Microarrays for Foodborne Virus Detection and Typing

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Microarrays for Foodborne Virus Detection and Typing

1. Description of colorimetric method for pathogen detection with low-density DNA microarrays

2. Description of microarray design for genotyping noroviruses

3. Validation of microarray specificity
DNA Microarrays for Pathogen Detection

DNA Microarrays:
- Sequence-specific probes are uniformly attached to a glass surface

Microarray Detection Methods:

Fluorescent Assays- Enzymatic incorporation of fluorescent dye during DNA amplification
  - Direct labeling with Cy3/Cy5 nucleotides
  - Indirect labeling with aminoallyl-dUTP and fluorescent dye
    - Inconsistent incorporation of dye-label
    - Expensive non-portable microarray scanners

Colorimetric Assays- Use a streptavidin-conjugated substrate with biotin-labeled target on the microarray
  - Silver staining
  - Alkaline phosphatase
    - Unstable reagents requiring controlled temperatures
    - Variable development time leading to overexposure
Photopolymerization: A New Method for Microarray Detection

Photoinduced Signal Amplification (ampliPHOX®)

Microarray Hybridization
- Attach probe sequences to slides
- Add single stranded biotin (●)-labeled DNA target

Microarray Labeling
- Add streptavidin (●)-labeled photoinitiator (P)
- Add monomer mix

Signal Amplification
- Colorless polymer forms after irradiating at λ=532 nm

Polymer Staining

Cooperative Research Agreement
InDevR, Inc., Boulder, Colorado, USA
Overview of Photopolymerization

Biotin-labeled targets hybridized on microarray (30-60 min)

1) Incubate with streptavidin-conjugated photo-label, 5 min
2) Wash, 1-2 min

Labeled microarray

3) Add monomer mix
4) Photoactivate, ~1 min
5) Stain, ~2 min
6) Image, ~1 min

- Has been estimated to cost $5 USD per assay.
- Uses a small and portable scanner that is $4000 USD, 15× more cost-effective than fluorescence scanners.

InDevR's ampliPHOX Detection System

InDevR, Inc., Boulder, Colorado, USA
- Designed probes to have >90% sequence similarity to target strains
  <75% sequence similarity to excluded strains
  - region B: two different 25-mer probes for genogroup identification
  - region C: 25-mer probe (S) and 35-mer probe (L) for genogroup typing

- Targeted region C probes for typing of NoV strains commonly associated with foodborne illness
Sample preparation for microarray analysis

RNA Sample
↓
Reverse transcription-PCR using biotinylated and phosphorylated primers
Region B: Mon431/Mon432/Mon433/Mon434 primers (Anderson, et al., 2003 J Inf. Dis.)
Region C: G1SKF/G1SKR; G2SKF/G2SKR primers (Kojima, et al., 2002 J. Virol. Methods)
↓
PCR product purification
↓
Enzymatic digestion of dsDNA
↓
Sample hybridization to microarray
↓
Microarray labeling and signal amplification (ampliPHOX assay)
Genogroup I Detection (Region B)

- **Genogroup I Detection** (Region B)
- **Array layout**

- **Polymer formed** where the GI-region B probes were spotted on the array when testing RNA from NoV GI strains
Genogroup II Detection (Region B)

Average SNR values

Genogroup II strain

Detection Threshold (SNR=3)

Polymer formed where the GII-region B probes were spotted on the array when testing RNA from NoV GII strains
Genogroup I Typing (Region C)

Average SNR Value

- **Gl.2**
- **Gl.3A**
- **Gl.3B**
- **Gl.4**
- **Gl.6A**

Probes

Array layout

- **Detection Threshold (SNR=3)**

Patterns of polymer formation correlated with the genotype of the NoV GI strains

- **Gl.2**
- **Gl.3A**
- **Gl.3B**
- **Gl.4**
- **Gl.6A**
The shorter (25-mer) region C-probes detected most GII strain types.
Conclusions

- Use of photopolymerization (ampliPHOX colorimetric method) with low density microarrays enabled the detection of NoV genogroups (GI and GII).

- Shorter probes (25-mer) allowed a more accurate genotyping of norovirus strains.

Future Studies

- Re-design probes to improve detection of GII.4 strains, targeting region D [3’-end of capsid gene (ORF2)].

- Test using biotinylated dNTPs to increase array signal detection.
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