SELF-AVOIDING MOLECULAR RECOGNITION SYSTEMS (SAMRS) PRIMERS FOR IMPROVED SPECIFICITY AND SENSITIVITY IN A MICROARRAY

Sara B. Griesemer[1], Darrell P. Chandler[2], Kirsten St.George[1]
[1]Laboratory of Viral Diseases, Wadsworth Center, Albany, NY; [2]Akonni Biosystems, Frederick, MD

NEW YORK STATE OF OPPORTUNITY. Department of Health | Wadsworth Center

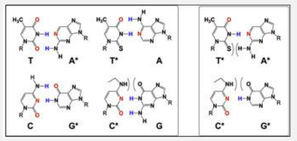
Akonni Biosystems

Background

- The use of multiplexed panel assays has wide appeal for the efficient diagnosis of numerous diseases and syndromes
- Despite the availability of commercial panels for common agents, additional targets continue to be needed as new agents and situations emerge
- Development of multiplexed assays are challenging due to primer-primer interactions and amplification artifacts, which can reduce assay sensitivity and specificity
- The use of recently described “self-avoiding molecular recognition systems”, SAMRS, in amplification primers have demonstrated benefits when multiplexing
- To investigate their utility in a diagnostic array, the performance of SAMRS was compared to standard primers in a multiplexed microarray for the detection of encephalitic viral agents on the Akonni Biosystems TruArray.
- Multiplexed panels provide efficient encephalitis testing for small CSF sample volumes
- However, viral loads are commonly low and very good assay sensitivity must be maintained

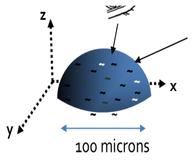
SAMRS Concept

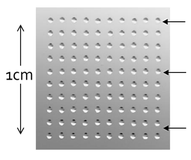
- DNA-like nucleotides, built from four different nucleotide analogs (A*, T*, G*, and C*)
- Strategically removed hydrogen bonds allow SAMRS to bind to their natural DNA partner, but not to each other
- Standard Primer: 5’ ACGAGCTAGCCGATCG**TGCT**A 3’
- SAMRS Primer: 5’ ACGAGCTAGCCGATCG**T*G*C*T***A 3’



Akonni Biosystems Encephalitis TruArray Panel

- Low-density, “gel-drop” microarray
- Gel elements are copolymerized with specific target capture probe and have a 3D structure
- 3D structure allows for increased hybridization efficiency over planar arrays
- Project goal is to develop a closed, sample-to-answer microarray system in which amplification and hybridization occur in a lateral flow cell in the presence of the gel element array





Encephalitis 8-plex Viral Panel
HSV1 HSV2
VZV CMV
HHV-6 Enterovirus
WNV Internal Control

Methods

Asymmetric PCR

- Performed in single or multiplexed format, using target-specific standard primers and SAMRS primers **(Table 1)**
- Standard and SAMRS primers were identical in sequence, except that SAMRS primers contained four SAMR nucleotides at the 3’ end, corresponding to the four natural DNA nucleotides in the standard primer
- 10:1 ratio of reverse to forward primer concentrations, creating an excess of ssDNA amplicons
- For microarray analysis, reverse primers were labeled at 5’end with cy3 fluorophore, creating labeled amplicons that hybridize to target-specific probes embedded in gel elements of array

Table 1: Multiplexed asymmetric PCR amplification parameters

Parameter	Tube	Lateral Flow Cell
Thermocycler	Conventional	Modified Flat Block
4-plex primer mix	HSV1/HSV2/VZV/CMV (All SAMRS vs. all standard)	HSV1/HSV2/VZV/CMV (All SAMRS vs. all standard)
8-plex primer mix	HSV1/HSV2/VZV/CMV (SAMRS) with HHV6/Enterovirus/WNV/IC (Standard) vs. all standard	All SAMRS vs. all Standard
Analysis	Gel electrophoresis	Microarray hybridization and detection

Microarray Hybridization, Imaging and Analysis

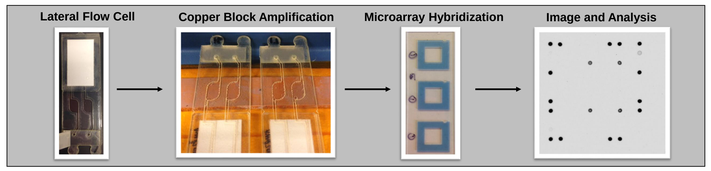
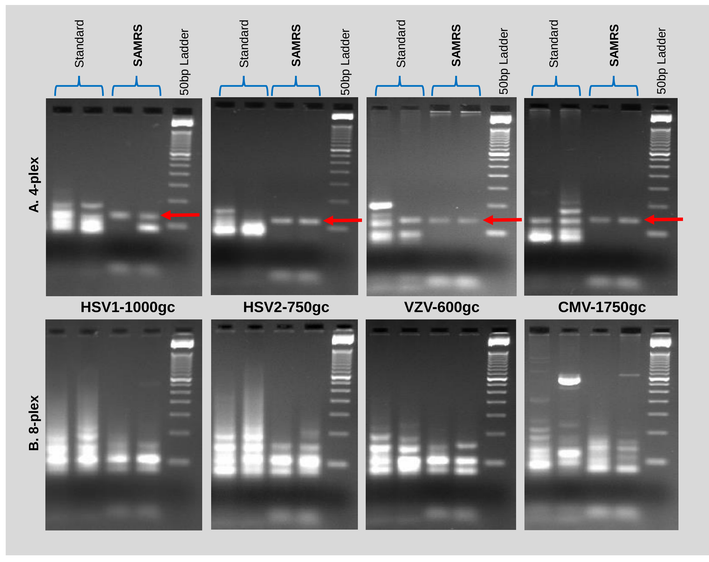


Figure 2: Amplification workflow for Akonni’s TruArray platform. Samples are loaded into a lateral flow cell and placed on a flat block modified with a copper strip to isolate heat transfer. After asymmetric PCR amplification, PCR products are removed and cy3-labeled amplicons are hybridized to a microarray containing target-specific probes. Arrays are washed, imaged and analyzed.

Results

Conventional Tube PCR

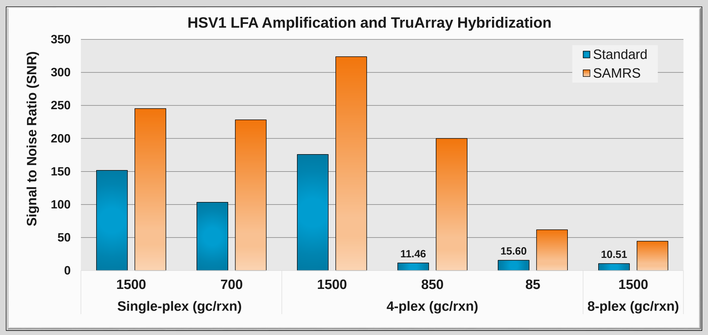
- **Figure 4: SAMRS reduces nonspecific PCR products in a conventional tube, asymmetric multiplex PCR. A.** Single-target amplification with SAMRS in a 4-plex assay dramatically reduced nonspecific products for all targets, as compared to the equivalent assay with standard primers. Arrows indicate expected amplicon. **B.** Combination of the SAMRS 4-plex assay with another 4-plex standard assay (HHV-6/Entero/WNV/IC) reduced unwanted amplification artifacts, as compared to the all standard primer 8-plex assay.



Lateral Flow Cell

- SAMRS primers increased the HSV1 target signal after amplification for all concentrations tested, in all formats **(Figure 5A)**
- HSV1 signal with SAMRS technology was 4X higher than standard primer technology. **(Figure 5A)**
- HSV1 DNA was detected in a 4-plex assay at less than 100gc/rxn. **(Figure 5A)**
- SAMRS primers dramatically increased HSV2 target signal by more than 10X, in all experiments at all concentrations tested **(Figure 5B)**
- HSV2 assay sensitivity improved by approximately 2 log with SAMRS technology, from 12,000gc/rxn to 100gc/rxn in single-plex format, and 9000gc/rxn to 100gc/rxn in a 4-plex format. **(Figure 5B)**
- SAMRS also improved overall HSV2 assay performance in an 8-plex format compared with standard primers **(Figure 5B)**

A.



B.

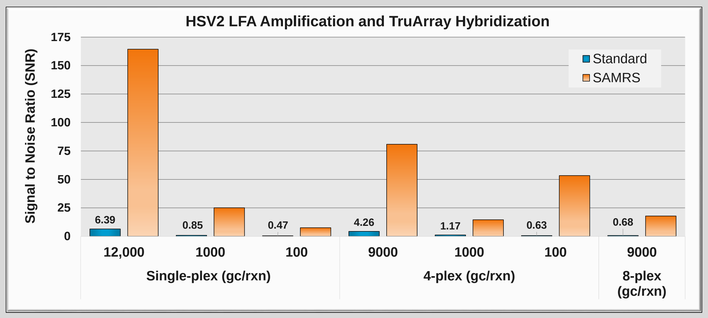


Figure 5: SAMRS improves amplification and detection of HSV1 and HSV2 DNA in Akonni’s TruArray microarray system. HSV1 and HSV2 DNA was amplified in a lateral flow cell, separately hybridized to a microarray, and analyzed. **(Fig. 2).** Comparison of standard and SAMRS technology was performed in singleplex, 4-plex, and 8-plex formats. **A**. HSV1 **B**. HSV2

Conclusions/Discussion

- SAMRS improved amplification specificity of HSV1, HSV2, VZV, and CMV DNA in conventional tube, asymmetric 4-plex PCR reactions
- The replacement of a 4-plex standard primer mix with an equivalent 4-plex SAMRS primer mix in an 8-plex PCR reaction reduced nonspecific PCR products seen with an all standard 8-plex primer mix
- SAMRS primers dramatically increased HSV1 and HSV2 target signals when amplified in a lateral flow cell
- SAMRS primers improved HSV2 detection sensitivity on Akonni’s Encephalitis TruArray system, in both single and multiplexed formats.
- This data highlights the potential of SAMRS technology to simplify assay design, improve assay performance, and streamline multiplexed diagnostic test development
- Despite the wide availability of viral panel diagnostics on the market today, additional viral targets of interest are outside the spectrum of what is available, as new agents continue to emerge
- SAMRS technology can be used to support new avenues in viral diagnostics, such as development of combined viral and cellular host biomarker arrays for expanded investigations

Works Cited

- Glushakova LG, Bradley A, Bradley KM, Alto BW, Hoshika S, Hutter D, Sharma N, Yang Z, Kim M, and Benner SA (2015): High-throughput multiplexed xMAP Luminex array panel for detection of twenty two medically important mosquito-borne arboviruses based on innovations in synthetic biology. *Journal of Virological Methods* 214: 60-74.
- Glushakova LG, Sharma N, Hoshika S, Bradley AC, Bradley KM, Yang Z, and Benner SA (2015): Detecting respiratory viral RNA using expanded genetic alphabets and self-avoiding DNA. *Analytical Biochemistry* 489: 62-72.
- Hoshika S, Chen F, Leal NA, and Benner SA (2010). Artificial Genetic Systems: Self-avoiding DNA in PCR and multiplexed PCR. *Angewandte Chemie International Edition English* 49: 5554-5557.

Acknowledgements

- The authors would like to thank Dr. Steven Benner and Linda Jackson of Firebird Biomolecular Sciences, LLC, and Dr. Shuichi Hoshika from the Foundation for Applied Molecular Evolution, for technical and customer support with design and production of SAMRS primers.
- SAMRS primer sets are commercially available from Firebird Biomolecular Sciences (http://www.firebirdbio.com)
- The authors would also like to thank Daryl Lamson at the Wadsworth Center, and Christopher Knickerbocker and Steven Garber at Akonni Biosystems, for their helpful discussions, assistance and technical support during the development and implementation of the lateral flow cell amplification system.
- This work was supported by National Institutes of Health, SBIR Phase 2 Grant, 5 R44 AI085650-04