Genetic diversity of Hepatitis C virus within a chronically infected HCV patient

Shanann Hoyos[1], Jayme Parker[1, 2], and Jack Chen[1, 2]

[1]Department of Biology and Wildlife, Institute of Arctic Biology, University of Alaska Fairbanks, and [2]Alaska State Public Health Virology Laboratory

Introduction

The global epidemic of hepatitis C virus (HCV) is among the leading causes of preventable death worldwide. In the United States, an estimated 2.7 to 2.9 million people have chronic HCV infection [1].

As a positive stranded RNA virus, HCV has high mutation rates and can be classified into six major genotypes and approximately 80 subtypes. HCV shows a genetic diversity even in one individual.

Circulating Hepatitis C virus (HCV) genotypes in Alaska have not been characterized. Genotyping HCV isolates is of specific clinical interest because genotyping is a significant predictor of the response to antiviral therapy and serves as a guideline for therapy duration.

Objective

To examine hepatitis C virus (HCV) genome variation within a single patient by targeting two conserved regions for genotypic sequencing and phylogenetic analysis.

Model

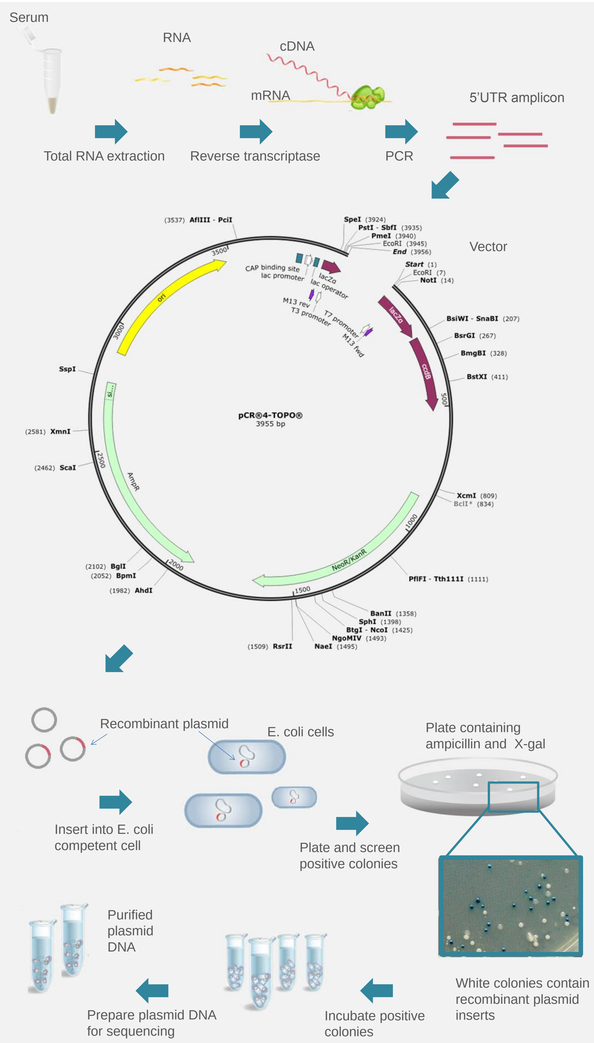
HCV viral genome is approximately 9.6 kb in length. The two regions selected for genotyping are the 5'untranslated region (UTR), a highly conserved region, and non-structural 5B (NS5B); both are often used for genotyping analysis.

Figure 1 Genotyping region of HCV: the most suitable region for genotyping are the 5'UTR, Core (C), E1 and NS5B [3]. In this study, target regions are the 5'UTR and NS5B region.

Methods

Serum from a chronically infected HCV-naïve patient was collected from the Alaska State Virology laboratory, from which multiple hepatitis C virus sequences covering the 5' untranslated region (UTR) region were sequenced and aligned.

Figure 2 Schematic of methodology: (i) RNA extraction, (ii) cDNA synthesis, (iii) cloning, and (iv) plasmid DNA preparation for sequencing [4,5].



Preliminary Results

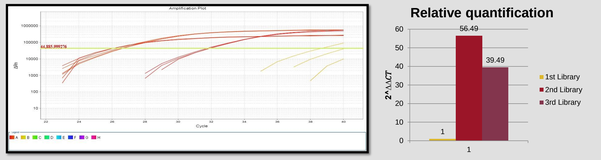


Figure 3 Quantitative real time PCR amplification plot comparing three cDNA libraries. With respect to threshold cycle (CT=26.59), we evaluated efficiency of our cDNA library (3rd Library), which was 39x more effective than the reference library.

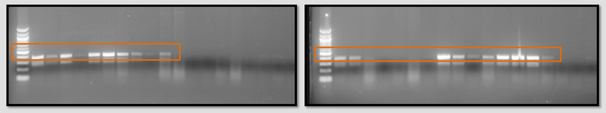


Figure 4 M13 PCR was utilized to screen colonies indicating positive plasmid insertion following TA cloning. A 216-bp fragment covering the 5'UTR region was amplified and cloned, generating several hundred TA colonies. Expected fragment size was 372 bp, which is indicated above.

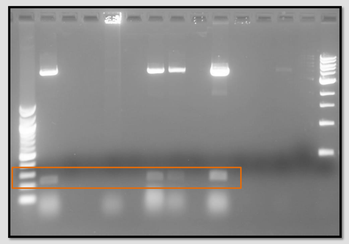


Figure 5 EcoR1 restriction enzyme digestion was employed following plasmid DNA preparation to verify purification and insert (216 bp).

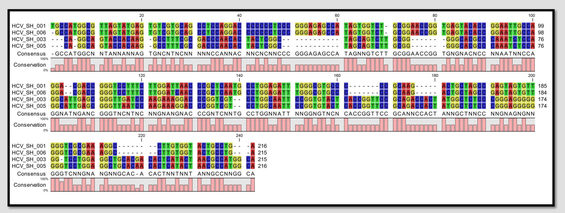


Figure 3 Alignment of partial HCV 5'UTR sequences. Four amplicons in the 5'UTR (216 bp) region were subject to Sanger Sequencing. Sequence data were aligned using CLC workbench software. Consensus between sequences 1 and 6 are nearly 100%. A similar relationship is observed between sequences 3 and 5. However, overall consensus is approximately 50%. Identity sequences were compared using the basic local alignment search tool (BLAST) database, which provided 98-100% shared identity to subtype 1a.

Discussion & Future Direction

We have begun aligning individual sequence data obtained from a fragment covering the 5'UTR region.

Results currently indicate that there are two primary sequence variations with about 50% conservation. This suggests high levels of HCV genetic diversity within this patient sample.

Future Direction

Our goal is to complete screening and alignment of the 5'UTR region and repeat the process at the NS5B region to obtain a more comprehensive representation of HCV genome variation. Once alignment is complete, a phylogenetic tree analysis can be conducted.

We aim to utilize next-generation sequencing technology to sequence the patient HCV genome as a comparison to our phylogenetic analysis.

Reference

1. Ward. *Topics in Antiviral Med*. 2013, *21,* 15.
2. Raju et al. *Int.l J. of Adv. Research*. 2013, *1*, 439.
3. Abbott Laboratories. In *Abbott Mol*. 2014.
4. Create a cDNA library. 2014, www.sithomas.net
5. PCR4-TOPO. 2014, www.snapgene.com/resources

Acknowledgements

This research was supported in part by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number P20GM103395 and by an equipment grant of Alaska State Public Health Laboratories. The clinical sample was collected and diagnosed in Alaska State Public Health Virology Laboratory (ASVL). I would like to thank Jack Chen and Jayme Parker for all of their support.

Contact Information

Shanann Hoyos
sehoyos@alaska.edu

UNIVERSITY OF ALASKA FAIRBANKS