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中华鳖4个 基因保守区的序列分析

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(1 ½ê = S v Ð 3 þ " , î µ 241000;
2 2 Ø M¡ D Ð ý M¡ † s 0 3 þ Ð 7 b L i , 2 Ø 210097)

摘要: • ¨ PCR / Œ, ” 9 „ X H Ï ¿ W Sox y (TSSox) b Ü DNA ½ s A U, Sox y " d É Ä E s o, Ï TSSox4 Ð T Ë LF4 y I ' ¥ ¼ Ö ½ › † M] aÐ ¦ Ë SOX4 „ I Š Sox4 I ' ¥ ½ Ç B ñ ¼ Ö ¥ µ s ; TSSox5 Ð T Ë ¥ LF5 y ¥ I ' 9 Ç B ñ ¼ Ö ? 3 ¿ M; TSSox2 Ð Z C ¥ TSox2 M » Ÿ K Ú b 4 H TSSox ½ Ï , TSSox Ð ¦ SRY y ½ M » Ÿ K Ú, r 75% ; ½ ¥ M » Ÿ V ? À U ñ Ì ÿ ? ¥ o Ÿ b

关键词: Ï ¿ W; Sox y ; ½ s ; PCR
中图分类号: S966. 5 文献标识码: A 文章编号: 1000- 3207(2001) 03- 0245- 06

Ð ¦ Ë „ • Ä î þ ,] , • › î þ ¥ Ÿ Y % ç Å µ y ˜ Ÿ Y % ç (Genotype sex determination, GSD) „ Ì â Ÿ Y % ç (Environment sex determination, ESD) © Õ Ë ˜ , 7 ª € » [Ñ G ˜ Ÿ Y % ç (Temperature dependent sex determination, TSD) ¹ ” b Â • › î þ Ÿ Y % ç Å ¥ ù î V ü , T ø 1 " Í ¥ Í ø Õ W, Ÿ Y % ç Å ¥ Ë ˜ V C µ Õ " (Â XY á ZW á TSD ©) , N À U ¾ Ë î þ ,] Ÿ Y % ç Å ¥ s 0 ' É 9 V ? E s M Í [1,2] b • › Ë ^ • Ä î þ „ T Ë ¥] C 5 , y N ñ Ì ^ ù î ¦ Ë „ Ú © î þ Ÿ Y % ç Å ¥ z T 3 þ [3] b Ï ¿ W^ B Õ $ < W É › ¦ ý ! ² ¥ • › î þ , T € [4] Y V Ï ¿ W x ¥ ¦ ý ' Ä , V ü Ï ¿ W ¥ Ÿ Y % ç ‹ ¿ TSD Å b Œ µ 1 Ÿ Y % ç s 0 Å ¥ ù î , S = “ Ã n ¡ b ' Ó • ¨ PCR Á þ ° ¤ X H E , X H Ï ¿ W Sox y ¥ o u , i É › ½ © ç , [ù £ U Ï ¿ W(Wiegmann) ¥ Ÿ Y % ç s 0 Å , É 7 ¹ ª M V ® © , î þ ž ¦ Ë Ÿ Y % ç ¥ Å 4 L ì b

材料与方法

试验动物 的提取和 扩增 基因保守区 Ï ¿ W( ) 2 á , 1 £ Á g Æ, Ÿ Y ¿ 8 ³ é ' ç , (¹ Ÿ î ƒ ñ 8 b DNA 4 | • v Ó D [5] b PCR ” 9 Sox y „ © ½ ¨ „ þ • v Ó D [2] , ® Z 3 ý ³ † î , PAGE B Ä b „ þ ½ ¹ : Rg- 3 5 - GGTCAAGCGACCCATGAA(C/ T) GC(AGCT) TT- 3 Rg- 4: 5′- AGGTCGGTACTT(AG) TA(AG) T(CT) (AGCT) GG(AG) TA- 3′ b¾ „ þ V + s ” 9

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作者简介: W> « (1962—) , 3; ½ê 8 . ö E ¦ ; ö 1 V Y L . Ð „ s 0 3 þ Ð ¥ • Ð „ S ù

¦ SRY　y HMG- box = ¥　o ½ , ” 9 Ë　É ¹ 217bp b

PCR ” 9 8 " ¹ 100uL, c 1. 0ug " Ö DNA, 25pmoL•L^{-1} Ä „ þ, 2. 0mmoL•L^{-1} MgCl$_2$, 4U Taq ½ b ” 9 H q ¹ : 94℃ 1min, 42℃ 1. 5min, 72℃ 1min, 5 ñ Ö Ì , ¤ " , 94℃ 1min, 52℃ 1min, 72℃ 1min, 35 ñ Ö Ì ,　72℃ ü %15min, 4℃　i b

PCR ² • ª , | 5uL PCR Á þ ¨ 2% j «　` ‹ É › _ ©, …v b Å / Á þ ¨ DNA B Ä k 4 ‡ B Ä, B Ä Z E • v Á Ö m ¨ É › b

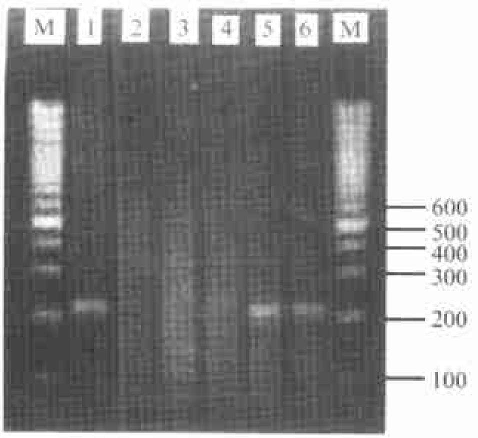
产物直接克隆　† T4 DNA õ ¤ ½ apEGM- T　8 á PCR B Ä Á þ ©, 4℃ b Q Ï õ ¤ Q ‹ 14h b %) ¥　Ä • v s 0 X H Z E[6] b G | â ä) ‹ ,　M ž 6 B p µ X-gal ¥ Ü ð　, 37℃ ¡ ! 12h, V n Ä ì 3 É B ñ) ‹ b　G | â ä) ‹　¸ , Â Eppendorf 5 Ï (Ä B) ‹ G B 5), F 20uL í) • < £, 100℃ m £ ò 10min, 1500r/ min Ö • 5min, |　b A S　ð É › PCR ” 9 , H q] - , 2% j «　` ‹ _ ©, µ ” 9 Á þ € V j ¹　Ÿ X H b　Ÿ X H Ê　ª ,　s Y G | B Ì ¤ Õ ž 5mL LB A 8 ¡ !　Ï , 37℃ (5 (250r/ min) ¡ ! 12h b ¡ ! Á þ ¨ l Å ! E 4 | É ì b

基因片段的筛选和测序　• ¨ SSCP(Single Strand Conformation Polymorphism) E ñ Ê ,] ¥ Sox　y Ë　, s　Z E • v Ó D[5] b • ¨ PE　³ ¥ © ½ k 4 ‡ , ABI 1 î © ½ N(373 ˜)　É › DNA © ½ b

基因的分析　ñ Ê　¥ 4 ñ ,] Sox　y ½　æ ¨ Pc gene È q 5 É › ¼ Ö ½　w • („ þ WVI ' ¼　Ö 56 ñ),　| N 4 H ¼　Ö ý { ÆGenBank, Ð X ? V ¥　ð 32 H Sox/ SOX　y ¥ I ' ý É ›] ÷ Ÿ s　, • ¨ MEGA È q, ¨　¿ ¼ Ö µ s ä s 1 T ¹ L .　Ö, NJ E　y　Ë Ž b ® N　Ï ¿ WSox　y (Sox of　-　, e • ¹ TSSox) b

结果

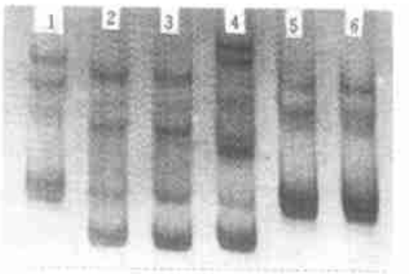
中华鳖　基因的　扩增



m 1　Ï ¿ WSox　y PCR ” 9 ² T

Fig. 1　The amplyfication result of Sox of

M pGEM3(+) marker; 1. ¦ SOX　y ; 2, 3, 4. | Ÿ　v ; 5, 6. ¦ Ÿ „ l Ÿ ñ 8



m 2　SSCP E ñ Ê　,] ¥ Sox　y Ë

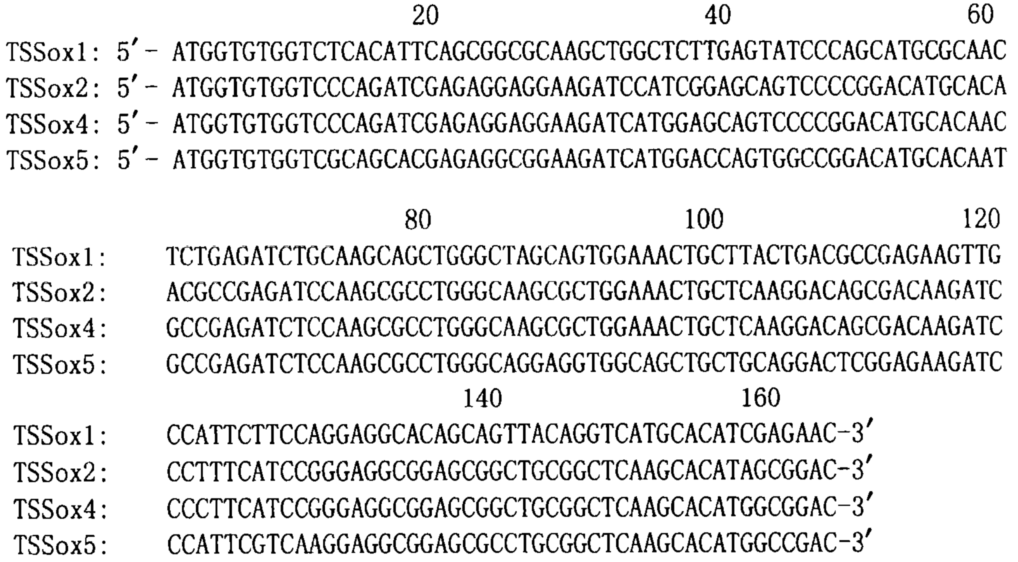
Fig. 2　The different Sox fragments were selected by SSCP

1. TSSox- 2; 2, 3. TSSox- 1; 4. TSSox- 3; 5, 6. TSSox- 4

• ¨ á ’ 0 » 3 Ê › † e i ¥ „ þ ” 9 Sox y, [L ¤ ž ¥ M » Ÿ PCR Á þ b ” 9 ² T A U, I ¦ ñ 8 (C B H ” 9 { , v l Ð¦ SRY y ” 9 Á þ M] , ÿ ¹ 220bp(m1) b

. 阳性克隆的测序

1205 ñ Ÿ X H Ï , ñ Ê 4 ñ µ µ s ¥ ¬ ÆË (m2), ñ Ê ¥ 4 ñ µ µ s ¥ Ÿ X H É › ©½, ² T n m3:



20 40 60

TSSox1: 5′- ATGGTGTGGTCTCACATTCAGCGGCGCAAGCTGGCTCTTGAGTATCCCAGCATGCGCAAC
TSSox2: 5′- ATGGTGTGGTCCCAGATCGAGAGGAGGAAGATCCATCGGAGCAGTCCCCGGACATGCACA
TSSox4: 5′- ATGGTGTGGTCCCAGATCGAGAGGAGGAAGATCATGGAGCAGTCCCCGGACATGCACAAC
TSSox5: 5′- ATGGTGTGGTCGCAGCACGAGAGGCGGAAGATCATGGACCAGTGGCCGGACATGCACAAT

80 100 120

TSSox1: TCTGAGATCTGCAAGCAGCTGGGCTAGCAGTGGAAACTGCTTACTGACGCCGAGAAGTTG
TSSox2: ACGCCGAGATCCAAGCGCCTGGGCAAGCGCTGGAAACTGCTCAAGGACAGCGACAAGATC
TSSox4: GCCGAGATCTCCAAGCGCCTGGGCAAGCGCTGGAAACTGCTCAAGGACAGCGACAAGATC
TSSox5: GCCGAGATCTCCAAGCGCCTGGGCAGGAGGTGGCAGCTGCTGCAGGACTCGGAGAAGATC

140 160

TSSox1: CCATTCTTCCAGGAGGCACAGCAGTTACAGGTCATGCACATCGAGAAC-3′
TSSox2: CCTTTCATCCGGGAGGCGGAGCGGCTGCGGCTCAAGCACATAGCGGAC-3′
TSSox4: CCCTTCATCCGGGAGGCGGAGCGGCTGCGGCTCAAGCACATGGCGGAC-3′
TSSox5: CCATTCGTCAAGGAGGCGGAGCGCCTGCGGCTCAAGCACATGGCCGAC-3′

m3 Ï ¿ WSox y HMG- box u DNA ½

Fig. 3 The HMG- box DNA sequence of Sox gene of

w© ¥ I ’ ¼ Ö ¥ ½ n V 1 b

表 中华鳖 基因 条 编码的序列

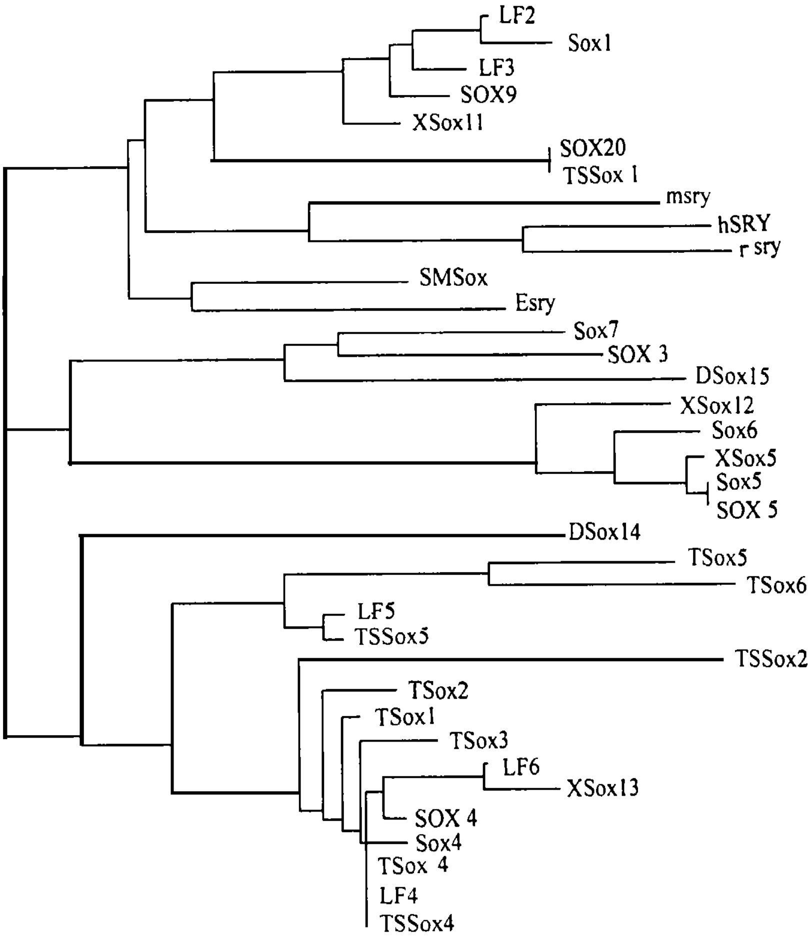
Tab. 1 The amino acid sequence of Sox gene of

TSSox1:	MVWSHIQRRK	LALEYPSMRN	SEICKQLGKQ	WKLLTDAEKL	PFFQEAQQLQ	QMHIEN
TSSox2:	MVWSQIERRK	IHRSSPRTCT	TPRSKRLGKR	WKLLKDSDKI	PFIREAERLR	LKHIAN
TSSox4:	MVWSQIERRK	IMEQSPDMHN	AEISKRLGKR	WKLLKDSDKI	PFIREAERLR	LKHMAD
TSSox5:	MVWSQHERRK	IMDQWPDMHN	AEISKRLGRR	WQLLQDSEKI	PFVKEAERLR	LKHMAD

进化程度不同的物种 基因家族的相似性分析

Ë s ¥ ² T n m4 b ² T V ü, Ï ¿ WTSSox4 y Ð T Ë LF4 aZ C ¥ TSox4 y I ’ ¥ ¼ Ö½ › † M] , Ð¦ SOX4 „ I Š Sox4 y ¥ I ’ ½ Ç B ñ ¼ Ö ¥ µ s; TSSox5 Ð T Ë ¥ LF5 y ¥ I ’ 9 º µ B ñ ¼ Ö ? 3 9 Ð, # s Y ¹ TSSox4 „ TSSox5 b ® m4 V n , Sox E B V s ¹ Ø v Ë • , » B v Ë • “ ¦ SRY, SOX9, SOX20; l Š mSry, Sox1, E t rSry [# • Š ESry, • Ë XSox11, T Ë LF2—3 ©, Ï ¿ WTSSox1 9 Ë ƒ B B, Ð¦ SOX20 ¥ M » Ÿ Ú, Ð¦ SRY y HMG- box

¥ M» Ÿ¹ 75%, # ¹ TSSox1 b » = v Ë • 5 ¹ ¦ Ë SOX5, SOX8, l Š ¥ Sox5—7, • Ë ¥ XSox5, XSox12, T ‘ ¥ DSox15 b » Ø v Ë • “ ¦ SOX4, Š Sox4, T Ë LF4—6 [# Z C ¥ TSox1—TSox6, • Ë ¥ XSox13 „ T ‘ ¥ DSox14, Ï ¿ W¥ TSSox2, TSSox4, TSSox5 (ƒ B B, Ï , TSSox2 Ð Z C ¥ TSox2 M» Ÿ K Ú, # N b



m4 Sox y E B ¥] ÷ Ÿ s

Fig. 4 The phylogenetics analysis of SOX/Sox gene family

讨论

ô T €¥ ù î , Ï ¿ WI ¦ ñ 8 (Ã n µ s ˜ Ÿ ™ä 8 ¥ s Ä, B î ª ¹, À µ Ÿ ™ä 8 ¥ s Ä ^ TSD Å ¥ ÕË] + ˜ ; s Ú x ¥ ¦ ý ’ Ä L 9 £ L Ÿ Y %ç Ð ’ Ä Ñ µ 1, # Ï ¿ WŸ Y %ç ‹ ¿ TSD Å[4] b Ÿ Y %ç s 0 Å ¥ ù î V ü, Â] • Ä î þ B " , Ï ¿ W9 µ SRY ¥] ÷ y —Sox, N V ? Q‹ GSD „ TSD ÕË˜ ŸY %ç Å ¥ ¦ & s 0 £ Ü ¥ B Á Ÿ b - ¦ ¥ ù î ª ¹ , ¦ „ • Ä î þ

¥ Ÿ Y %ç V ? ^ [SRY y ¹ ö • B " y • Ð ¥ x Ø V r ¥) ó Q ‹ ¥ ² T, SRY y ¥ Á þ ^ B Ë c y 0, Y V ð y V r ¥ Ø « 7 3 Ÿ Ÿ Y s Ä " 7 1 e Å ¥ T ¨ [7, 8], N “ , SOX5 a6 a9 a17 © 9 Ð Ÿ Y %ç „ Ú 0 ™î µ 1[9] b SOX y ¥ ÿ ? ù î Î V ü, SRY „ SOX y ø ? â V ñ Ï Î µ Ø e T ¨ , Â SOX1 a2 a3 a11 © Ð * Ü " d ? â µ 1; SOX4 a6 Ð 8 % s Ä µ 1 b ' ù î X H ž ¥ Ï ¿ W4 H SOX y, TSD Å ¥ þ Õ Ï ¥ ÿ ? ^ Ž Ð GSD î þ ¥ SRY ð SOX y M] , [# y V r ¥ ? p ^ B ñ µ i l ¥ ª Ã [5, | É B „ ù î b

' Ó s Ö ¤ ž ¥ Ï ¿ W4 H Sox y Ï , TSSox4 Ð T Ë LF4 a¦ Ë ¥ SOX4 „ l Š ¥ Sox4 y l ' ¥ ¼ Ö½ › † M] Ç B ñ ¼ Ö ¥ µ s, s A U ƒ B y º, i " d É Ä E s o, y ¹ * ù ¥ • › î þ 3 a M[- ' s Ä ™î T Ë „ • Ä î þ b l ' ¥ ½ M] V ? À U ÿ ? ¥ B Á Ÿ b Í M ¦ Ë „ Ú ©î þ ¥ ù î V ü, Sox4 y ? \ É £ F % ¥ s Ä „ ? â[10]; ÷ µ • ¥ ^, Sox4 y ® Hep3B % s 8 M ± ¥ Š Š Ã / 3 É ? Ï v V r , A U Sox y V ? Ð • Á % Ó § Ö ? ¥ ? 3 µ 1[11] b y N, Sox4 y % 3 î Ï V ? µ × 1 ¥ T ¨ b

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! " # # *TRIONYX SINENSIS*

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and LU Xiao- xuan[2]
*1 + # % & + 241000,
2$ - " ! + % . + 210009/

$%&()& The Sox genes of were amplified and cloned using PCR. The four different fragments of Sox gene HMG- box DNA were selected and sequenced. The results indicated that the Sox gene is conservative in phylogeny, in which the amino acid sequence encode by TSSox4 (The Sox of $) is identical to those encode by LF4 (bird) or differ from SOX4(human) and Sox4(mouse) only on one amino acid; The amino acid sequences encode by TSSox5 have an amino acid difference from those encode by LF5 (bird); TSSox1 has about 75% identical to SRY HMG- box at the nucleotide level, and is most resemblance among four TSSox. Phylogenetic analysis of available 32 Sox sequences including SRY/Sry sequence suggests that there wolud be a high degree of divergence between any possible immediate common ancestor of the $ Sox sequence and the SRY (Sry) sequences.

+,-. '/% ; Sox gene; Sequence analysis; PCR