

Production of the Novel Anti-hypertensive Peptide by Oocytes of Zebrafish(*Danio rerio*)

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ABSTRACT

In Taiwan, hypertension is one of the ten leading causes of death and is the major controllable risk factor associated with cardiovascular disease. Angiotensin converting enzyme (ACE) inhibitory peptides have attracted particular attention and have been studied widely for their applications to prevent hypertension among the bioactive peptides derived from milk proteins. A novel anti-hypertensive peptide (AP1), which derived from milk protein fermented by a nature symbiotic microbial starter-kefir and isolated by Dr. Chen at National Chung-Hsing University and Dr. Chen at Da-Yeh University, has a significant effect on reducing systolic and diastolic blood pressure in spontaneously hypertensive rat. Though AP1 can be produced by *E coli* and yeast, its post-translational modification and bioactivity is still not clear. The AP1 fragment (202 bp) was constructed with the promoters of vitellogenin, ovarian tumour or female-specific zebrafish zona pellucida genes into pAAV-IRES-VTG/OTU/ZPC-hrGFP and pEGFP-N1 vectors, and these vectors were transfected into tilapia ovarian (TO-2) cells or microinjected into the zebrafish oocytes to establish the transgenic fish line. RT-PCR or western blot analysis revealed that green fluorescent protein (GFP, 55 kDa) and AP1 peptide (8 kDa) could be expressed, observed, and detected after transfection or microinjection. Eggs produced by the transgenic fish showed green fluorescence and lasted to 2 months post fertilization. Only 6 eggs of 96 eggs (6.2%) from founder showed green fluorescence, and whose bioactivity would be further studied. The application of the platform techniques can be an alternative for the development of blood pressure controlling health food or even pharmaceuticals, and can be patented for commercial purpose or for the purpose of biosafety and biomedical researches.

Keywords : hypertension、anti-hypertensive peptide、tilapia ovarian cells、zebrafish、oocytes

Table of Contents

封面內頁 簽名頁 中文摘要.....	iii	英文摘要.....	v	致謝.....	vi	目錄.....
圖目錄.....	viii	表目錄.....	xiii	附錄.....	xvi	1. 前言.....
12. 文獻討論.....	3	2.1 高血壓 (Hypertension) 疾病.....	3	2.2 血管緊縮素轉化? (Angiotensin converting enzyme) 與血壓調控之相關性.....	3	2.2.1 血管緊縮素轉化?抑制劑 (Angiotensin converting enzyme inhibitor) 之簡介.....
2.2.2 抗高血壓勝? (Anti-hypertensive peptide) 之簡介.....	4	2.2.2.1 血管緊縮素轉化?抑制劑 (Angiotensin converting enzyme inhibitor) 之簡介.....	4	2.3 卵巢腫瘤 (Ovarian tumor, otu) 基因之簡介.....	5	2.3.1 高血壓 (Hypertension) 疾病.....
2.3.2 斑馬魚卵黃蛋白前質基因 (Vitellogenin, vtg) 之簡介.....	7	2.3.2.1 血管緊縮素轉化?抑制劑 (Angiotensin converting enzyme inhibitor) 之簡介.....	7	2.3.2.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.3 斑馬魚卵黃蛋白前質基因 (Vitellogenin, vtg) 之簡介.....
2.3.2.4 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.4.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.4.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.4.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.5 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.5.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.5.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.5.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.6 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.6.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.6.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.6.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.7 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.7.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.7.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.7.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.8 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.8.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.8.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.8.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.9 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.9.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.9.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.9.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.10 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.10.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.10.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.10.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.11 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.11.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.11.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.11.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.12 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.12.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.12.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.12.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.13 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.13.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.13.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.13.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.14 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.14.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.14.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.14.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.15 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.15.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.15.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.15.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.16 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.16.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.16.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.16.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.17 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.17.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.17.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.17.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.18 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.18.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.18.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.18.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.19 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.19.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.19.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.19.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.20 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.20.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.20.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.20.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.21 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.21.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.21.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.21.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.22 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.22.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.22.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.22.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.23 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.23.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.23.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.23.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.24 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.24.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.24.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.24.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.25 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.25.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.25.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.25.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.26 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.26.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.26.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.26.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.27 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.27.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.27.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.27.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.28 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.28.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.28.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.28.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.29 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.29.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.29.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.29.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.30 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.30.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.30.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.30.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.31 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.31.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.31.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.31.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.32 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.32.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.32.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.32.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.33 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.33.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.33.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.33.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.34 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.34.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.34.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.34.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.35 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.35.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.35.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.35.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.36 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.36.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.36.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.36.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.37 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.37.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.37.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.37.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.38 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.38.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.38.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.38.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.39 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.39.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.39.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.39.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.40 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.40.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.40.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.40.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.41 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.41.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.41.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.41.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.42 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.42.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.42.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.42.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.43 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.43.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.43.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.43.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.44 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.44.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.44.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.44.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.45 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.45.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.45.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.45.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.46 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.46.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.46.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.46.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.47 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.47.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.47.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.47.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.48 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.48.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.48.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.48.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.49 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.49.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.49.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.49.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.50 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.50.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.50.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.50.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.51 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.51.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.51.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.51.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.52 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.52.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.52.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.52.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.53 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.53.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.53.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.53.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.54 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.54.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.54.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.54.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.55 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.55.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.55.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.55.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.56 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.56.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.56.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.56.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.57 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.57.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.57.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.57.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.58 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.58.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.58.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.58.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.59 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.59.1 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.3.2.59.2 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	8	2.3.2.59.3 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....
2.3.2.60 斑馬魚 (Zebrafish, <i>Danio rerio</i>) 之簡介.....	7	2.				

細胞 RNA 之萃取.....	27
3.2.17 反轉錄聚合?連鎖反應 (Reverse transcription polymerase chain reaction,RT-PCR)	28
3.3 統計分析.....	29
第四章 結果.....	30
4.1 質體 pAAV-VTG-AP1 及 pEGFP-N1-AP1 之構築.....	30
4.1.1 抗高血壓勝? (AP1) 構築於 pAAV 及 pEGFP-N1 之表現載體.....	30
4.1.2 質體 pAAV-VTG-AP1 及 pEGFP-N1-AP1 之驗證.....	30
4.3 抗高血壓勝? (AP1) 及 VTG 啟動子基因於 pAAV 載體之序列分析.....	31
4.4 抗高血壓勝? 基因質體之功能活性分析.....	31
4.4.1 TO-2 細胞轉染.....	31
4.4.2 反轉錄聚合?連鎖反應 (RT-PCR) 及西方墨點反應 (Western blot) 之分析.....	31
4.4.3 Coomassie Brilliant Blue 染色法分析.....	32
4.5 斑馬魚卵顯微注射之分析與觀察.....	32
4.6 斑馬魚第一子代基因轉殖分析.....	33
第五章 討論.....	33
第六章 結論.....	34
參考文獻.....	37
	66

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