

Detection of Carcinoembryonic antigen with sandwich-biosensor



Aims

- To obtain aptamers against carcinoembryonic antigen (CEA)
- To express streptavidin on yeast and bacterial cell surfaces
- To make sandwich-biosensor to detect different types of cancer at early stages

Introduction

Carcinoembryonic antigen (CEA) is one of the examples of the biomarker which appears at early stages of such types of cancer as colorectal, gastric, pancreatic, lung, and breast carcinoma. We propose to generate a biosensor for detection of CEA at early stages^[1]. The first part of the study is designed to select ssDNA aptamers with strong affinity for CEA during 12 cycles of SELEX (Systematic Evolution of Ligands by Exponential Enrichment) procedure^[2].

The model organisms for creating the biosensor are *E. Coli* and *S. cerevisiae*. *E. coli* will express the streptavidin through Lpp-Omp expression system.



Mating type of *S. Cerevisiae* expresses Aga 1 protein on its surface which acts like signaling protein. Another protein Aga 2 forms disulfide bonds with Aga 1. This Aga 1/Aga2 expression system can be used to express streptavidin on its surface^[3,4].

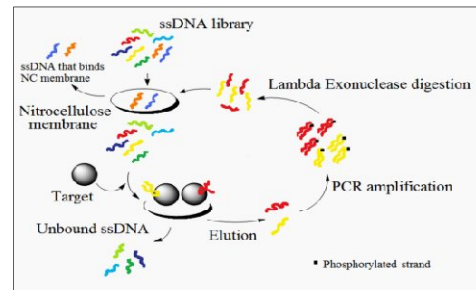


aptamers will be used to make a sandwich biosensor for CEA detection.

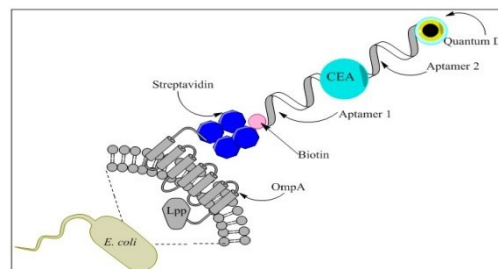
In this project, it is planned to use CdSe/ZnS QDs for conjugation to aptamers to create biosensor of sandwich manner^[5].

Design Overview

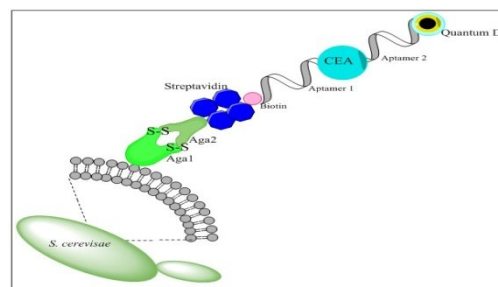
1. SELEX of aptamers



2. Bacterial Expression System



3. Yeast Expression System



Results

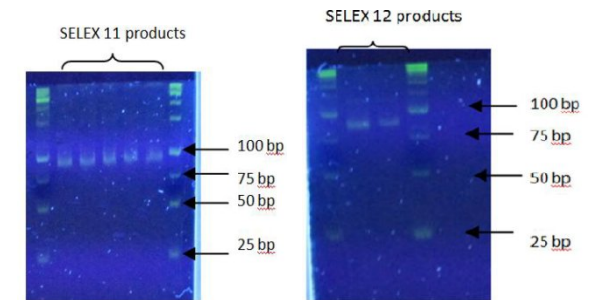


Figure 1. SELEX 11 gel run

Figure 2. SELEX 12 gel run

12 cycles of SELEX were successfully finished, at the moment characterization step of aptamers from cycles 11 and 12 is ongoing. The optimization of SPR, Dot Blot, EMSA conditions is also on the process.

Current work

- TOPO TA cloning of aptamers for sequencing
- Dot Blot Analysis of aptamers
- Construction of parts

Reference

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- 2) Mascini, M. (2009). Aptamers in bioanalysis. John Wiley and Sons
- 3) Lim, H. K., Hwang, I., Sh., Park. (2011). Biotin-Assisted Folding of Streptavidin on the Yeast Surface. American Institute of Chemical Engineers. Retrieved from: <http://www.cbe.buffalo.edu/people/pdfPub.jsp?id=2887>
- 4) Huang, D. & E., Shusta. (2005). Secretion and Surface Display of Green Fluorescent Protein Institute of Chemical Engineers. Vol 21 No2. (349-357); Retrieved from: <http://46.38.63.192/mail/g.php?doi=10.1021/bp0497482&url=aHR0cDovL2xpYmldbi5vcmcvc2NpbWFnMy8xMC4xMDIxL2JwMDO5NzQ0Ml5wZGY%3D>
- 5) Tan, A., Yildirim, L., Rajadas, J., De La Peña, H., Pastorin, G., Seifalian, A. (2011). Quantum Dots and Carbon Nanotubes in Oncology. Nanomedicine. 6(6):1101-1114. Retrieved from http://www.medscape.com/viewarticle/749698_2