# It's in the chips: Development of a microarray GeneChip approach to detect and type waterborne viruses

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## Environmental/ Public Health Issue

- In response to the 1996 Amendments to the Safe Drinking Water Act, the EPA has published a Contaminant Candidate List (CCL, see Table 1) to aid in setting priorities of unregulated chemical and microbiological contaminants in the Agency's drinking water program.
- Members of the *Norovirus* genus in the *Caliciviridae* family, are a genetically diverse group of viruses consisting of many distinct strains that cause acute gastroenteritis in humans of all ages.
- The Centers for Disease Control and Prevention report that noroviruses are the leading cause of non-bacterial gastroenteritis outbreaks in the United States.
- Microarrays provide a platform to identify norovirus strain types using DNA probes to sequences that are unique to each strain type.

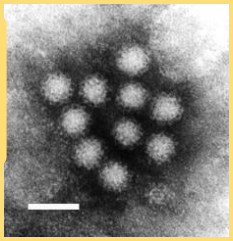


**Figure 1.** Transmission electron micrograph of Norwalk virus, the type strain for the genus Norovirus. Bar = 50 nanometers. Photo Credit: F.P. Williams, U.S. EPA

**Table 1. Drinking Water Contaminate Candidate List (Microbiological Contaminants)**

Acanthamoeba
Adenoviruses
*Aeromonas hydrophila*
Caliciviruses
Coxsackieviruses
Cyanobacteria (blue-green algae), other freshwater algae, and their toxins
Echoviruses
*Helicobacter pylori*
Microsporidia (*Enterocyotzoon* and *Encephalitozoon*)
Mycobacterium Avium Complex (MAC)

## Environmental Approach

- The Affymetrix GenFlex GeneChip (Figure 2) is a flexible array with the ability to hybridize 2000 nucleic acid sequences.
- Tag-probes are made so that the 5' end is complementary to a capture-probe sequence on the chip and the 3' end will bind to the sequence of the individual norovirus subtypes (Figure 3).
- Probes are labeled if their sequence anneals to the amplified viral sequences. The pool of probes (labeled and/or unlabeled) are then hybridized to their respective sequences on the chip (Figure 4).
- Labeled probes are detected (Figure 5) and their signal intensity quantified .

**Figure 2.** Affymetrix GenFlex GeneChip

**Figure 4.**
Flowchart of probe labeling and hybridization to the chip. A) RT-PCR amplifies norovirus templates ( ) for use with the tag-probes ( ) in a labeling reaction. B) At a specific annealing temperature, the tag-probes can anneal to the viral templates. C) If the tag-probe matches the viral template perfectly, the tag probe will be extended by a labeled nucleotide ( ). D) At the end of the labeling reaction, the tag-probes are pooled then added to the chip. E) The tag-probes hybridize to their respective capture-probes on the chip for detection.

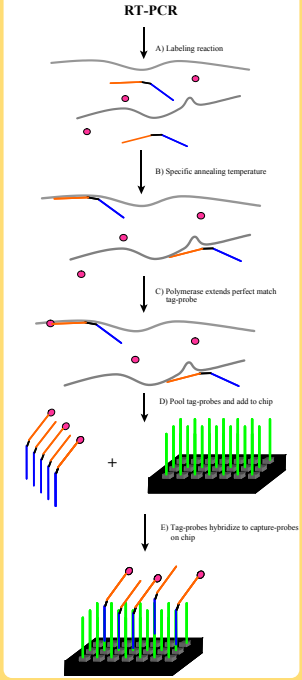



**Figure 3.**
Design of tag-probes for detection of norovirus strains. The probe sections in blue represent the complementary sequences to the capture-probes on the chip. The black regions represent a 4 base pair hinge to allow flexibility. The regions of other colors represent the sequences that match the individual norovirus strains.

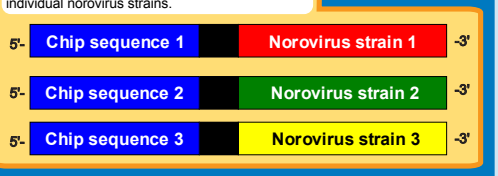
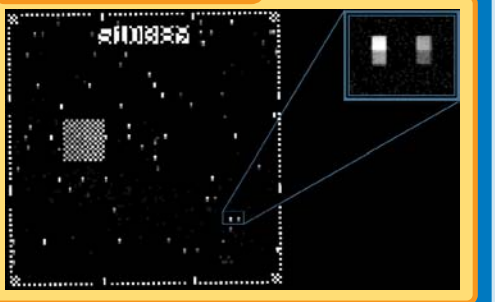



**Figure 5.** Results of scanned chip.



## Preliminary Data

- Table 2 shows the mean hybridization intensity values for two tag-probes designed to have a perfect match (5 and 225) and two tag-probes designed to have mismatches (214 and 226) to the sequence of our Norwalk virus reference strain.
- The perfect match tag-probes, 5 and 225, show a higher mean signal intensity value for the labeled tag-probes as compared to the unlabeled tag-probes.
- The mismatched tag-probes, 214 and 226, show no difference in mean signal intensity value between the labeled and unlabeled tag-probes.

| Table 2. | Hybridization Intensity Values (Mean ± standard deviation) | |
|---|---|---|
| | Control - unlabeled tag-probes | Experimental - labeled tag-probes |
| Tag-probe 5 | 5.5 ± 2.65 | 199.00 ± 29.51 |
| Tag-probe 214 | 4.0 ± 0.82 | 4.6 ± 2.19 |
| Tag-probe 225 | 5.0 ± 1.41 | 222.00 ± 26.51 |
| Tag-probe 226 | 2.67 ± 2.08 | 5.0 ± 2.0 |

## Potential Applications

- The ability to subtype noroviruses can provide useful information for:
  - Identifying the source of contamination in outbreak situations by comparing waterborne and clinical isolates.
  - Occurrence data regarding the genetic diversity of noroviruses circulating in the environment.
  - Assessing risk posed by these viruses (after virulence properties are determined).
- Since the genetic code is universal, this approach can be adapted for detection and identification of other viruses, bacteria, parasites, algae - all the microorganisms on the CCL - in a single analysis.