High-Throughput Silver NanoCluster Beacons Activator Sequence Selection

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Introduction: Activatable fluorescent probes make a great impact on chemistry, biology, and medicine field. However, these probes are usually suffering from several drawbacks, such as high manufacturing cost, low enhancement upon activation. Silver NanoCluster Beacons (NCB) are one of a kind[1-3]. Interacting with a proximal DNA sequence termed activator sequence, a non-emissive silver nanocluster can be activated and strongly emit fluorescence from visible to near-infrared range. The fluorescence activatable property and low manufacturing cost make NCB an ideal probe for DNA detection and cancer cells detection. However, previous research does not have a wide range of sequence library to test with. A high-throughput selection on next-generation sequencing chip (Illumina *MiSeq®*) using Chip-Hybridized Association Mapping Platform (CHAMP) algorithm[4] could overcome this dilemma[4]. This technique facilitates us to study the interaction between NCB and millions of DNA sequences simultaneously. Here we demonstrate the potential of this platform identifying the outcome of different activator sequences from an immense probable sequence.

Materials and Methods: To perform a high-throughput selection of our NCB probes, we first evaluate our system by recording the fluorescent images by Olympus X71 Epifluorescence microscope and apply CHAMP algorithm to register the images. We obtained several used chips from GSAF, Genomic Sequencing and Analysis Facility, at UT Austin. ATTO 647-DNA from IDT (Integrated DNA Technology) will be introduced and hybridized to the sequence synthesized on the chip evaluating the setup capability. To record the images automatically, we developed Graphical User Interface (GUI) designed for Micro-Manager to control and align the stage. This GUI obtains 244 fields of view (FOV) over the 4 rows of the chip, each FOV having a length and width of 220 µm. After we add handler sequences which link with activator sequence, ordered from IDT to the chip, NCB can hybridize with the handler sequences and generate fluorescent signal which could be recorded through the microscope.

Results and Discussion: The results of alignment are obtained from the sequencing file coordinates of fastq data and the alignment marker of ATTO 647 fluorophore (**Figure. 1A**). Since not every sequence coordinates are recorded inside fastq data, the best alignment rate is 84% for a specific row or 70% in total. Currently, the signal to noise ratio is not acceptable for us to do the alignment for NCB handler-PhiX label (**Figure. 1B**). However, we should expect similar results using a handler sequence to label PhiX cluster in the future. With the GUI facilitating image acquisition, to record a great amount of image data using less time (**Figure. 1C**).

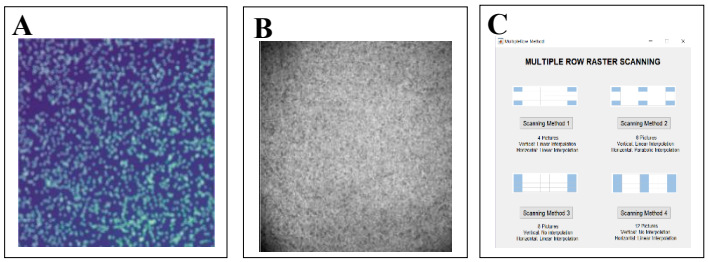

Figure 1. (**A**) represents the alignment of fastq data as white circle and the NCB activation on green dots, further analysis could obtain sequence results from the PhiX cluster 512 × 512 pixels. (**B**) Full FOV with high signal to noise ratio from epifluorescence microscope, labeled with NCB-handler-PhiX. **(C)** GUI algorithm for an automatic image scanning method to obtain the 244 FOVs.

Conclusion: For the first time we repurpose the next generation sequencing chips for the nanomaterial synthesis and selection. Proper alignment of fluorescent images is crucial for optimal design and quantification of NCB. We performed a high-throughput selection algorithm with 70% alignment rate in total, and 84% for a specific row. Due to the poor signal-to-noise ratio for NCB-handler-PhiX labeling, the NCB alignment feasibility validation still needs to be tested. The GUI, a data visualization platform, will be an open source in the future.

Acknowledgments: Research reported in this paper was supported by the National Institute of General Medical Sciences of the National Institutes of Health under linked Award Numbers RL5GM118969, TL4GM118971, and UL1GM118970. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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