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Microarray Techniques for Evaluation of Genetic Stability of Live Viral Vaccines

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1. Introduction

Recent advances in biotechnology gave rise to a set of microarray technologies that became ubiquitous in research, medicine, and industry for tens of applications. Microarray technology has centered on providing platforms for analyzing, in a single experiment, tens or even hundreds of samples from different biologic sources. Its rapid and global adoption has been predicated on its simplicity and efficiency in quickly providing relevant data generated by simultaneous testing of biological samples with a large number of probes. In this chapter, we describe new microarray approaches that have considerably simplified the characterization of viral genes and genomes with specific emphasis on analysis of the genetic stability of live viral vaccines.

There are different types of vaccines that immunize against viruses: whole viral vaccines (either live attenuated or inactivated), subunit vaccines; purified or recombinant viral antigen vaccines, and DNA vaccines.

Genetic instability and plasticity of genomes are inherent properties of viruses, especially RNA viruses, with many profound implications for their replication, evolution, and pathogenesis. Because of the presence of a large number of mutants, populations of viruses are often described as quasispecies (Domingo et al., 1985; Hansen et al., 2004). Most mutants are present at a relatively low level, making them difficult to detect using conventional sequencing methods.

Genetic stability of live viral vaccines, including recombinant virus vaccines, is a key element of their safety and protective efficacy. Assessment of genetic stability is an important part of pre-licensure evaluation and quality control of a live viral vaccine, both during its manufacturing and after its administration. Spontaneous mutations easily emerge during viral replication and accumulation of mutants must be identified to ensure the safety of live vaccines.

To ensure maximum genetic stability and to optimize genetic structure of prospective live vaccine strains, it is important to identify the mutations that accumulate both during manufacturing and replication in vaccine recipients. Incorporation of mutations that increase virus fitness and do not affect its attenuation into the genetic makeup of the new vaccine strain may increase its potency and contribute to genetic stability

Most new viral vaccines are produced by propagation in cell cultures that do not necessarily represent the natural substrate for the virus, raising the possibility of introducing undesirable mutations in the course of virus adaptation. RNA viral vaccines mutate easily upon passage in cell cultures, which can change the phenotype (Amexis et al., 2001), leading to increased pathogenicity. That occurred with pSPBNGA-GA, a live rabies virus recombinant vaccine candidate, which was obtained via reverse genetics (Dietzschold et al., 2004). Additionally, it was demonstrated that some deletions in HIV-1 vaccine strains can evolve into fast-replicating variants by multiplication of remaining sequence motifs, and their safety is therefore not guaranteed (Berkhout et al., 1999), and the presence of even a small fraction of viral mutants in an oral poliovirus vaccine can have negative effect on its safety (Chumakov et al., 1991), suggesting that genetic consistency must be carefully monitored to ensure that accumulated mutants do not adversely impair the safety and efficacy of the vaccine.

In addition, vaccines have been recently developed to serve as live viral vectors expressing heterologous host genes. Examples of such live viral vectors include herpesviruses (such as pseudorabies and bovine herpesvirus type 1, and 2), poxviruses (Blancou et al., 1986; Fekadu et al., 1991; Taylor et al., 1991), human adenovirus 5, (Prevec et al., 1990) and flaviviruses (Arroyo et al., 2001; Monath et al., 1999; Pletnev et al., 2001; Pletnev et al., 1992; Pletnev et al., 2000; Pletnev and Men, 1998; Pletnev et al., 2002; Pletnev et al., 2006). The recombination in genomes of chimeric viruses probably plays an important role in the reduction of viral fitness that leads to attenuation. This creates a selective pressure to accumulate mutants that restore viral fitness by adapting heterologous genomic parts to each other, potentially leading to a loss of attenuation. The accumulation of mutations and genetic stability of flaviviruses were previously reported (Dunster et al., 1999; Laassri et al., 2011; Pugachev et al., 2004; Pugachev et al., 2002; Pugachev et al., 2007).

Of paramount importance is the need to demonstrate the genetic stability of the recombinant construct and confirm the fidelity of the heterologous gene inserted into the vector genome (WHO, 1990). An important consideration for the licensure and use of any genetically engineered live vaccine is the stability of the vector and that of the recombinant construct. In addition, genetic stability is an important safety concern, since predictions of vaccine behavior rely heavily on the knowledge of the genetic makeup of the recombinant. If a recombinant vaccine is to be useful, it should undergo no substantial mutation either during production of the vaccine by passage of working seed or after administration to the target species.

Therefore, it is essential to identify genomic loci that are prone to mutations and determine their phenotypes. If mutations in unstable genomic loci increase virulence, then methods to prevent their emergence and control their presence in vaccine preparations must be developed. On the other hand, if the fitness-restoring mutants do not lead to de-attenuation, then it may be desirable to incorporate them into the genetic makeup of the vaccine strain.

Additionally, increased yields of such viruses during vaccine production may help stabilize the genome by relieving selective pressure, thereby preventing random and potentially undesirable mutations from being passively selected through the "passenger effect".

Conventional assays of genetic stability of viruses by combined sequencing and sequence analysis are generally too insensitive to detect small proportion of mutant viruses in a quasispecies, and are laborious. Thus, sensitive high-throughput microarray techniques including microarray for resequencing and sequence heterogeneity (MARSH), microarray analysis of viral recombination (MAVR) assay, and microarray for quantitation of known virulent mutations (MQNVM) have been developed and applied as valuable tools to evaluate the genetic stability of live viral vaccines (Cherkasova et al., 2003; Laassri et al., 2011; Laassri et al., 2005; Laassri et al., 2007).

These microarray approaches allow large-scale full-genome mutational screening of live viral vaccines from various sources including cell culture, humans, monkeys, and mice. They can be used to improve quality control and to accelerate development of safer and more effective vaccines. The study of genetic stability also contributes to our understanding of live viral vaccine evolution.

2. DNA microarray: An overview

DNA microarray is a high-throughput hybridization technology used for quantitative and qualitative assessments of gene-expression, chromosomal aberrations, and mutations in molecular biology and biotechnology. It consists of an arrayed series of tens or thousands of micro-spots of oligonucleotides of specific DNA sequence, known as probes. Probes can be short regions of a gene or other DNA elements used to hybridize DNA or RNA samples (targets) under high-stringency conditions. Probe-target hybridizations are usually detected and quantified using fluorophore-labeled targets to determine the relative quantities of nucleic acid sequences in the target. Since an array can contain tens of thousands of probes, a microarray experiment enables analysis of many genes simultaneously. Therefore, arrays have dramatically accelerated many types of investigations.

In standard microarrays, the probes are attached via their engineered chemical group to a solid surface by a covalent bond to a chemical matrix (via epoxy - silane, amino-silane, lysine, polyacrylamide, or others). The solid surface can be glass, a silicon chip, or microscopic beads.

DNA microarrays can be used to measure changes in expression level, to detect and quantify single nucleotide polymorphisms (SNPs), to genotype and resequence mutant genomes, or to determine recombinant nucleotide sequences. Microarrays vary in fabrication, operating protocols, accuracy, and efficiency. Additional factors affecting microarray experiments are experimental design and methods for analyzing the data.

3. Microarray for resequencing and sequence heterogeneity

Several approaches based on hybridization of viral probes with oligonucleotide microarrays have been applied for rapid analysis of genetic variations during the microevolution of viruses. Microarray for resequencing and sequence heterogeneity (MARSH) was used to

identify mutations in vaccine viruses and their derivatives, revealing the degree of their evolutionary divergence and quantifying mutant genome proportions present.

The MARSH assay was based on the hybridization of fluorescently-labeled RNA produced from the virus genome with microarrays of oligonucleotide probes that are complementary to and cover the entire viral genome or specific genes. Quantitative comparison of hybridization data produced for a test sample with the data for homogeneous reference RNA reveal mutations that have emerged and accumulated during the replication of vaccine strains *in vitro* or *in vivo* (Figure 1).

MARSH (Figure 1) microchips were fabricated using a set of short oligonucleotides (T_m ~ 50°C) overlapping at half length, matching genomic sequences of virus strains, and covering a specific viral region of interest in the genome or the entire viral genome. Each oligonucleotide probe was synthesized with an aminolink group at its 5′ end for immobilization on a specific platform and purified after automated synthesis. Microarrays were printed on sialylated (aldehyde-coated) glass slides by using a contact microspotting robot equipped with a microspotting pin. Each oligonucleotide probe was spotted several times within a single microarray for redundancy to increase the reliability of results.

For RNA viruses, cDNA was prepared with reverse transcriptase using a specific reverse primer at the 3′ end region of the genome. Microarrays of immobilized oligonucleotide probes were hybridized with fluorescently-labeled RNA transcribed by T7 RNA polymerase from PCR-amplified viral cDNA. First, the viral genome was amplified using the specific primers (the reverse primer contains T7 promotor) to produce the needed DNA segments. RNA for hybridization was produced by *in vitro* transcription of the PCR products with a T7 RNA polymerase kit. Each RNA product (~10 μg) can be fluorescently labeled with a Cy3 RNA Labeling Kit. Labeled RNA samples were purified using spin columns.

Microarray hybridization was performed as follow: fluorescently-labeled RNA samples were vacuum-dried prior to hybridization, reconstituted in Hybridization Buffer, and denatured by incubation for one minute at 95°C. The final concentration of each fluorescent target in the hybridization solution should not exceed 0.1 μM. An aliquot of the hybridization mixture (~10 μl) was applied to the microarray area and covered with an individual plastic cover slip. Hybridization was performed in an incubation chamber for one hour at 45°C. Fluorescent images of processed microarray slides were captured using a ScanArray 5000, and the images were analyzed using ScanArray Express software.

In the experiments presented on Figure 1, each microarray contained 4 identical sub-arrays that were simultaneously hybridized in order to assess reproducibility of the hybridization results and to eliminate outlier data points. Hybridization signals from individual sub-array elements that differ from the average value were calculated for all 4 replicates of the oligonucleotide probes by more than two standard deviations were discarded; the number of such invalidated data points should not exceed 0.1%. Next, average values from the 4 sub-arrays were normalized by the total fluorescence signal from the entire array. Finally, normalized signals from the reference sample (homogeneous RNA) were divided by the respective normalized signals from the test samples, and the results were expressed as a fluorescence ratio (Figure 1C). Regions with no mutations should have ratios close to one, while test samples with mutations reduced hybridization with some oligonucleotide probes and therefore, produce ratios greater than one.

The MARSH assay was first used to analyze mutations that accumulate in the region coding for VP1, the most variable capsid protein of poliovirus (Cherkasova et al., 2003; Laassri et al., 2005). Later, this microarray approach was expanded to discriminate between vaccinia strains and to evaluate genetic stability of the vaccinia virus Ankara (MVA) B5R gene following propagation of a cloned isolate of MVA in Vero and MRC-5 cell lines (Laassri et al., 2007), to analyze the variability of the structural region of West Nile (WN) virus (Grinev et al., 2008), and to evaluate stability of the entire genome of a WN/Dengue 4 chimeric virus under study as a new candidate of WN vaccine (Laassri et al., 2011).

The MARSH microarray approach facilitates rapid analysis of viral genes and genomes, and circumvents traditional more laborious methods. With the microarray method, many samples can be analyzed simultaneously within a few hours. Furthermore, test samples do not need to be cloned, thus preserving the natural composition of viral gene populations. This method permits large-scale full genome screening of viral isolates, useful for epidemiological surveillance, vaccine quality control, and analysis of genetic changes in viruses that may occur in response to drug treatment.

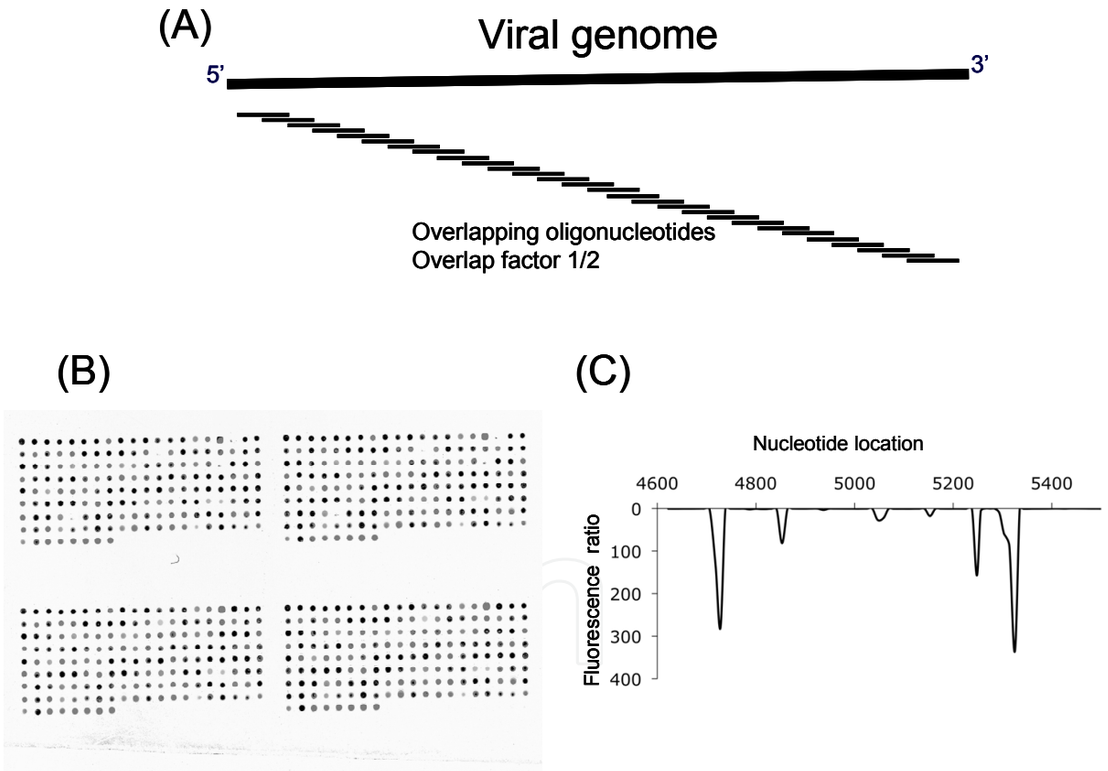

Fig. 1. Schematic overview of the MARSH assay: (A) Oligonucleotide microarray containing short oligonucleotides overlapped at half-length covering the entire viral genome. Each microarray has four identical sub-arrays hybridized at the same time. (B) Images of 4 individual identical sub-arrays hybridized with control sample. (C) Ratio of hybridization signals from reference and test sample preparations was plotted to reveal peaks indicating the presence of mutations. The hybridization images and the ratio plot are cartoons given for illustration purposes only.

4. Microarray analysis of viral recombination assay

Microarray analysis of viral recombination (MAVR) assay is, in essence, an extension of the genotype-specific oligonucleotide microarrays previously used to identify different viruses and bacteria (Laassri et al., 2003; Volokhov et al., 2003). The MAVR assay was developed to detect recombinations between the three serotypes of oral poliovirus vaccine (OPV) (Cherkasova et al., 2003; Laassri et al., 2005). The locations of oligonucleotide spots within the poliovirus MAVR microarray produce an image that graphically reveals genomic recombination patterns and crossover regions (Figure 2).

The MAVR microarray was composed of genotype-specific oligonucleotide probes selected to identify Sabin strains of OPV (GenBank accession nos. AY184219–AY184221); the selected sequences were spaced ~150 bases from each other in the viral genome, they contain a moderate amount of GC (T_m between 41 and 57°C), spots were printed in three rows according to their location in the genome. Each slide accommodates 5 individual microarrays for MAVR microarrays. Microarrays of immobilized oligonucleotide probes were hybridized with fluorescently-labeled viral cDNA prepared with hexanucleotide random primers and Superscript III reverse transcriptase. Each viral cDNA product (10 μg) was fluorescently labeled with a Cy3 RNA Labeling Kit and purified using spin columns. Hybridization between microarray oligonucleotide probes and fluorescently-labeled cDNA was performed as follow: vacuum-dried fluorescently-labeled cDNA samples were reconstituted in Hybridization Buffer and denatured by incubation for one minute at 95°C. A 10-μl aliquot of the hybridization mixture was applied to the microarray area and covered with an individual plastic cover slip. Hybridization was proceeded in an incubation chamber for two hours at 45°C. Fluorescent images of processed microarray slides were captured using ScanArray 5000. Any recombination in the analyzed viruses was detected as a change of fluorescent patterns of spots in the rows (clinical sample, Figure 2).

One potential caveat regarding MAVR analysis is that it can positively identify recombinations only in regions derived from strains represented on the microarray. If one recombination partner is unknown, the microarray reveals a "gap" or an irregular pattern, calling for nucleotide sequencing as a tool of last resort. Alternatively, conserved oligonucleotide probes with broader specificity might be included in MAVR microarrays to tentatively identify the origin of "orphan" genomic segments.

MAVR mapping of poliovirus genomes has an advantage over the more detailed complete nucleotide sequencing because it can determine more than one recombinant in the same samples without a need for cloning and has an extraordinary throughput. Restriction fragment length polymorphism (RFLP), also used for this purpose, is more time-consuming and less informative than MAVR. In addition, MAVR analysis allows the genotyping of naturally heterogeneous populations. For example, the MARV analysis of a clinical sample (coded 18058) (Laassri et al., 2005) revealed a mixture of poliovirus strains composed of at least two and probably several more different types of recombinants. MAVR combined with cDNA preparation, coping of viral genome directly from clinical specimens (Laassri et al., 2005), opened the possibility of studying natural heterogeneity of viral populations *in vivo*.

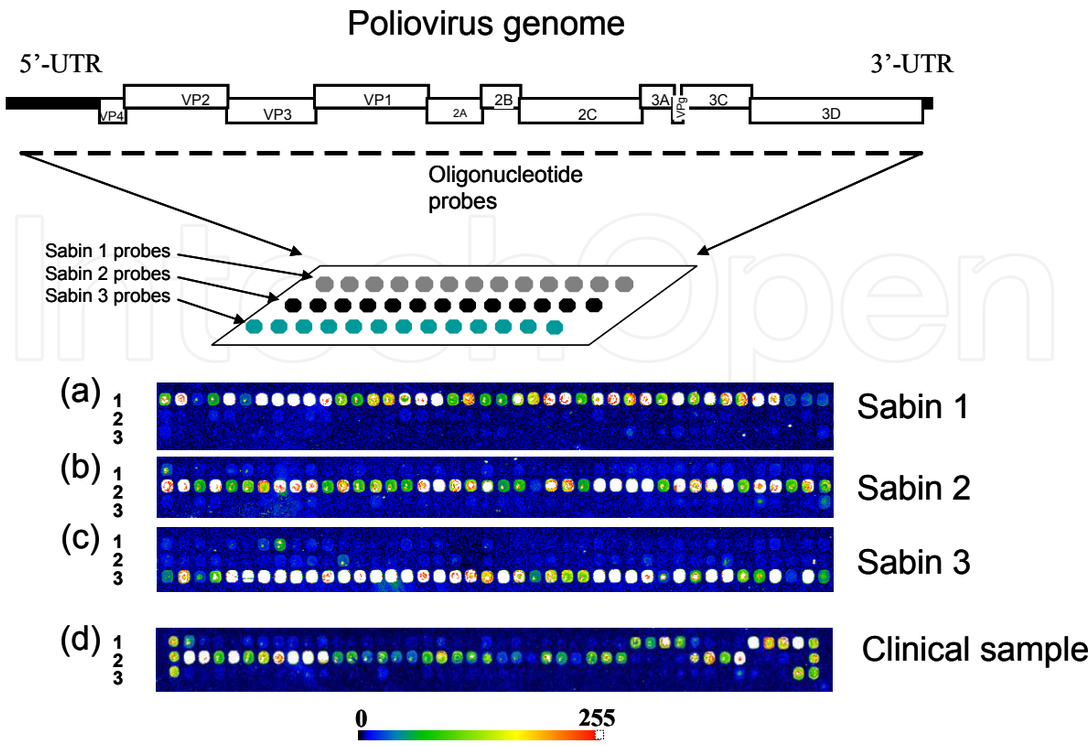

Fig. 2. MAVR analysis of the genome structure of poliovirus. Three rows (coded 1, 2, and 3 in the left of microarrays) of oligonucleotide probes in each microarray are specific to 3 serotypes of poliovirus were spotted according to their location in the genome. Sample names are shown on the right. (a); Hybridization pattern of Sabin 1 genome with the MAVR microarray, (b); Represent the hybridization pattern of Sabin 2 genome with the MAVR microarray, (c); Hybridization pattern of Sabin 3 genome with the MAVR microarray, (c); Hybridization pattern of poliovirus genome extracted from a clinical sample obtained from acute flaccid paralysis (AFP).

5. Microarray assay for quantitation of known virulent mutations

Microarray assay for quantitation of known virulent mutations (MQNVM) was developed to quantify the virulent mutations in the genomes of the three serotypes of oral poliovirus vaccine (OPV) isolated from clinical specimens (Laassri et al., 2005; Laassri et al., 2006).

Sabin strains of OPV mutate rapidly *in vitro* and *in vivo*. Some of these mutations are direct reversions to the alleles of wild-type progenitors of the vaccine strains, whereas others are second-site suppressors of the attenuated phenotype or are incidental changes. Among the best characterized attenuating mutations in the OPV Sabin strains are mutations located in the Internal Ribosome Entry Site (IRES) of the 5'-untranslated region (5'-UTR) (Minor, 1992) (Figure 3). These mutations have been identified in Sabin type 3 poliovirus (472U→C) (Cann et al., 1984), as well as type 2 (481A→G) (Macadam et al., 1993), and type 1 (480G→A and 525U→C) (Otelea et al., 1993); they are believed to selectively affect initiation of translation of viral polyprotein in neuronal cells (Guest et al., 2004; Svitkin et al., 1990). Previously was shown that the content of these revertants

was low in vaccine batches that failed the monkey neurovirulence test (Chumakov et al., 1991). Sensitive mutant analysis by PCR and restriction enzyme cleavage (MAPREC) method is used to monitor the quantity of neurovirulent revertants in batches of oral poliovirus vaccine (Chumakov et al., 1991). However, the method is relatively labor-intensive, and is not amenable to analysis of a large number of clinical samples that is needed for studies of genetic stability of vaccine viruses *in vivo*. Therefore, the development of high throughput methods to quantitate revertants in attenuated poliovirus remains a high priority for evaluation of existing and new vacines.

Recently, an MQNVM assay (Figure 4A) was created to identify and quantitate the 4 known reversions in the 5′-UTR of the 3 poliovirus strains (mutations located at nucleotides 480 and 525 for Sabin type 1, nucleotide 481 for Sabin type 2, and nucleotide 472 for Sabin type 3); the assay has been described in detail elsewhere (Laassri et al., 2005; Laassri et al., 2006).

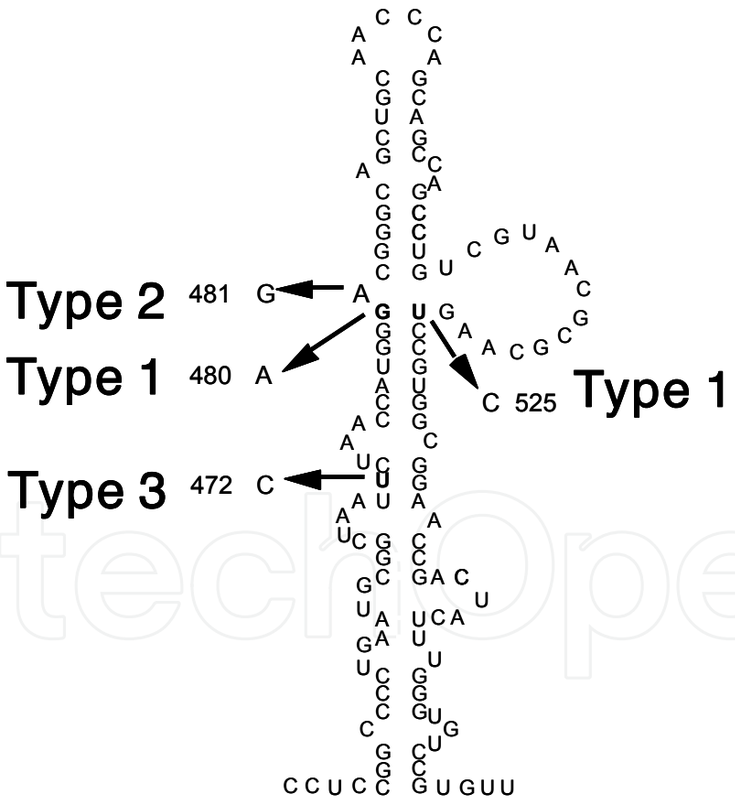

Fig. 3. Locations of the most important primary attenuating mutations of Sabin poliovirus in the IRES region of 5′-UTR of the genome. There are two known attenuating mutations for Sabin 1 (at nucleotides 480 and 525), one for Sabin 2 (at nucleotide 481) and one for Sabin 3 (at nucleotide 525).

To prepare viral genomes directly from stool samples and to quantitate the reversions by MQNVM, 1 g of frozen stool was suspended in 10 ml of Dulbecco's PBS, vortexed, centrifuged, and supernatants aliquoted and stored at -70°C. Viral RNA was isolated from a total of 140 µl of stool supernatant. The extracted RNA was eluted in a final volume of 60 µl of sterile RNase-free water.

For viral cDNA preparation, 10 µl of RNA was added to a reaction mixture containing 1 mM dithiothreitol, 2.5 µg/ml concentrations of each primer (A7-sabin1, 3 and A7-sabin2 (Laassri et al., 2005)), 0.5 mM dNTP mix, and 1x first-strand RT buffer. The final volume of the reaction mix is 50 µl. The mixture was heated for 5 min at 65°C and then quickly chilled on ice. Superscript II reverse transcriptase (12 U/µl) was added to the mixture and incubated for two hours at 42°C, then additional Superscript II reverse transcriptase (4 U/µl) was added, and the mixture incubated for another 3 hours at 42°C.

Full-length poliovirus genome from stool specimens was amplified by PCR (Laassri et al., 2005). The reaction was performed with an XL-PCR kit. The viral full-length amplicons obtained from this PCR amplification were used for MQNVM analysis.

The MQNVM microarrays (Figure 4A) contain 10 spots of oligonucleotide probe for a specific Sabin strain and 10 spots of oligonucleotide probe specific to the revertant virus. They also contain two oligonucleotides specific to a conserved region as control. Each control oligonucleotide was spotted 5 times in the last row. The redundant spotting of oligonucleotide probes was used to improve the quantitation accuracy. Ten individual MQNVM microarrays were spotted on each slide. Hybridization probes are single-stranded DNA (ssDNA) prepared by asymmetric PCR (Laassri et al., 2005). The ssDNA was purified with a PCR purification kit, and diluted in 50 µl of water. Aliquots containing 0.2 µM were labeled with a Cy5 or Cy3 RNA Labeling Kit and purified using spin columns.

Several microarrays spotted on the same slide were simultaneously hybridized for 30 min at 45°C with fluorescently-labeled ssDNA samples prepared from the reference Sabin strain, reference Sabin revertant (or wild-type poliovirus), and one or more test strains. The microarray was then washed for 2 min in 2x standard saline citrate (SSC) with 0.1% sodium dodecyl sulfate (SDS), followed by one min in 2x SSC.

Microarray images were taken with confocal fluorescent scanner ScanArray 5000 equipped with green and red HeNe lasers (543 nm and 632 nm that excite Cy3 and Cy5, respectively). Images were then analyzed using QuantArray software. The values obtained from MQNVM microarrays were normalized, and the percentage of reversion was calculated by dividing the normalized signal from each revertant oligonucleotide probe by the total signal (signal obtained from both revertant and vaccine oligonucleotide probes). The values obtained from 10 replicates of each oligonucleotide probe (vaccine, revertant) were averaged, and the standard deviation calculated.

To study the linearity of MQNVM assay to quantify mutants, another candidate vaccine virus such as West Nile (WN)/Dengue 4 chimeric virus, was used. An MQNVM microarray was developed as described above to analyze the mutation 2337G→C in West Nile/Dengue 4 virus (Laassri et al., 2011), and samples to contain different percentages of the mutant were spiked and analyzed by MQNVM assay, the detected percentages of mutants were plotted against the expected percentages of mutants (Figure 4B). Results demonstrate that mutant quantitation by MQNVM assay is linear, indicating that this assay is suitable to quantitate mutants.

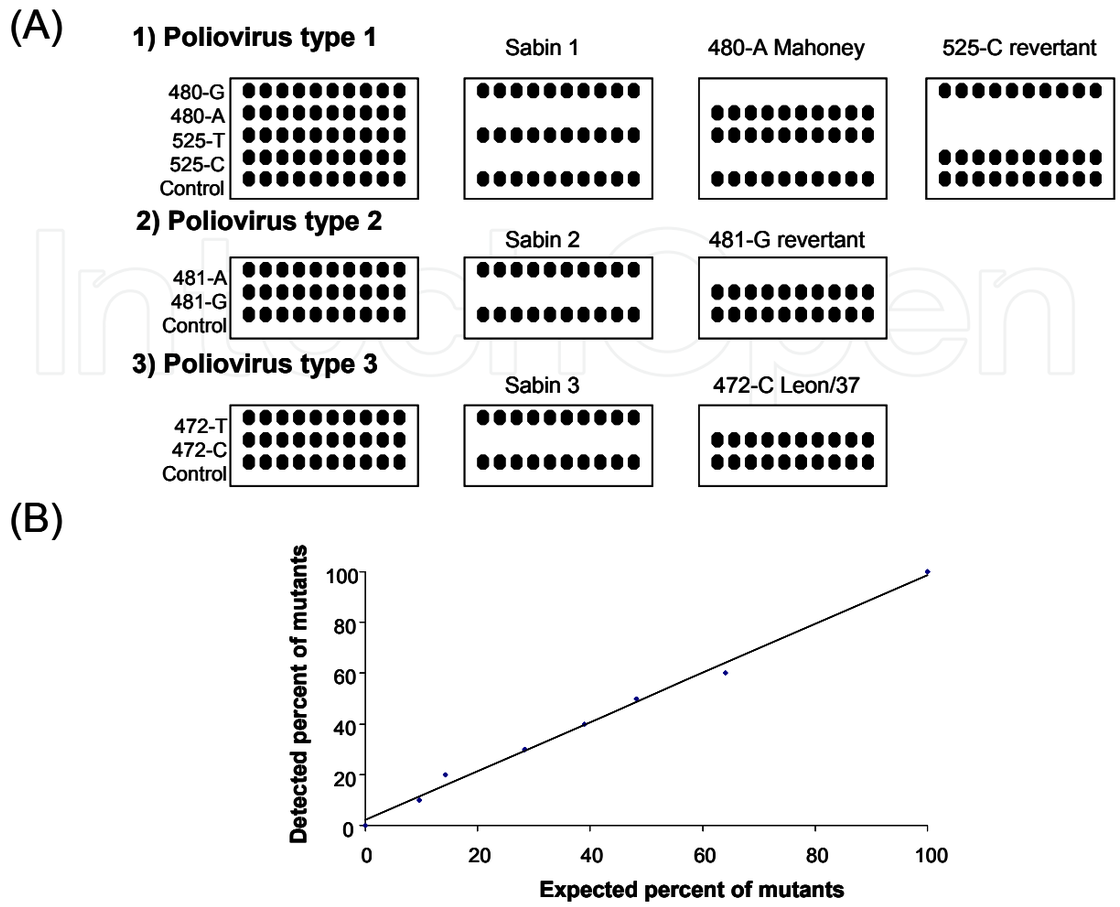

Fig. 4. Layout of a microarray for quantitation of known virulent mutations (MQNVM) in Sabin strains, the hybridization pattern of Sabin strains and their revertants, and the linearity of mutants quantitation with MQNVM assay.

(A): 1) Detection of the 480G→A and 525U→C revertants in the Sabin strain type-1 of poliovirus. The first microarray shows the layout of oligonucleotide probes: 10 spots each of 4 allele-specific oligonucleotide probes were spotted into the top 4 rows; the bottom row contains 5 spots each of a universal oligonucleotide probe and of a Sabin1-specific oligonucleotide probe. The second, third and fourth microarrays show patterns of hybridization of, respectively, Sabin 1, Mahoney (wild-type poliovirus type 1), and revertant strain 11262 (poliovirus type 1). 2) Detection of 481A→G revertants in Sabin 2 strain. The first microarray shows the layout of oligonucleotide probes: 10 spots each of two allele-specific oligonucleotide probes were spotted onto the top 2 rows; the bottom row contains 5 spots each of universal oligonucleotide probe and Sabin 2-specific oligonucleotide probe. The second and third microarrays show patterns of hybridization of, respectively, Sabin 2 strain, and revertant strain 154 (poliovirus type 2). 3) Detection of the 472U→C revertants in Sabin 3 strain. The first microarray shows the layout of oligonucleotide probes: 10 spots each of two allele-specific oligonucleotide probes were spotted into the top two rows; the bottom row contains 5 spots each of universal oligonucleotide probe and Sabin3-specific oligonucleotide probe. The second and third microarrays show patterns of hybridization of, respectively, Sabin 3 strain, and Leon/37 (wild type poliovirus type 3). (B): Evaluation of the

linearity of a quantitative MQNVM assay. Samples containing different amounts of WN/Dengue 4 virus 2337G→C mutant were analyzed by MQNVM assay. The results were plotted as observed versus the expected mutant contents. This result shows that mutants quantitation with MQNVM assay is linear with R-squared value (R^2) equal 0.99.

Also, MQNVM assay was used to characterize poliovirus in about 300 stool specimens obtained from children vaccinated with different combinations of OPV and inactivated polio vaccine (IPV) (Laassri et al., 2005; Laassri et al., 2006). The PCR-amplified viral cDNA prepared directly from the stool specimens was used to quantitate reversions in the 5′-UTR of each of the 3 poliovirus serotypes. Fluorescently-labeled ssDNA for hybridization was prepared form each poliovirus serotype as described above and elsewhere (Laassri et al., 2005). Results of our study (Laassri et al., 2006) show that many stool samples from healthy children one week after OPV vaccination contained different percentages of revertants, consistent with earlier observations based on conventional methodology (Cann et al., 1984; Kew et al., 2002; WHO, 2002). The oligonucleotide microarrays simultaneously detected and discriminated between vaccine and revertant sequences and allowed the quantitation of reversions in the 5′-UTRs of all 3 serotypes of poliovirus.

6. Conclusion

Microarray technology is a sensitive and versatile method for genetic analysis that allows screening of mutations in genetic materials and readily detecting single-point mutations. Viral nucleic acid hybridization with immobilized oligonucleotides in microarrays that encompass thousands of individual probes offers a rapid method suitable for simultaneous analysis of a large number of markers distributed over the whole viral genome. The technique generates instant genetic maps of mutant strains and reveals evolutionary divergence and mutational profiles of individual viral stocks.

The simplicity and high throughput of microarray-based analyses might also assist in improving genetic stability of candidate vaccine strains by incorporating mutations conferring better replicative properties. They also facilitate monitoring of molecular consistency in a new viral vaccine during its manufacturing. The same approach can be applied in future development of new live viral vaccines and used as a new paradigm for better quality control tests of vaccines against other pathogens.

The oligonucleotide microarrays described in this chapter have already facilitated the analysis of the genetic diversity of viruses and live virus vaccines at the levels of genomic recombination, nucleotide sequence heterogeneity, and quantitation of single-point mutations. They facilitate rapid analysis of viral genes and genomes, circumventing traditional methods that usually involve much more laborious efforts. Microarray methods can analyze a very large number of samples simultaneously, within few hours. Furthermore, cloning of nucleic acids is not required, thus preserving the natural genomic composition of viral gene populations. Microarray methods open the possibility of a large-scale full-genome screening of viral isolates needed for improved epidemiological surveillance and better vaccine quality control; for example, MQNVM microarrays rapidly, simultaneously, and unambiguously identified viral vaccines and their revertants and quantified the amounts of single-point mutations.

Unlike direct DNA sequencing, the MARSH assay determines only the approximate location of mutations within a single oligonucleotide probe. However, this limitation has the

advantage of increasing the sensitivity of detecting genomic changes by microarray, since it reveals several adjacent mutations on different molecules, even mutations present in quantities too low to detect by conventional sequencing (Cherkasova et al., 2003).

Besides having a high throughput capacity the MARV assay easily demonstrates naturally heterogeneous viral populations, even in the same sample, without the need to separate or clone them.

Microarray-based assays for genetic stability of live viral vaccines should greatly assist in evaluating safety. The information obtained from such microarray methods will not only expedite regulatory review of the prospective recombinant vaccines but also provide a method suitable for monitoring consistency of vaccine production as part of routine quality control. Microarray techniques also offer the possibility for a large-scale full-genome screening of viral isolates to improve epidemiological surveillance, and better vaccine quality control.

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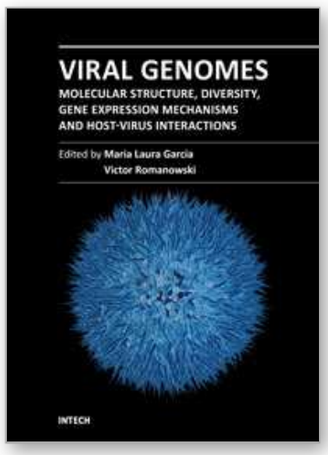

Viral Genomes - Molecular Structure, Diversity, Gene Expression Mechanisms and Host-Virus Interactions

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Viruses are small infectious agents that can replicate only inside the living cells of susceptible organisms. The understanding of the molecular events underlying the infectious process has been of central interest to improve strategies aimed at combating viral diseases of medical, veterinary and agricultural importance. Some of the viruses cause dreadful diseases, while others are also of interest as tools for gene transduction and expression and in non-poluting insect pest management strategies. The contributions in this book provide the reader with a perspective on the wide spectrum of virus-host systems. They are organized in sections based on the major topics covered: viral genomes organization, regulation of replication and gene expression, genome diversity and evolution, virus-host interactions, including clinically relevant features. The chapters also cover a wide range of technical approaches, including high throughput methods to assess genome variation or stability. This book should appeal to all those interested in fundamental and applied aspects of virology.

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