

pPIC9

hFSH β

Pichia pastoris

(Ph.D)

(Ph.D)

(M.Sc)

(Ph.D)*

-
-
-

(FSH)

:

FSH β

AOX1

pPIC9

:

pPIC9-F1

5'

kb

pTV-

FSH β

:

pPIC9F1

AOX1

PCR Restriction analysis .

2019

pPIC9F1

:

AOX1

FSH β (+sig E2-IVS2-E3)

FSH β .

FSH

-

FSH β

Follicle Stimulating Hormone pPIC9 Pichia pastoris :

[]

(FSH)

FSH

[]

FSH ()

Jameson JL

FSH hFSH β

[]

RNA Shoham

cDNA Cys28-Ala-Gly-Tyr31 (CAGY)

[] FSH- β

Tekeno Saneyoshi []

cDNA RNA

FSH β E-coli

[] CHO E-coli

FSH β)

(

AOX1 pPIC9

Invitrogen Pichia pastoris

Pfu DNA E-coli

DNA DNA marker polymerase

InsT/AcloneTM (DNA Extraction Kit;#K0513)

PCR product cloning kit (#K1214)

Fermentase pTZ57R/T

DNA Taq DNA Polymerase . []

(DNGTM-Plus DNA Extraction Solution))

(High Pure () FSH (

Roche Plasmid Isolation Kit)

Invitrogen pPIC9

[]

DNA . DNA Invitrogen

DNGTM-Plus DNA Extraction Solution

(*) KEX2
 FSHB XhoI
 FSHE2F: **.FSH**
 5'-AGT-TTC-TAG-TGG-GCT-TCA-TTG-TTT-G-3'
 FSHE3R: 5'-GTA-TGT-GGC-CTG-AAA-TGT-
 ~ kb CC-3'
 FSHβ
 PICSR: 5'-CCC-TCT-AGA-GAA-TTC-
TTA-TTC-TTT-CAT-TTC-ACC-AAA-GG
 EcoRI
 dNTP
 Taq DNA
 () polymerase
 PCR Mg
 °C
 Mg
 ()
 pTV- 2019
 dNTP
 Taq:pfu DNA
 / / Mg
 / Annealing
 /
 PCR
 2020 pTV-2019 pTV-2011 PstI
 2020 pTV-2019 pTV-
 Dideoxy chain termination pTV-
 pPIC9 F1 (+sig E2-IVS2-E3)
 FSHβ - -
 (+sig E2-IVS2-E3)
 EcoRI XhoI pPIC9 **.FSHβ (+sig E2-IVS2-E3)**
 PCR FSHβ
 EcoRI XhoI α-factor
 Ligation mix pPIC9 pPIC9
 E-coli XL1 blue
 PicxsigF1: 5'-CCC-CTC-GAG-AAA-AGA-
GAG*-GCT-GAA-GCT-ATG-AAG-ACA-
CTC-CAG-TTT-TT-C- 3'
 ()
 Leu-Glu-Lys-Arg*Glu-Ala-Glu-Ala-Met



FSH β

XhoI/NotI NcoI PstI

PCR

pPIC9F1/2

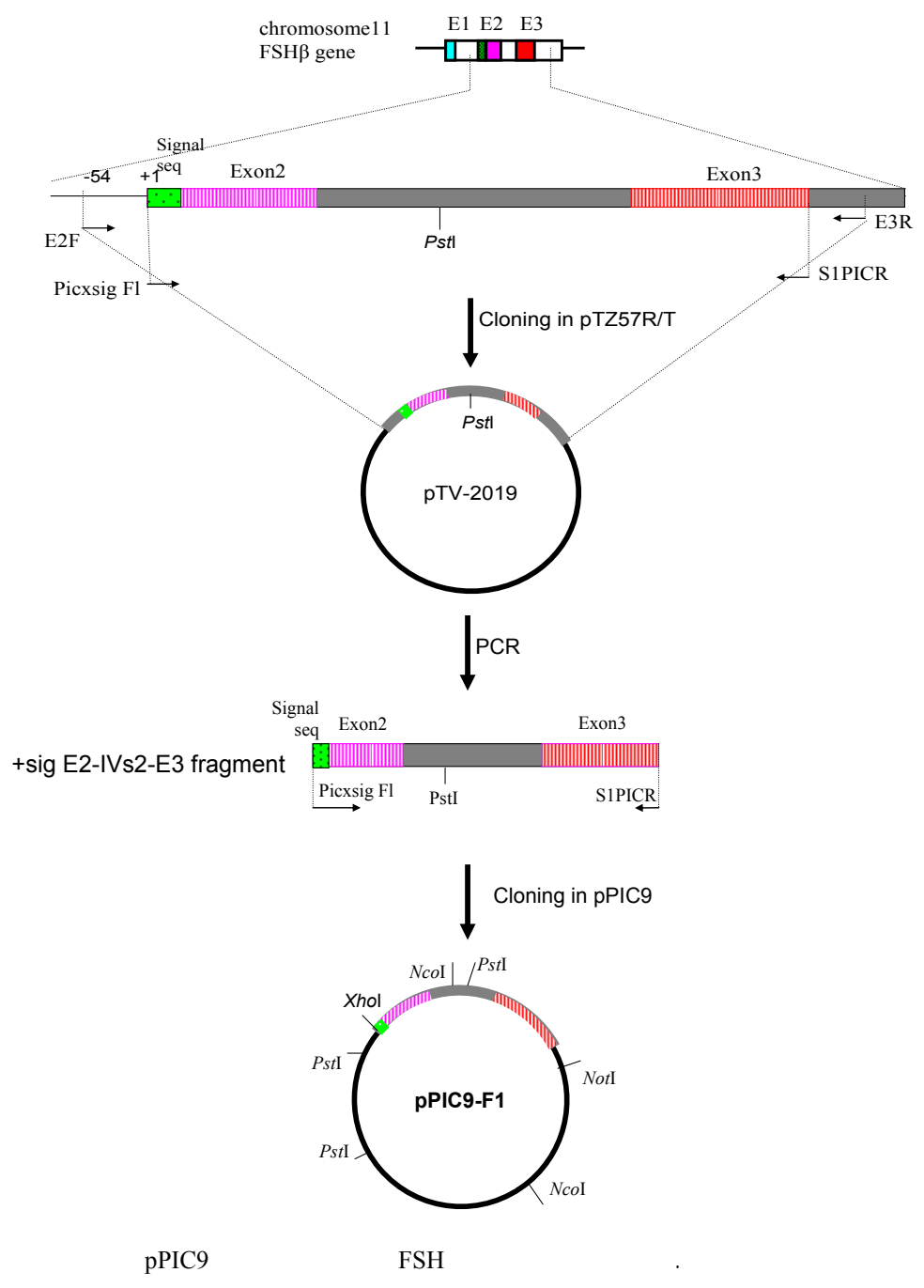
pPIC9F1/1

()

AOX1

PCR

[]



-)

FSH β ()

(Flanking

(-

FSHE2F FSHE3R

.() ' ' regions)

pTV-2020 pTV-2019

pTRZ57R

M13/pUC

TV-2020 pTV-2019 pTV-2011

PstI

GenBank

.www.ncbi.nlm.nih.gov

~ kb / kb

()

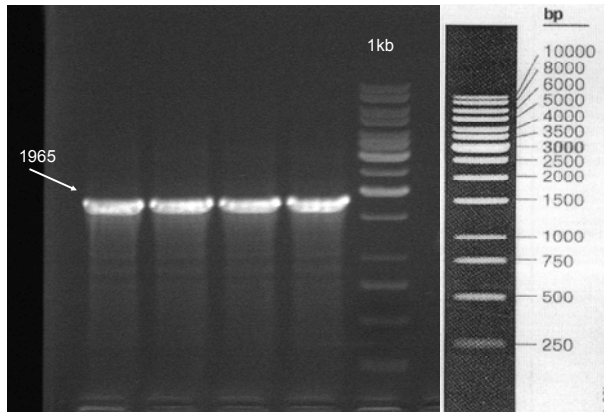
PICSR PicxsigF1

pTV-2019

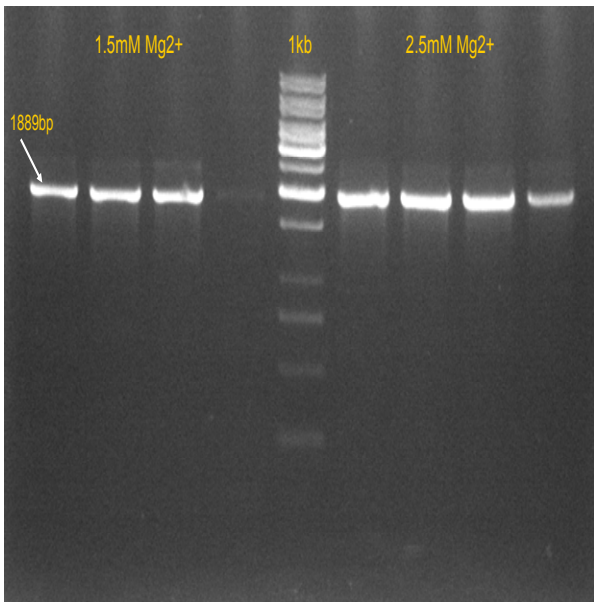
-) FSH β ()

(-

() ' '



.E2F E3R FSH
FSH PCR : -
/ / / / /
() 1kb ladder :

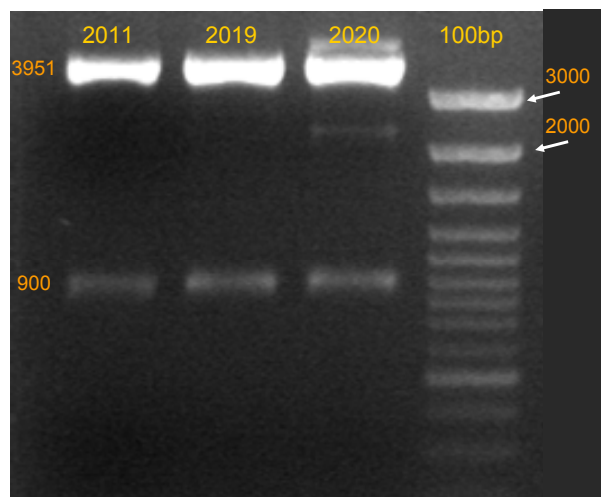


FSH (+sig E2-IVS2-E3)
.PICSR PICsigF1

Mg /
/ / / /

kb

Mg /

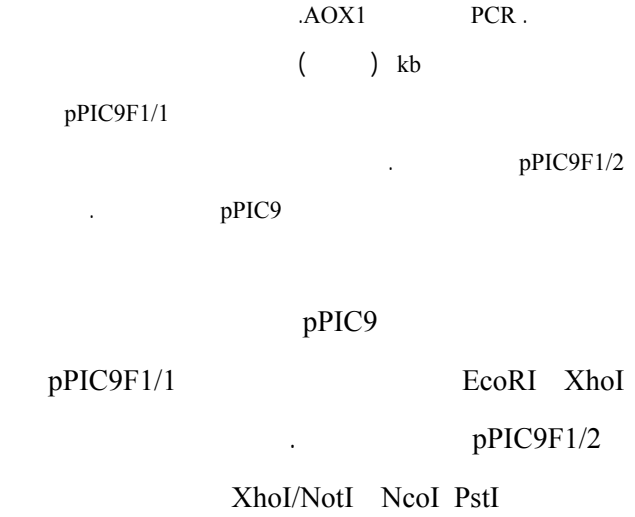
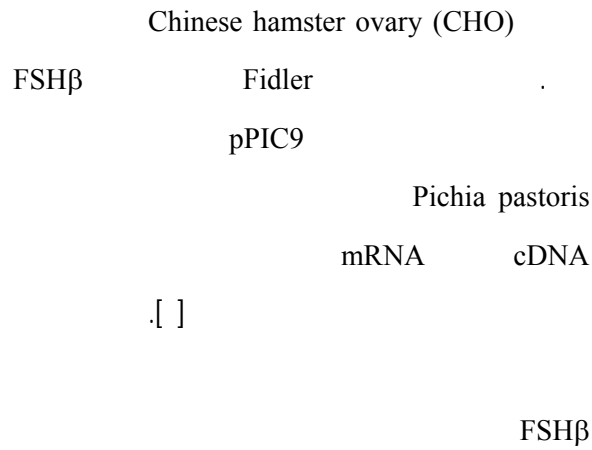
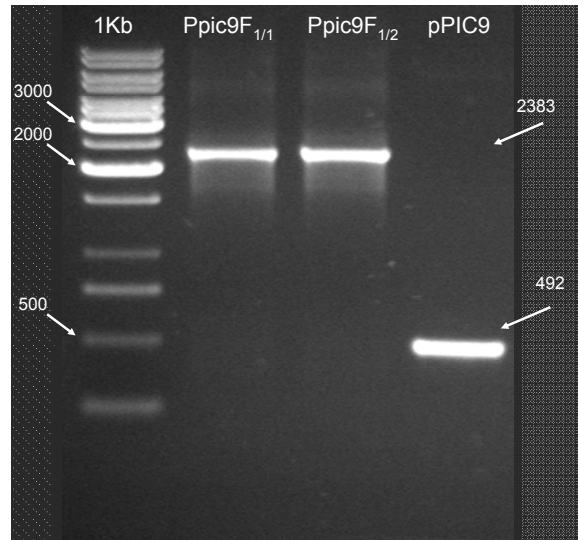
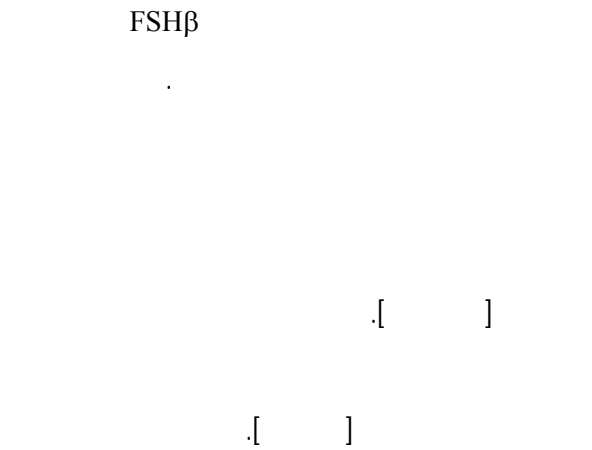
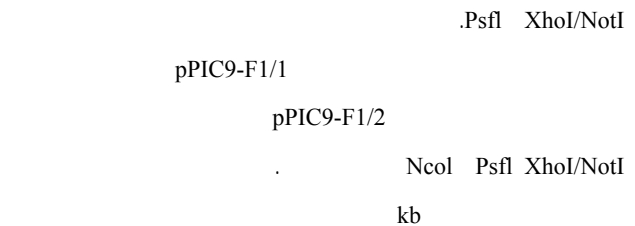
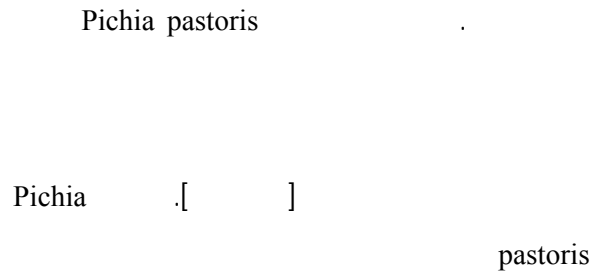
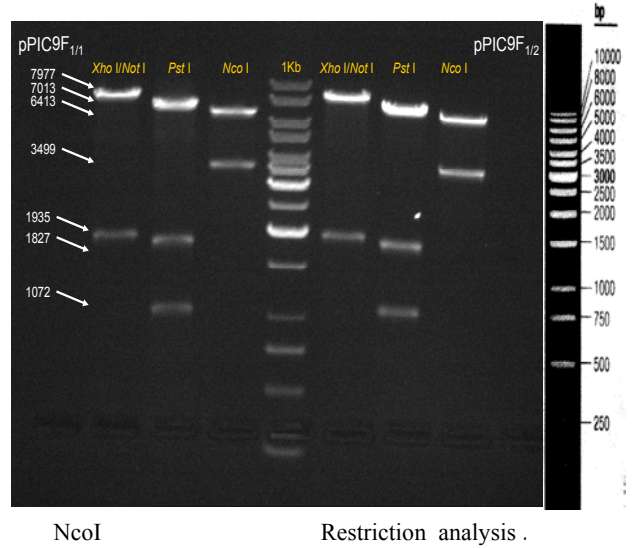
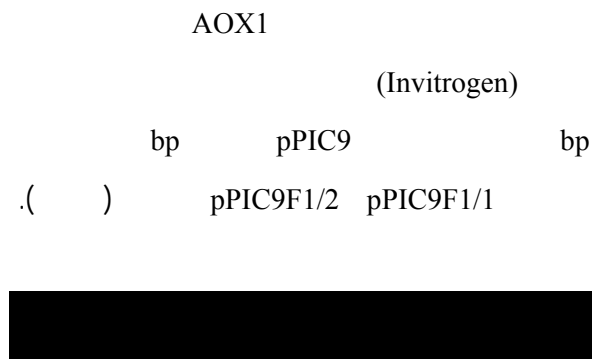


.PstI Restriction analysis .

PTV-2011

pTV-2020 pTV-2019

() bp :



	Pichia pastoris	
FSHβ (+sig E2-IVS2-E3)		AOX1
		Restriction mapping
FSH -		(Invitrogen) AOX1
	GenBank	
	Splicing	
	(AG TG)	
		PicxsigF1
		FSHB
	KEX2	
		STE13
	*	Glu-Lys-Arg*-Glu-Ala-Glu-Ala
		Glu-Ala
		rFSHβ
	Pichia pastoris	
		Samadder .[]
	MFα	FSHβ
		Cognate s.p.
	Fidler .[]	
	MFα	
	Splicing	Pichia pastoris
		FSH
	FSH	pPIC9-F1
		MFα
	Pichia pastoris	Splicing



[1] Ulloa-Aguirre A, Timossi C. Structure-function relationship of follicle-stimulating hormone and its receptor. Hum Reprod Update, 1998; 4(3):260-83.

[2] Keene JL, Matzuk MM, Otani T, Fauser BC, Galway AB, Hsueh AJ, et al. Expression of biologically active human follitropin in Chinese hamster ovary cells. J Biol Chem, 1989; 9:4769-75.

[3] Sugahara T, Sato A, Kudo M, Ben-Menahem D, Pixley MR, Hsueh AJ, et al. Expression of biologically active fusion genes encoding the common alpha subunit and the follicle-stimulating hormone beta subunit. Role of a linker sequence. J Biol Chem, 1996; 271(18):10445-8.

[4] Aizen J, Kasuto H, Golan M, Zakay H, Levavi-Sivan B. Tilapia Follicle Stimulating Hormone (FSH): Immunochemistry, stimulation by GnRH, and effect of biologically active recombinant FSH on steroid secretion. Biol Reprod, 2006 Dec 27; on-line.

[5] Invitrogen. Pichia Expression Kit, Protein Expression, A Manual of Methods for Expression of Recombinant Proteins in Pichia pastoris. Catalog no. K1710-01. 2005. <http://www.invitrogen.com>

[6] Fidler AE, Lin JS, Lun S, Chie WNg, Western A, Stent V, McNatty KP. Production of biologically active tethered ovine FSHβα by the methylotrophic yeast Pichia pastoris. J Mol Endocrinol, 2003; 30:213–25.

[7] Jameson JL, Becker CB, Lindell CM, Habener JF. Human follicle-stimulating hormone beta-subunit gene encodes multiple messenger ribonucleic acids. Mol Endocrinol, 1988; 2:806-15.

[8] Shoham Z, Insler V. Recombinant technique and gonadotropins production: new era in reproductive medicine Fertil Steril, 1996; 66(2):187-201.

[9] Saneyoshi T, Min KS, Jing Ma X, Nambo Y, Hiyama T, Tanaka S, et al. Equine follicle-stimulating hormone: molecular cloning of beta subunit and biological role of the asparagine-linked oligosaccharide at asparagine(56) of alpha subunit. Biol Reprod, 2001; 65(6):1686-90.

[10] Gadkari R, Deshpande R, Dighe RR. Hyperexpression and purification of biologically active human luteinizing hormone and human chorionic gonadotropin using the methylotrophic yeast, Pichia pastoris. Protein Expression and Purification, 2003; 32(2):175-84.

[11] Kamei H, Ohira T, Yoshiura Y, Uchida N, Nagasawa H, Aida K. Expression of a biologically active recombinant follicle stimulating hormone of Japanese Eel *Anguilla japonica* using methylotrophic yeast, Pichia pastoris, 2003; 134(3):244-54.

[17] Kanda M, Jablonka-Shariff A, Sato A, Pixley MR, Bos E, Iro'oka T, et al. Genetic fusion of an α -subunit gene to the follicle-stimulating hormone and chorionic gonadotropin- β subunit genes: Production of a bifunctional protein. *Mol Endocrinol*, 1999; 11:1873-81.

[18] Recombinant Human FSH Product Development Group. Recombinant follicle stimulating hormone: development of the first biotechnology product for the treatment of infertility. Recombinant Human FSH Product Development Group. *Hum Reprod Update*, 1998; 4(6):862-81.

[19] Xiong AS, Peng RH, Li X, Fan HQ, Yao QH, Guo MJ, et al. Influence of signal peptide sequences on the expression of heterogeneous proteins in *Pichia pastoris*. *Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao (Shanghai)*, 2003; 35(2):154-60.

[20] Samaddar M, Catterall JF, Dighe RR. Expression of biologically active beta subunit of bovine follicle-stimulating hormone in the methylotrophic yeast *Pichia pastoris*. *Protein Expr Purif*, 1997; 10(3):345-55.

[12] Kasuto H, Levavi-Sivan B. Production of biologically active tethered tilapia LHbetaalpha by the methylotrophic yeast *Pichia pastoris*. *Gen Comp Endocrinol*, 2005; 140(3):222-32.

[13] Setlur SR, Dighe RR. Single chain human chorionic gonadotropin, hCG $\alpha\beta$: Effects of mutations in the α subunit on structure and bioactivity. *Glycoconjugate J*, 2007; 24(1):97-106.

[14] Layman LC, Lee EJ, Peak DB, Nannoum AB, Vu KV, van Lingen BL, et al. Delayed puberty and hypogonadism caused by mutations in the follicle-stimulating hormone beta-subunit gene. *N Engl J Med*, 1997; 337(9):607-11.

[15] Layman LC, Porto ALA, Xie J, Da Motta LACR, Da Motta LDC, Weiser W, et al. FSH-beta gene mutations in a female with partial breast development and a male sibling with normal puberty and azoospermia. *J Clin Endocr Metab*, 2002; 87:3702-7.

[16] Phillip M, Arbelle JE, Segev Y, Parvari R. Male hypogonadism due to a mutation in the gene for the beta-subunit of follicle-stimulating hormone. *N Engl J Med*, 1998; 338(24):1729-32.