UNIVERSITI PUTRA MALAYSIA

A DRAFT GENOME SEQUENCES AND MOLECULAR CHARACTERISATION OF GLYCOPROTEIN B AND LOWER MATRIX PHOSPHOPROTEIN OF RAT CYTOMEGALO VIRUS ALL-03 STRAIN

QUAH YI WAN

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By

QUAH YI WAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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July 2013

Chairman: Zeenathul Nazariah Allaudin, PhD

Faculty: Veterinary Medicine

Congenital cytomegalovirus (CMV) infection causes neurological damages to newborn infants and persists over the years in infants. Rat CMV (RCMV) ALL-03 strain (RCMV ALL-03) was the first RCMV isolated from the placenta and uterus of rat. This RCMV ALL-03 was hypothesised to mimic congenital human CMV (HCMV) infection. Overall, the study signifies the genome characterisation of a novel local transplacental strain RCMV ALL-03, as well as the glycoprotein B (gB) and lower matrix phosphoprotein (pp65) RCMV ALL-03 gene characterisation. The study was commenced by propagating RCMV ALL-03 extensively to a large amount alongside with the reference RCMV English strain (RCMV-E) in rat embryonic fibroblast (REF) cells. Infected cells exhibiting advance cytopathic effect were harvested and concentrated by 8% (w/v) PEG6000. Concentrated viruses were then purified through sucrose gradient. Based on the similarity between HCMV and

RCMV ALL-03, monoclonal antibody against gB of HCMV was used to immunoprecipitate RCMV ALL-03 gB through Western blot and immunoperoxidase assay. Likewise, monoclonal antibody pp65 of HCMV was able to immunoprecipitate pp65 of RCMV ALL-03 by immunoperoxidase assay, indicating there are shared homology between HCMV and RCMV ALL-03. The Illumina Genome Analyzer IIx generated a total of 3.8 million paired end sequence reads from multiplexing of both genomic viral DNA libraries. The raw sequencing results were assembled via *de novo* approach into contigs, producing a total of 6 large CMV-related contigs for RCMV ALL-03 and 11 large CMV-related contigs for RCMV-E. These contigs were aligned and arranged based on reference RCMV Maastricht strain (RCMV-M), followed by scaffolding into draft genomes. The estimated size of RCMV ALL-03 was 198,895 bp while RCMV strain English was estimated to be 175,071 bp. Phylogenetic tree of RCMV ALL-03 draft genome with other reference CMVs revealed that RCMV ALL-03 belongs to the *Muromegalovirus* genus and that it is closest to RCMV-E, with an estimated difference of 8.2 substitution per 1 kbp from RCMV-E. Based on gene prediction software GeneMarkS, there were 136 genes predicted for RCMV ALL-03 and 112 genes predicted for RCMV-E. All predicted genes were subjected to BlastX analysis, followed by mapping and annotation of the genes using Blast2Go software. For the RCMV ALL-03, there were 5 genes without any blast hits and a total of 46 genes were annotated. The gB and pp65 genes were among the annotated genes. Phylogenetic analysis of RCMV ALL-03 gB and pp65 showed that RCMV ALL-03 branched individually from other reference CMVs despite having almost similar branch distance as the reference CMVs. By designing and examining the RCMV gB and pp65 specific primers for polymerase chain reaction (PCR) via *in silico* and lab verification, RCMV ALL-03

gB and pp65 gene were amplified. This study revealed the genome sequence of RCMV ALL-03, which is essential in the understanding of shared homology between RCMV ALL-03 and HCMV. The relatedness of RCMV ALL-03 to RCMV-E showed the conserve regions of rodent CMV but their divergence revealed potential RCMV ALL-03 unique transplacental characteristics. Future exploitation study on the RCMV ALL-03 could help provide a better understanding of congenital HCMV.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

DRAF URUTAN GENOM DAN CIRI-CIRI MOLEKUL GLIKOPROTEIN B DAN FOSFOPROTEIN MATRIX RENDAH STRAIN ALL-03 SITOMEGALOVIRUS TIKUS

Oleh

QUAH YI WAN

Julai 2013

Pengerusi: Zeenathul Nazariah Allaudin, PhD

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Jangkitan kongenital sitomegalovirus (CMV) boleh mengakibatkan kerosakan sistem saraf pada bayi baru lahir dan ia akan berkekalan pada bayi tersebut. Strain CMV tikus (RCMV) ALL-03 adalah RCMV pertama yang dipencilkan daripada uri dan rahim tikus. Jangkitan RCMV ALL-03 ke atas tikus menyerupai jangkitan kongenital CMV manusia (HCMV). Secara keseluruhannya, kajian ini adalah berkaitan pencirian genom virus transplasenta tempatan, strain RCMV ALL-03, serta pencirian gen glikoprotein B (gB) dan gen fosfoprotein matriks rendah (pp65). Kajian ini dimulakan dengan pembiakkan RCMV ALL-03 pada jumlah yang besar secara

intensif bersama-sama dengan RCMV rujukan daripada strain English (RCMV-E) di dalam sel fibroblas embrio tikus (REF). Sel terjangkit yang mempamerkan kesan sitopatik telah dituai dan dipekatkan dengan menggunakan 8% (w/v) PEG6000. Virus yang pekat kemudiannya ditulenkan melalui proses kecerunan sukrosa. Berdasarkan persamaan di antara HCMV dan RCMV ALL-03, antibodi monoklonal terhadap gB HCMV telah digunakan untuk pemendakkan tindak balas imun RCMV ALL-03 gB melalui Western blot dan ujian immunoperoksidase. Antibodi monoklonal pp65 daripada HCMV mampu memendakkan tindak balas immun pp65 daripada RCMV ALL-03 melalui ujian immunoperoksidase. Ini menunjukkan perkongsian homologi di antara HCMV dan RCMV ALL-03. Penjujuk Illumina Genome Analyzer IIx menjana sebanyak 3.8 juta jujukan akhir berpasangan daripada koleksi multipleks kedua-dua genom DNA virus. Hasil jujukan dibentuk melalui kaedah *de novo* kepada kontig yang menghasilkan 6 kontig berkaitan CMV untuk RCMV ALL-03 dan 11 kontig berkaitan CMV untuk RCMV-E. Kesemua kontig disusun dan dipadan berdasarkan RCMV rujukan strain Maastricht (RCMV-M), dan kemudian diperancahkan menjadi draf genom. Saiz anggaran RCMV ALL-03 adalah 198,895 bp manakala saiz RCMV-E dianggarkan berjumlah 175,071 bp. Pokok filogenetik di antara draf genom RCMV ALL-03 dengan CMV rujukan lain menunjukkan RCMV ALL-03 tergolong di bawah genus *Muromegalovirus* dan ia mempunyai persamaan terdekat dengan RCMV-E (anggaran perbezaan sebanyak 8.2 penggantian pada setiap 1 kbp). Berdasarkan gen ramalan pada perisian GeneMarkS, terdapat 136 gen yang dijangkakan untuk RCMV ALL-03 dan 112 gen untuk RCMV-E. Semua gen ramalan dianalisis dengan BlastX, diikuti dengan pemetaan dan anotasi gen menggunakan perisian Blast2Go. Terdapat 5 gen RCMV ALL-03 yang tidak dapat dikenal pasti dan sebanyak 46 gen RCMV ALL-03 yang dianotasi.

Gen-gen gB dan pp65 adalah di antara gen yang dianotasi. Analisis filogenetik RCMV ALL-03 gB dan pp65 menunjukkan bahawa RCMV ALL-03 mempunyai jarak cabang yang hampir sama dengan rujukan CMV lain tetapi bercabang sendirian dan jauh daripada rujukan CMV lain. Pencetus khas bagi RCMV ALL-03 gB and pp65 telah direka dan diuji melalui tindak balas berantai polymerase (PCR) *in silico* dan gen RCMV ALL-03 gB dan pp65 telah berjaya diamplifikasi. Urutan genom RCMV ALL-03 yang dihasilkan adalah penting bagi memahami persamaan di antara RCMV ALL-03 dengan HCMV. Perkaitan antara RCMV ALL-03 dengan RCMV-E menunjukkan kawasan pemuliharaan pada CMV tikus tetapi perbezaannya adalah ciri transplasenta yang unik bagi RCMV ALL-03. RCMV ALL-03 boleh dieksploitasikan untuk kajian masa depan bagi pemahaman yang lebih mendalam mengenai tabiat kongenital HCMV.

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I certify that a Thesis Examination Committee has met on 5th July 2013 to conduct the final examination of Quah Yi Wan on her thesis entitled "**A draft genome sequences and molecular characterisation of glycoprotein B and lower matrix phosphoprotein of Rat Cytomegalovirus ALL-03 strain**" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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QUAH YI WAN

Date: 5 July 2013

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