New Methods for Rapid Isothermal Amplification and Detection of Short DNA Sequences

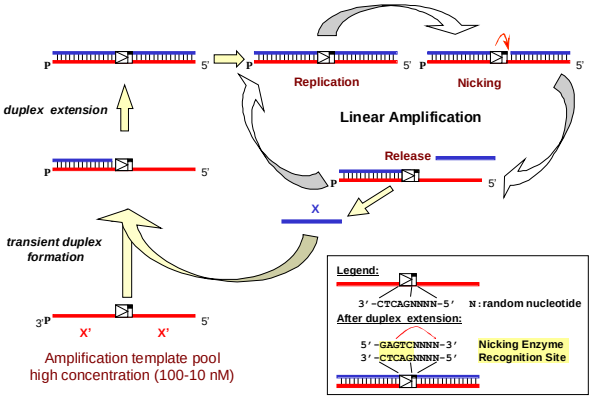
Eric Tan, Megan Buechel, Ekaterina Kniazeva, Jennifer Wong, Krisanu Bandyopadhyay, Angelika Niemz
Keck Graduate Institute, Claremont, CA

Goal:

- Rapid, sensitive, specific, low tech, portable DNA diagnostic device
- Detection of clinical pathogens: SARS pathogen, Streptococcus pneumoniae, HSV I & II and biothreat agents: Bacillus anthracis , Brucella species
- Detection of single nucleotide polymorphisms (SNPs)
- Multiplexed detection format

Isothermal Amplification of Oligonucleotides via EXPAR

- Developed by Galas Group (Keck Graduate Institute)
- Isothermal 10^6-10^9 fold amplification of short oligonucleotides at 55°C within minutes

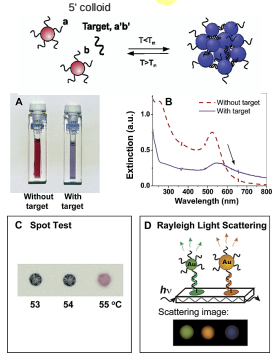


Van Ness, J.; Van Ness, L. K.; Galas, D. J. *PNAS* **2003**, *100*, 4504-4509.

Colorimetric DNA Detection through Nanosphere Aggregation

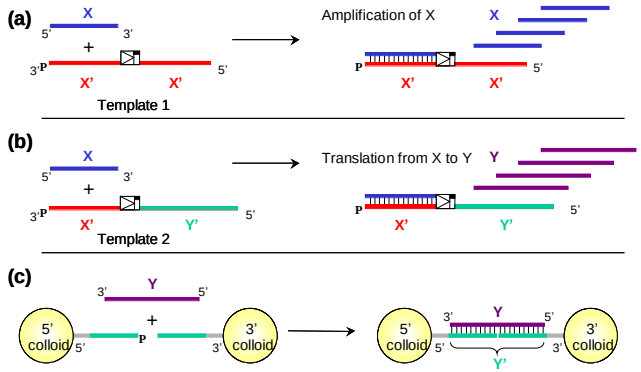
- Developed by Mirkin Group (Northwestern University)
- DNA nanosphere aggregation through bridging target oligonucleotides:

- Color change from red to blue through shift in plasmon resonance band
- Color enhancement through spotting onto C18-modified silica plates (spot test): simple, rapid, low tech visual DNA detection. However: lacks sensitivity (10 nM)
- Alternative detection methods: Silver reduction coupled to optical or electronic detection, surface enhanced Raman scattering: high sensitivity. However: multiple steps, requires instrumentation



Figures taken from:
Jin, R.; Wu, G.; Li, Z.; Mirkin, C. A.; Schatz, G. C.; *J. Am. Chem. Soc.* **2003** *125(6); 1643-1654*

Two Stage EXPAR Amplification with Colorimetric Detection



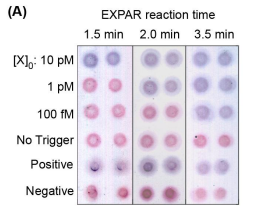
Sequence-specific DNA-detection:

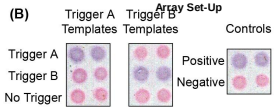
- Assay discriminates trigger sequences, based on template design
- Same set of colloids can be used to detect different trigger sequences

Format of overall assay:

- Two-stage EXPAR amplification: 1.5 to 3.5 min at 55°C, depending on desired detection limit
- Add colloids, incubate at room temperature for 2 min, spot onto plate

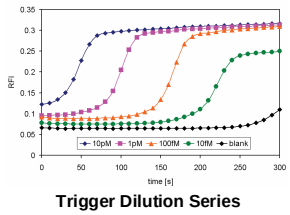
Overall assay time less than 10 min





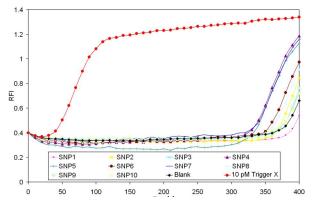
Eric Tan et al. "Isothermal DNA Amplification Coupled with DNA Nanosphere-Based Colorimetric Detection"; *Anal. Chem.* **2005**, *77*, 7984-7992

SNP Detection with EXPAR



Trigger Dilution Series

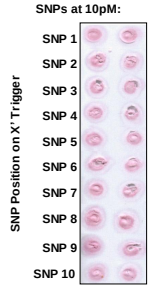
- EXPAR mastermix containing X'-X' template only, plus SYBR green dye
- Sigmoidal increase in fluorescence intensity: conversion of template from ss to the partially ds form
- Amplification time is dependent on concentration of trigger.
- Background amplification (no trigger X) observed for long times

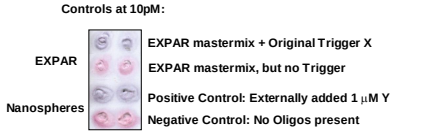


SNP Discrimination Assay

- SNPs were introduced at each position of the 10mer trigger oligonucleotide X
- Amplification using X'-X' template only, SYBR green dye and 10 pM of each trigger followed in real time
- Amplification of the original trigger X is faster than amplification of SNP-containing triggers and of the blank.

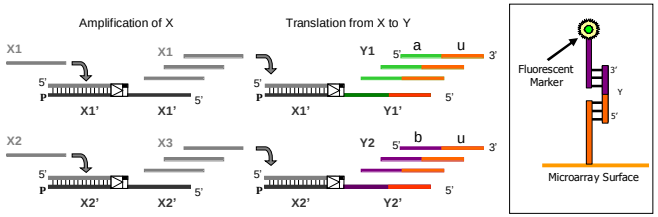
Results of two stage EXPAR plus colorimetric detection



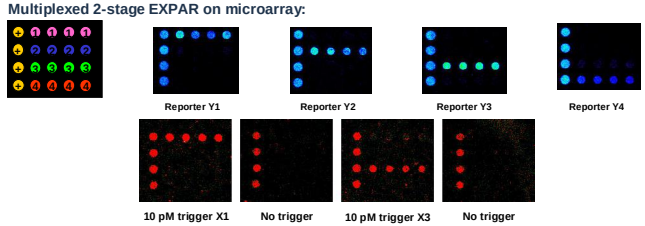


- Assay discriminates between original trigger X and all ten SNP-containing trigger sequences
- SNP variations of original trigger sequence will not result in a positive readout demonstrating the sensitivity of the assay.

Multiplexed 2-stage EXPAR on microarray



Multiplexed 2-stage EXPAR on microarray:



- Simultaneous amplification and detection of multiple sequences
- Uses existing microarray materials and techniques

EXPAR Detection through DNA Nanosphere Aggregation: Next Steps

- Optimize sensitivity of the assay: detection of atomolar DNA concentrations
- Generate specific oligonucleotide triggers from genomic DNA of pathogens
- Integrate trigger generation, EXPAR amplification and colorimetric detection into a closed system assay for point of care applications
- Expand to multiplexed surface based optical and electronic detection

Acknowledgements

Doris Nguyen, Yolanda Zhang, Barbara Erwin, Lori Van Ness, Jeff Van Ness, David Galas, Shenda Baker

Funding: NSF-NER Grant # 0304675; NSF-REU Grant# 0243910 (KGI); Keck Graduate Institute