I. INTRODUCTION

Dengue viruses are members of the Family *Flaviviridae*. There are four distinct serotypes of dengue virus. Dengue virus virion consists of a single-stranded linear RNA molecule, which is capped at the 5' end but lacks a poly(A) tract at the 3' end (Putnak and Henchal, 1990). The RNA genome is about 11 kb in length, surrounded by an icosahedral nucleocapsid of about 30 nm in diameter and covered by a lipid envelope of about 10 nm in thickness. The complete virion is about 50 nm in diameter. Dengue virus is transmitted between human hosts by infected mosquitoes and *Aedes aegypti* is the most important vector. Dengue virus infection continues to cause major public health problems in Asia, the Caribbean, and Central and South America. Disease manifestations among dengue virus infected humans are various, from classical dengue fever to dengue hemorrhagic fever (DHF), which can progress to dengue shock syndrome (DSS) and death. In man, all of the four serotypes of dengue virus can cause classical dengue fever and dengue hemorrhagic fever (Putnak and Henchal, 1990) and there is no long term cross-protective immunity between the dengue serotypes, so persons may have as many as four dengue infections during their life.

In Thailand, the first outbreak of dengue hemorrhagic fever occurred in 1958 in Bangkok-Thonburi and nearby areas. Almost 2,500 cases and a 10% case fatality rate were recorded. Most of the affected people were children under 10 years old (reviewed by Thongcharoen and Jatanasen, 1993). In 1962, the disease started to spread to other big cities and thereafter to throughout the country (Nimmannitya, 1987). The highest record of DHF in Thailand was reported in 1987 (174,285 cases with 1,007 deaths) (Ungchusak, 1987; Ungchusak and Kunasol, 1988; Thongcharoen and Jatanasen, 1993).

The determination that what dengue serotype caused severe illness over time in Thailand revealed that dengue serotype 2 viruses accounted for most of the cases of DHF observed in Bangkok (Pang, 1987; Burke et al., 1988; Nisalak et al., 1990).

Molecular epidemiologic study of dengue serotype 2 viruses in Thailand was done by several workers. Walker and his colleague (1988) analyzed dengue serotype 2 virus isolated in Bangkok in 1980 by employing the oligonucleotide fingerprinting technique and restriction enzyme mapping, they found that one dengue serotype 2 strain, D80-141, produced distinctly different T1 oligonucleotide fingerprint and restriction enzyme map when compared with other isolates. This isolate also lacked an epitope on the NS1 protein which was commonly present in other Bangkok isolates (Walker et al., 1988). This is the first indication of the presence of the minor subtype of dengue serotype 2 which co-circulated with the major subtype in Bangkok in 1980. This result was confirmed by Blok et al (1989) when they determined and compared the sequences of the envelope glycoprotein gene of five Thai dengue serotype 2 viruses. They found that Thai isolates could be divided into two groups (Blok et al., 1989). These were also evident when the NS1 sequences of this group of viruses were compared (Blok et al., 1991). Furthermore, phylogenetic studied by Lewis et al, in 1993, indicated that viruses from Thailand belonged to subtype III which was divided into subtype IIIa and IIIb (Lewis et al., 1993).

To further delineate the genetic relationship and evolution of dengue serotype 2 viruses in Bangkok, Sittisombut et al (1997) determined the E gene sequences of 19 dengue serotype 2 viruses isolated during the 1987 epidemic season. The comparison using the maximum likelihood method revealed that the subtype IIIa viruses represented the majority (18/19) of dengue serotype 2 viruses in this year. Furthermore, a comparison of the E gene sequences of the 1987 strains with earlier isolates revealed the

substitutions that were common to the subtype IIIa and subtype IIIb viruses of both the 1980 and 1987 epidemic seasons and the substitutions that were shared by all subtype IIIa or subtype IIIb viruses of the 1987 epidemic seasons but were absent from the corresponding viruses in 1980. This type of substitution was suggestive of the occurrence of population (genetic) bottleneck in Bangkok between 1980 and 1987 (Sittisombut et al., 1997). Moreover, the studies of dengue serotype 2 viruses from other parts of Thailand indicated that 1/1 dengue serotype 2 viruses isolated from Kanchanaburi in 1987 (Sittisombut et al., 1997), 3/3 that isolated from Chiang Mai in 1991 (Sistayanarain et al., 1996) and 4/4 that isolated from Nakhon Phanom in 1993 (Thant et al., 1995) were all subtype IIIa strains. In contrast, a study of dengue serotype 2 viruses that circulated in Maha Sarakham by Duangchanda et al (1994) revealed that 3/3 viruses isolated in 1986-1987 were all subtype IIIb strains.

Data from several studies indicated that there was an uneven distribution of dengue serotype 2 subtypes in various localities of Thailand. However, the sample size used in the previous studies was too small, that lead to the non-accuracy in the proportion between the subtype IIIa and IIIb. From this reason, the objective of this study is to determine the proportion between dengue serotype 2 virus subtype IIIa and IIIb that circulate in Bangkok, Thailand in 1994 by employing the appropriate sample size and analyze by using the oligonucleotide probe that specific to the nucleotide sequence that different between the two subtypes of the viruses. This analysis should be useful in understanding the proportion of dengue serotype 2 virus subtype IIIa and IIIb in Bangkok which is importance in the vaccine production.