).

http://dx.doi.org/10.1016/j.biotechadv.2017.01.003
0734-9750/

1. Introduction

Jayasena predicted in 1999 that aptamers would rival antibodies in diagnostics and therapeutics (Jayasena, 1999), and despite a few clear successes in the forms of Macugen and ultrasensitive aptamers (Vance and Sandros, 2014), or SOMAmer-based proteomic chips (Kraemer et al., 2011) and a few other commercial assays (Penner, 2012), the potential of aptamers is yet to be reached. However, aptamers offer clear advantages over antibodies including, but not limited to, greater batch to batch reproducibility, speed, lower cost, ease of development, obviating the use of host animals (Chopra et al., 2014; Lange et al., 2012; Sharma and Shukla, 2014a, 2014b). Researchers continue to innovate the SELEX (Systematic Evolution of Ligands by EXponential enrichment) process itself as well as developing novel assay formats in which only aptamers can work or work clearly better than their antibody competitors (Chopra et al., 2014; Lange et al., 2012; Sharma and Shukla, 2014a, 2014b). An example of a system in which aptamers can exclude antibodies in competition are conformation-dependent electrochemical systems in which the changes in aptamer 3-dimensional conformations lead to detectable changes in conduction or voltammetry (Catanante et al., 2016). Another example of aptamers working better than antibodies is multiplexed FRET assays in which aptamer-quantum dot (QD) conjugates adsorbed onto gold nanoparticles (GNPs) are liberated by greater affinity for target analytes in solution leading to multicolored or multiple fluorescence emission wavelengths with a single ultraviolet light source (Kim and Jurng, 2011; Zhang et al., 2010).

While many researchers continue to use aptamers in conventional antibody-based immunoassay formats such as ELISA-like assays or lateral flow chromatographic test strips, the more creative approaches may lead to the real future commercial product breakthrough. Though there are several published reviews underlining the importance of SELEX and application of aptamers, none, comprehensively summarize every single step of SELEX from library design to post-SELEX optimization to assay development. In the following work, the authors have endeavored to summarize the basic steps and more recent advanced developments in SELEX and aptamer assays to aid current and future innovators and to help aptamers realize their full potential especially in the diagnostics area. An overall picture of aptamer technology (SELEX) is portrayed in Fig. 1.

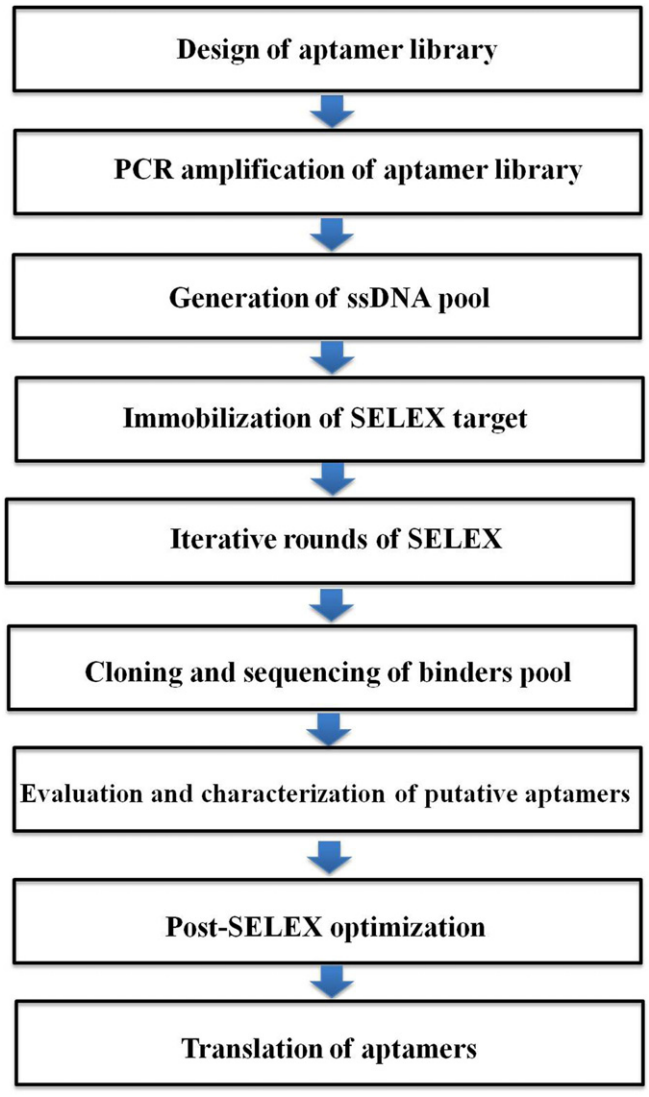

Fig. 1. Flow chart showing overall picture of SELEX technology.

2. Library design

2.1. First generation

The design of the starting randomized nucleic acid library provides the basis for SELEX. A variety of SELEX libraries are used across the