Rapid isolation of high affinity aptamers through microfluidic SELEX technology and high-throughput sequencing analysis

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Summary: Microfluidic selection with high-throughput DNA sequencing technology is developed for rapid and efficient discovery of nucleic acid aptamers. Sequencing data and measured binding characteristics are presented.

SELEX is an in vitro method for generating aptamers that possess high affinity and specificity to the target molecule [1, 2]. We recently described the use of microfluidics technology to accelerate the selection process [3]; due to multiple advantages that occur at the micro-scale, specific aptamers that bind target proteins with nanomolar affinity can be generated within 1–3 rounds. In this work, we combine the microfluidic selection technique with high throughput sequencing to rapidly and quantitatively discover high affinity aptamers. Our method - Quantitative Selection of Aptamers through Sequencing (QSAS) – simultaneously tracks the copy number and enrichment-fold of more than 10 million individual sequences through multiple selection rounds, enabling the identification of high-affinity aptamers without the need for the pool to fully converge to a small number of sequences (Fig. 1a). This is a significant improvement over traditional cloning and sequencing approaches, which can only sample a small portion of the sequence space. Importantly, our method enables the quantitative measurement of enrichment-fold of an individual sequence as a function of selection rounds, thereby discriminating those sequences that arise from experimental biases rather than true target binding.

Using the QSAS method, we have identified aptamers that specifically bind to platelet derived growth factor BB (PDGF-BB) protein with $K_d < 3$ nM within 3 rounds. Furthermore, the aptamers identified by QSAS have ~3–8-fold higher affinity and ~2–4-fold higher specificity relative to those discovered through conventional cloning methods (Fig. 1b). Given that many biocombinatorial libraries are encoded with nucleic acids, we believe that our method may be generalized for the quantitative selection of other types of libraries, including tagged small molecules, phage display, cell surface display, as well as ribosome and mRNA displays, and we extrapolate that the method may ultimately be extended for the isolation of molecules with useful functions beyond binding, such as cooperative assembly, enzymatic activity and binding-induced folding.