Influenza Genome Sequencing Project: Reagent Request Pre-Proposal

Date of Pre-Proposal (MM/DD/YY): ____June 9, 2009_____
Reagent Request: Sequence selected genomes of measles, mumps, rubella, varicella and rotaviruses_____
Purpose/Objective:

Currently there are major gaps in knowledge concerning the genetic characteristics of viral agents that are controlled by vaccination. In many cases, only partial genomic sequences are available and these are from well characterized laboratory strains. In addition, there is little information available about the genetic properties of currently circulating strains. CDC proposes to sequence the genomes of selected Vaccine Preventable Disease agents to close this gap in information. Obtaining such genetic information will facilitate the monitoring of vaccine safety and stability, studies of transmission, vaccine development, and disease pathogenesis. Complete sequences for these organisms will help inform the development of improved or new diagnostic assays to diagnose disease in vaccinated individuals and to differentiate between vaccine-associated disease and natural infection. These sequences will also be useful in studies of virus nomenclature and tracking the origin of viruses.

Measles and mumps: Though the nomenclature for describing the genetic characteristics of wild-type measles viruses was established over 10 years ago, in many cases, only partial genomic sequences are available from each of the recognized genotypes. Complete genomic sequences are available from only a few well characterized laboratory and vaccine strains. There is very little complete genomic information available for the genotypes currently in circulation. There are even fewer genomic sequences available for mumps virus and only partial sequences are available for viruses that have been proposed as reference strains. To close this gap in information, CDC will sequence the complete genomes of selected measles and mumps strains from the genotypes that are currently associated with the greatest number of cases globally.

Rotavirus: To develop improved/new genotyping assays for monitoring the U.S. rotavirus vaccine program, a genomic sequence database of currently circulating rotavirus strains is needed to discriminate the genes of common wild-type rotaviruses from new vaccine strains. We propose to conduct genomic sequencing of common strains collected during routine surveillance from 1996-2009 to provide an improved dataset for assay development. Over time, these studies will also help assess the impact of new rotavirus vaccines licensed since 2006 on rotavirus evolution and the emergence of strains that could evade vaccine immunity.

Rubella: Partial genome sequences of rubella reference viruses form the basis for the nomenclature of wild-type rubella viruses that is crucial for tracking viruses for epidemiologic purposes. As new strains are discovered, the nomenclature must be modified to accommodate them. Complete genomic sequences of the reference viruses will allow the modification/expansion of the nomenclature. In addition, viruses of some genotypes are widely distributed and subdividing them into groups useful for tracking the origin of viruses will require more sequence information (whole genomes) of a number of viruses in these widely distributed genotypes.

Varicella: Seven genotypes of varicella-zoster virus (VZV) are currently circulating in the world and, in common with other viral agents, have distinctive geographic distributions. Two or more complete genomic sequences have been determined for only three of those genotypes at this time, a single complete sequence is available for two more, and two provisional genotypes have no complete sequences available to date. Genotypic analysis of VZV isolates has already proven important in a number of varicella outbreak investigations, and filling in the gaps (at least two complete genome sequences for all 7 genotypes) is expected to provide fundamental insights into the

evolutionary history of VZV. In addition, we recently identified and characterized two isolates from cases of zoster in vaccinated children that proved to be recombinants between vaccine and wild-type virus. This phenomenon could reasonably be expected to become more common with the introduction of the zoster vaccine, which is administered to persons known to have latent infections with wild-type VZV.

Principal Investigator:

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Organization/Company:

Name:	Centers for Disease Control and Prevention
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Description of Reagents Available for Sequencing:

- Number of Isolates: 10 measles, 1 varicella, 16 rubella, 22 rotavirus

- Subtype: **rotavirus**, G1P[8], G2P[4] , G3P[8], G9P[8], G12P[8]
- Host: Rotavirus: humans
- Collection Date: Rotavirus, each year from1996-2009
- Storage Method: -80C
- Amplification Method: Rotavirus, RT-PCR

- Subtype: **measles** 10 samples will include representatives of genotypes B2, B3, D4, D5, D6, D8, D9, G2, G3, H1
- Host: Humans
- Collection Date: from 1/1/00 to 6/9/09
- Storage Method: -80 C
- Amplification Method: long RT-PCR, RT-PCR

- Subtype: **mumps** (genotypes to be determined)
- Host: humans
- Collection Date: from 1/1/00 to 6/9/09
- Storage Method: -80 C
- Amplification Method: long RT-PCR, RT-PCR

- Subtype: **varicella**
- Host: human
- Collection Date: 2004 isolate Spain 4242 (genotype M4)
- Storage Method: -80C
- Amplification Method: long PCR, 10 approximately 14Kb amplimers with overlaps

- Subtype: **rubella virus.** Clade 1 [11], Clade 2 [5] Total of 16 viruses.
- Host: rubella virus: humans
- Collection Date: 1985-2009
- Storage Method: short and long term storage at -70 C
- Amplification Method: RT-PCR

Expectations for Collaboration: (please include: (a) number of isolates to be processed during project period; and (b) rate of processing; i.e. – how many isolates per month can collaborator ship to TIGR)

1. Rotavirus: ship >=11 Genomic RNA extracts/month (22 total)
2. Varicella (currently working on protocol for template generation)
3. Measles: ship 3/month (10 total)
4. Rubella virus; ship 4/month (16 total)

Mumps would be added in year 2 if project funds available

Agree to NIAID's Microbial Sequencing Center Data Release Policy:
Yes ___X_____ No _____

Principal Investigator Signature:

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