

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

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
 : / unit Taq)
 Taq: Pfu (/ unit pfu
 Mg
 ()
 pTV- 2019
 dNTP
 Taq:pfu DNA
 / / Mg
 / Annealing
 PCR
 pTZ57R
 pTZ57R K0513 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

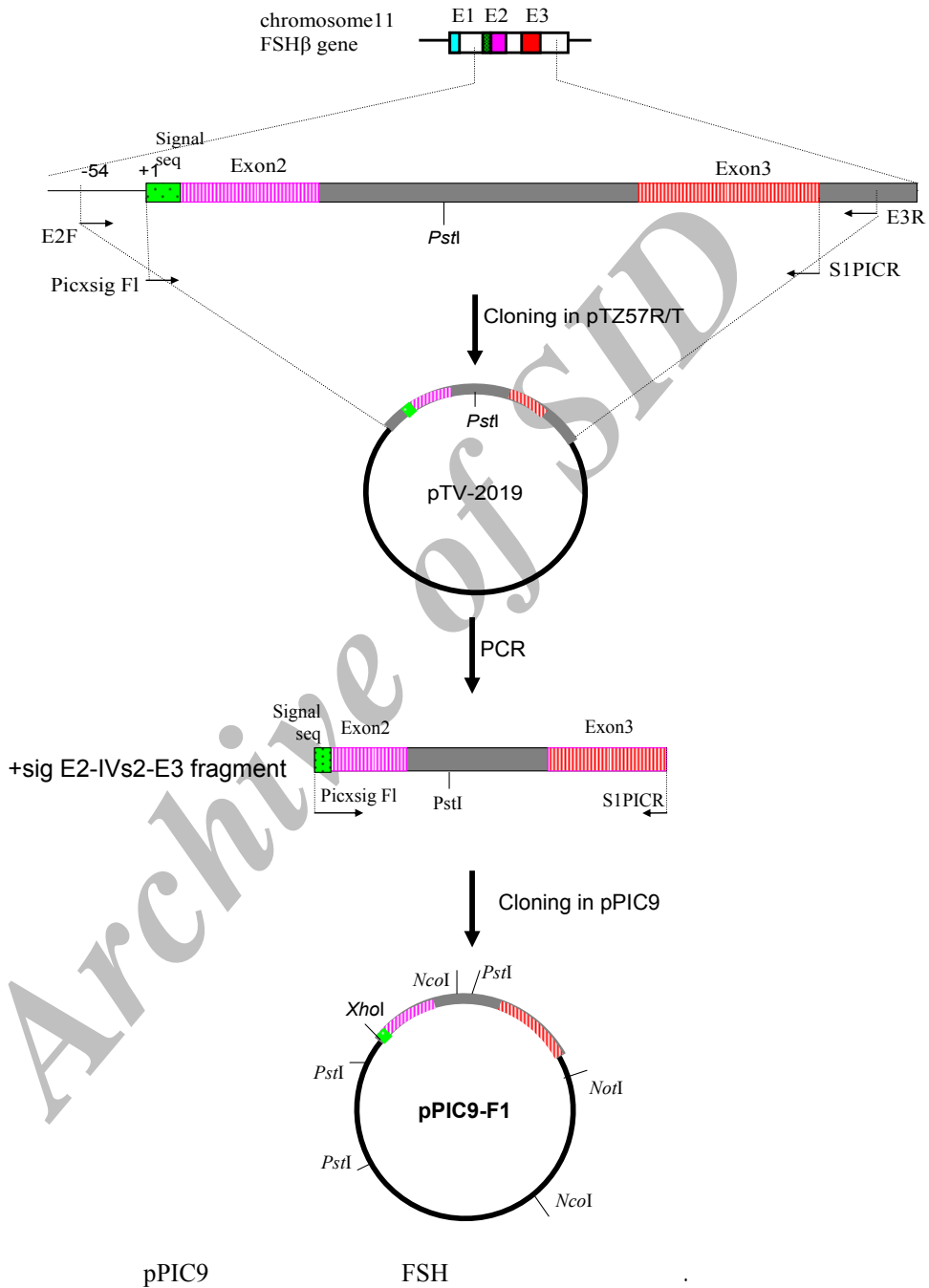
pPIC9F1/1

()

AOX1

PCR

[]



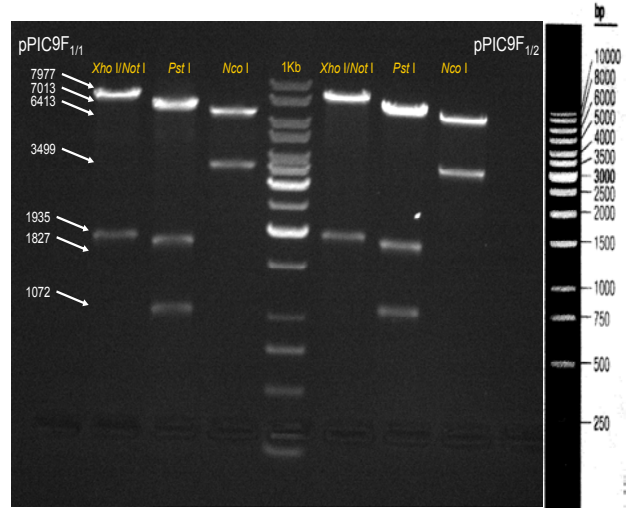
-)
(Flanking

FSH β ()

FSHE2F FSHE3R

.() ' ' regions)

AOX1
(Invitrogen)
bp pPIC9 bp
() pPIC9F1/2 pPIC9F1/1

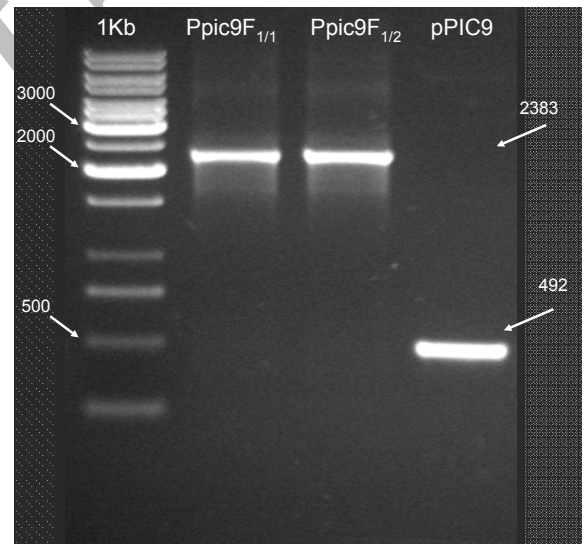


NcoI Restriction analysis .
.PstI XhoI/NotI

Pichia pastoris
Pichia []
pastoris

FSHβ

pPIC9-F1/1
pPIC9-F1/2
NcoI PstI XhoI/NotI
kb



.AOX1 PCR .

() kb

Chinese hamster ovary (CHO)

FSHβ

Fidler

pPIC9

pPIC9F1/1

pPIC9

pPIC9F1/2

Pichia pastoris

mRNA

cDNA

pPIC9

[]

pPIC9F1/1

EcoRI XhoI

pPIC9F1/2

FSHβ

XhoI/NotI NcoI PstI

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



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