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DEVELOPMENT OF A MONOLITH BASED - CHROMATOGRAPHIC PURIFICATION SYSTEM FOR PLASMID DNA VACCINE AGAINST THE GROUPER IRIDOVIRUS

TAMAR KANSIL

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Tamar Kansil

(Dr. Clarence M. Ongkudon)

Tarikh: 6 September 2016

(Dr. Kenneth F. Rodrigues)

DECLARATION

I hereby declare that the content in this thesis is my own except for quotations, equations, summaries and references, which have been duly acknowledged.

6th September 2016

Tamar Kansil
MZ1311005T

CERTIFICATION

NAME	:	**TAMAR KANSIL**
MATRIC NO.	:	**MZ1311005T**
TITLE	:	**DEVELOPMENT OF A CHROMATOGRAPHIC PURIFICATION SYSTEM FOR GROUPER'S IRIDOVIRUS PLASMID DNA VACCINE**
DEGREE	:	**MASTER OF SCIENCE**
DATE OF VIVA	:	**18th JULY 2016**

DECLARED BY;

1. **SUPERVISOR**
   Dr. Clarence M. Ongkudon

   Signature

2. **CO-SUPERVISOR**
   Assoc. Prof. Dr. Kenneth F. Rodrigues

CERTIFICATION

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MATRIC NO.	:	**MZ1311005T**
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   Dr. Clarence M. Ongkudon

   Signature

   _____

2. **CO-SUPERVISOR**
   Assoc. Prof. Dr. Kenneth F. Rodrigues

   _____

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ABSTRACT

Routine research-scale production of plasmid DNA vaccine relies heavily on commercial kits that are expensive due to the application of RNAse, including the use of multiple elution steps and single-use columns which are subjected to lot-to-lot quality deficiency. This research focused on developing an innovative plasmid DNA purification strategy by using a non-functionalized polymethacrylate monolith in centrifugal-based purification technique coupled with a modified lysis procedure. The effect of temperature on pore size distribution was first analyzed to determine the suitability of the monolith for plasmid DNA purification. Initially, the plasmid DNA was purified via monolithic chromatography using an automated chromatography system based on ionic interaction to determine the optimal chromatographic conditions such as flow rate and elution condition. The centrifugal-based monolithic purification was then conducted by mimicking the elution conditions used in the automated chromatography system. The main obstacle encountered during the research was the limited versatility when using non-functionalized polymethacrylate monoliths to purify plasmid DNA from crude cell extracts. In most cases, a reasonable amount of RNA was found to co-elute with plasmid DNA. This greatly affected the purification efficiency as the separation was presumably relied on size exclusion as the only mean of separation. However, we discovered that the monolith was inherently charged even without a functional group immobilization. Apparently, an electrostatic interaction between plasmid DNA and charged matrix had occurred. This was evident by the gel electrophoresis of samples obtained after several elutions using deionized water. There was no noticeable RNA or plasmid DNA observed on the gel indicating that an interaction between nucleic acids and the monolith had taken place. The overall purification methodology comprised pretreatment of lysed cells with sulphate ions followed by step-wise elution of plasmid DNA using monolithic column via centrifugation (300 x g) with NaCl as the elution buffer. The first elution step involved the use of low concentration of saline buffer (0.2 M NaCl) to remove the remaining RNA from the pretreated cell extracts with some of the plasmid DNA were being compromised. The second elution step involved the use of high concentration (1.0 M NaCl) buffer to elute the remaining bound plasmid DNA. The study showed that 70% porogen content in the polymerization mixture gave a minimum heat build-up of 64°C and a homogenous pore size distribution of 3µm – 4µm. In this research, it was evident that the pretreatment of lysed cells with 0.8 M sulphate salt solution resulted in the removal of more than 80% RNAs with a minimal amount of plasmid DNA being compromised. This method allowed the removal of the remaining RNA with a high recovery of plasmid DNA during the first step elution using monolithic centrifugation technique (two-step elution). The discovery of a chemically induced RNA removal method based on the bias selection of sulphate salt ion and the establishment of a centrifugal-based monolithic purification platform would have a great impact on the production of plasmid DNA vaccine for grouper's Iridovirus. The developed method eliminates the use of an expensive enzyme RNAase making it economically favourable. The monolithic purification column can also be used repeatedly with consistent outputs thus enabling a high-throughput production of Iridovirus plasmid DNA vaccine for *in vivo* testing.

ABSTRAK

Perkembangan Sistem Penulinan Kromatografi untuk Iridovirus Plasmid Vaksin DNA Kerapu

Penghasilan rutin vaksin DNA plasmid berskala penyelidikan banyak bergantung kepada kit komersil yang mahal disebabkan oleh penggunaan 'RNA-ase', di samping ia juga melibatkan pelbagai langkah pengeluatan dan penggunaan kolum kromatografi kolom yang terhad kepada sekali sahaja dan ini mendorong kepada penurunan dari segi kualiti produk. Kajian ini tertumpu kepada pembangunan strategi untuk penulinan DNA plasmid secara inovatif dengan menggunakan monolit poli(metakrilat) tanpa kumpulan berfungsi di dalam teknik penulinan berasaskan pengemparan yang digabungkan dengan prosedur lisis yang terubahsuai. Kesan suhu ke atas taburan saiz liang dianalisa untuk menentukan kesesuaian monolit bagi penulinan DNA plasmid. Pada mulanya, penulinan menggunakan monolit berasaskan pengemparan telah dijalankan dengan mengaplikasikan kadar aliran dan parameter pengeluatan yang digunakan dalam sistem kromatografi-automatik. Dalam kebanyakan kajian, sejumlah RNA didapati mengeluat bersama DNA selepas proses penulinan. Keadaan ini telah menghadkan kaedah-kaedah penulinan kepada penulinan berasaskan saiz molekul sebagai prinsip pengasingan yang tunggal. Hasil kajian ini menunjukkan bahawa tiada RNA atau plasmid DNA yang kelihatan pada gel selepas beberapa langkah pengeluatan menggunakan air tanpa ion dan ini menerangkan bahawa terdapat interaksi antara asid nukleik dan monolit. Penulinan keseluruhan terdiri daripada rawatan awal sel-sel terlisis dengan ion sulfat diikuti dengan langkah berperingkat, pengeluatan DNA plasmid menggunakan kolum monolit melalui pengemparan (300 x g) dengan NaCl sebagai larutan penimbal. Langkah pertama pengeluatan melibatkan penggunaan penimbal yang mempunyai kepekatan garam rendah (0.2 M NaCl) untuk mengeluarkan sisa RNA dari ekstrak sel yang telah melalui rawatan awal dan sebahagian DNA plasmid yang turut hilang pada peringkat ini. Langkah pengeluatan kedua melibatkan penggunaan penimbal yang mempunyai kepekatan garam tinggi (1.0 M NaCl) untuk mengeluarkan sisa DNA plasmid yang terikat. Kajian menunjukkan 70% kandungan porogen dalam campuran pempolimeran memberikan bacaan haba terbentuk yang minimum sebanyak 64°C dan taburan saiz liang yang sekata dalam julat antara 3 µm – 4 µm. Hasil keputusan juga menunjukkan rawatan awal sel lisis dengan 0.8 M ion sulfat dapat menyingkirkan sebanyak 80 % RNA dengan sedikit kehilangan DNA plasmid. Kaedah ini dapat menyingkirkan sisa RNA dengan pengumpulan semula DNA plasmid yang tinggi semasa langkah pengeluatan pertama menggunakan teknik pengemparan monolitik. Penemuan kaedah penyingkiran RNA aruhan secara kimia berasaskan pemilihan ion sulfat secara berat sebelah dan pembangunan kaedah penulinan menggunakan monolit berasaskan emparan akan memberi impak yang besar ke atas penghasilan vaksin DNA plasmid terhadap Iridovirus kerapu. Kaedah yang dibangunkan ini akan dapat menggantikan penggunaan enzim 'RNA-ase' yang mahal dan menyebabkan ianya menjadi pilihan. Kolum penulinan monolit ini juga boleh digunakan berulang kali dengan hasil yang konsisten, dan dengan itu, membenarkan penghasilan vaksin DNA plasmid Iridovirus dengan daya pemprosesan yang tinggi untuk ujian in vivo.

TABLE OF CONTENTS

		Page
TITLE		i
DECLARATION		ii
CERTIFICATION		iii
ACKNOWLEDGEMENT		iv
ABSTRACT		v
ABSTRAK		vi
TABLE OF CONTENTS		vii
LIST OF TABLES		xii
LIST OF FIGURES		xv
LIST OF ABBREVIATION		xx
CHAPTER 1: INTRODUCTION		1
1.1	Grouper Iridovirus	1
1.2	Aquaculture Vaccine	1
1.3	Monolithic Purification of Plasmid DNA	3
1.4	Problem Statements	3
1.5	Hypotheses	5
1.6	Objectives	5
1.7	Significance of the Study	5
CHAPTER 2: LITERATURE REVIEW		6
2.1	Evolution of Vaccines	6
2.2	First Generation Vaccines	7
2.3	Second Generation Vaccines	8
2.4	Third Generation Vaccines	9
2.5	Antibody and Cellular Immune Responses	10
2.6	External Factors Affecting Immune Efficiency in Fish	11
2.7	Advantages of DNA Vaccines	11
2.8	Aquaculture Vaccines Administration via Injection Response	12
2.9	Aquaculture Vaccines Administration via Immersion Response	13
2.10	Challenges of Aquaculture Vaccines	13

2.11	Biomanufacturing of Plasmid DNA Vaccines	14
2.12	Biomanufacturing of Subunit Vaccines	15
2.13	Plasmid DNA Vaccines Purification	16
2.14	Subunit Vaccines Purification	17
2.15	Conventional Chromatographic Supports	17
2.16	The Properties of Chromatographic Supports	18
2.17	Monolith Properties	19
2.18	Fabrication of Polymer Monoliths	20
2.19	Polymethacrylate as the Building Block of Monoliths	21
2.20	Challenges in Monolith Upscaling	21
2.21	Heat Expulsion Method	22
2.21.1	Gradual Addition of Feedstock	22
2.21.2	Frontal Polymerization	22
2.22	Wall Channel Elimination	23
CHAPTER 3: MATERIALS AND METHODOLOGY		24
3.1	List of Material	24
3.2	Model Plasmid Vaccine	24
3.3	pUC-based Plasmid DNA Semi-defined Medium (PDM) Preparation	24
3.4	Fermentation	25
3.5	Preparation of pMCS2.1-IRIDOVIRUS of *E. coli* Cleared Lysate	25
3.6	Synthesis of Poly (GMA-EDMA) Monolithic Column	26
3.7	Temperature Profile Distribution	26
3.8	Pore Size Analysis	26
3.9	Chromatographic Purification of Plasmid Iridovirus	27
3.10	Centrifugal Purification of Plasmid Iridovirus	27
3.11	Plasmid DNA and RNA Analysis	29
CHAPTER 4: RESULTS		30
4.1	*E. coli* Growth Optimization	30
4.1.1	Cultivation in LB Broth via Conventional Shaking Vs Bench-top Bioreactor	31

4.1.2	LB Broth Vs pUC-based Plasmid DNA Semi-defined Medium (Bench-top Bioreactor)	32
4.2	Pore Morphology Analysis of Polymethacrylate Monolith	32
4.2.1	The morphology of the Polymethacrylate Monoliths is Illustrated on Scanning Electron Microscope (SEM)	33
4.2.2	SEM Analysis on Each Partitioned Section Of Monolith	34
4.2.3	Mean Pore Size	37
4.2.4	Pore Size Distribution Studies (Quantitative)	39
4.3	Temperature Profile Analysis	42
4.4	Automated Chromatographic Purification of Plasmid Iridovirus (Two Steps Elution)	43
4.4.1	Optimization of Elution Buffer Concentration without Pretreating of Crude Lysate with Sulphate Salt Solution	45
4.4.2	First Step Elution Solution (NaCl) Optimization for the Complete Removal of RNAs	46
4.4.3	Optimization of the Concentration of Sulphate Salt Solution in Crude Lysate Pretreatment to Significantly Reduce the Amount of RNAs	47
4.5	Centrifugal Purification of Plasmid Iridovirus	51
4.5.1	Effect of Wall Channel	53
4.5.2	Plasmid DNA Extraction using Single-Step Elution Solution	54
	a. Screening of Relative Centrifugal Force (3000 x g – 8000 x g)	54
	b. Screening of Relative Centrifugal Force (600 x g – 1000 x g)	56
	c. Screening of Relative Centrifugal Force (100 x g – 300 x g)	58
	d. Inclusion of Sulphate Salt Solution in Pretreatment of Crude Lysate (0.6 M – 1.0 M) with Fixed Relative Centrifugal Force (200 x g)	60
4.5.3	Plasmid DNA Extraction using a Two-Step Elution Solution (Dual Solution Mode)	63
	a. Screening of Relative Centrifugal Force (200 x g – 400 x g) at Fixed Concentration of Sulphate Salt Solution	63
	b. Screening of the Volume of First Elution Solution (3	65

ml, 6 ml and 9 ml) at Fixed Relative Centrifugal Force (300 x g) and Concentration of Sulphate Salt Solution

4.5.4 Plasmid DNA Extraction using a Two-Step Elution Solution (Dual Alternating [NaCl] Solution) 67

a. Screening of the Concentration of First Elution Solution (0.1 M – 0.3 M NaCl) at Fixed Relative Centrifugal Force (300 x g) and Volume of Elution Solution 67

b. Plasmid DNA Extraction at Fixed Concentration of First Elution Solution (0.2 M NaCl) 69

CHAPTER 5: DISCUSSION 73

5.1 Cultivation of *E. coli* Carrying Plasmid Iridovirus (pMCS2.1 IRIDOVIRUS of *E. coli)* 73

5.1.1 Cell Density of pMCS2.1-IRIDOVIRUS of *E. coli* via Conventional Shaking Vs Bench-top Bioreactor in LB Broth 73

5.1.2 Cell Density of pMCS2.1-IRIDOVIRUS of *E. coli* in LB Broth Vs Semi-defined using Bench-top Bioreactor 74

5.2 Synthesis of Poly (GMA-EDMA) Monolithic Column: Pore Size Selection 75

5.2.1 Pore Structure of the Monolith 75

5.2.2 Pore Size Distribution of 70 %, 75 % and 80 % Porogen Content 77

5.3 Temperature Profile Analysis 78

5.3.1 Effect of Porogen Content on Heat Build-up during Polymerization 78

5.4 Optimization of Chromatographic Conditions using an Automated Chromatography System 79

5.4.1 Inherently-charged Polymethacrylate Resin 79

5.4.2 Optimization of Sample Loading and Elution 80

5.4.3 Inclusion of Sulphate Salt Solution in the Optimization of Sample Loading and Elution 81

5.5 Centrifugal Purification of pMCS2.1-IRIDOVIRUS of *E. coli* 82

5.5.1 Effect of Wall Channel 82

5.5.2 Optimization of Sample Loading and Elution 83

a. pMCS2.1-IRIDOVIRUS of *E. coli* Elution using 83

Deionized Water
b. pMCS2.1-IRIDOVIRUS of *E. coli* Elution with Inclusion of Sulphate Salt Solution 84
c. pMCS2.1-IRIDOVIRUS of *E. coli* elution via dual solution mode (two-step elution) 84
d. pMCS2.1-IRIDOVIRUS of *E. coli* elution via dual alternating [NaCl] solution 85

CHAPTER 6: CONCLUSION 86

REFERENCES 89

LIST OF TABLES

		Page
Table 2.2:	Theoretical binding capacities of polymethacrylate monoliths.	20
Table 3.1:	Medium components and concentrations (g/L) of semi-defined medium.	25
Table 4.1:	pMCS2.1-IRIDOVIRUS of *E. coli* isolated using non-functionalized monolithic centrifugation adsorbent and eluted using deionized water at 500µl/spin. Centrifugal force was tested at 3000 x g - 8000 x g at fixed volume of elution solution for 5 minutes/spin. Analysis was performed in 1% agarose of 60 mL TBE x 1 buffer at 100 V for 0.7 h.	54
Table 4.2:	pMCS2.1-IRIDOVIRUS of *E. coli* isolated using non-functionalized monolithic centrifugation adsorbent and eluted using deionized water at 500 µl/spin. Centrifugal force was tested at 600 x g - 1000 x g at fixed volume of elution solution for 1 minute/spin to increase the sensitivity of coverage. Analysis was performed in 1% agarose of 60 mL TBE x 1 buffer at 100 V for 0.7 h.	56
Table 4.3:	pMCS2.1-IRIDOVIRUS of *E. coli* isolated using non-functionalized monolithic centrifugation adsorbent and eluted using deionized water at 500 µl/spin. Centrifugal force was tested at 100 x g - 300 x g at fixed volume of elution solution for 1 minute/spin. Analysis was performed in 1% agarose of 60 mL TBE x 1 buffer at 100 V for 0.7 h.	58
Table 4.4:	pMCS2.1-IRIDOVIRUS of *E. coli* isolated using non-functionalized monolithic centrifugation adsorbent via pretreatment with 0.6 M - 1.0 M of sulphate salt solution concentrations and eluted using deionized water at 500 µl/spin. Centrifugal force was fixed at 200 x g and at fixed volume of elution solution for 1 minute/spin. Analysis was	60

performed in 1% agarose of 60 mL TBE x 1 buffer at 100 V for 0.7 h.

Table 4.5: pMCS2.1-IRIDOVIRUS of *E. coli* isolated using non-functionalized monolithic centrifugation adsorbent via pretreatment with 0.8 M of sulphate salt solution concentration and eluted using deionized water at 500 µl/spin. Centrifugal force was tested at 200 x g - 400 x g for 2 minutes/spin. Analysis was performed in 1% agarose of 60 mL TBE x 1 buffer at 100 V for 0.7 h. 63

Table 4.6: pMCS2.1-IRIDOVIRUS of *E. coli* isolated using non-functionalized monolithic centrifugation adsorbent via pretreatment with 0.8 M of sulphate salt solution concentration and eluted using deionized water at 500µl/spin. Different volumes of the first elution solution were tested and the centrifugal force was fixed at 300 x g for 2 minutes/spin. Analysis was performed in 1% agarose of 60 mL TBE x 1 buffer at 100 V for 0.7 h. 65

Table 4.7: pMCS2.1-IRIDOVIRUS of *E. coli* isolated using non-functionalized monolithic centrifugation adsorbent via pretreatment with 0.8 M of sulphate salt solution concentrations and eluted using 1 M NaCl at 500 µl/spin. Centrifugal force was fixed at 300 x g and volume of elution solution for 2 minutes/spin. Analysis was performed in 1% agarose of 60 mL TBE x 1 buffer at 100 V for 0.7 h. 67

Table 4.8: pMCS2.1-IRIDOVIRUS of *E. coli* isolated using non-functionalized monolithic centrifugation adsorbent via pretreatment with 0.8 M of sulphate salt solution concentration and eluted using 1 M NaCl at 500 µl/spin. Centrifugal force was fixed at 300 x g and volume of elution solution at 9 ml for 2 minutes/spin. Analysis was performed in 1% agarose of 60 mL TBE x 1 buffer at 100 V for 0.7 h. 69

Table 4.9: Recovery percentage and purity of pMCS2.1-IRIDOVIRUS of *E. coli* extracted at fixed concentration of first elution solution (0.2 M NaCl) and second elution solution (1.0 M NaCl). Analysis was performed with Nanodrop 2000 UV-Vis spectrophotometer. Agarose of 60 mL TBE x 1 buffer at 100 V for 0.7 h. 72

LIST OF FIGURES

		Page
Figure 2.1:	The strategy in downstream processing of rotavirus VLP	15
Figure 3.1:	A thorough qualitative SEM analysis was conducted by horizontally and vertically partitioning monolith into 9 different sections.	25
Figure 3.2:	DNA extraction via two - step elution strategy using single reagent.	29
Figure 4.1:	Growth curve of *E. coli* plasmid iridovirus cultured in incubator shaker at 180 rpm, 37 °C	31
Figure 4.2:	Growth curve of *E. coli* plasmid iridovirus cultures in bench-top bioreactor at loop controlled rpm, 37 °C.	31
Figure 4.3:	Growth curve of *E. coli* plasmid iridovirus on different media.	32
Figure 4.4:	Surface morphology of the poly (GMA-EDMA) monoliths prepared at different porogen contents (%). Images were taken from the scanning electron microscope (SEM), Hitachi S-3400, 15kV, x5.50 (50 %) x5.00 (60 %) x4.00 (70 %) x5.00 (80 %). The monoliths were prepared from GMA:EDMA ratio of 21:9; porogen concentrations of 50 %, 60 %, 70 % and 80 %; polymerisation temperature of 60 °C; AIBN concentration of 1 % (w/v) with respect to monomers.	33
Figure 4.5:	Pore morphology across the 70 % porogen content polymethacrylate monolith. The monolith was prepared from GMA:EDMA ratio of 21:9; porogen concentrations of 70 %; polymerisation temperature of 60 °C; AIBN concentration of % (w/v) with respect to monomers. Microscopic analysis was performed at 15 kV.	34

Figure 4.6: Pore morphology across the 75 % porogen content polymethacrylate monolith. The monolith was prepared from GMA:EDMA ratio of 21:9; porogen concentrations of 75%; polymerisation temperature of 60 °C; AIBN concentration of 1 % (w/v) with respect to monomers. Microscopic analysis was performed at 15 kV. 35

Figure 4.7: Pore morphology across the 80 % porogen content polymethacrylate monolith. The monolith was prepared from GMA:EDMA ratio of 21:9; porogen concentrations of 80%; polymerisation temperature of 60 °C; AIBN concentration of 1 % (w/v) with respect to monomers. Microscopic analysis was performed at 15 kV. 36

Figure 4.8: Mean pore size of each section of the polymethacrylate monolith. The monolith was prepared from GMA:EDMA ratio of 21:9; porogen concentrations of 70 %; polymerisation temperature of 60 °C; AIBN concentration of 1 % (w/v) with respect to monomers. 37

Figure 4.9: Mean pore size of each section of the polymethacrylate monolith. The monolith was prepared from GMA:EDMA ratio of 21:9;porogen concentrations of 75 %; polymerisation temperature of 60 °C; AIBN concentration of 1 % (w/v) with respect to monomers. 37

Figure 4.10: Mean pore size of each section of the polymethacrylate monolith. The monolith was prepared from GMA:EDMA ratio of 21:9; porogen concentrations of 80 %; polymerisation temperature of 60 °C; AIBN concentration of 1 % (w/v) with respect to monomers. 38

Figure 4.11: Pore size distribution of 70 % porogen content of polymethacrylate monolith. The monolith was prepared from GMA:EDMA ratio of 21:9; porogen concentrations of 70 %; 39

polymerisation temperature of 60 °C; AIBN concentration of 1 % (w/v) with respect to monomers.

Figure 4.12: Pore size distribution of 75 % porogen content of polymethacrylate monolith. The monolith was prepared from GMA:EDMA ratio of 21:9; porogen concentrations of 75 %; polymerisation temperature of 60 °C; AIBN concentration of 1 % (w/v) with respect to monomers. 40

Figure 4.13: Pore size distribution of 80 % porogen content of polymethacrylate monolith. The monolith was prepared from GMA:EDMA ratio of 21:9; porogen concentrations of 80 %; polymerisation temperature of 60 °C; AIBN concentration of 1 % (w/v) with respect to monomers. 41

Figure 4.14: Exothermic heat build-up during polymerization at different porogen contents (%). T_{poly}: 60 °C. 42

Figure 4.15: Effect of porogen content (%) on T_{max}. Polymerisation temperature was 60 °C. Each value was taken from the highest peak of the temperature (T_{max}) distribution data. 43

Figure 4.16: Gel electrophoresis of pMCS2.1-IRIDOVIRUS of *E. coli* isolated using non-functionalized monolithic chromatography adsorbent and eluted with 1 M NaCl. Analysis was performed in 1 % agarose of 60 mL TBE x 1 buffer at 100 V for 0.7 h. 45

Figure 4.17: Origin of two steps elution. Chromatogram of *E. coli* plasmid DNA extraction using Next Generation Chromatography System (BIORAD) via alkaline lysis. Deionized water was used during the first peak elution followed by 1.0 M NaCl for second peak elution. Flow rate of 1.0 mL/min. 45

Figure 4.18: Narrowed effective concentration range of NaCl (0.2M - 0.3M) in RNAs removal. Second step elution was carried out using 1 M 46

NaCl. Gel electrophoresis of pMCS2.1-IRIDOVIRUS of *E. coli* isolated via monolithic (non-functionalized) chromatography purification with 0.1 M - 1.0 M NaCl as the 1st step elution solution, and 1.0 M as the 2nd step elution solution. Analysis was performed in 1 % agarose at 100 V for 0.7 h.

Figure 4.19: Gel electrophoresis of *E. coli* plasmid DNA extraction via alkaline lysis with chemical modification of 0.6 M – 1.0 M sulphate salt solution. Analysis was performed in 1 % agarose of 60 mL TBE x 1 buffer at 100 V for 0.7 h. 47

Figure 4.20: Gel electrophoresis of pMCS2.1-IRIDOVIRUS of *E. coli* purified isolated using non-functionalized monolithic chromatography adsorbent and with 0.01 M - 0.10 M NaCl as the 1st step elution solution coupled with 0.6 M sulphate salt solution. Plasmid DNA elution was carried out using 2nd step elution solution 1 M NaCl. Analysis was performed in 1 % agarose of 60 mL TBE x 1 buffer at 100 V for 0.7 h. 48

Figure 4.21: Optimal concentration of sulphate salt solution for pretreatment of crude lysate. Gel electrophoresis of pMCS2.1-IRIDOVIRUS of *E. coli* isolated via monolithic chromatography purification from 0.6 M - 0.9 M sulphate salt solution treated crude lysate with 0.1 M NaCl as the 1st step elution solution, and 1.0 M as the 2nd step elution solution. Analysis was performed in 1 % agarose of 60 mL TBE x 1 buffer at 100 V for 0.7 h. 49

Figure 4.22: Optimal concentration of first step elution solution. Gel electrophoresis of pMCS2.1-IRIDOVIRUS of *E. coli* isolated via monolithic (non-functionalized) chromatography purification with 0.2 M – 0.3 M NaCl as the 1st step elution solution, and 1.0 M as the 2nd step elution solution. Analysis was performed in 1 % agarose at 100 V for 0.7 h. 50

Figure 4.23: Chromatograms of 0.2 M – 0.3 M NaCl runs. Chromatogram of *E. coli* plasmid DNA extraction using Next Generation 50

Chromatography System (BIORAD) via alkaline lysis. 0.2 M – 0.3 M NaCl are used during the first peak elution followed by 1.0 M NaCl for second peak elution. Flow rate of 1.0 mL/min.

Figure 4.24: Recovery percentage (%) of plasmid DNA (pDNA) and RNA at 0.21 M, 0.25 M and 0.29 M NaCl during second peak elution. 51

Figure 4.25: The comparison of number of frits used in the conical column. Preliminary results of gel electrophoresis of pMCS2.1-IRIDOVIRUS of *E. coli* isolated with innovative conical monolithic column in centrifugal format. Plasmid DNA elution was carried out using deionized water. Analysis was performed in 1 % agarose of 60 mL TBE x 1 buffer at 100 V for 0.7 h. 53

LIST OF ABBREVIATIONS

GI	-	Grouper iridovirus
HSV	-	Hemorrhagic septicemia virus
IPNV	-	Infectious pancreatic necrosis virus
IHNV	-	Infectious haematopoietic necrosis virus
ISAV	-	Infectious salmon anaemia virus
Nm	-	Nanometre
RPS-	-	Relative percentage survival
DNA	-	Deoxyribonucleic acid
RNA	-	Ribonucleic acid
CMV	-	Cytomegalovirus
KHV	-	Koi herpes virus
IgM	-	Immunoglobulin M
MHC	-	Major histocompatibility complex
CD8+	-	Cluster of differentiation 8 +
CPG	-	5'-C-phosphate-G-3'
GM-CSF	-	Granulocyte-macrophage colony-stimulating factor
µg	-	Microgram
µl	-	Microliter
IM	-	Intramuscular injection
IFN	-	Interferon
VHS	-	Viral hemorrhagic septicemia
IL	-	Interleukin
Th2	-	Type 2 T helper
H	-	Hyperosmotic infiltration
DI	-	Direct immersion
gDNA	-	Genomic DNA
pDNA	-	Plasmid DNA
VLP	-	Virus-like particles
BEVS	-	Baculovirus expression vector
IC	-	Insect cell
SEC	-	Size exclusion

AC	-	Affinity chromatographic
AEX	-	Anion-exchange chromatography
D_{pore}	-	Pore radius
ε_p	-	Particle porosity
$A_{internal}$	-	Specific surface area
CIM	-	Convective interaction media
mg	-	Miligram
mL	-	Mililitre
L	-	Litre
cm	-	centimetre
Min	-	Minute
EDMA	-	Ethylene glycol dimethacrylate
GMA	-	Glycidyl methacrylate
AIBN	-	Azobisisobutyronitrile
NaCl	-	Sodium chloride
SDS	-	Sodium dodecyl sulfate
MeOH	-	Methanol
EDTA	-	Ethylenediaminetetraacetic acid
RPM	-	Revolutions per minute
Tris-HCL	-	Tris-Hydrochloride
CH_3COOK	-	Potassium acetate
SEM	-	Scanning electron microscope
NGC	-	Next generation chromatography
RCF	-	Relative centrifugal force
Kb	-	Kilobase
TBE	-	Tris-borate-EDTA
PDM	-	pUC-based plasmid DNA semi-defined medium
OD	-	Optical density
LB	-	Lysogeny broth
Hr	-	Hour
Vs	-	Versus
kV	-	Kilovolt
T_{poly}	-	Polymerization temperature
T_{max}	-	Maximal temperature

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GMA	-	Glycidyl methacrylate
AIBN	-	Azobisisobutyronitrile
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SDS	-	Sodium dodecyl sulfate
MeOH	-	Methanol
EDTA	-	Ethylenediaminetetraacetic acid
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