

記錄 編號	6332
狀態	NC094FJU00105032
助教 查核	
索書 號	
學校 名稱	輔仁大學
系所 名稱	生命科學系
舊系 所名 稱	
學號	492546197
研究 生 (中)	廖智凱
研究 生 (英)	Chih-Kai Liao
論文 名稱 (中)	基因轉殖蕃茄表現單鏈人類介白素 12 之基因
論文 名稱 (英)	Expression of Single-chain human interleukin-12 gene in transgenic tomato plants
其他 題名	
指導 教授 (中)	蘇睿智
指導 教授 (英)	Ruey-Chih Su
校內 全文 開放 日期	不公開

校外全文開放日期	不公開
全文不開放理由	
電子全文送交國圖	同意
國圖全文開放日期	2008.08.01
檔案說明	電子全文
電子全文	01
學位類別	碩士
畢業學年度	94
出版年	
語文別	中文
關鍵字(中)	介白素 12 連接子 單鏈人類介白素 12 基因轉殖蕃茄 農桿菌轉型作用
關鍵字(英)	interleukin-12, IL-12 linker single-chain human IL-12, schIL-12 transgenic tomato plants Agrobacterium-mediated transformation
摘要(中)	相較其他的表現系統，利用植物生產醫療用蛋白具有價格低廉、安全與可長期貯存等品質與經濟上的利益，本研究目的是利用可食用的蕃茄植物生產人類介白素-12 號蛋白質（human interleukin-12, hIL12），IL-12 為 p40 與 p35 次單位藉由雙硫鍵摺疊形成的異型雙隅體蛋白質，是一種促發炎與多效性的細胞激素，在引發細胞免疫中佔有重要的地位，目前已應

用於癌症治療、呼吸道發炎反應，以及病毒感染的治療與研究上，也常被利用來當作疫苗用的佐劑。為了避免在轉基因植物內形成 p40 同型雙體而抑制 IL-12 的生物活性，我們利用 18 個胺基酸序列的連接子 (linker) 將人類 IL-12 的 p40 與 p35 次單位構築成單鏈的融合基因，送交定序確定無誤後，利用 pET 系統於大腸桿菌 BL21 (DE3) 品系中表現，經 IPTG 誘導後，以人類的 IL-12 單株抗體可以偵測到約 70 kDa 的蛋白質。將 IL-12 的融合基因構築於 CaMV 35S 啟動子驅動的基因表現匣中，利用農桿菌 (Agrobacterium) 轉殖的方式把 IL-12 的融合基因表現匣送入蕃茄細胞中，在含有植物生長激素 Zeatin 與抗生素 hygromycin 的培養基中進行癒傷組織的篩選、分化與再生。經 PCR 檢測確認 10 株含有單鏈人類 IL-12 的基因轉殖蕃茄，其中 4 株以南方氏墨點法分析顯示僅含有一個轉殖基因的插入位置。而 ELISA 定量結果顯示在 10 株基因轉殖蕃茄中單鏈人類 IL-12 蛋白的表現量均很低。此外，本實驗中亦發現表現單鏈人類 IL-12 蛋白可能對植物體具有毒性。

摘要 (英)

The production of biopharmaceutical proteins in plants has many economic benefits compared to traditional expression system, including low cost, high safety, and long term storage. The aim of this study is to produce a disulfide-bonded heterodimeric cytokine, human Interleukin-12 (hIL-12) in transgenic tomato plants. IL-12 is consisting of p35 and p40 subunits. It is an proinflammatory and pleiotropic cytokine that plays a crucial role in cell-mediated immunity. IL-12 has been extensively employed in clinical treatments for cancer immunotherapy, airway inflammation, infectious disease, and also used as a vaccine adjuvant. In order to avoid the antagonism of p40 homodimers, we have constructed a human single-chain IL-12 (schIL-12) fusion gene in which p40 and p35 subunits were linked by a 18 amino acid linker. The hschIL-12 gene has been sequenced and expressed in the pET E.coli system. Upon the IPTG induction, the expression of about 70 kDa human IL-12 protein can be detected by anti-human IL-12 monoclonal antibody. Subsequently, we constructed the pC02B-schIL12 recombinant plasmid which harboring schIL-12 gene that was driven by CaMV35S promoter, and introduced into tomato explants via Agrobacterium-mediated transformation. After transformation, transgenic tomato plants were selected and regenerated in culture medium containing Zeatin and hygromycin. The PCR results indicated that 13 transgenic plants containing the schIL12 fusion gene, and the Southern blot analysis showed that 4 transgenic plants contained only one copy transgene in genomic DNA. ELISA quantizative analysis of hIL-12 in transgenic tomato plants revealed that schIL-12 accumulates in very low levels both in leaves and fruits. It was suspected that the expression of schIL-12 in transgenic plants might be toxic to plant cells.

論文目次

摘要..... I
 Abstract..... II 目
 錄..... III 表目
 錄..... VII 圖目
 錄..... VIII 前

言.....	1
介白素-12 的發現與免疫功能相關研究.....	1
醫療用蛋白的發展.....	4
重組蛋白表現系統之介紹.....	6
植物基因轉殖的方式.....	8
農桿菌轉型法 (Agrobacterium-mediated transformation) 之原理.....	9
植物表現醫療用蛋白之發展.....	11
植物表現細胞激素之相關研究.....	12
本論文研究之目的.....	13
材料與方法.....	14
1. 菌種、植物與引子.....	14
2. 菌種的培養.....	14
2.1 細菌的培養條件.....	14
2.2 細菌的保存方式.....	14
3. 少量抽取細菌質體 DNA.....	14
4. DNA 之酵素處理.....	15
4.1 限制酵素之作用.....	15
4.2 DNA 去磷酸化之製備.....	15
4.3 DNA 的接合作用.....	16
5. DNA 洋菜膠體電泳分析.....	16
6. 洋菜膠體 DNA 之分離與回收.....	16
6.1 膠膜透析法.....	16
6.2 GFXTM PCR DNA 與膠體 DNA 條帶純化套組.....	17
7. 細菌的轉型作用.....	17
7.1 大腸桿菌之轉型作用.....	17
7.2 農桿菌之轉型作用.....	18
8. 人類介白素-12 基因重組質體之製備.....	18
8.1 單鏈人類介白素-12 基因之構築.....	18
8.2 植物表現載體之製備.....	19
8.3 植物表現單鏈人類介白素-12 基因的重組質體之構築.....	19
8.4 大腸桿菌表現單鏈人類介白素-12 基因的重組質體之構築.....	20
9. 大腸桿菌蛋白表現之分析.....	20
9.1 誘導單鏈人類介白素-12 基因的表現.....	20
9.2 SDS-聚丙烯胺膠體 (SDS-polyacrylamide) 之製備.....	20
9.3 大腸桿菌蛋白質之粗萃取.....	21
9.4 SDS-聚丙烯胺蛋白質電泳分析之進行.....	21
10 西方墨點法 (Western blot) 與免疫點漬法 (Immuno-dot blot)	21
11. 蕃茄轉型、篩選與再生.....	22
12. 蕃茄轉植株的分子檢測.....	24
12.1 植物基因組 DNA (genomic DNA) 之萃取.....	24
12.2 Genomic DNA 之定量.....	25
12.3 以 PCR 確認蕃茄轉植株.....	25
13 南方氏墨點法 (Southern blot)	26
14.植物表現重組人類 IL-12 的測量.....	28
14.1 植物可溶性蛋白的粗萃取.....	28
14.2 蛋白質的定量.....	29
14.3 酵素連結免疫吸附分析法 (ELISA).....	29
15.統計方法.....	30
結 果.....	31
一、單鏈人類 IL-12 基因之構	

	<p>築.....31 1、帶有連接子 (Linker) 片段的人類 p40 與 p35 質體之構築.....31 2、重組質體 pCRII-schIL12 的構築.....31 二、大腸桿菌表現單鏈人類 IL-12 蛋白.....32 三、表現單鏈人類 IL-12 的植物重組質體之構築.....32 四、蕃茄植株的轉型作用.....33 五、擬轉殖植物之分子檢測.....34 六、基因轉殖蕃茄葉片中單鏈人類 IL-12 蛋白之表現.....35 七、基因轉殖蕃茄果實中單鏈人類 IL-12 蛋白之表現.....36 八、培養瓶中擬轉殖蕃茄表現單鏈人類 IL-12 蛋白之情形.....36 討論.....38 單鏈人類介白素-12 基因的構築.....38 植物表現重組質體的構築.....40 蕃茄植株的轉型作用.....40 人類 IL-12 於植物表現的情形.....42 轉殖 IL-12 基因對植物的可能影響.....44 未來的工作.....45 參考文獻.....46 附錄一、本實驗使用的菌種與植物名稱和特性.....81 附錄二、本實驗使用之質體.....82 附錄三、本實驗使用的引子與 PCR 程式.....83 附錄四、細菌生長之培養基.....84 附錄五、SDS-聚丙烯胺蛋白質電泳的藥品成分.....85 附錄六、植物組織培養的培養基.....86 附錄七、植物馴化的培養基.....87 附錄八、單鏈老鼠 IL-12 基因的構築流程.....88 附錄九、hIL-12 基因在人類與蕃茄細胞的 condon usage 之比較.....89 作者簡歷.....90</p>
<p>參考文獻</p>	<p>邱麗慧。1999。農桿菌轉殖阿拉伯芥 CBF1 基因於番茄之研究。國立師範大學生物研究所碩士論文。黃建豪。2002。基因轉殖植物生產人類血清蛋白。國立東華生物技術研究所碩士論文。葉怡鈴。2002。介白素 12 基因在過敏疾病治療及免疫調節機制之探討。國立台灣大學免疫學研究所博士論文。Boggio K, Nicoletti G, Di Carlo E, Cavallo F, Landuzzi L, Melani C, Giovarelli M, Rossi I, Nanni P, De Giovanni C, Bouchard P, Wolf S, Modesti A, Musiani P, Lollini PL, Colombo MP, Forni G (1998) Interleukin 12-mediated prevention of spontaneous mammary adenocarcinomas in two lines of Her-2/neu transgenic mice. <i>J Exp Med</i> 188: 589-596 Bretthauer RK (2003) Genetic engineering of <i>Pichia pastoris</i> to humanize N-glycosylation of proteins. <i>Trends Biotechnol</i> 21: 459-462 Chan SH, Perussia B, Gupta JW, Kobayashi M, Pospisil M, Young HA, Wolf SF, Young D, Clark SC, Trinchieri G (1991) Induction of interferon gamma production by natural killer cell stimulatory factor: characterization of the responder cells and synergy with other inducers. <i>J Exp Med</i> 173: 869-879 Chen TL, Lin YL, Lee YL, Yang NS, Chan MT (2004) Expression of bioactive human interferon-gamma in transgenic rice cell suspension cultures. <i>Transgenic Res</i> 13: 499-510 Clerici M, Shearer GM (1993) A TH1-->TH2 switch is a critical step in the etiology of HIV infection. <i>Immunol Today</i> 14: 107-111 Cortina C, Culianez-Macia FA (2004) Tomato transformation and transgenic plant</p>

production. *Plant Cell Tissue Organ Cult* 76: 269-275 Costa MGC, Noguera FTS, Figueira ML, Ontoni WC, Brommonshenkel SH, Cecon PR (2000) Influence of the antibiotic timentin on plant regeneration of tomato (*Lycopersicon esculentum* Mill) cultivars. *Plant Cell Rep* 19: 327-332 Coyle AJ, Le Gros G, Bertrand C, Tsuyuki S, Heusser CH, Kopf M, Anderson GP (1995) Interleukin-4 is required for the induction of lung Th2 mucosal immunity. *Am J Respir Cell Mol Biol* 13: 54-59 D'Andrea A, Rengaraju M, Valiante NM, Chehimi J, Kubin M, Aste-Amezaga M, Chan SH, Kobayashi M, Young D, Nickbarg E, Chizzonite R, Wolf SF, Trinchieri G (1992) Production of nature killer cell stimulatory factor (Interleukin 12) by peripheral blood mononuclear cells. *J Exp Med* 176: 1387-1398 Daniell H, Chebolu S, Kumar S, Singleton M, Falconer R (2005) Chloroplast-derived vaccine antigens and other therapeutic proteins. *Vaccine* 23: 1779-1783 Daniell H, Streatfield SJ, Wycoff K (2001) Medical molecular farming: production of antibodies, biopharmaceuticals and edible vaccines in plants. *Trends Plant Sci* 6: 219-226 Douglas CJ, Halperin W, Nester EW (1982) *Agrobacterium tumefaciens* mutants affected in attachment to plant cells. *J Bacteriol* 152: 1265-1275 Eklund KK, Ghildyal N, Austen KF, Stevens RL (1993) Induction by IL-9 and suppression by IL-3 and IL-4 of the levels of chromosome 14-derived transcripts that encode late-expressed mouse mast cell proteases. *J Immunol* 151: 4266-4273 Filichkin SA, Gelvin S (1993) Formation of a putative relaxation intermediate during T-DNA processing directed by the *Agrobacterium tumefaciens* VirD1,D2 endonuclease. *Mol Microbiol* 8: 915-926 Fischhoff DA, Bowdish KS, Perlak FJ, Marrone PG, McCormick SM, Niedermeyer JG, Dean DA, Kusano-Kretzmer K, Mayer EJ, D.E. R, Rogers SG, Fraley RT (1987) Insect Tolerant Transgenic Tomato Plants. *Bio Technology* 5: 807-813 Flynn JL, Goldstein MM, Triebold KJ, Sypek J, Wolf S, Bloom BR (1995) IL-12 increases resistance of BALB/c mice to *Mycobacterium tuberculosis* infection. *J Immunol* 155: 2515-2514 Fraley RT, Rogers SG, Horsch RB, Sanders PR, Flick JS, Adams SP, Bittner ML, Brand LA, Fink CL, Fry JS, Galluppi GR, Goldberg SB, Hoffmann NL, Woo SC (1983) Expression of bacterial genes in plant cells. *Proc Natl Acad Sci U S A* 80: 4803-4807 Frary A, Earle ED (1996) An examination of factor affecting the efficiency of *Agrobacterium*-mediated transformation of tomato. *Plant Cell Rep* 16: 235-240 Galvao da Silva AP, de Almeida Abrahamsohn I (2001) Interleukin-12 stimulation of lymphoproliferative responses in *Trypanosoma cruzi* infection. *Immunology* 104: 349-354 Gavett SH, O'Hearn DJ, Li X, Huang SK, Finkelman FD, Wills-Karp M (1995) Interleukin 12 inhibits antigen-induced airway hyperresponsiveness, inflammation, and Th2 cytokine expression in mice. *J Exp Med* 182: 1527-1536 Gelvin SB (1998) The introduction and expression of transgenes in plants. *Curr Opin Biotechnol* 9: 227-232 Gelvin SB (2003) *Agrobacterium*-mediated plant transformation: the biology behind the "gene-jockeying" tool. *Microbiol Mol Biol Rev* 67: 16-37 Gerngross TU (2004) Advances in the production of human therapeutic proteins in yeasts and filamentous fungi. *Nat Biotechnol* 22: 1409-1414 Giddings G, Allison G, Brooks D, Carter A (2000) Transgenic plants as factories for biopharmaceuticals. *Nat Biotechnol* 18: 1151-1155 Goeddel DV, Kleid DG,

Bolivar F, Heyneker HL, Yansura DG, Crea R, Hirose T, Kraszewski A, Itakura K, Riggs AD (1979) Expression in *Escherichia coli* of chemically synthesized genes for human insulin. *Proc Natl Acad Sci U S A* 76: 106-110 Goldsby RA, Kindt TJ, Osborne BA (2000) *Kuby immunology* 4th ed: New York: W.H. Freeman and company Gomord V, Denmat LA, Fitchette-Laine AC, Satiat-Jeunemaitre B, Hawes C, Faye L (1997) The C-terminal HDEL sequence is sufficient for retention of secretory proteins in the endoplasmic reticulum (ER) but promotes vacuolar targeting of proteins that escape the ER. *Plant J* 11: 313-325 Gosset P, Tscopoulos A, Wallaert B, Joseph M, Capron A, Tonnel AB (1992) Tumor necrosis factor alpha and interleukin-6 production by human mononuclear phagocytes from allergic asthmatics after IgE-dependent stimulation. *Am Rev Respir Dis* 146: 768-774 Gubler U, Chua AO, Schoenhaut DS, Dwyer CM, McComas W, Motyka R, Nabavi N, Wolitzky AG, Quinn PM, Familletti PC (1991) Coexpression of two distinct genes is required to generate secreted bioactive cytotoxic lymphocyte maturation factor. *Proc Natl Acad Sci U S A* 88: 4143-4147 Gutierrez-Ortega A, Avila-Moreno F, Saucedo-Arias LJ, Sanchez-Torres C, MA. G-L (2004) Expression of a single-chain human interleukin-12 gene in transgenic tobacco plants and functional studies. *Biotechnol Bioeng* 85: 734-740 Gutierrez-Ortega A, Sandoval-Montes C, de Olivera-Flores TJ, Santos-Argumedo L, Gomez-Lim MA (2005) Expression of functional interleukin-12 from mouse in transgenic tomato plants. *Transgenic Res* 14: 877-885 Hamza S, Chupeau Y (1993) Re-evaluation of Conditions for Plant Regeneration and *Agrobacterium*-Mediated Transformation from Tomato (*Lycopersicon esculentum*). *J Exp Bot* 269: 1837-1845 Haq TA, Mason HS, Clements JD, Arntzen CJ (1995) Oral immunisation with a recombinant bacterial antigen produced in transgenic plants. *Science* 268: 714-716 Hiatt A, Cafferkey R, Bowdish K (1989) Production of antibodies in transgenic plants. *Nature* 342: 76-78 Hiei YK, T., Kubo T (1997) Transformation of rice mediated by *Agrobacterium tumefaciens*. *Plant Mol Biol* 35: 205-218 Hsieh TH, Lee JT, Chang YY, Chan MT (2002) Tomato plants ectopically expressing *Arabidopsis* CBF1 show enhanced resistance to water deficit stress. *Plant Physiol* 130: 618-626 Huang Z, Dry I, Webster D, Strugnell R, Wesselingh S (2001) Plant-derived measles virus hemagglutinin protein induces neutralizing antibodies in mice. *Vaccine* 19: 2163-2171 Itakura K, Tadaaki H, Crea R, Riggs AD, Heyneker HL, Bolivar F, Boyer HW (1977) Expression in *Escherichia coli* of a chemically synthesized gene for the hormone somatostatin. *Biotechnology* 24:84-91 Johnson IS (1983) Human insulin from recombinant DNA technology. *Science* 219: 632-637 Kang BY, Kim E, Kim KTS (2005) Regulatory mechanisms and their therapeutic implications of interleukin-12 production in immune cells. *Cell Signal* 17: 665-673 Kanno T, Naito S, Shimamoto K (2000) Post-transcriptional gene silencing in cultured rice cells. *Plant Cell Physiol* 41: 321-326 Kincy-Cain T, Clements JD, Bost KL (1996) Endogenous and exogenous interleukin-12 augment the protective immune response in mice orally challenged with *Salmonella dublin*. *Infect Immun* 64: 1437-1440 Ko K, Koprowski H (2005) Plant biopharming of monoclonal antibodies. *Virus Res* 111: 93-100 Kobayashi M, Fitz L, Ryan M,

Hewick RM, Clark SC, Chan S, Loudon R, Sherman F, Perussia B, Trinchieri G (1989) Identification and purification of natural killer cell stimulatory factor (NKSF), a cytokine with multiple biologic effects on human lymphocytes. *J Exp Med* 170: 827-845 Kost TA (1997) Expression systems: Gene expression systems in the genomics era. *Curr Opin Biotechnol* 8: 539-541 Kwon TH, Seo JE, Kim J, Lee JH, Jang YS, Yang MS (2003) Expression and secretion of the heterodimeric protein interleukin-12 in plant cell suspension culture. *Biotechnol Bioeng* 81: 870-875 Larrick JW, Thomas DW (2001) Producing proteins in transgenic plants and animals. *Curr Opin Biotechnol* 12: 411-418 Lee YL, Ye YL, Yu CI, Wu YL, Lai YL, Ku PH, Hong RL, Chiang BL (2001) Construction of single-chain interleukin-12 DNA plasmid to treat airway hyperresponsiveness in an animal model of asthma. *Hum Gene Ther* 12: 2065-2079 Lee YW, Jin S, Sim WS, Nester EW (1996) The sensing of plant signal molecules by *Agrobacterium*: genetic evidence for direct recognition of phenolic inducers by the VirA protein. *Gene* 179: 83-88 Leonard P, Sur S (2003) Interleukin-12: potential role in asthma therapy. *BioDrugs* 2003: 1-7 Ling P, Gately MK, Gubler U, Stern AS, Lin P, Hollfelder K, Su C, Pan YC, Hakimi J (1995) Human IL-12 p40 homodimer binds to the IL-12 receptor but does not mediate biologic activity. *J Immunol* 154: 116-127 Lopez AF, Sanderson CJ, Gamble JR, Campbell HD, Young IG, Vadas MA (1988) Recombinant human interleukin 5 is a selective activator of human eosinophil function. *J Exp Med* 167: 219-224 Ma S, Huang Y, Yin Z, Menassa R, Brandle JE, Jevnikar AM (2004) Induction of oral tolerance to prevent diabetes with transgenic plants requires glutamic acid decarboxylase (GAD) and IL-4. *Proc Natl Acad Sci U S A* 105: 5680-5685 Magnuson NS, Linzmaier PM, Reeves R, An G, HayGlass K, Lee JM (1998) Secretion of biologically active human interleukin-2 and interleukin-4 from genetically modified tobacco cells in suspension culture. *Protein Expr Purif* 13: 45-52 Mason HS, Ball JM, Shi JJ, Jiang X, Estes MK, Arntzen CJ (1996) Expression of Norwalk virus capsid protein in transgenic tobacco and potato and its oral immunogenicity in mice. *Proc Natl Acad Sci U S A* 93: 5335-5340 Mason HS, Haq TA, Clements JD, Arntzen CJ (1998) Edible vaccine protects mice against *Escherichia coli* heat-labile enterotoxin (LT): potatoes expressing a synthetic LT-B gene. *Vaccine* 16: 1336-1343 Mason HS, Lam DM, Arntzen CJ (1992) Expression of hepatitis B surface antigen in transgenic plants. *Proc Natl Acad Sci U S A* 89: 11745-11749 Mason HS, Warzecha H, Mor T, Arntzen CJ (2002) Edible plant vaccines: applications for prophylactic and therapeutic molecular medicine. *Trends Mol Med* 8: 324-329 Matsuo R, Kobayashi M, Herndon DN, Pollard RB, Suzuki F (1996) Interleukin-12 protects thermally injured mice from herpes simplex virus type 1 infection. *J Leukoc Biol* 59: 623-630 McLane MP, Haczku A, van de Rijn M, Weiss C, Ferrante V, MacDonald D, Renauld JC, Nicolaidis NC, Holroyd KJ, Levitt RC (1998) Interleukin-9 promotes allergen-induced eosinophilic inflammation and airway hyperresponsiveness in transgenic mice. *Am J Respir Cell Mol Biol* 19: 713-720 Ohya K, Itchoda N, Ohashi K, Onuma M, Sugimoto C, Matsumura T (2002) Expression of biologically active human tumor necrosis factor- α in transgenic potato plant. *J Interferon Cytokine Res* 22: 371-378

Ohya K, Matsumura T, Ohashi K, Onuma M, Sugimoto C (2001) Expression of two subtypes of human IFN-alpha in transgenic potato plants. *J Interferon Cytokine Res* 21: 595-602

Ou-Lee TM, Turgeon R, Wu R (1986) Expression of a foreign gene linked to either a plant-virus or a Drosophila promoter, after electroporation of protoplasts of rice, wheat, and sorghum. *Proc Natl Acad Sci U S A* 83: 6815-6819

Pansegrau W, Schoumacher F, Hohn B, Lanka E (1993) Site-specific cleavage and joining of single-stranded DNA by VirD2 protein of *Agrobacterium tumefaciens* Ti plasmids: analogy to bacterial conjugation. *Proc Natl Acad Sci U S A* 90: 11538-11542

Perlak FJ, Fuchs RL, Dean DA, McPherson SL, Fischhoff DA (1991) Modification of the coding sequence enhances plant expression of insect control protein genes. *Proc Natl Acad Sci U S A* 88: 3324-3328

PhRMA (2004) survey: Medicines in development biotechnology. www.phrma.org

Portielje JE, Kruit WH, Eerenberg AJ, Schuler M, Sparreboom A, Lamers CH, Gratama JW, Stoter G, Huber C, Hack CE (2005) Subcutaneous injection of interleukin 12 induces systemic inflammatory responses in humans: implications for the use of IL-12 as vaccine adjuvant. *Cancer Immunol Immunother* 54: 37-43

Rai M, Padh H (2001) Expression systems for production of heterologous protein. *Curr Science* 80: 1121-1128

Reuhs BL, Kim JS, Matthyse AG (1997) Attachment of *Agrobacterium tumefaciens* to carrot cells and *Arabidopsis* wound sites is correlated with the presence of a cell-associated, acidic polysaccharide. *J Bacteriol* 179: 5372-5379

Rossol S, Marinos G, Carucci P, Singer MV, Williams R, Naoumov NV (1997) Interleukin-12 induction of Th1 cytokines is important for viral clearance in chronic hepatitis B. *J Clin Invest* 99: 3025-3027

Roy EJ, Gawlick U, Orr BA, Rund LA, Webb AG, Kranz DM (2000) IL-12 treatment of endogenously arising murine brain tumors. *J Immunol* 165: 7293-7299

Saldarriaga OA, Perez LE, Travi BL, Melby PC (2006) Selective enhancement of the type 1 cytokine response by expression of a canine interleukin (IL)-12 fused heterodimeric DNA. *Vet Immunol Immunopathol* 110: 377-388

Sanford JC, Klein TM, Wolf ED, Allen N (1987) Delivery of substances into cells and tissue using a particle bombardment process. *J Plant Sci Tech* 5:27-37

Sgadari C, Angiolillo AL, Tosato G (1996) Inhibition of angiogenesis by interleukin-12 is mediated by the interferon-inducible protein 10. *Blood* 87: 3877-3882

Sgadari C, Farber JM, Angiolillo AL, Liao F, Teruya-Feldstein J, Burd PR, Yao L, Gupta G, Kanegane C, Tosato G (1997) Mig, the monokine induced by interferon-gamma, promotes tumor necrosis in vivo. *Blood* 89: 2635-2643

Sheng J, Citovsky V (1996) *Agrobacterium*-plant cell DNA transport: have virulence proteins, will travel. *Plant Cell* 8: 1699-1710

Sieburth D, Jabs EW, Warrington JA, Li X, Lasota J, LaForgia S, Kelleher K, Huebner K, Wasmuth JJ, Wolf SF (1992) Assignment of genes encoding a unique cytokine (IL12) composed of two unrelated subunits to chromosomes 3 and 5. *Genomics* 14: 59-62

Smart V, Foster PS, Rothenberg ME, Higgins TJ, Hogan SP (2003) A plant-based allergy vaccine suppresses experimental asthma via an IFN-gamma and CD4+CD45RBlow T cell-dependent mechanism. *J Immunol* 171: 2116-2126

Stachel SE, Messens E, Van Montagu M, Zambryski P (1985) Identification of the signal molecules produced by wounded plant cells that activate

T-DNA transfer in *Agrobacterium tumefaciens*. *Nature* 318: 624-629 Staub JM, Garcia B, Graves J, Hajdukiewicz PT, Hunter P, Nehra N, Paradkar V, Schlittler M, Carroll JA, Spatola L, Ward D, Ye G, Russell DA (2000) High-yield production of a human therapeutic protein in tobacco chloroplasts. *Nat Biotechnol.* 18: 330-338 Steinberg FM, Raso J (1998) Biotech pharmaceuticals and biotherapy: an overview. *J Pharm Pharm Sci* 1: 48-59 Stern AS, Podlaski FJ, Hulmes JD, Pan YC, Quinn PM, Wolitzky AG, Familletti PC, Stremlo DL, Truitt T, Chizzonite R (1990) Purification to homogeneity and partial characterization of cytotoxic lymphocyte maturation factor from human B-lymphoblastoid cells. *Proc Natl Acad Sci U S A* 87: 6808-6812 Stylianou E, Aukrust P, Nordoy I, Muller F, Froland SS (2000) Enhancement of lymphocyte proliferation induced by interleukin-12 and anti-interleukin-10 in HIV-infected patients during highly active antiretroviral therapy. *APMIS* 108: 601-607 Tang W, Samuels V, Whitley N, Bloom N, T. D, Newton RJ (2004) Post-transcriptional gene silencing induced by short interfering RNAs in cultured transgenic plant cells. *Genomics Proteomics Bioinformatics* 2: 97-104 Teixeira LK, Fonseca BP, Barboza BA, Viola JP (2005) The role of interferon-gamma on immune and allergic responses. *Mem Inst Oswaldo Cruz* 100 Suppl 1: 137-144 Temann UA, Geba GP, Rankin JA, Flavell RA (1998) Expression of interleukin 9 in the lungs of transgenic mice causes airway inflammation, mast cell hyperplasia, and bronchial hyperresponsiveness. *J Exp Med* 188: 1307-1320 Thomashow MF, Karlinsey JE, Marks JR, Hurlbert RE (1987) Identification of a new virulence locus in *Agrobacterium tumefaciens* that affects polysaccharide composition and plant cell attachment. *J Bacteriol* 169: 3209-3216 Trinchieri G (1994) Interleukin-12: a cytokine produced by antigen-presenting cells with immunoregulatory functions in generation of T-helper cells type 1 and cytotoxic lymphocytes. *Blood* 84: 4008-4027 Trinchieri G (1995) Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. *Annu Rev Immunol* 13: 251-276 Trinchieri G (2003) Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nature Rev Immunol* 3: 133-146 Tzfira T, Li J, Lacroix B, Citovsky V (2004) *Agrobacterium* T-DNA integration: molecules and models. *Trends Genet* 20: 375-383 van den Eede G, Aarts H, Buhk HJ, Corthier G, Flint HJ, Hammes W, Jacobsen B, Midtvedt T, van der Vossen J, von Wright A, Wackernagel W, Wilcks A (2004) The relevance of gene transfer to the safety of food and feed derived from genetically modified (GM) plants. *Food Chem Toxicol* 42: 1127-1156 Vaucheret H, Beclin C, Elmayan T, Feuerbach F, Godon C, Morel JB, Mourrain P, Palauqui JC, Vernhettes S (1998) Transgene-induced gene silencing in plants. *Plant J* 16: 651-659 Vaucheret H, Beclin C, Fagard M (2001) Post-transcriptional gene silencing in plants. *J Cell Sci* 114: 3083-3091 Vaucheret H, Fagard M (2001) Transcriptional gene silencing in plants: targets, inducers and regulators. *Trends Genet* 17: 29-35 Velcheva M, Faltin Z, Flaishman M, Eshdat Y, Perl A (2004) A liquid culture system for *Agrobacterium*-mediated transformation of tomato (*Lycopersicon esculentum* L. Mill). *Plant Sci* 168: 121-130 Verwecken W, Kaigorodov V, Callewaert N, Geysens S, De Vusser

	<p>K, Contreras R (2004) In vivo synthesis of mammalian-like, hybrid-type N-glycans in <i>Pichia pastoris</i>. <i>Appl Environ Microbiol</i> 70: 2639-2646 Walsh G (2005) Therapeutic insulins and their large-scale manufacture. <i>Appl Microbiol Biotechnol</i> 67: 151-159 Whitlow M, Bell B.A., Feng S.L., Filpula D., Hardman K.D., Hubert S.L., Rollence M.L., Wood J.F., Schott M.E., Milenic D.E. (1993) An improved linker for single-chain Fv with reduced aggregation and enhanced proteolytic stability. <i>Protein Eng</i> 6: 989-995 Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL, Donaldson DD (1998) Interleukin-13: central mediator of allergic asthma. <i>Science</i> 282: 2258-2261 Zambryski P, Tempe J, J. S (1989) Transfer and function of T-DNA genes from agrobacterium Ti and Ri plasmids in plants. <i>Cell</i> 56: 193-201 Zhang B, Yang YH, Lin YM, Rao Q, Zheng GG, Wu KF (2003) Expression and production of bioactive human interleukin-18 in transgenic tobacco plants. <i>Biotechnol Lett</i> 25: 1629-1635 Zheng LY, Mou L, Lin S, Lu RM, Luo EJ (2005) Enhancing DNA vaccine potency against hantavirus by co-administration of interleukin-12 expression vector as a genetic adjuvant. <i>Chin Med J (Engl)</i> 118: 313-319 Zhou GY, Weng J, Zeng Y, Huang SQ, Liu G (1983) Import of exogenous DNA into cotton embryos. <i>Plant Cell</i> 13: 369-384 Zupan JR, Zambryski P (1995) Transfer of T-DNA from <i>Agrobacterium</i> to the plant cell. <i>Plant Physiol</i> 107: 1041-1047</p>
論文頁數	90
附註	
全文點閱次數	
資料建置時間	
轉檔日期	
全文檔存取記錄	
異動記錄	M admin Y2008.M7.D3 23:18 61.59.161.35