

# **Identification of Hepatitis B Virus Genotypes in the Filipino Population Using Restriction Fragment Length Polymorphism**

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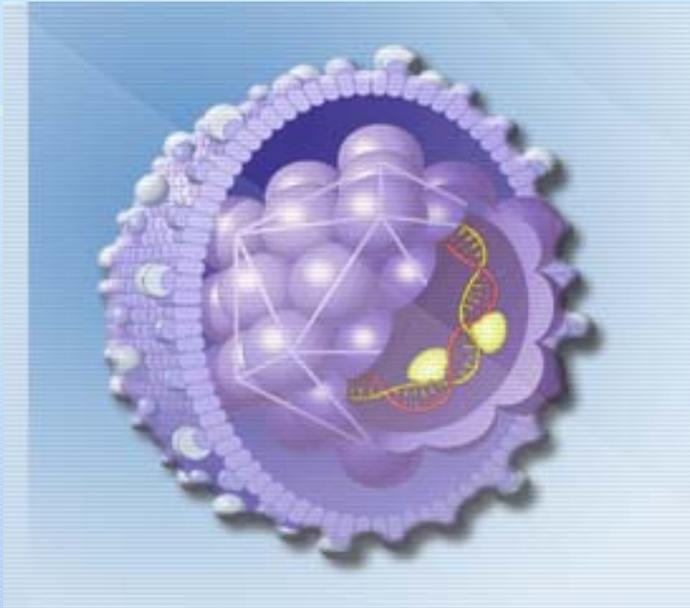
# Presentation Outline

- Introduction
- Materials and Methods
- Results and Discussion
- Conclusion

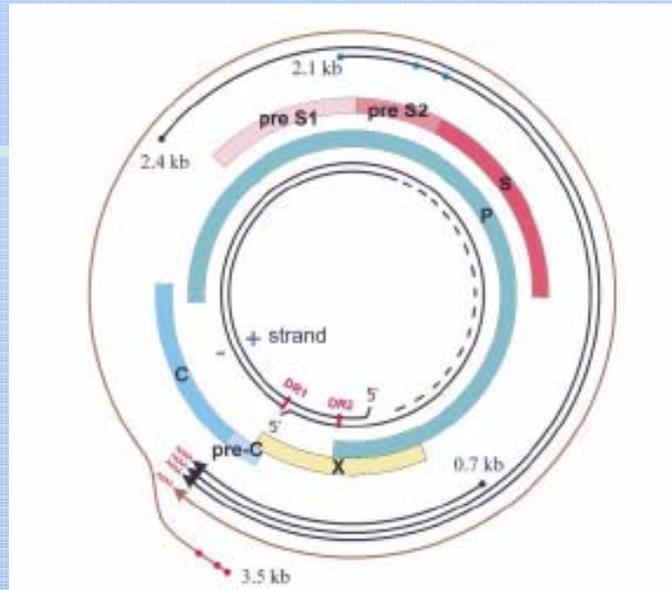
# Introduction

- 350 million people are infected with hepatitis B virus (HBV) worldwide
- HBV infections develop to chronic hepatitis, cirrhosis and hepatocellular carcinoma
- HBV is endemic in Southeast Asia
- Causes 90% of liver cancer in the Philippines

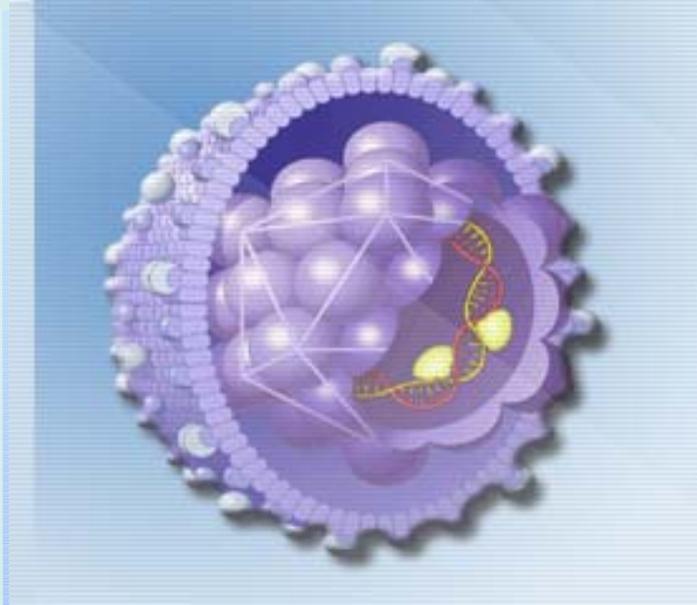
# Hepatitis B Virus



- Family *Hepadnaviridae*
- Partially double-stranded, circular DNA
- Genome size: 3.2 kb
- With 4 partially overlapping ORF's
- Hepatotropic virus

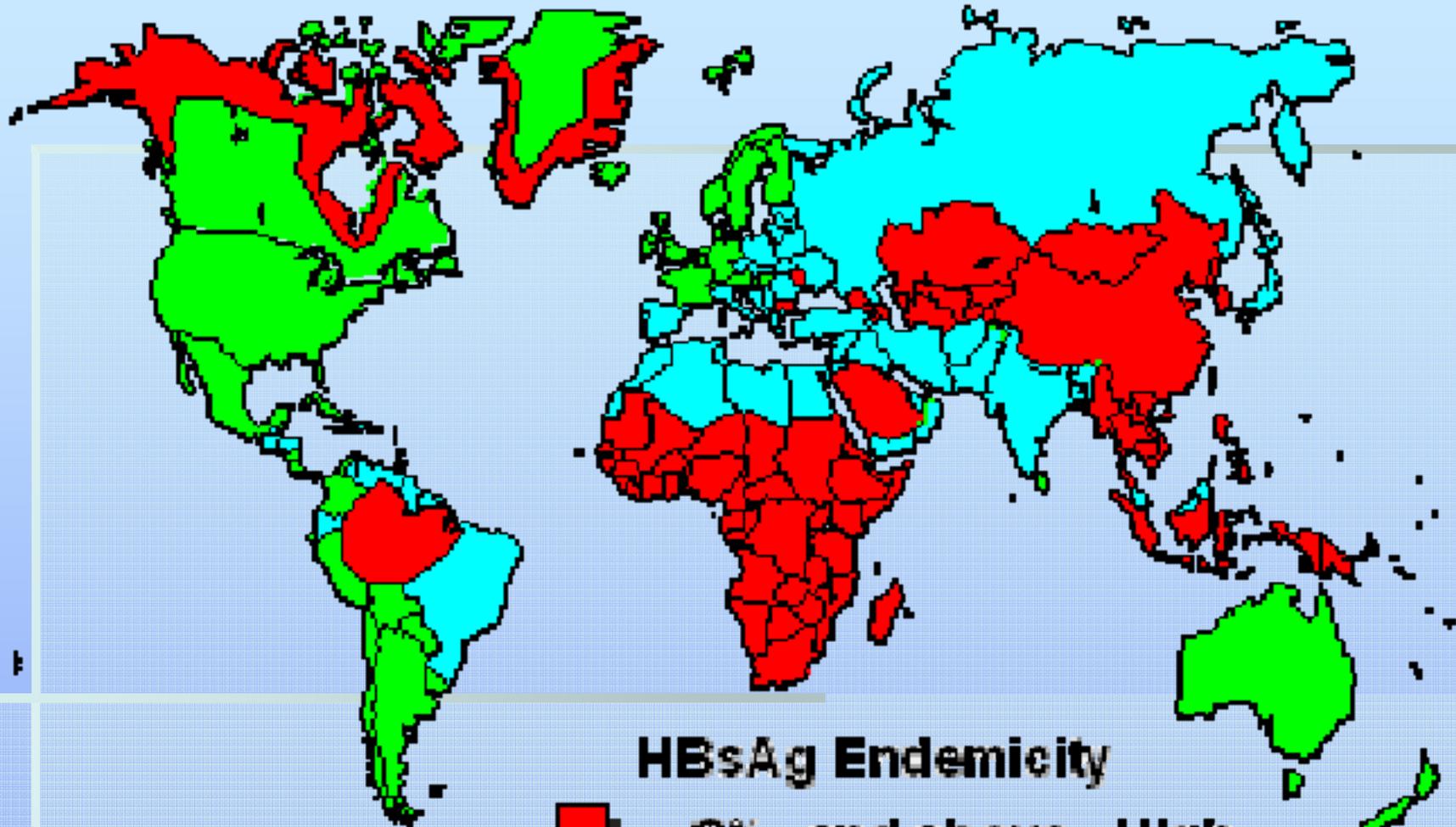


# Hepatitis B Virus Genotypes



- Classified into 8 genotypes to date (A-H) based on 8% or more DNA sequence differences
- HBV genotypes were found to have varied geographic distributions

# HBV World Distribution Map



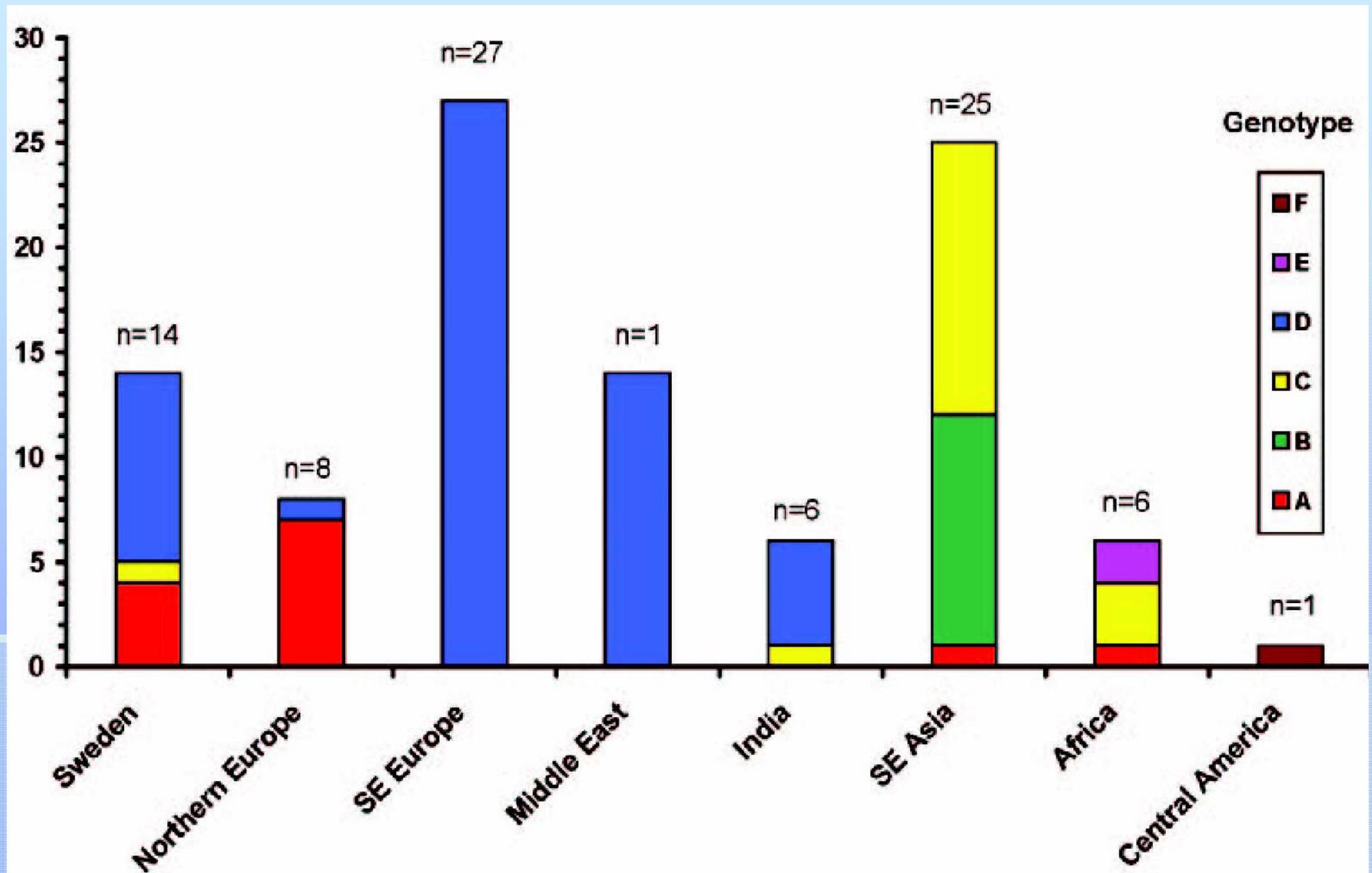
## HBsAg Endemicity

-  8% and above - High
-  2% - 8% - Intermediate
-  Below 2% - Low

# HBV Genotypes Distribution

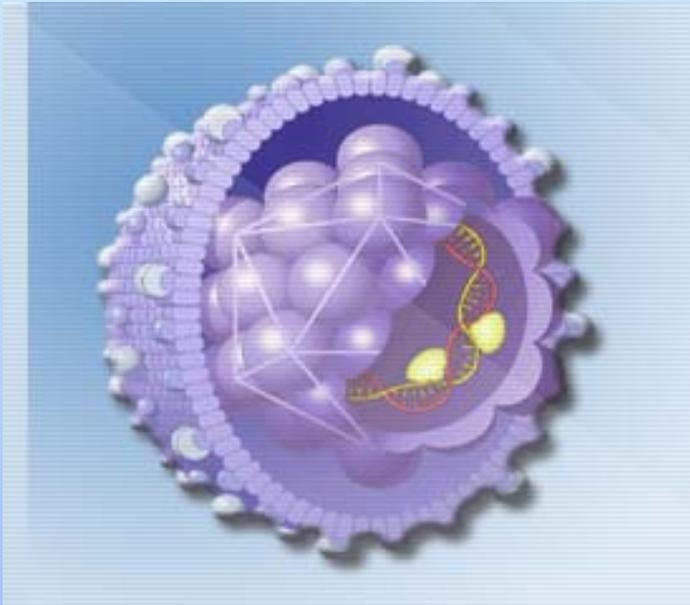


# HBV Genotypes Distribution



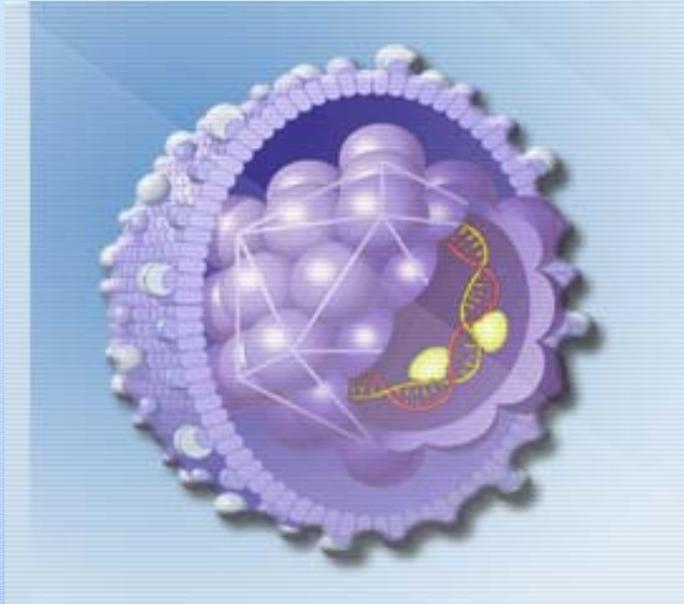
Ljunggren, KD et al. 2004. Clinical and Serological Variation between Patients Infected with Different Hepatitis B Virus Genotypes. *J Clin Microbiol.* 42(12): 5837-5841

# Objective



- To determine genotypes of HBV from patients seen at St. Luke's Medical Center, Philippines using restriction endonuclease cleavage of the PCR-amplified S-gene of HBV genome

# MATERIALS AND METHODS



# General Flowchart

Serum or plasma collection  
from SLMC patients 2002 to 2007



HBV DNA extraction  
Qiagen DNA Blood Mini Kit



HBV S-gene PCR amplification

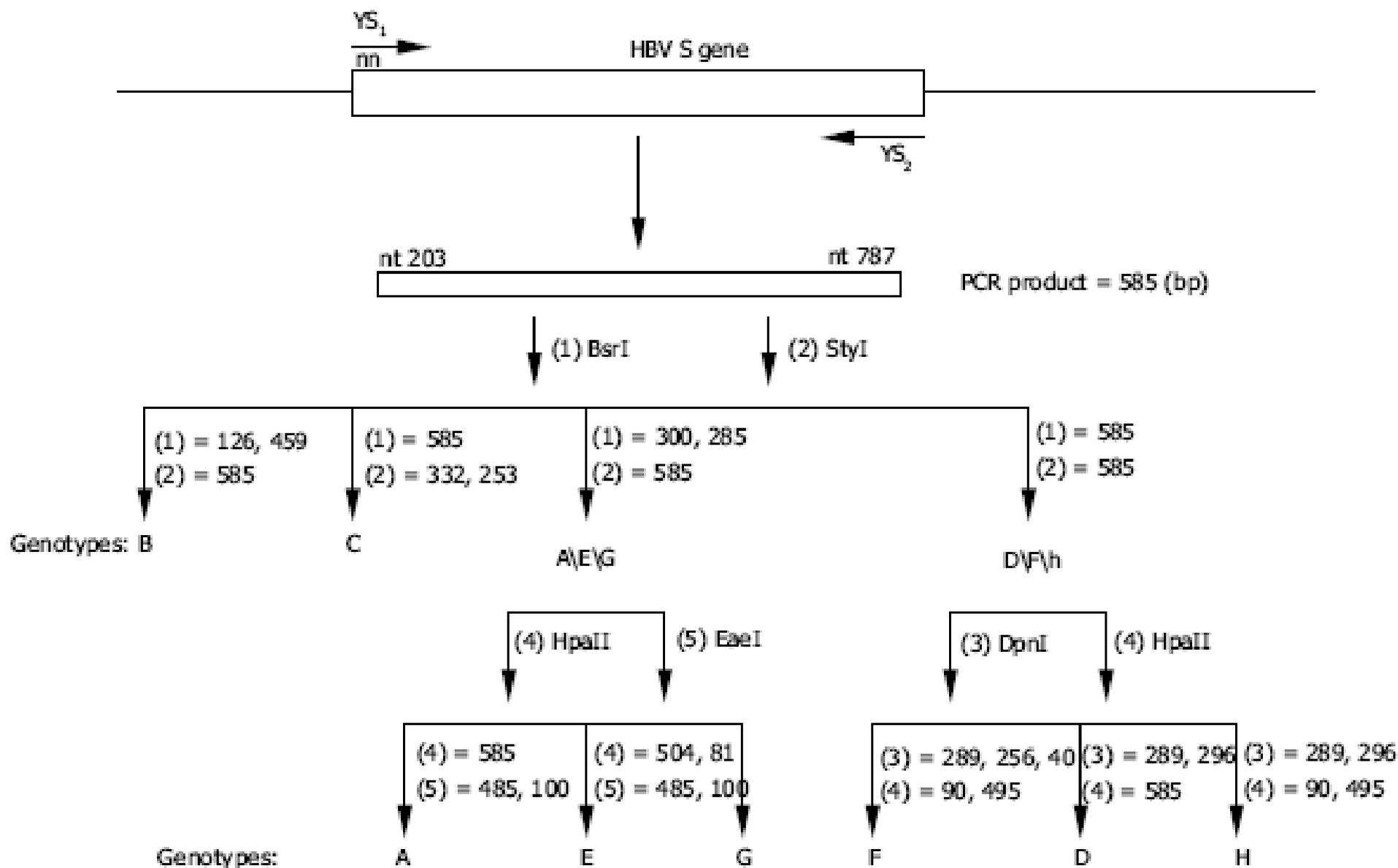


Restriction enzyme digestion

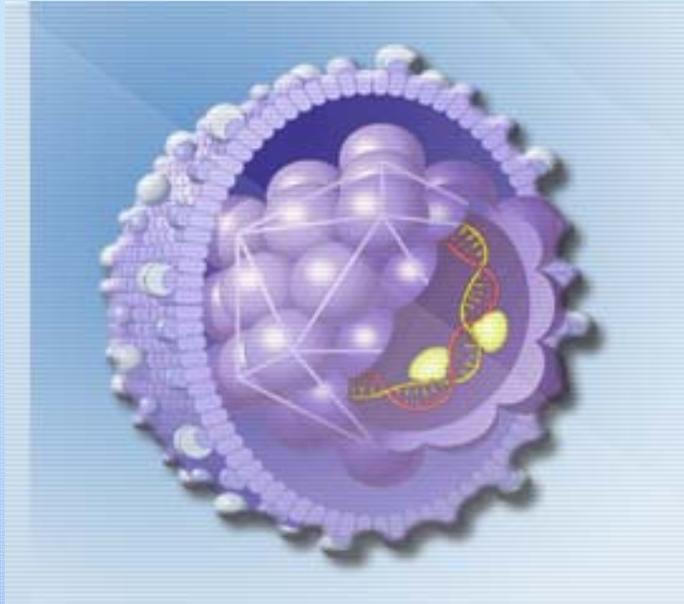


Polyacrylamide gel electrophoresis

# Genotyping Scheme

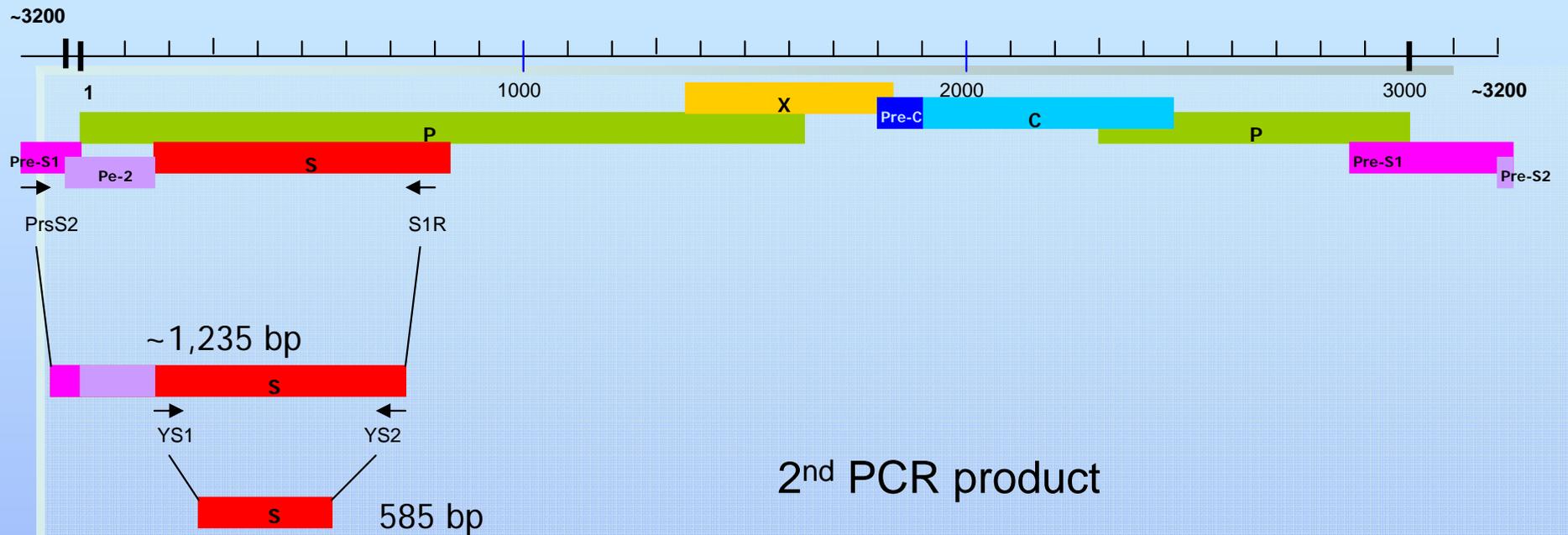


# RESULTS AND DISCUSSION

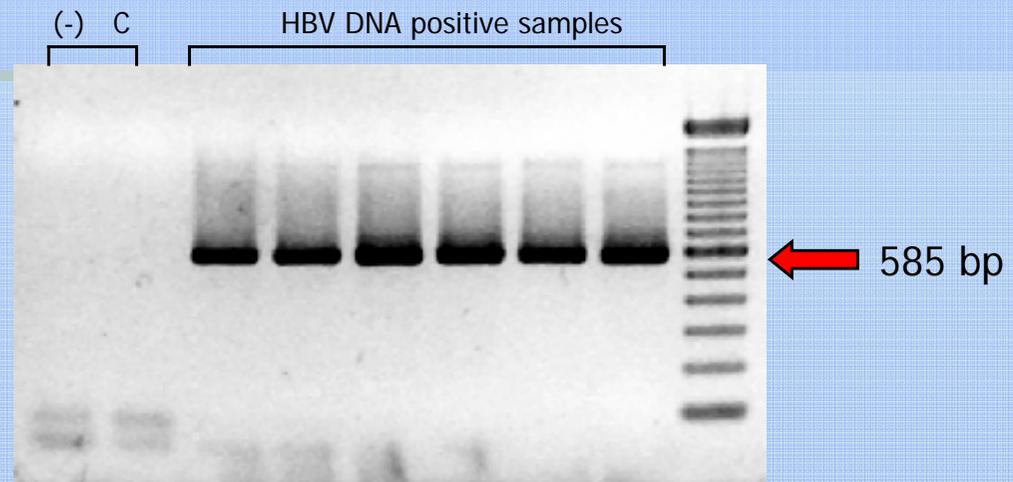


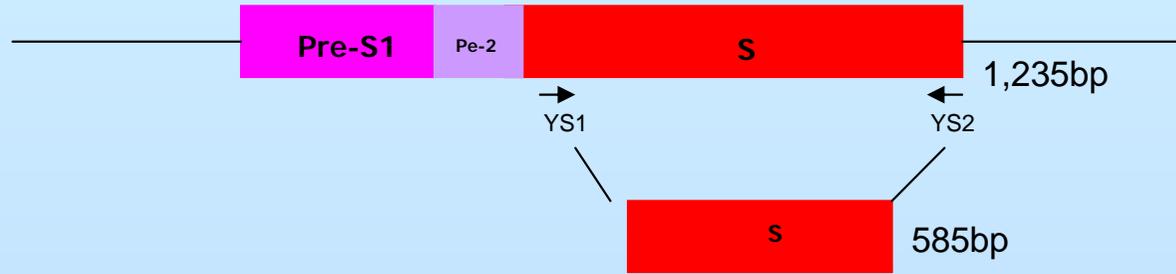
# PCR-RFLP

## PCR Amplification of HBV S-gene

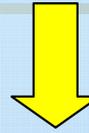


2<sup>nd</sup> PCR product





RE Digestion



BsrI



126bp 459bp

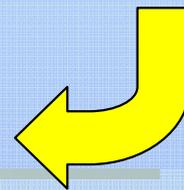
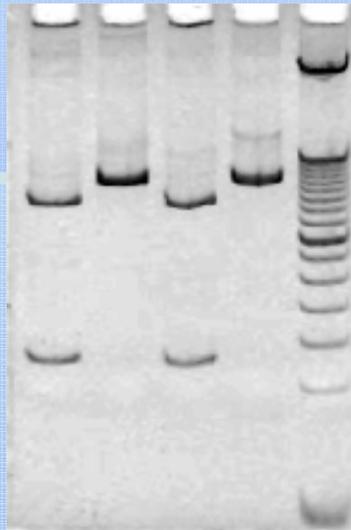
StyI



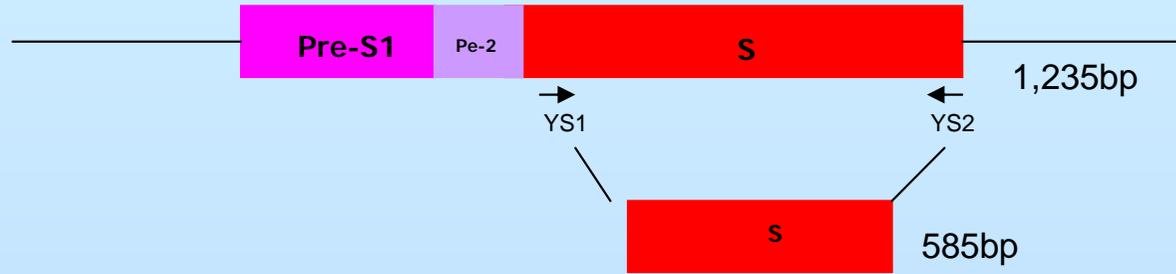
585bp

## HBV Genotype B

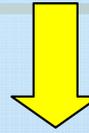
**B**      **B**  
BsrI StyI BsrI StyI MW



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RE Digestion



Bsrl



585bp

Styl



332bp 253bp

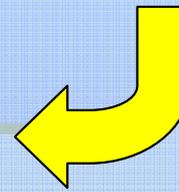
# HBV Genotype C

C

MW Styl Bsrl

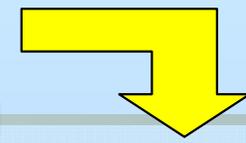
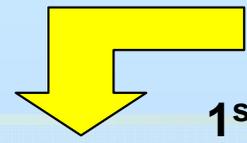
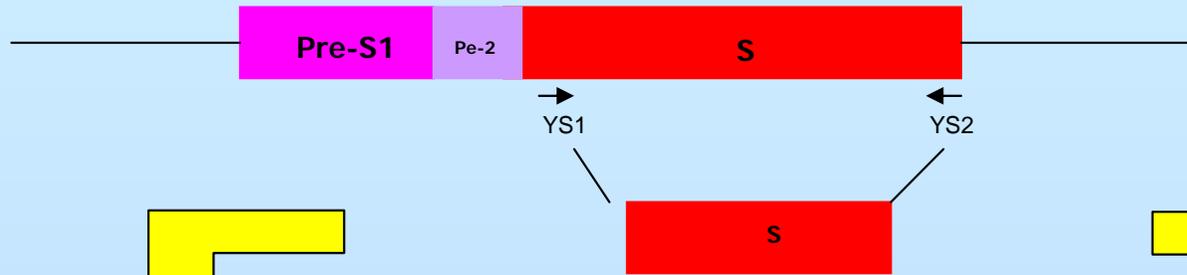


350bp



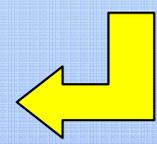
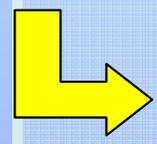
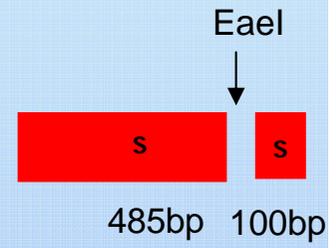
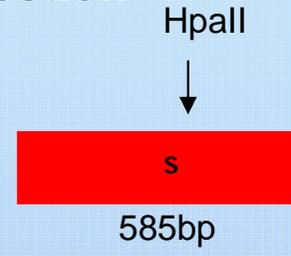
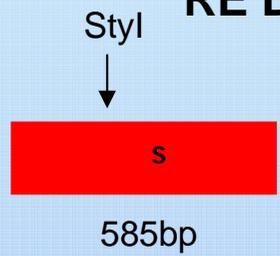
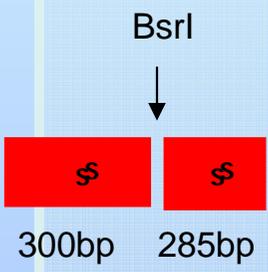
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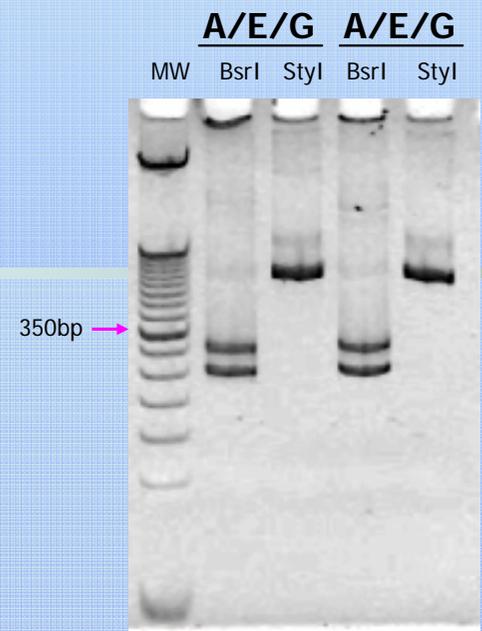


**1<sup>st</sup> round  
RE Digestion**

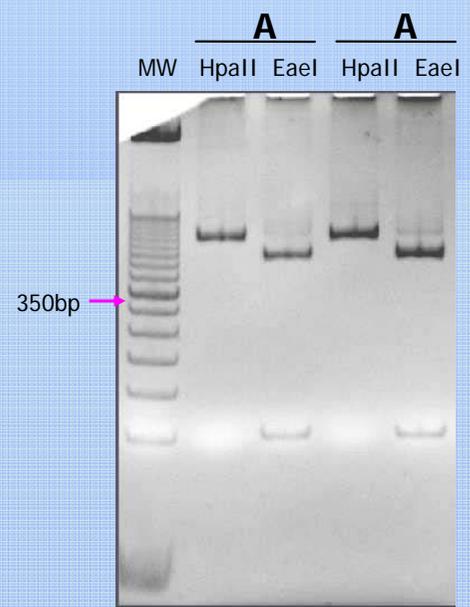
**2<sup>nd</sup> round  
RE Digestion**



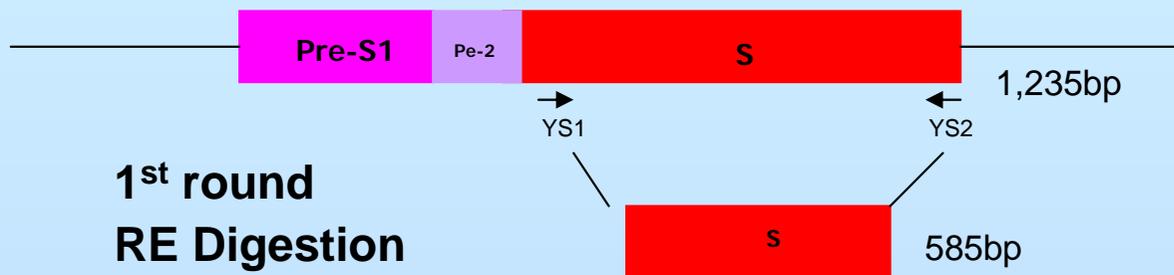
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**HBV Genotype A**

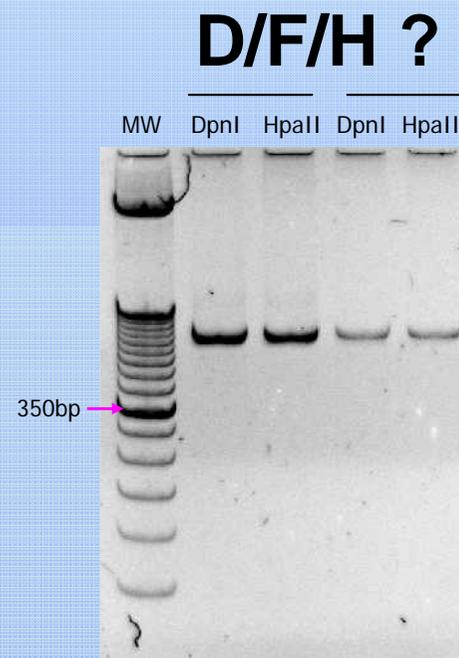
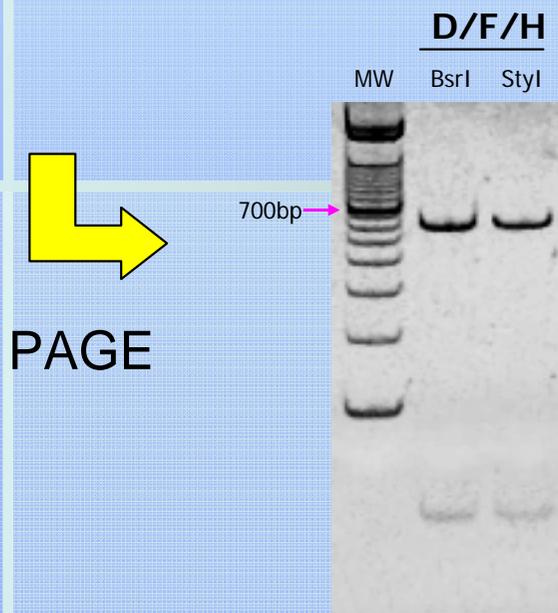
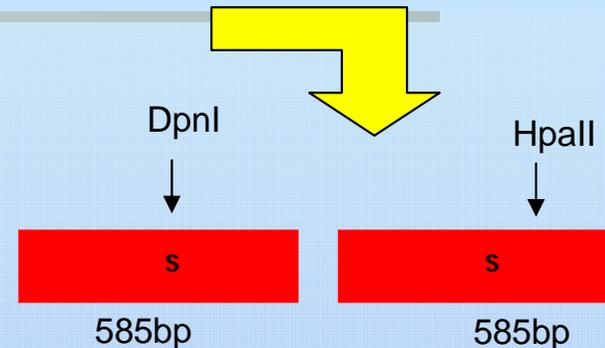
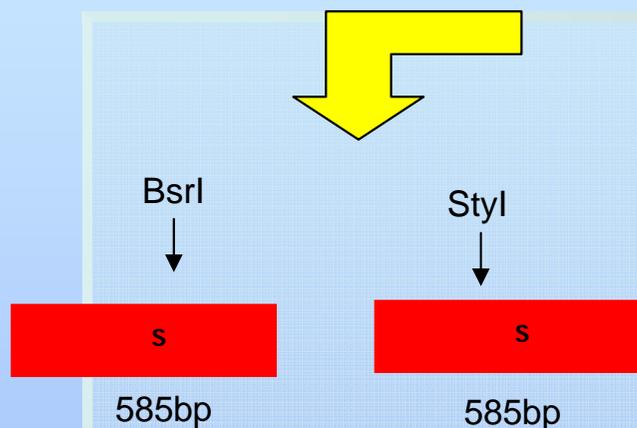


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### 1<sup>st</sup> round RE Digestion

### 2<sup>nd</sup> round RE Digestion



# Distribution of HBV genotypes among patients seen at St. Luke's Medical Center from January 2002 to August 2007

**N = 436**

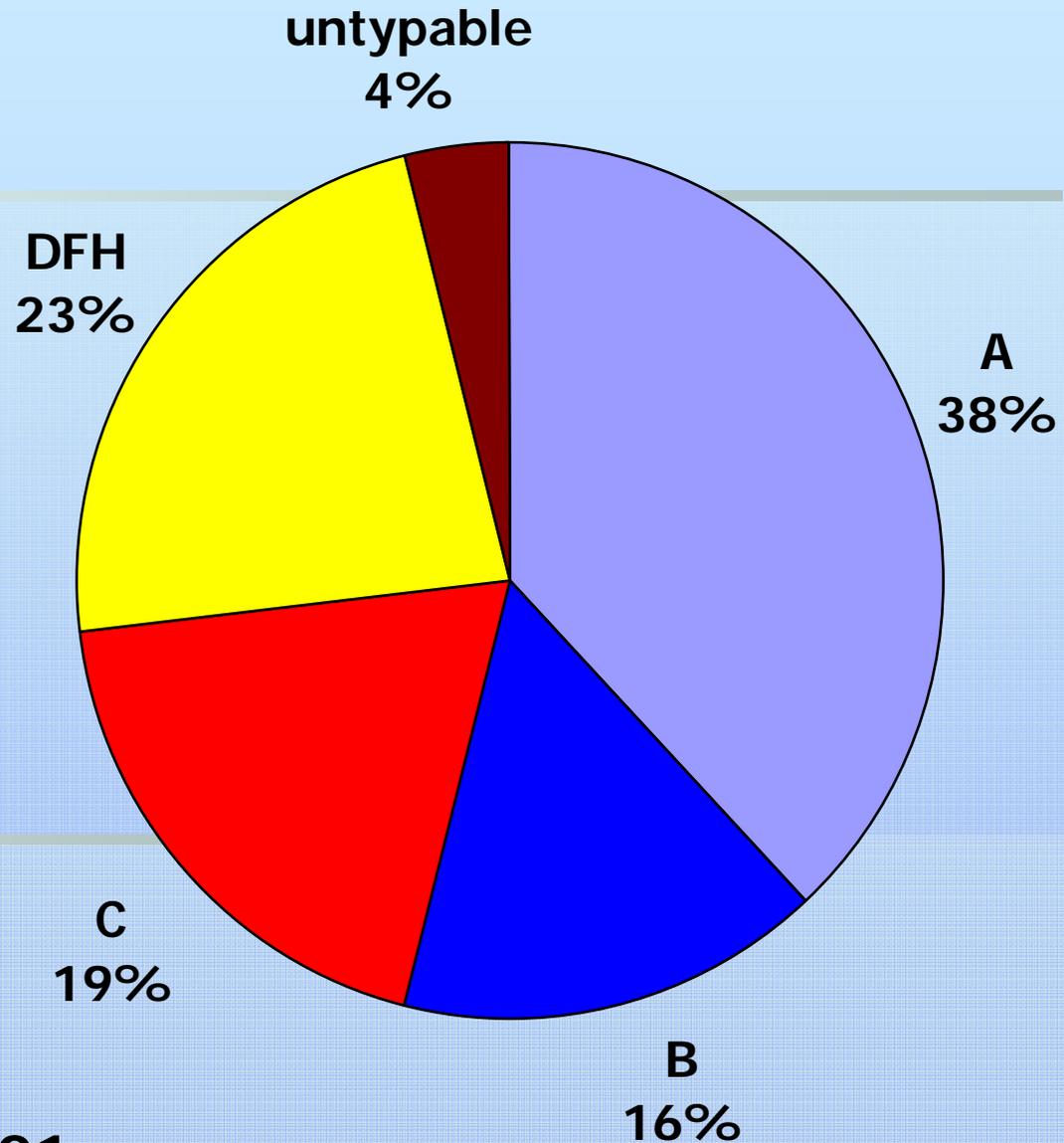
**A = 166**

**B = 69**

**C = 85**

**DFH = 99**

**Untypable = 17**



**Chi-square test,  $P < 0.001$**

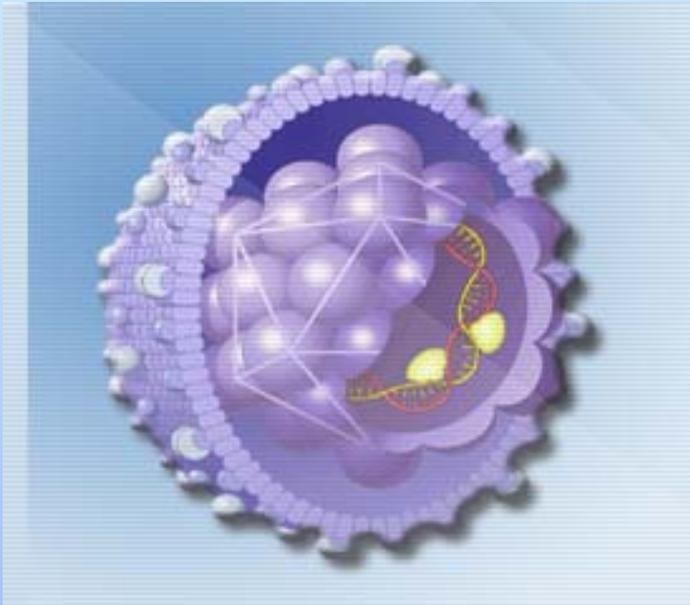
# HBV/A subtyping

- Subtype-specific PCR assay in the X/precore/core region, combined with *Bgl* II restriction endonuclease digestion

HBV/A samples processed	85
HBV/Aa (African and asian type)	80 (94%)
HBV/Ae (European and US type)	5 (6%)

- This confirms that the HBV/A strain in the Philippines is similar to those in Southeast Asian countries

# Conclusion



- HBV genotype A (subtype Aa) is the predominant genotype in the Filipino population
- Results of this study will provide a base for correlating HBV genotype with clinical outcomes and response to antiviral treatment

**Thank You !**