

# A cell SELEX assay for detection of *Neisseria meningitidis*

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## Abstract

Despite advances in controlling infectious diseases, *Neisseria meningitidis*, the causative agent of epidemic meningitis and sepsis worldwide, has inflicted rapid death, disability and fear on disparate human populations. Biosensors show great potential for rapid detection of pathogens. In turn, aptamers have great potential for biosensor development. Their small size, ease of synthesis and labeling, lack of immunogenicity, lower cost of production, and high target specificity are their advantages over antibodies. SsDNA aptamers specific to *N.meningitidis* were obtained by a whole bacterium-based systematic evolution of ligands by exponential enrichment (SELEX) procedure. The aptamers that bound to *N.meningitidis* were isolated using centrifugation, PCR amplification followed by purification to yield an enriched ssDNA pool suitable for further rounds of selection. After 9 rounds of selection with *N.meningitidis* as the target and *Neisseria lactamica*, *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus*, *Acinetobacter baumannii*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, as counter targets, the highly enriched oligonucleic acid pool was sorted using flow cytometry. Our work demonstrates that whole-cell SELEX is a promising technique for detection of microbial pathogens.

Keywords: *N.meningitidis*; DNA aptamers; Cell SELEX; FACS analysis

## Introduction

Despite advances in controlling infectious diseases, *Neisseria meningitidis*, the causative agent of epidemic meningitis and sepsis worldwide, has inflicted rapid death, disability and fear on disparate human populations[1,2]. Biosensors show great potential for rapid detection of pathogens. In turn, aptamers have great potential for biosensor development. Their small size, ease of synthesis and labeling, lack of immunogenicity, lower cost of production, and high target

specificity are their advantages over antibodies[3,4,5]. SsDNA aptamers specific to *N.meningitidis* were obtained by a whole bacterium-based systematic evolution of ligands by exponential enrichment (SELEX) procedure.

## Materials & Methods

The aptamer selection procedure, whole cell SELEX was performed targeting *N.meningitidis* ATCC 13090. Other bacteria used in this study were obtained from the American Type Culture Collection (ATCC) and included *Escherichia coli* serovar O1:k1:H7, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Listeria monocytogenes*, *Neisseria lactamica*, *Acinetobacter baumannii*, *Haemophilus influenzae* were used for counter SELEX[6]. The whole cell SELEX aptamer selection protocol used in this study was adapted from the work of Dwivedi(2010) with modifications. Six rounds of positive-SELEX targeting *N.meningitidis* were performed, while two rounds of negative (counter)-SELEX were also done. The aptamer candidates were cloned and the plasmid DNA extracted from the clones prior to sequencing. Flow cytometry was performed to measure the percent fluorescent cells and fluorescence intensity using a FACSCalibur™ flow cytometer[7].

## Results

The most abundant PCR product was obtained using an annealing temperature of 59°C, and a single 80-bp band was observed after DNA gel electrophoresis (Fig.1). The results of agarose gel electrophoresis indicated that a single and correct band was obtained when using 30 cycles. Thus, annealing at 59°C followed by 30 cycles was selected as the standard temperature and cycles in this work. Six rounds of positive-SELEX and two rounds of negative (counter)-SELEX were performed targeting *N.meningitidis* and a mixture of meningitis bacteria respectively. In preliminary screening using flow cytometry (n = 200,000), binding affinity for these aptamers ranged from 4.46 to 7.94 %. Based on the high binding affinity of K1 and K4, these aptamers were chosen for further characterization (Fig.2).



Fig.1: PCR-amplified nucleic acid fractions of initial DNA library. Lane 1 is the DNA ladder (50 bp). Lanes 2 is the initial DNA library. Lanes 3 is the seventh round of aptamer pool

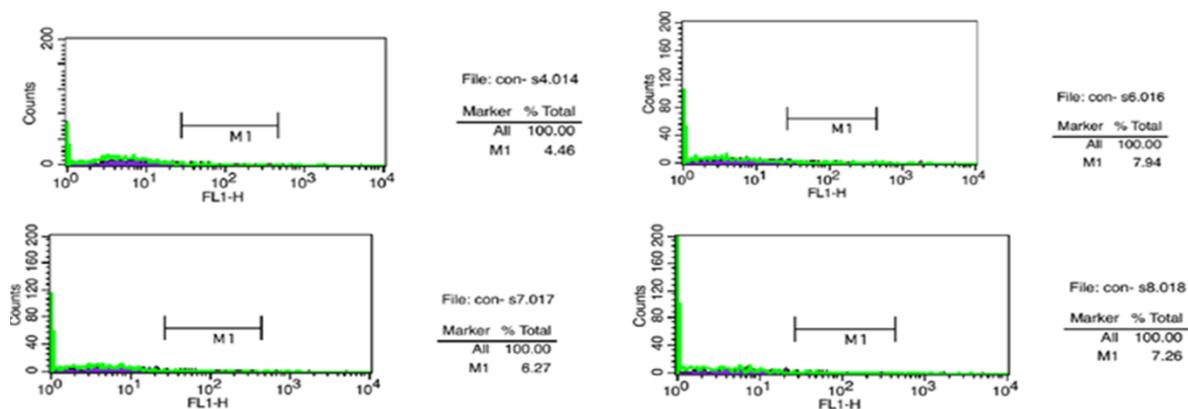


Fig.2: Flow cytometry assay to monitor the affinity of FITC-labeled aptamer with N.meningitidis in forth,sixth,seventh and eighth rounds of SELEX.

## Discussion & Conclusion

Nucleic acid aptamers are novel ligands with unique structural folding that have been reported to have a high degree of binding affinity and specificity to cell surface targets of N.meningitidis as well as bacteria of meningitis[8].The whole-cell SELEX approach was the ability to use flow cytometry to sort cell-bound aptamers into pools based on fluorescence intensity. From the sequenced transformants,60 unique aptamer sequences were selected for preliminary screening of binding affinity. Aptamers K1 and K4 demonstrated the highest

binding affinity in terms of percent fluorescent cells[4]. This study is the first report on the use of whole-cell SELEX to identify DNA aptamers specific for *N.meningitidis*. Furthermore, we developed a method for the rapid detection of *N.meningitidis* based on aptamers K1 and K4. The work described in this study demonstrates the ability of this method to select a good aptamer probe for the detection of *N.meningitidis* and has the potential to contribute greatly to the development of rapid and sensitive detection methods for other pathogenic bacteria.

## References

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