454 (1000) 16 00

EEDC L ...

# The zebrafish genome contains two distinct selenocysteine tRNA

genes

Xue-Ming Xu<sup>a</sup>, Xuan Zhou<sup>a</sup>, Bradley A. Carlson<sup>a</sup>, Lark Kyun Kim<sup>b</sup>, Tae-Lin Huh<sup>c</sup>, Byeong Jae Lee<sup>b</sup>, Dolph L. Hatfield<sup>a,\*</sup>

<sup>a</sup>Section on the Molecular Biology of Selenium, Basic Research Laboratory, Division of Basic Sciences, National Cancer Institute,

National Institutes of Health, Building 37, Room 2D09, Bethesda, MD 20892, USA

<sup>b</sup>Laboratory of Molecular Genetics, Institute for Molecular Biology and Genetics, Seoul National University, Seoul 151-742, South Korea <sup>c</sup>Department of Genetic Engineering, Kyungpook National University, Taegu 702-702, South Korea

Received 29 March 1999; received in revised form 31 May 1999

Abstract The zebrafish is widely used as a model system for studying mammalian developmental genetics and more recently, as a model system for carcinogenesis. Since there is mounting evidence that selenium can prevent cancer in mammals, including humans, we characterized the selenocysteine tRNA<sup>[Ser]Sec</sup> gene and its product in zebrafish. Two genes for this tRNA were isolated and sequenced and were found to map at different loci within the zebrafish genome. The encoding sequences of both are identical and their flanking sequences are highly homologous for several hundred bases in both directions. The two genes likely arose from gene duplication which is a common phenomenon among many genes in this species. In addition, zebrafish tRNA<sup>[Ser]Sec</sup> was isolated from the total tRNA population and shown to decode UGA in a ribosomal binding assay.

© 1999 Federation of European Biochemical Societies.

*Key words:* Zebrafish; Selenocysteine; tRNA; Gene; Selenium

#### 1. Introduction

The zebrafish is becoming a prominent model organism for studying mammalian genetics and development [1] and more recently, as a model for studying oncogenes and carcinogenesis (see [1-4] and references therein). Selenium suppresses cancer in rodents, and possibly in humans, and selenoproteins are the most likely candidates responsible for the chemopreventive effect of this element (see [5,6] and references therein). As selenocysteine (Sec) tRNA<sup>[Ser]Sec</sup> is the central component in the selenoprotein biosynthesis [7], we examined this critical molecule as the initial step in determining if zebrafish may also serve as a model organism for studying selenium as a chemopreventive agent in cancer. We found that zebrafish, unlike any other known animal in which the tRNA<sup>[Ser]Sec</sup> gene has been sequenced, encodes two copies of this gene. All other higher and lower animals, including several mammals, chickens, frogs, fruit flies and worms, in which the  $tRNA^{\left[Ser\right]Sec}$  gene has been sequenced, contain this gene only in a single copy (reviewed in [8]). Both zebrafish genes have identical encoding sequences, contain highly homologous sequences for several hundred bases upstream and downstream of the gene and map at different loci. The two genes likely result from gene duplication which is a common occurrence with many genes of the zebrafish lineage [9,10] and they may

have resulted from genome duplication in an early ancestor [11].

### 2. Materials and methods

#### 2.1. Materials

The zebrafish genomic library cloned in  $\lambda$  (EMBL 3 SP6/T7) was purchased from Clontech (Palo Alto, CA, USA) and all reagents were commercial products of the highest grade available. Adult zebrafish were quickly frozen in liquid nitrogen and stored at  $-80^{\circ}$ C until ready for use.

#### 2.2. Library screening, gene isolation and sequencing

All molecular cloning procedures including plaque lifting, Southern blotting, subcloning, restriction enzyme digestions and gel electrophoresis followed standard techniques [12] or the manufacturer's protocol unless otherwise mentioned. The zebrafish genomic library (18 plates with approximately  $2 \times 10^4$  phages/plate) was screened with a 193 bp fragment encoding the human tRNA<sup>[Ser]Sec</sup> gene [13] labelled with  $[\alpha^{-32}P]dCTP$  as probe. Following hybridization for 2 h, membranes were washed twice for 10 min each in SSC (1×SSC was used during library screening and 0.2×SSC in all subsequent hybridizations) and 0.5% SDS at room temperature and exposed to a phosphur screen for 0.5–2 h or to X-ray films for 2–10 h. Bacteriophages giving positive signals were amplified and phage DNA was isolated using Qiagen columns. Fragments encoding the Sec tRNA<sup>[Ser]Sec</sup> gene obtained by restriction endonuclease digestion were subcloned into pUC 19 for sequencing. GCG-Lite+Clustalw in the NIH Network was used for sequence alignment and analysis.

#### 2.3. Genomic mapping

Genomic mapping of the two ZStR genes was performed using a zebrafish-hamster radiation hybrid panel (Research Genetics). PCR analyses were carried out in a final volume of 10  $\mu$ l containing 1×PCR buffer (Perkin Elmer), 2  $\mu$ M of each primer, 200  $\mu$ M of each dNTP, 1  $\mu$ l of template DNA (25 ng) and 0.5 U of AmpliTaq Gold DNA polymerase (Perkin Elmer). After an initial activation of polymerase at 95°C for 10 min, 36 cycles at 95°C for 15 s, 58°C for 15 s and 72°C for 40 s were carried out on a GeneAmp PCR System 9600 (Perkin Elmer). PCR products were separated on a 2.5% agarose gel (Gibco BRL). Analysis of the radiation hybrid panel data was performed by Dr Robert Geisler of the Max-Planck Institute.

## 2.4. Isolation, fractionation and codon recognition studies of $tRNA^{[Ser]Sec}$

Total tRNA was isolated from 45 g of frozen adult zebrafish as described [13]. 315  $A_{260}$  U of total tRNA were applied to a RPC-5 column in 0.45 M NaCl, 0.01 M Mg(OAc)<sub>2</sub>, 0.01 M NaOAc and 0.001 M EDTA, pH 4.5, and the attached tRNA eluted in a linear 0.50 M–0.70 M NaCl gradient as described [14]. Sec tRNA<sup>[Ser]Sec</sup> was identified in fractionated tRNA by dot blotting 5 µl of every other eluted fraction onto nitrocellulose filters and hybridizing with a labelled 193 bp human DNA fragment encoding the Sec tRNA<sup>[Ser]Sec</sup> gene as probe. Fractions containing tRNA<sup>[Ser]Sec</sup> were pooled, amino-acylated with [<sup>3</sup>H]serine [13], the resulting [<sup>3</sup>H]seryl-tRNA<sup>[Ser]Sec</sup> was fractionated on a RPC-5 column and individual peaks of [<sup>3</sup>H]seryl-tRNA were isolated, prepared for encoding studies and encoding

<sup>\*</sup>Corresponding author. Fax: (1) (301) 435 4957. E-mail: hatfield@dc37a.nci.nih.gov

<sup>0014-5793/99/\$20.00 © 1999</sup> Federation of European Biochemical Societies. All rights reserved. PII: S 0 0 1 4 - 5 7 9 3 (9 9 ) 0 0 7 6 7 - X



Fig. 1. Restriction map of zebrafish ZStR1 and ZStR2. The shaded regions were sequenced including the hatched areas that correspond to the encoding sequences of the tRNA<sup>[Ser]Sec</sup> genes as shown in Fig. 2. Arrows in the hatched boxes indicate the transcription orientation. X designates *Xba*I, P, *Pst*I and A, *Acc*I. The *Pst*I fragment of ZStR1 was subcloned and sequenced. The sequence of an additional 171 bp upstream of the *Pst*I site within ZStR1 was obtained by subcloning a 1 kb *Acc*I fragment of recombinant DNA that was cloned in  $\lambda$  near the 3'-terminus of the tRNA<sup>[Ser]Sec</sup> gene and contained an *Acc*I site inside the vector DNA. The 2.1 kb *Xba*I fragment of ZStR2 was subcloned and 1031 bp sequenced.

studies were carried out by the procedure of Nirenberg and Leder [15] as described [14].

#### 3. Results

## 3.1. Restriction analysis of recombinant and genomic DNAs

The genomic library of zebrafish was screened and six positive clones were isolated. Digestion of each with endonucleases showed that they fell into two classes, designated ZStR1 and ZStR2 (see restriction map in Fig. 1). ZStR1, ZStR2 and zebrafish genomic DNA were digested with *XbaI* and with *PstI* and the resulting fragments analyzed by Southern blotting (see Fig. 2). Genomic DNA yielded two fragments in both digests as shown in lanes 4 and 5. One fragment in each digest corresponded to those generated from ZStR1 (lanes 2 and 6), while the other corresponded to those generated from ZStR2 (lanes 3 and 7). Thus, ZStR1 and ZStR2 are located at different loci within the zebrafish genome.

#### 3.2. Sequencing of recombinant DNAs

A total of 1031 bp in ZStR1 and 1006 bp in ZStR2 were sequenced and aligned with the corresponding tRNA<sup>[Ser]Sec</sup> gene and flanking regions in *Xenopus* as shown in Fig. 3. The encoding sequences of the two zebrafish genes are identical and differ by 5 bp from the tRNA<sup>[Ser]Sec</sup> gene in *Xenopus*. The flanking sequences of both zebrafish genes are highly homologous (97.5% in the 5'-flank and 93.6% in the 3'-flank). The upstream sequence encodes three regulatory regions, a TATA box at -30, a proximal sequence element (PSE) at -66 and an activator element (AE) at -205. These regulatory elements correspond to similar elements in *Xenopus* (see Fig. 3) that govern expression of the tRNA<sup>[Ser]Sec</sup> gene [8].

## 3.3. Genomic mapping of the zebrafish Sec tRNA genes

3.4. Identification and codon recognition properties of the gene products

Total tRNA from zebrafish was fractionated on a RPC-5



Fig. 2. Hybridization of zebrafish genomic and recombinant DNAs. Genomic DNA or recombinant DNA was digested, electrophoresed on an agarose gel, transblotted to a filter and hybridized with a 193 bp fragment of human DNA encoding the tRNA<sup>[Ser]Sec</sup> gene (see Section 2). Lanes 2 and 6 contain ZStR1, lanes 3 and 7 contain ZStR2 and lanes 4 and 5 contain genomic DNA (designated gDNA) digested with *XbaI* and *PstI*, respectively. Lane 1 contains labelled DNA markers digested with *Hind*III.

	-600									-501
ZStR1										
ZStR2	AAAGTATITA	AAGTCTTCTT	GTATAATACT	TGTTGTATCA	GATATITIAC	TTAATAACAA	TICAGCIICI	TITICATIGI	AAGGGICIGA	AGIAIAIAGA
AIRSP										
	-500									-401
ZStR1										
ZStR2	TTCTTATAAA	ACTGTGTGAC	ATATTCAGCA	ATTTCTTTAT	TACTTTCACA	TATTCTGTTA	TTAATACTGA	GTTTACACAT	AGATGACATT	TCATTTTTAC
XTRSP			CC	CGCGCTCTAT	CCTTTGCTCC	CTGTGA	CTCCTTCCCT	CATTACACTG	ATTTCTCACC	CCCATTCTCC
	-400							Unive	rsal FW Prim	mer -301
ZStR1	400					GT	CTACTTACAA	AGGCACCTTT	AGAAGTCTTG	AAGATGTCAT
ZStR2	GTTTTTCTAA	АТААААААА	ATATTTAGTA	TTTTTTTCTC	CTTGTTCAAT	CCACTTTTGT	CTACTTACAA	ATGCACCTTT	AGAAGTCTTG	AAGATGTCAT
XTRSP	GTCACCTCCT	CTGACGCGGA	AACTCT.GTG	TGACTCAGGC	TGACCCCATT	GTCACTGTAA	AGACCAACAG	CAGCACAGC.	.GCAGTGATG	TATCCCACAT
										0.01
801 D 1	-300	2022002	022000202000	N N N N OH O C N C		ACCANUACCA	7707	3.0033.000C	CARCCCCR C	-201
ZSTRI	TAATTAACAA	ACAACGAA	CAATGACATA	AAAACICCAC	CCAAGICICA	ACCAATAGCA	AACA	ATTAATCIG	CATGCGGI.G	CAGCGCTGTG
XTRSP	CCACTAACAA	ACAGCAACCA	CACGCCCCTC	CTCCCTCTTC	CCGTTTTTTC	ATTGAAAATA	AACCGAAGCG	TATTGTTATG	GAAGTACCAG	CATGCCTCGC
	0.0110.111.101.81								1	ΛE
	-200									-101
ZStR1	TTTAATGCAG	GATTTCTGCT	AAACGTGTAT	TTCCCTGCAG	TTCAT	ACTTTACGGG	GGACTAATAC	ATCATAAGGA	GATGATGATA	AGATGATGAT
ZStR2	TTTAATGCAG	GATTTCTGCT	AAACGTGTAT	TTCCCTGCAG	TTCAT	ACTITACGGG	GGACTAATAC	ATCATAAGGA	GATGATGATA	AGACGATGAT
XTRSP	GCGCGTGTAT	GCTATGCGAT	CTTGTTTGAT	TTCCCTCGAT	TTCACGTTAG	ACTAGTCGGG	GTATGTAAGC	GGCGATACGT	T. TAACTAGA	AAAGGAAI
	-100									-1
ZStR1	ATGCTGCCAA	AT.ATAGTTT	TAGAAATATC	CCCTCACCAC	AGATGTAAAG	CACATCACTG	CCACGACCTG	TATATAAGGG	TGGTTTCCGC	TGCTTCGAAT
ZStR2	ATGCTGCCAA	AT.ATAGTTT	TAGAAATTTC	CTCTCACCAC	AGATGTAAAG	CACATCACTG	CCACGACCTG	TATATAG GGG	TGGTTTCAGC	TGCTTCGAAT
XTRSP	AGTCAGATTT	TTGATACCAG	AAACAATTTT	TTGTCACCCC	A.ATATATAA	TATAATGGTG	GGAGGGGG <u>TA</u>	<u>TAAAA</u> GGAAA	TGGGAGTA.C	TGAGGTATTT
					PSE		·	FATA		. 1 0 0
20+D1	+1	1.00moccmcc	magagagamag	ACCOMMONAN	COTTOTACOTO	COURTCOCCCT	CACTCOTTCA	ATTCCACCTT	TCCCCCC	+100 #TCACTC T
ZStRI	GCCCGGATGA	ACCTCGGTGG	TCCGGGGTGC	AGGCTTCAAA	CCTGIAGCIG	CCTAGCGGCA	CACTCOTTCA	ATTCCACCTT	TCGGGCGACC	ATCACTC T
ZSTRZ VEDOD	GCCCGGATGA	ACCICGGIGG	TCCGGGGTGC	AGGCIICAAA	CCIGIAGCIG	TCTAGCGGCA	CACTCOTTCA	ATTCCACCTT	TCGGGCGACI	COTTOT
ATRSP	GUUUGGATGA	CCCTCAGIGG	ICIGGGGIGC	AGGUIICAAA	CCIGIAGCIG	ICIAGCGACA	GAGIGGIICA	AIICCACCII	1000000400	Gerrereerr
		* *	*			* *				
	+101	* *	* Specific FV	√ Primers		* ^				+200
ZStR1	+101 CATCACTACA	* * AAAA.TA <b>TTT</b>	* Specific FW AAGAAATTAC	N Primers ACAAAACAGA	<b>C</b> GATACTGAA	AATAATAAAC	GTTACTTATA	AAATACTTTG	CGTCATGTGC	+200 CGCTTTATAA
ZStR1 ZStR2	+101 CATCACTACA CATTACTACA	* * AAAA.TA <b>TTT</b> AAAAATA <b>CTT</b>	* Specific FW AAGAAATTAC AAGAAATTTC	<pre>V Primers ACAAAACAGA ACTAAACAGC</pre>	CGATACTGAA AGATGCTGAA	AATAATAAAC AATAATAAAC	GTTACTTATA GTTACTTCTA	AAATACTTTG	CGTCATGTGC CATCATTTGC	+200 CGCTTTATAA CGCTTTATAA
ZStR1 ZStR2 XTRSP	+101 CATCACTACA CATTACTACA TTTCATCACT	* * AAAA.TA <b>TTT</b> AAAAATA <b>CTT</b> TCTATCCTCT	* Specific FV AAGAAATTAC AAGAAATTTC GATAAATAGC	<pre>V Primers ACAAAACAGA ACTAAACAGC TCATCTCAGT</pre>	<b>C</b> GATACTGAA <b>A</b> GATGCTGAA GGCTA	AATAATAAAC AATAATAAAC TATAATGCGC	GTTACTTATA GTTACTTCTA TTAGCA	AAATACTTTG AAATACTTTG ATATTCTTTA	CGTCATGTGC CATCATTTGC CCGAAAC	+200 CGCTTTATAA CGCTTTATAA GGCTTTCTAT
ZStR1 ZStR2 XTRSP	+101 CATCACTACA CATTACTACA TTTCATCACT +201	* * АААА. ТА <b>ТТТ</b> АААААТА <b>СТТ</b> ТСТАТССТСТ	* Specific FV AAGAAATTAC AAGAAATTTC GATAAATAGC	<pre>V Primers ACAAAACAGA ACTAAACAGC TCATCTCAGT</pre>	CGATACTGAA AGATGCTGAA GGCTA	AATAATAAAC AATAATAAAC TATAATGCGC	GTTACTTATA GTTACTTCTA TTAGCA Univer	AAATACTTTG AAATACTTTG ATATTCTTTA sal RV Prim	CGTCATGTGC CATCATTTGC CCGAAAC	+200 CGCTTTATAA CGCTTTATAA GGCTTTCTAT +300
ZStR1 ZStR2 XTRSP ZStR1	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA	* * AAAA.TA <b>TT</b> AAAAATA <b>CTT</b> TCTATCCTCT	* Specific FV AAGAAATTAC AAGAAATTAC GATAAATAGC .CAGCCACGC	V Primers ACAAAACAGA ACTAAACAGC TCATCTCAGT GAACAACCCA	GGATACTGAA AGATGCTGAA GGCTA ATCAGCGC	AATAATAAAAC AATAATAAAC TATAATGCGC ATTAAACACT	GTTACTTATA GTTACTTCTA TTAGCA Univer. C <b>AACTGGACC</b>	AAATACTTTG AAATACTTTG ATATTCTTTA sal RV Prime AATC	CGTCATGTGC CATCATTTGC CCGAAAC er ACAGCGCTCA	+200 CGCTTTATAA CGCTTTATAA GGCTTTCTAT +300 CTGAAGTGTC
ZStR1 ZStR2 XTRSP ZStR1 ZStR1 ZStR2	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA TTTTCATAAA	* * AAAA.TA <b>TT</b> AAAAATA <b>CTT</b> TCTATCCTCT CCCGGAAGCG CCCGGAAGCG	* Specific FV AAGAAATTAC AAGAAATTAC GATAAATAGC .CAGCCACGC .CAGCCACGC	V Primers ACAAAACAGA ACTAAACAGC TCATCTCAGT GAACAACCCA GAACAACCCA	CGATACTGAA AGATGCTGAA GGCTA ATCAGCGC ATCAGCGC	AATAATAAAAC AATAATAAAC TATAAATGCGC ATTAAACACT ATTAAACACT	GTTACTTATA GTTACTTCTA TTAGCA Univer. C <b>AACTGGAAC</b> CAACTGGAAC	AAATACTTTG AAATACTTTG ATATTCTTTA sal RV Prim AATC	CGTCATGTGC CATCATTTGC CCGAAAC er ACAGCGCTCA ACAGCGCTCA	+200 CGCTTTATAA CGCTTTATAA GGCTTTCTAT +300 CTGAAGTGTC CTGAAGTGTC
ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA TTTTCATAAA TTTACATTCA	* * AAAA.TA <b>TTT</b> AAAAATA <b>CTT</b> TCTATCCTCT CCCGGAAGCG CCCGGAAGCG GTCACAGTAT	* Specific FV AAGAAATTAC AAGAAATTTC GATAAATAGC .CAGCCACGC .CAGCCACGC ACAGTATCCA	V Primers ACAAAACAGA ACTAAACAGC TCATCTCAGT GAACAACCCA GAACAACCCA TAACAACCAG	CGATACTGAA AGATGCTGAA GGCTA ATCAGCGC ATCAGCGC GTTCCATTT	AATAATAAAC AATAATAAAC TATAATGCGC ATTAAACACT ATTAAACACT ATTAAACACT ATTAAACACT	GTTACTTATA GTTACTTCTA TTAGCA Univer C <b>AACTGGACC</b> CAACTGGAAC TCATTTCTGA	AAATACTTTG AAATACTTTG ATATTCTTTA sal RV Primu AATC TATCTTTAGT	CGTCATGTGC CATCATTTGC CCGAAAC er ACAGCGCTCA ACAGCGCTCA ACATGGGTAA	+200 CGCTTTATAA GGCTTTCTAT +300 CTGAAGTGTC CTCCAG.GTG
ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA TTTTCATAAA TTTACATTCA	* * AAAA. TA <b>TTT</b> AAAAATA <b>CTT</b> TCTATCCTCT CCCGGAAGCG CCCGGAAGCG GTCACAGTAT	* Specific FV AAGAAATTAC AAGAAATTTC GATAAATAGC .CAGCCACGC .CAGCCACGC ACAGTATCCA	V Primers ACAAACAGA ACTAAACAGC TCATCTCAGT GAACAACCCA GAACAACCCA TAACAACCAG	CGATACTGAA AGATGCTGAA GGCTA ATCAGCGC ATCAGCGC GTTCCATTT	AATAATAAAC AATAATAAAC TATAATGCGC ATTAAACACT ATTAAACACT ATTAAACACT ATTATGTTTG	GTTACTTATA GTTACTTCTA TTAGCA Univer C <b>AACTGGACC</b> CAACTGGAAC TCATTTCTGA	AAATACTTTG AAATACTTTG ATATTCTTTA sal RV Prim AATC AATC TATCTTTAGT	CGTCATGTGC CATCATTTGC CCGAAAC er ACAGCGCTCA ACAGCGCTCA ACATGGGTAA	+200 CGCTTTATAA GGCTTTCTAT +300 CTGAAGTGTC CTCCAG.GTG
ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA TTTTCATAAA TTTTCATAAA TTTACATTCA +301	* * AAAA.TA <b>TTT</b> AAAATAC <b>TT</b> TCTATCCTCT CCCGGAAGCG CCCGGAAGCG GTCACAGTAT	* specific FI AAGAAATTAC AAGAAATTAC GATAAATAGC .CAGCCACGC .CAGCCACGC ACAGTATCCA	Primers ACAAACAGA ACTAAACAGA TCATCTCAGT GAACAACCCA GAACAACCCA TAACAACCAG	GGATACTGAA AGATGCTGAA GGCTA ATCAGCGC ATCAGCGC GTTCCATTT	AATAATAAAC AATAATAAAC TATAAATGCGC ATTAAACACT ATTAAACACT ATTATGTTTG CATTCAACACT Spe	GTTACTTATA GTTACTTCTA TTAGCA Univer CAACTGGACC CAACTGGACC TCATTTCTGA ecific RV P CTTACTACCO	AAATACTTTG AAATACTTTG ATATTCTTTA sal RV Prime AATC AATC TATCTTTAGT rimers	CGTCATGTGC CATCATTTGC CCGAAAC er ACAGCGCTCA ACAGCGCTCA ACATGGGTAA	+200 CGCTTTATAA CGCTTTATAA GGCTTTCTAT +300 CTGAAGTGTC CTCAAGTGTC CTCCAG.GTG +400
ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR1 ZStR2	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA TTTTCATAAA TTTACATTCA +301 TGTCATACGA TGTCATACGA	* * AAAA.TA <b>TTT</b> AAAATAC <b>TT</b> TCTATCCTCT CCCGGAAGCG CCCGGAAGCG GTCACAGTAC AATGACGTAC	* AGAAATTAC AAGAAATTAC AAGAAATTTC GATAAATAGC .CAGCCACGC .CAGCCACGC ACAGTATCCA AGTGCCCTTG	V Primers ACAAAACAGA ACTAAACAGC TCATCTCAGT GAACAACCCA GAACAACCCA TAACAACCAG GTTAGCTTCA	CGATACTGAA AGATGCTGAA GGCTA ATCAGCGC ATCAGCGC GTTCCATTTT TGCA.TGCTG	AATAATAAAC AATAATAAAC TATAAATGCGC ATTAAACACT ATTAAACACT ATTATGTTG GATTGAGAGG GATTGAGAAG	GTTACTTATA GTTACTTCTA TTAGCA Univer: CAACTGGACC CAACTGGAAC TCATTTCTGA ecific RV P CTAGCA.GGC CTAACAAGGC	AAATACTTTG AAATACTTTG ATATTCTTTA sal RV Primu AATC TATCTTTAGT rimers TAACATTTAT TAACATTTAT	CGTCATGTC CATCATTTCC CCGAAAC er ACACGCGCTCA ACAGCGCTCA ACATGGGTAA	+200 CGCTTTATAA CGCTTTATAA GGCTTTCTAT +300 CTGAAGTGTC CTGAAGTGTC CTCCAG.GTG +400 CAATTTATAA
ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR1 ZStR2 XTRSP	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA TTTTCATAAA TTTACATTCA +301 TGTCATACGA TGTCATACGA GCCAGTCTA	* * AAAA.TA <b>TTT</b> AAAAATAC <b>TT</b> TCTATCCTCT CCCGGAAGCG CCCGGAAGCG GTCACAGTAT AATGACGTAC AATGACGTAC TCAGTCATAA	* Specific FV AAGAAATTAC AAGAAATTTC GATAAATAGC .CAGCCACGC .CAGCCACGC ACAGTATCCA AGTGCCCTTG AGTGCCCTTG GGTGCCCTTG	V Primers ACAAAACAGA ACTAAACAGA TCATCTCAGT GAACAACCCA GAACAACCCA TAACAACCAG GTTAGCTTCA GTTAGCTTCA TATATCTTGC	CGATACTGAA AGATGCTGAA GGCTA ATCAGCGC ATCAGCGC GTTCCATTTT TGCA.TGCTG TCCAGTGAAC	AATAATAAAC AATAATAAAC TATAAACACT ATTAAACACT ATTAAACACT ATTATGTTG GATTGA <b>CACG</b> GATTGA <b>CACG</b> TTATATATCA	GTTACTTATA GTTACTTCTA TTAGCA Univer: CAACTGGACC CAACTGGAAC TCATTTCTGA ecific RV P CTAGCA.GGC CTAACAAGGC GTAACAGTTA	AAATACTTTG AAATACTTTG ATATTCTTTA sal RV Prim AATC TATCTTTAGT rimers TAACATTAT ATACAGTTAA	CGTCATGTCC CATCATTTGC CCGAAAC er ACAGCGCTCA ACAGCGCTCA ACATGGGTAA CATGTA.AAA AAGGGATAAA	+200 CGCTTTATAA CGCTTTATAA GGCTTTCTAT +300 CTGAAGTGTC CTCCAG.GTG +400 CAATTTATAA AAAAACATAT
ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA TTTTCATAAA TTTACATTCA +301 TGTCATACGA .GCCAGTCTA	* * AAAA.TA <b>TTT</b> AAAAATAC <b>TT</b> TCTATCCTCT CCCGGAAGCG CCCGGAAGCG GTCACAGTAT AATGACGTAC AATGACGTAC TCAGTCATAA	* AAGAAATTAC AAGAAATTAC GATAAATAGC .CAGCCACGC .CAGCCACGC ACAGTATCCA AGTGCCCTTG GCTGCCCTTG	V Primers ACAAAACAGA ACTAAACAGC TCATCTCAGT GAACAACCCA GAACAACCCA TAACAACCAG GTTAGCTTCA GTTAGCTTCA TATATCTTGC	CGATACTGAA AGATGCTGAA GGCTA ATCAGCGC ATCAGCGC GTTCCATTTT TGCA.TGCTG TGCA.TGCTG TCAAGTGAAC	AATAATAAAC AATAATAAAC TATAAATGCGC ATTAAACACT ATTAAACACT ATTAAACACT ATTATGTTTG GATTGA <b>CACG</b> GATTGA <b>CACG</b> TTATATATCA	GTTACTTATA GTTACTTCTA TTAGCA Univer. CAACTGGACC CAACTGGAAC TCATTTCTGA ecific RV P CTACCA.GGC CTAACAAGGC GTAACAGTTA	AAATACTTTG AAATACTTTG ATATTCTTTA sal RV Prim AATC TATCTTTAGT rimers TAACATTTAT TAACATTTAT ATACAGTTAA	CGTCATGTGC CATCATTTGC CCGAAAC er ACAGCGCTCA ACAGCGCTCA ACATGGGTAA CATGTA.AAA CGTGTA.AAA AAGGGATAAA	+200 CGCTTTATAA CGCTTTATAA GGCTTTCTAT +300 CTGAAGTGTC CTCAAGTGTC CTCCAG.GTG +400 CAATTTATAA AAAAACATAT
ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA TTTTCATAAA TTTACATTCA +301 TGTCATACGA TGTCATACGA .GCCAGTCTA +401	* * AAAA.TA <b>TTT</b> AAAAATAC <b>TT</b> TCTATCCTCT CCCGGAAGCG CCCGGAAGCG GTCACAGTAT AATGACGTAC AATGACGTAC TCAGTCATAA	* AAGAAATTAC AAGAAATTAC GATAAATAGC .CAGCCACGC .CAGCCACGC ACAGTATCCA AGTGCCCTTG AGTGCCCTTG GCTGCTCCCT	V Primers ACAAAACAGA ACTAAACAGC TCATCTCAGT GAACAACCCA GAACAACCCA TAACAACCAG GTTAGCTTCA GTTAGCTTCA TATATCTTGC	CGATACTGAA AGATGCTGAA GGCTA ATCAGCGC ATCAGCGC GTTCCATTTT TGCA.TGCTG TGCA.TGCTG TCAAGTGAAC	ААТААТАААС ААТААТАААС ТАТАААСАСТ АТТАААСАСТ АТТАААСАСТ АТТАААСАСТ АТТАААСАСТ АТТАААСАСТ ЭЛТСАСС САТТСАССС САТССС САТТСАССС САТТСАССС САСТСАСС САСТСАСС САСТСАСС САСТСАСССС САССС САСССС САССС САСТСС САСССС САССС САСТС САССС САСССС САССС	GTTACTTATA GTTACTTCTA TTAGCA Univer. CAACTGGAAC CAACTGGAAC TCATTTCTGA ecific RV P CTAGCA.GGC CTAACAAGGC GTAACAAGTTA	AAATACTTTG AAATACTTTG ATATTCTTTA sal RV Prime AATC TATC.TTAGT rimers TAACATTTAT TAACATTTAT ATACAGTTAA	CGTCATGTGC CATCATTTGC CCGAAAC Pr ACAGCGCTCA ACAGCGCTCA ACATGGGTAA CATGTA.AAA CGTGTA.AAA AAGGGATAAA	+200 CGCTTTATAA GGCTTTATAA GGCTTTCTAT +300 CTGAAGTGTC CTCAAGTGTC CTCCAG.GTG +400 CAATTTATAA AAAAACATAT +500
ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA TTTTCATAAA TTTTCATACA +301 TGTCATACGA TGTCATACGA .GCCAGTCTA +401 CATTTCC	* * AAAA.TA <b>TTT</b> AAAAATAC <b>TT</b> TCTATCCTCT CCCGGAAGCG CCCGGAAGCG GTCACAGTAT AATGACGTAC AATGACGTAC TCAGTCATAA CGATCGCGTC	* AGAAATTAC AAGAAATTAC AAGAAATTAC GATAAATAGC .CAGCCACGC .CAGCCACGC ACAGTATCCA AGTGCCCTTG AGTGCCCTTG GCTGCTCCCT AATCCCCCGGT	V Primers ACAAAACAGA ACTAAACAGC TCATCTCAGT GAACAACCCA GAACAACCCA TAACAACCAG GTTAGCTTCA GTTAGCTTCA TATATCTTGC AAGAGTGCTG	CGATACTGAA AGATGCTGAA GGCTA ATCAGCGC ATCAGCGC GTTCCATTTT TGCA.TGCTG TGCA.TGCTG TCAAGTGAAC AATTGTTTGC	ААТААТАААС ААТААТАААС ТАТАААСАСТ АТТАААСАСТ АТТАААСАСТ АТТАТАТТТ GATTGA <b>CACG</b> GATTGA <b>CACG</b> GATTGA <b>CACG</b> TTATATATCA TAATATTAGT	GTTACTTATA GTTACTTCTA TTAGCA Univer CAACTGGACC CCAACTGGACC TCATTTCTGA ecific RV P CTAGCA.GGC CTAACAAGGC GTAACAGTTA ATATCGATAT	AAATACTTTG AAATACTTTG ATATTCTTTA sal RV Prime AATC AATC TATCTTTAGT TAACATTTAGT TAACATTTAT ATACAGTTAA TATAGTTGTA	CGTCATGTC CATCATTTGC CCGAAAC er ACAGCGCTCA ACAGCGCTCA ACATGGGTAA CATGTA.AAA CGTGTA.AAA AAGGGATAAA GGATCAGATG	+200 CGCTTTATAA CGCTTTATAA GGCTTTCTAT +300 CTGAAGTGTC CTCCAG.GTG CTCCAG.GTG CAATTTATAA AAAACATAT +500 ATTTCATGTG
ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA TTTTCATAAA TTTTCATACA +301 TGTCATACGA .GCCAGTCTA +401 CATTTCC CATTTCC	* * AAAA.TATTT AAAAATACTT TCTATCCTCT CCCGGAAGCG GTCACAGTAT AATGACGTAC AATGACGTAC AATGACGTAC CGATCGCGTC CGATCG	* AGAAATTAC AAGAAATTAC AAGAAATTAC GATAAATAGC .CAGCCACGC .CAGCCACGC ACAGTATCCA AGTGCCCTTG GCTGCTCCCT AATCCCCCGGT	Primers ACAAACAGA ACTAAACAGA ACTAAACAGC TCATCTCAGT GAACAACCCA GAACAACCCA TAACAACCAG GTTAGCTTCA GTTAGCTTCA TATATCTTGC AAGAGTGCTG	GGATACTGAA AGATGCTGAA GGCTA ATCAGCGC ATCAGCGC GTTCCATTTT TGCA.TGCTG TCCA.TGCTG TCCAGTGAAC	AATAATAAAC AATAATAAAC AATAATAAAC TATAAACACT ATTAAACACT ATTATGTTTG GATTGAGAGG GATTGAGAGG GATTGAAAAG TTATATATCA TAATATTAGT	GTTACTTATA GTTACTTCTA TTAGCA Univer CAACTGGACC TCATTTCTGA ecific RV P CTAGCA.GGC GTAACAGGC GTAACAGTA ATATCGATAT	AAATACTTTG AAATACTTTG ATATTCTTTA sal RV Prime AATC TATCTTTAGT rimers TAACATTTAT TAACATTTAT ATACAGTTAA TATAGTTGTA	CGTCATGTC CATCATTTGC CCGAAAC er ACAGCGCTCA ACAGCGCTCA ACATGGGTAA CATGTA.AAA CGTGTA.AAA AGGGATAAA GGATCAGATG	+200 CGCTTTATAA CGCTTTATAA GGCTTTCTAT +300 CTGAAGTGTC CTCCAG.GTG CTCCAG.GTG CTCCAG.GTG CAATTTATAA AAAACATAT +500 ATTTCATGTG
ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA TTTTCATAAA TTTACATTCA +301 TGTCATACGA .GCCAGTCTA +401 CATTTCC GTGCAGTACA	* * AAAA.TATTT AAAAATACTT TCTATCCTCT CCCGGAAGCG GTCACAGTAT AATGACGTAC AATGACGTAC TCAGTCATAA CGATCGCGTC CGATCGCGTC CGATCGCGT TGATCACAGT	* AAGAAATTAC AAGAAATTAC GATAAATAGC .CAGCCACGC .CAGCCACGC ACAGTATCCA AGTGCCCTTG GCTGCTCCCT AATCCCCGGT 	<ul> <li>Primers</li> <li>ACAAAACAGA</li> <li>ACTAAACAGA</li> <li>ACTAAACAGC</li> <li>TCATCTCAGT</li> <li>GAACAACCCA</li> <li>GAACAACCCA</li> <li>TAACAACCAG</li> <li>GTTAGCTTCA</li> <li>GTTAGCTTCA</li> <li>TATATCTTGC</li> <li>AAGAGTGCTG</li> <li>GAGTGTAAGA</li> </ul>	GGATACTGAA AGATGCTGAA GGCTA ATCAGCGC ATCAGCGC GTTCCATTT TGCA.TGCTG TGCA.TGCTG TCCAGTGAAC AATTGTTTGC 	AATAATAAAC AATAATAAAC AATAATAAAC TATAAACACT ATTAAACACT ATTATGTTTG GATTGAAAAG GATTGAAAAG TTATATATCA TAATATTAGT GAAGTTGCAT	GTTACTTATA GTTACTTCTA TTAGCA Univer CAACTGGACC TCATTTCTGA ecific RV P CTAGCA GGC GTAACAGGC GTAACAGGTA ATATCGATAT TTTCCTTTAG	AAATACTTTG AAATACTTTG ATATTCTTTA sal RV Primu AATC TATCTTTAGT rimers TAACATTTAT TAACATTTAT ATACAGTTAA TATAGTTGTA TATAGTTGTA	CGTCATGTC CATCATTTGC CCGAAAC PT ACAGCGCTCA ACATGGGTAA CATGTA.AAA CGTGTA.AAA AAGGGATAAA GGATCAGATG TTATGGACTA	+200 CGCTTTATAA GGCTTTATAA GGCTTTCTAT +300 CTGAAGTGTC CTCCAG.GTG CTCCAG.GTG CTCCAG.GTG CTCCAG.GTG ATTTATAA AAAACATAT +500 ATTTCATGTG ATTGTGCTTG
ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA TTTACATACA TTTACATACA +301 TGTCATACGA .GCCAGTCTA +401 CATTTCC GTGCAGTACA +501	* * AAAA.TA <b>TTT</b> AAAAATAC <b>TT</b> TCTATCCTCT CCCGGAAGCG CCCGGAAGCG GTCACAGTAT AATGACGTAC AATGACGTAC TCAGTCATAA CGATCGCGTC CGATCGCGTC CGATCGCATC	* AAGAAATTAC AAGAAATTAC GATAAATAGC .CAGCCACGC .CAGCCACGC ACAGTATCCA AGTGCCCTTG GCTGCTCCCT AATCCCCGGT 	Primers ACAAACAGA ACTAAACAGA TCATCTCAGT GAACAACCCA GAACAACCCA GAACAACCCA GTAGCTTCA GTTAGCTTCA GTTAGCTTCA AAGAGTGCTG AAGAGTGCTG GAGTGTAAGA	CGATACTGAA AGATGCTGAA GGCTA ATCAGCGC ATCAGCGC GTTCCATTTT TGCA.TGCTG TGCA.TGCTG TCAAGTGAAC AATTGTTTGC 	ААТААТААА ААТААТАААС ААТАААТААС ТАТАААСАСТ АТТАААСАСТ АТТАТАТТТG САТТGА <b>ГАСС</b> САТТGА <b>ГАСС</b> САТТGА <b>ГАСС</b> ТТАТАТАТСА ТААТАТТАСА СААТСТСАСА СААСТСАСА	GTTACTTATA GTTACTTCTA TTAGCA Univer CAACTGGAAC TCATTTCTGA ecific RV P CTAGCA GGC GTAACAGGC GTAACAGGTA ATATCGATAT 	AAATACTTTG AAATACTTTG ATATTCTTTA sal RV Primu AATC TATCTTTAGT rimers TAACATTTAT TAACATTTAT ATACAGTTAA TATAGTTGTA  TTTAATTCTA	CGTCATGTC CATCATTTCC CCGAAAC PT ACAGCGCTCA ACAGCGCTCA ACATGGGTAA CGTGTA.AAA CGTGTA.AAA AGGGATAAA GGATCAGATG 	+200 CGCTTTATAA CGCTTTATAA GGCTTTCTAT +300 CTGAAGTGTC CTCCAG.GTG CTCCAG.GTG CTCCAG.GTG CTCCAG.GTG ATTTATAA AAAACATAT +500 ATTTCATGTG 
ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA TTTACATTCA +301 TGTCATAGGA TGTCATACGA .GCCAGTCTA +401 CATTTCC GTGCAGTACA +501 ACATAAAG	* * AAAA.TA <b>TTT</b> AAAAATAC <b>TT</b> TCTATCCTCT CCCGGAAGCG CCCGGAAGCG GTCACAGTAT AATGACGTAC AATGACGTAC TCAGTCATAA CGATCGCGTC CGATCGCGTC CGATCGCGTC AGTAGAGCTG	* AAGAAATTAC AAGAAATTAC GATAAATAGC .CAGCCACGC .CAGCCACGC ACAGTATCCA AGTGCCCTTG GCTGCCCTTG AATCCCCGGT 	Primers ACAAACAGA ACTAAACAGA TCATCTCAGT GAACAACCCA GAACAACCCA GAACAACCCA GTTAGCTTCA GTTAGCTTCA TATATCTTGC AAGAGTGCTG GAGTGTAAGA AGCTTTAAAC	CGATACTGAA AGATGCTGAA GGCTA ATCAGCGC GTT.CATTTT TGCA.TGCTG TGCA.TGCTG TCAAGTGAAC AATTGTTTGC TTCTTTTCTG CACCGGATAA	AATAATAAAC AATAATAAAC AATAATAAAC TATAAACACT ATTAAACACT ATTATGTTTG GATTGAGAGG GATTGAGAAG TTATATATCA TAATATTAGT GAAGTTGCAT AATGACAATA	GTTACTTATA GTTACTTCTA TTAGCA Univer CAACTGGACC CCAACTGGAAC TCATTTCTGA ecific RV P CTAGCA.GGC GTAACAGGC GTAACAGGC GTAACAGGTA ATATCGATAT TTTCCTTTAG	AAATACTTTG AAATACTTTG ATATTCTTTA sal RV Prime AATC TATCTTTAGT rimers TAACATTAT TAACATATAT ATACAGTTAA TATAGTTGTA TATAGTTGTA AACATGGTGG	CGTCATGTC CATCATTTCC CCGAAAC T ACAGCGCTCA ACATGGGTAA CGTGTA.AAA CGTGTA.AAA AAGGGATAAA GGATCAGATG 	+200 CGCTTTATAA GGCTTTATAA GGCTTTCTAT +300 CTGAAGTGTC CTCCAG.GTG CTCCAG.GTG CTCCAG.GTG CAATTTATAA AAAACATAT +500 ATTTCATGTG ATTGTGCTTG +600 GTTTTAAGTA
ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA TTTACATTCA +301 TGTCATACGA .GCCAGTCTA +401 CATTTCC GTGCAGTACA +501 ACATAAAG	* * AAAA.TA <b>TTT</b> AAAAATAC <b>TT</b> TCTATCCTCT CCCGGAAGCG CCCGGAAGCG GTCACAGTAT AATGACGTAC AATGACGTAC TCAGTCATAA CGATCGCGTC CGATCGC TGATCACAGT AGTAGAGCTG	* AAGAAATTAC AAGAAATTAC GATAAATAGC .CAGCCACGC .CAGCCACGC ACAGTATCCA AGTGCCCTTG GCTGCCCTTG AATCCCCGGT 	V Primers ACAAACAGA ACTAAACAGA TCATCTCAGT GAACAACCCA GAACAACCCA GTAGCTTCA GTTAGCTTCA TATATCTTGC AAGAGTGCTG GAGTGTAAGA AGCTTTAAAC	CGATACTGAA AGATGCTGAA GGCTA ATCAGCGC GTT.CAGCGC GTTCCATTTT TGCA.TGCTG TCAAGTGAAC AATTGTTTGC TTCTTTTCTG CACCGGATAA	AATAATAAAC AATAATAAAC TATAAACACT ATTAAACACT ATTAAACACT ATTAAACACT ATTATGTTG GATTGA <b>GAGG</b> GATTGA <b>GAAG</b> TTATATATCA TAATATTAGT GAAGTTGCAT AATGACAATA	GTTACTTATA GTTACTTCTA TTAGCA Univer: CAACTGGACC CAACTGGACC CTATTCTGA ecific RV P CTAGCA.GGC GTAACAGGTA ATATCGATAT TTTCCTTTAG TGCTGAGTAA	AAATACTTTG AAATACTTTG ATATTCTTTA sal RV Prim AATC TATCTTTAGT rimers TAACATTAT ATACATTAT ATACAGTTAA TATAGTTGTA TATAGTTGTA TATAATTCTA AACATGGTGG 	CGTCATGTGC CATCATTTGC CCGAAAC Pr ACAGCGCTCA ACAGCGCTCA ACATGGGTAA CATGTA.AAA CCTGTA.AAA AAGGGATAAA GGATCAGATG TTATGGACTA	+200 CGCTTTATAA GGCTTTATAA GGCTTTCTAT +300 CTGAAGTGTC CTCCAG.GTG +400 CAATTTATAA AAAAACATAT +500 ATTTCATGTG ATTGTGCTTG +600 GTTTTAAGTA
ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA TTTTCATAAA TTTACATTCA +301 TGTCATACGA TGTCATACGA .GCCAGTCTA +401 CATTTCC GTGCAGTACA +501 ACATAAAG TATTACAGGT	* * AAAA.TA <b>TTT</b> AAAAATAC <b>TT</b> TCTATCCTCT CCCGGAAGCG CCCGGAAGCG GTCACAGTAT AATGACGTAC AATGACGTAC TCAGTCATAA CGATCGCGTC CGATCGC TGATCACAGT AGTAGAGCTG ATGGGATCCG	* AAGAAATTAC AAGAAATTAC GATAAATAGC .CAGCCACGC .CAGCCACGC ACAGTATCCA AGTGCCCTTG GCTGCCCTTG GCTGCTCCT AATCCCCGGT TATCTTAT.T CTGAAGATTC .TTAACTGGAA	<ul> <li>Primers</li> <li>ACAAACAGA</li> <li>ACTAAACAGA</li> <li>ACTAAACAGA</li> <li>TCATCTCAGT</li> <li>GAACAACCCA</li> <li>GAACAACCCA</li> <li>GATAGCTTCA</li> <li>GTTAGCTTCA</li> <li>TATATCTTGC</li> <li>AAGAGTGCTG</li> <li>GAGTGTAAGA</li> <li>AGCTTTAACA</li> <li>ACCTNTTATC</li> </ul>	CGATACTGAA AGATGCTGAA GGCTA ATCAGCGC GTT.CATTTT TGCA.TGCTG TCAAGTGAAC AATTGTTTGC TCATTTTTCTG CACCGGATAA CAGAAGCTCA	AATAATAAAC AATAATAAAC TATAAACACT ATTAAACACT ATTAAACACT ATTAAACACT ATTATGTTG GATTGA <b>GAGG</b> GATTGA <b>GAGG</b> GATTGA <b>AAAG</b> TTATATATCA TAATATTAGT GAAGTTGCAT AATGACAATA GAATTACGGA	GTTACTTATA GTTACTTCTA TTAGCA Univer: CAACTGGACC CAACTGGACC CCAACTGGACC CTACAGGC GTACAGGC GTAACAGGTA ATATCGATAT TTTCCTTTAG TGCTGAGTAA AGCCCTTTTC	AAATACTTTG AAATACTTTG ATATTCTTTA sal RV Prim AATC TATCTTTAGT rimers TAACATTAT ATACATTAT ATACAGTTAA TATAGTTGTA TATAGTTGTA AACATGGTGG 	CGTCATGTC CATCATTIGC CCGAAAC Pr ACAGCGCTCA ACAGCGCTCA ACATGGGTAA CATGGATAAA CGTGTA.AAA CGTGTA.AAA AGGGATCAGATG  TTATGGACTA TTTATGGGTG CATGTATCTA	+200 CGCTTTATAA GGCTTTATAA GGCTTTCTAT +300 CTGAAGTGTC CTCAAGTGTC CTCCAG.GTG ATTTATAA AAAACATAT +500 ATTTCATGTG ATTTCATGTG GTTTTAAGTA ATAATTCATA
ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA TTTTCATAAA TTTTCATAAA TTTCATACA +301 TGTCATACGA TGTCATACGA .GCCAGTCTA +401 CATTTCC GTGCAGTACA +501 ACATAAAG TATTACAGGT	* * AAAA.TA <b>TTT</b> AAAAATAC <b>TT</b> TCTATCCTCT CCCCGGAAGCG GCCCGGAAGCG GTCACAGTAT AATGACGTAC AATGACGTAC TCAGTCATAA CGATCGCGTC CGATCGCGTC CGATCACAGT AGTAGAGCTG ATGGGATCCG	* * AAGAAATTAC AAGAAATTAC AAGAAATTAC AAGAAATAGC .CAGCCACGC .CAGCCACGC ACAGTATCCA AGTGCCCTTG GCTGCCCCTTG GCTGCTCCCT AATCCCCGGT  TATCTTAT.T CTGAAGATTC TTAACTGGAA	<ul> <li>Primers</li> <li>ACAAAACAGA</li> <li>ACTAAACAGC</li> <li>TCATCTCAGT</li> <li>GAACAACCCA</li> <li>GAACAACCCA</li> <li>GATAGCTTCA</li> <li>GATAGCTTCA</li> <li>GATAGCTTCA</li> <li>TATATCTTGC</li> <li>AAGAGTGCTG</li> <li>GAGTGTAAGA</li> <li>AGCTTTAAACA</li> <li>ACCTNTTATC</li> </ul>	CGATACTGAA AGATGCTGAA GGCTA ATCAGCGC GTTCCATTTT TGCA.TGCTG TGCA.TGCTG TCAAGTGAAC AATTGTTTGC TTCTTTTCTG CACCGGATAA CAGAAGCTCA	ААТААТАААС ААТААТАААС ТАТАААСАСТ АТТАААСАСТ АТТАААСАСТ АТТАТАТТ САТТСАСС САТТСАССС САТТСАСССС САТТСАСССССС САТТСАСССССССС	GTTACTTATA GTTACTTCTA TTAGCA Univer CAACTGGAC CCAACTGGAC TCATTTCTGA CTACCA.GGC GTAACAGGC GTAACAGGC GTAACAGTTA ATATCGATAT  TTTCCTTTAG TGCTGAGTAA AGCCCTTTTC	AAATACTTTG AAATACTTTG ATATTCTTTA sal RV Prime AATC AATC TATCTTTAGT TAACATTTAGT TAACATTTAT ATACAGTTAA TATAGTTGTA TATAGTTGTA AACATGGTGG AACATGGTGG CTATAGTCTC	CGTCATGTC CATCATTTGC CCGAAAC PT ACAGCGCTCA ACAGCGCTCA ACATGGGTAA CATGTA.AAA CCTGTA.AAA AGGGATCAGATG  TTATGGACTA TTTATGGACTA CATGTATCTA	+200 CGCTTTATAA GGCTTTATAA GGCTTTCTAT +300 CTGAAGTGTC CTCAAGTGTC CTCCAG.GTG CTCCAG.GTG CTCCAG.GTG ATTTATAA AAAACATAT +500 ATTTCATGTG ATTGTGCTTG ATTGTGCTTG GTTTTAAGTA ATAATTCATA
ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP	+101 CATCACTACA CATTACTACA TTTCATCACA +201 TTTTCATAAA TTTTCATAAA TTTTCATAAA TTTTCATACA +301 TGTCATACGA +301 TGTCATACGA .GCCAGTCTA +401 CATTTCC GTGCAGTACA +501 ACATAAAG TATTACAGGT +601 TATAAAGCAA	* * AAAA.TATTT AAAAATACTT TCTATCCTCT CCCCGGAAGCG GCCCGGAAGCG GTCACAGTAT AATGACGTAC AATGACGTAC TCAGTCATAA CGATCGCGTC CGATCGCGTC CGATCGCGTC AGTAGAGCTG AGTAGAGCTG AGTATAATTA	* * AGAAATTAC AAGAAATTAC AAGAAATTAC GATAAATAGC .CAGCCACGC .CAGCCACGC ACAGTATCCA AGTGCCCTTG GCTGCCCTTG GCTGCTCCCT AATCCCCGGT 	<ul> <li>Primers</li> <li>ACAAAACAGA</li> <li>ACTAAACAGC</li> <li>ACTAAACAGC</li> <li>GAACAACCCA</li> <li>GAACAACCCA</li> <li>GAACAACCCA</li> <li>GTTAGCTTCA</li> <li>GTTAGCTTCA</li> <li>TATATCTTGC</li> <li>AAGAGTGCTG</li> <li>AAGAGTGTAAGA</li> <li>ACCTNTTATC</li> <li>ACCTNTTATC</li> </ul>	CGATACTGAA AGATGCTGAA GGCTA ATCAGCGC GTTCCATTTT TGCA.TGCTG TGCA.TGCTG TGCA.TGCTG TCAAGTGAAC AATTGTTTGC TTCTTTTCTG CACCGGATAA CAGAAGCTCA	AATAATAAAC AATAATAAAC AATAATAAAC TATAAACACT ATTAAACACT ATTAAACACT ATTATGTTTG GATTGACAGG GATTGACAGG GATTGACAAG TAATATTAGT GAAGTTGCAT AATGACAATA GAATTACGGA	GTTACTTATA GTTACTTCTA TTAGCA Univer CAACTGGACC TCATTTCTGA ecific RV P CTAGCA.GGC CTAACAAGGC GTAACAAGGC GTAACAAGTA ATATCGATAT TTTCCTTTAG TGCTGAGTAA AGCCCTTTTC	AAATACTTTG AAATACTTTG ATATTCTTTA sal RV Prime AATC TATCTTTAGT rimers TAACATTTAGT TAACATTTAT ATACAGTTGTA TATAGTTGTA TATAGTTGTA AACATGGTGG CTATAGTCTC CG.CTTTTAA	CGTCATGTC CATCATTTGC CCGAAAC PT ACAGCGCTCA ACATGGGTAA CATGGATAA CGTGTA.AAA CGTGTA.AAA AGGGATCAGATG TTATGGGACTA TTTATGGACTA TTTATAGGTG CATGTATCTA	+200 CGCTTTATAA GGCTTTATAA GGCTTTCTAT +300 CTGAAGTGTC CTCCAG.GTG CTCCAG.GTG CAATTTATAA AAAACATAT +500 ATTTCATGTG ATTGTGCTTG GTTTTAAGTA ATAATTCATA +700 ACTATTAGT
ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA TTTTCATAAA TTTTCATACA +301 TGTCATACGA GCCAGTCTA +401 CATTTCC GTGCAGTACA +501 ACATAAAGCAA TATTACAGGT +601 TATAAAGCAA	* * AAAA.TATTT AAAAATACTT TCTATCCTCT CCCCGGAAGCG GCCCGGAAGCG GTCACAGTAT AATGACGTAC AATGACGTAC CGATCGCGTC CGATCGCGTC CGATCGCGTC AGTAGAGCTG ATGGGATCCG AGTATAATTA	* * AAGAAATTAC AAGAAATTAC AAGAAATTAC GATAAATAGC .CAGCCACGC .CAGCCACGC ACAGTATCCA AGTGCCCTTG GCTGCCCTTG GCTGCTCCCT AATCCCCGGT TATCTTAT.T CTGAAGATTC .TTAACTGGAA TTTAATCTTA	<ul> <li>Primers</li> <li>ACAAACAGA</li> <li>ACTAAACAGA</li> <li>ACTAAACAGA</li> <li>ACTAAACAGC</li> <li>GAACAACCCA</li> <li>GAACAACCCA</li> <li>GATAGCTTCA</li> <li>GTTAGCTTCA</li> <li>GATAGCTTCA</li> <li>TATATCTTGC</li> <li>AAGAGTGCTG</li> <li>GAGTGTAAGA</li> <li>AGCTTTAAACA</li> <li>ACCTNTTATC</li> <li>TTTAATTATT</li> </ul>	GGATACTGAA AGATGCTGAA GGCTA ATCAGCGC GTTCCATTTT TGCA.TGCTG TCCATGCTG TCCAGTGAAC AATTGTTTGC TTCTTTTCTG CACCGGATAA CAGAAGCTCA TGTTTCATGG	AATAATAAAA AATAATAAAC AATAATAAAC TATAAACACT ATTAAACACT ATTATGTTTG GATTGA <b>GAGG</b> GATTGA <b>GAGG</b> TTATATATACA TAATATTAGT GAAGTTGCAT AATGACAATA GAATTACGGA	GTTACTTATA GTTACTTCTA TTAGCA Univer CAACTGGACC TCATTTCTGA ecific RV P CTAGCA GGC GTAACAGGC GTAACAGGC GTAACAGGTA ATATCGATAT TTTCCTTTAG TGCTGAGTAA AGCCCTTTTC TTTAAATGGT	AAATACTTTG AAATACTTTG ATATTCTTTA sal RV Prime AATC TATCTTTAGT rimers TAACATTTAT TAACATTTAT ATACAGTTAA TATAGTTGTA TATAGTTGTA AACATGGTGG  CTATAGTCTC CG.CTTTTAA	CGTCATGTC CATCATTTGC CCGAAAC PT ACAGCGCTCA ACATGGGTAA CATGTA.AAA CGTGTA.AAA CGTGTA.AAA AGGGATAAA GGATCAGATG TTTATGGCACTA TTTATGGCTG CATGTATCTA TATGTCAGAA	+200 CGCTTTATAA GGCTTTATAA GGCTTTCTAT +300 CTGAAGTGTC CTCCAG.GTG CTCCAG.GTG CAATTTATAA AAAAACATAT +500 ATTTCATGTG ATTTCATGTG GTTTTAAGTA ATAATTCATA ATAATTCATA
ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP	+101 CATCACTACA CATTACTACA TTTCATCACA +201 TTTTCATAAA TTTTCATAAA TTTACATTCA +301 TGTCATACGA .GCCAGTCTA +401 CATTTCC GTGCAGTACA +501 ACATAAAG TATTACAGGT +601 TATAAAGCAA .TTTTCAAAAA	* * AAAA.TATTT AAAAATACTT TCTATCCTCT CCCGGAAGCG GCCCGGAAGCG GTCACAGTAT AATGACGTAC TCAGTCATAA CGATCGCGTC CGATCGCGTC CGATCGCGTC CGATCGCGTC AGTAGAGCTG ATGGGATCCG AGTATAATTA TGATTTCCTT	* AAGAAATTAC AAGAAATTAC GATAAATAGC .CAGCCACGC .CAGCCACGC ACAGTATCCA AGTGCCCTTG GCTGCCCTTG GCTGCTCCCT AATCCCCGGT  TATCTTAT.T CTGAAGATTC  TTTAATCTTA .TTTAATCTTA	<ul> <li>Primers</li> <li>ACAAACAGA</li> <li>ACTAAACAGA</li> <li>ACTAAACAGA</li> <li>ACTAAACAGC</li> <li>GAACAACCCA</li> <li>GAACAACCCA</li> <li>GATAGCTTCA</li> <li>GATAGCTTCA</li> <li>GATAGCTTCA</li> <li>AAGAGTGCTG</li> <li>AAGAGTGTAAGA</li> <li>AGCTTTAAACA</li> <li>ACCTNTTATC</li> <li>TTTAATTATT</li> <li>ATAATAAAACA</li> </ul>	CGATACTGAA AGATGCTGAA GGCTA ATCAGCGC ATCAGCGC GTTCCATTT TGCA.TGCTG TGCA.TGCTG TCCAGTGAAC AATTGTTTGC TTCTTTTCTG CACCGGATAA CAGAAGCTCA TGTTTCATGG AGTACTTTGG	AATAATAAAAC AATAATAAAC AATAATAAAC TATAAACACT ATTAAACACT ATTATGTTTG GATTGACACG GATTGACAGG GATTGACAAAG TTATATATCA TAATATTAGT GAAGTTGCAT AATGACAATA GAATTACGGA AAATGTGATC ACTTTATCTT	GTTACTTATA GTTACTTCTA TTAGCA Univer CAACTGGACC CAACTGGACC TCATTTCTGA ecific RV P CTACCA GGC GTAACAGGC GTAACAGGTA ATATCGATAT TTTCCTTTAG TGCTGAGTAA AGCCCTTTTC TTTAAATGGT	AAATACTTTG AAATACTTTG ATATTCTTTA sal RV Primu AATC TATCTTTAGT rimers TAACATTTAT TAACATTTAT TAACATTATA TATAGTTGTA AACATGGTGG  CTATAGTCTC CG.CTTTTAA	CGTCATGTC CATCATTTGC CCGAAAC PT ACAGCGCTCA ACATGGGTAA CATGTA.AAA CGTGTA.AAA CGTGTA.AAA AAGGGATAAA GGATCAGATG TTTATGGACTA TTTATGGACTA CATGTATCTA CATGTACAGAA CATGTCAGAA	+200 CGCTTTATAA GGCTTTATAA GGCTTTCTAT +300 CTGAAGTGTC CTCCAG.GTG CTCCAG.GTG CTCCAG.GTG CTCCAG.GTG ATTTATAA AAAAACATAT +500 ATTTCATGTG ATTTCATGTG ATTTCATGTG ATTAGTA ATAATTCATA ATAATTCATA ATAATTCATA ATAATTCATA
ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA TTTACATTCA +301 TGTCATACGA .GCCAGTCTA +401 CATTTCC GTGCAGTACA +501 ACATAAAG TATTACAGGT +601 TATAAAGCAA  TTTCCAAAAA	* * AAAA.TATTT AAAAATACTT TCTATCCTCT CCCGGAAGCG CCCGGAAGCG GTCACAGTAT AATGACGTAC AATGACGTAC TCAGTCATAA CGATCGCGTC CGATCGCGTC CGATCGCGTC AGTAGAGCTG AGTATAATTA TGATTTCCTT	* AAGAAATTAC AAGAAATTAC GATAAATAGC .CAGCCACGC .CAGCCACGC ACAGCACGC ACAGTATCCA AGTGCCCTTG GCTGCTCCCT AATCCCCGGT .TATCTTAT.T CTGAAGATTC .TTAACTGGAA TTTAATCTTA .TTCTCTGTA	Primers ACAAACAGA ACTAAACAGA ACTAAACAGC TCATCTCAGT GAACAACCCA GAACAACCCA GAACAACCCA GATAGCTTCA GTTAGCTTCA GTTAGCTTCA AAGAGTGCTG AAGAGTGCTG AAGAGTGTAAGA AGCTTTAAAC ACCTNTTATC TTTAATTATT 	GGATACTGAA AGATGCTGAA GGCTA ATCAGCGC GTTCCATTT TGCA.TGCTG TGCA.TGCTG TCAAGTGAAC AATTGTTTGC  TTCTTTTCTG CACCGGATAA CAGAAGCTCA TGTTTCATGG  AGTACTTTGG	AATAATAAAA AATAATAAAC AATAATAAAC TATAAACACT ATTAAACACT ATTAAACACT ATTATGTTG GATTGAAAG GATTGAAAG GATTGAAAG TTATATATCA TAATATTAGT 	GTTACTTATA GTTACTTATA TTAGCA Univer CAACTGGAAC TCATTTCTGA CAACTGGAAC TCATTTCTGA CTACCA GGC GTAACAGGC GTAACAGGC GTAACAGGTA ATATCGATAT  TTTCCTTTAG TGCTGAGTAA  AGCCCTTTTC TTTAAATGGT  AAGGAGAAAT	AAATACTTTG AAATACTTTG ATATTCTTTA sal RV Primu AATC TATCTTTAGT rimers TAACATTTAT TAACATTTAT ATACAGTTAA TATAGTTGTA AACATGGTGG  CTATAGTCTC CG.CTTTTAA	CGTCATGTC CATCATTTCC CCGAAAC PT ACAGCGCTCA ACATGGGTAA CATGTA.AAA CGTGTA.AAA AGGGATAAA GGATCAGATG  TTATGGACTA TTTATGGACTA CATGTATCTA TATGTCAGAA GGGGAAAAAA	+200 CGCTTTATAA GGCTTTATAA GGCTTTCTAT +300 CTGAAGTGTC CTCCAG.GTG CTCCAG.GTG CTCCAG.GTG CTCCAG.GTG CTCCAG.GTG ATTTATAA AAAACATAT +500 ATTTCATGTG ATTGTGCTTG 4600 GTTTTAAGTA ATAATTCATA ATAATTCATA ATAATTCATA ATAATTCATA ATAATTCATA
ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA TTTACATTCA +301 TGTCATAAGA TGTCATACGA .GCCAGTCTA +401 CATTTCC GTGCAGTACA +501 ACATAAAG TATTACAGGT +601 TATAAAGCAA .TTTTCAAAAA	* * AAAA.TATTT AAAAATACTT TCTATCCTCT CCCGGAAGCG CCCGGAAGCG GTCACAGTAT AATGACGTAC AATGACGTAC TCAGTCATAA CGATCGCGTC CGATCGCGTC CGATCGCGTC AGTAGAGCTG AGTATAATTA TGATTTCCTT	* AAGAAATTAC AAGAAATTAC GATAAATAGC .CAGCCACGC .CAGCCACGC ACAGTATCCA AGTGCCCTTG GCTGCTCCCT AATCCCCGGT .TATCTTAT.T CTGAAGATTC .TTAACTGGAA TTTAATCTTA .TTTCTCTGTA	Primers ACAAACAGA ACTAAACAGA ACTAAACAGC TCATCTCAGT GAACAACCCA GAACAACCCA GAACAACCCA GTTAGCTTCA GTTAGCTTCA TATATCTTGC AAGAGTGCTG AAGAGTGCTG AAGAGTGCTG AAGAGTGCTG AAGCTTTAACA ACCTNTTATC TTTAATTATT ATAATAAAAC	CGATACTGAA AGATGCTGAA GGCTA ATCAGCGC GTTCCATTTT TGCA.TGCTG TGCA.TGCTG TCAAGTGAAC AATTGTTTGC CACCGGATAA CAGAAGCTCA TGTTTCATGG AGTACTTTGG	AATAATAAAC AATAATAAAC AATAATAAAC TATAAACACT ATTAAACACT ATTAAACACT ATTATGTTTG GATTGAAAG GATTGAAAG GATTGAAAG TAATATTATCA TAATATTAGT GAAGTTGCAT AATGACAATA GAATTACGGA AAATGTGATC 	GTTACTTATA GTTACTTATA TTAGCA Univer CAACTGGACC CCAACTGGAAC TCATTTCTGA CTATTCTGA CTACCAGGC GTAACAGGC GTAACAGGC GTAACAGGTA ATATCGATAT TTTCCTTTAG TGCTGAGTAA AGCCCTTTTC TTTAAATGGT AAGGAGAAAT	AAATACTTTG AAATACTTTG ATATTCTTTA sal RV Prime AATC TATCTTTAGT rimers TAACATTTAT TAACATATAT ATACAGTTAA TATAGTTGTA AACATGGTGG  CTATAGTCTC CG.CTTTTAA	CGTCATGTGC CATCATTTGC CCGAAAC PT ACAGCGCTCA ACAGGGGTAA CATGGATA.AAA CGTGTA.AAA AGGGATAAAA GGATCAGATG 	+200 CGCTTTATAA GGCTTTATAA GGCTTTCTAT +300 CTGAAGTGTC CTCCAG.GTG +400 CAATTTATAA AAAAACATAT +500 ATTTCATGTG 
ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA TTTTCATAAA TTTTACATTCA +301 TGTCATACGA TGTCATACGA .GCCAGTCTA +401 CATTTCC GTGCAGTACA +501 ACATAAAG TATTACAGGT +601 TATAAAGCAA  TTTTCAAAAA +701 CCGTTCCACACA	* * AAAA.TATTT AAAAATACTT TCTATCCTCT CCCGGGAAGCG GCCCGGAAGCG GTCACAGTAT AATGACGTAC AATGACGTAC TCAGTCATAA CGATCGCGTC CGATCGCGTC CGATCGCGTC AGTAGAGCTG AGTAGAGCTG AGTATAATTA TGATCTCCTT	* * AAGAAATTAC AAGAAATTAC AAGAAATTAC AAGAAATAGC .CAGCCACGC .CAGCCACGC ACAGTATCCA AGTGCCCTTG GCTGCCCCT AATCCCCGGT 	<ul> <li>Primers</li> <li>ACAAAACAGA</li> <li>ACTAAACAGA</li> <li>ACTAAACAGA</li> <li>ACTAAACAGC</li> <li>GAACAACCCA</li> <li>GAACAACCCA</li> <li>GATAGCTTCA</li> <li>GATAGCTTCA</li> <li>TATACTTGC</li> <li>AAGAGTGCTG</li> <li>AAGAGTGTAAGA</li> <li>AGCTTTAAACA</li> <li>ACCTNTTATC</li> <li>ATAATAAAAAC</li> </ul>	CGATACTGAA AGATGCTGAA GGCTA ATCAGCGC GTTCCATTTT TGCA.TGCTG TGCA.TGCTG TCAAGTGAAC AATTGTTTGC  TTCTTTTCTG CACCGGATAA CAGAAGCTCA TGTTTCATGG AGTACTTTGG	AATAATAAAC AATAATAAAC AATAATAAAC TATAAATGCGC ATTAAACACT ATTAAACACT ATTATATTG GATTGA <b>GAGG</b> GATTGA <b>GAGG</b> TTATATATCA TAATATTAGT GAAGTTGCAT AATGACAATA GAATTACGGA AAATGTGATC ACTTTATCTT +760	GTTACTTATA GTTACTTCTA TTAGCA Univer CAACTGGAC CAACTGGAC TCATTTCTGA CIFIC RV P CTACA.GGC GTAACAGGC GTAACAGTTA ATATCGATAT  TTTCCTTTAG TGCTGAGTAA AGCCCTTTTC TTTAAATGGT AAGGAGAAAT	AAATACTTTG AAATACTTTG ATATTCTTTA sal RV Prime AATC AATC TATCTTTAGT TIMETS TAACATTTAT TAACATTAT ATACAGTTAA TATAGTTGTA AACATGGTGG CTATAGTCTC CG.CTTTTAA CAACCTGTAG	CGTCATGTC CATCATTTGC CCGAAAC PT ACAGCGCTCA ACAGCGCTCA ACATGGGTAA CATGTA.AAA CCTGTA.AAA AAGGGATAAA GGATCAGATG  TTATGGCACTA TTTATAGGTG CATGTATCTA TATGTCAGAA GGGGAAAAAAA	+200 CGCTTTATAA GGCTTTATAA GGCTTTCTAT +300 CTGAAGTGTC CTCCAG.GTG CTCCAG.GTG CTCCAG.GTG CTCCAG.GTG ATTTATAA AAAACATAT +500 ATTTCATGTG ATTGTGCTTG +600 GTTTTAAGTA ATAATTCATA ATAATTCATA ATAATTCATA ATAATTCATA
ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP	+101 CATCACTACA CATTACTACA TTTCATACA TTTCATACA TTTCATAAA TTTTCATAAA TTTTCATACA +301 TGTCATACGA TGTCATACGA .GCCAGTCTA +401 CATTTCC GTGCAGTACA +501 ACATAAAG TATTACAGGT +601 TATAAAGCAA TTTTCAAAAA +701 GCGTTCGACA	* * AAAA.TATTT AAAAATACTT TCTATCCTCT CCCGGAAGCG GCCCGGAAGCG GTCACAGTAT AATGACGTAC AATGACGTAC TCAGTCATAA CGATCGCGTC CGATCGCGTC CGATCGCGTC AGTAGAGCTG AGTATAATTA TGATTTCCTT TGAAGCTCAG	xpecific FI AAGAAATTAC AAGAAATTAC GATAAATAGC .CAGCCACGC .CAGCCACGC ACAGTATCCA AGTGCCCTTG GCTGCCCTTG GCTGCCCCTG AATCCCCGGT .TATCTTAT.T CTGAAGATTC TTAACTGGAA TTTAATCTTA TTTCTCTGTA CTGCAG	V Primers ACAAACAGA ACTAAACAGC TCATCTCAGT GAACAACCCA GAACAACCCA GATAGCTTCA GTTAGCTTCA GTTAGCTTCA TATATCTTGC AAGAGTGCTG AAGAGTGCTG AAGAGTGCTG AAGAGTGCTG AAGAGTGCTG AAGAGTGTAAGA ACCTNTTATC TTTAATTATT ATAATAAAAC	CGATACTGAA AGATGCTGAA GGCTA ATCAGCGC GTTCCATTTT TGCA.TGCTG TGCA.TGCTG TGCA.TGCTG TCAAGTGAAC AATTGTTTGC .TTCTTTTCTG CACCGGATAA CAGAAGCTCA TGTTTCATGG AGTACTTTGG	AATAATAAAC AATAATAAAC AATAATAAAC TATAAACACT ATTAAACACT ATTAAACACT ATTATATTG GATTGACACG GATTGACACG GATTGACAACA TAATATTAGT GAAGTTGCAT AATGACAATA GAATTACGGA AAATGTGATC ACTTTATCTT +760	GTTACTTATA GTTACTTCTA TTAGCA Univer CAACTGGACC CCAACTGGACC TCATTTCTGA CIFIC RV P CTAGCA GGC GTAACAGGC GTAACAGGTA ATATCGATAT TTTCCTTTAG TGCTGAGTAA AGCCCTTTTC TTTAAATGGT 	AAATACTTTG AAATACTTTG ATATTCTTTA sal RV Prime AATC AATC TATCTTTAGT TIMETS TAACATTTAT ATACATTTAT ATACAGTTAA TATAGTTGTA AACATGGTGG CTATAGTCTC CG.CTTTTAA	CGTCATGTC CATCATTTGC CCGAAAC PT ACAGCGCTCA ACAGCGCTCA ACATGGGTAA CATGTA.AAA CGTGTA.AAA AGGGATCAGATG  TTATGGACTA TTTATAGGTG CATGTATCTA TATGTCAGAA GGGGAAAAAA	+200 CGCTTTATAA GGCTTTATAA GGCTTTCTAT +300 CTGAAGTGTC CTCCAG.GTG CTCCAG.GTG CTCCAG.GTG CTCCAG.GTG CATTTATAA AAAAACATAT +500 ATTTCATGTG ATTGTGCTTG GTTTTAAGTA ATAATTCATA ATAATTCATA ATAATTCATA ACCCGTACCC

Fig. 3. Alignment of zebrafish recombinant DNA sequences and a *Xenopus* DNA sequence encoding the  $tRNA^{[Ser]Sec}$  gene. The encoding sequences of the  $tRNA^{[Ser]Sec}$  genes of zebrafish and *Xenopus* (boxed region) and the flanking sequences of ZStR1, ZStR2 and *Xenopus* are shown. The sequences from *Xenopus* were taken from Lee et al. [19]. Base differences within the encoding sequences are indicated with an asterisk. TATA boxes at -30 in ZStR1 and ZStR2 and at -31 in XtRSP, the PSE at -66 in ZStR1 and ZStR2 and at -64 in XStR and the AE at -205 in ZStR1 and ZStR2 and at -209 in XStR are underlined. Base differences in the flanking sequences of ZStR1 and ZStR2 are shaded. To maximize homology between sequences, dots were used to indicate gaps with the exception of the 3'- and 5'-ends of sequences where dots indicate unidentified bases. Specific and universal primers used for PCR analysis of the radiation hybrid panel are shown in bold. GenBank accession numbers for ZStR1 and ZStR2 are AF135236 and AF135237, respectively.

column and Sec tRNA<sup>[Ser]Sec</sup> identified in the eluted fractions by Northern hybridization (see Fig. 4A). Two peaks were observed that were pooled as shown in the figure, amino-acylated with [<sup>3</sup>H]serine and refractionated over the column (Fig. 4B and C, respectively). Most of the serine tRNA<sup>Ser</sup> eluted with the earlier eluting peak of tRNA<sup>[Ser]Sec</sup>. Hence, the amount of [<sup>3</sup>H]seryl-tRNA<sup>[Ser]Sec</sup> relative to [<sup>3</sup>H]seryl-tRNA<sup>Ser</sup> is much less in the earlier eluting Sec tRNA<sup>[Ser]Sec</sup> isoacceptor than the latter one. Both seryl-tRNA<sup>[Ser]Sec</sup> isoacceptors decode UGA in a ribosomal binding assay.

18



Fig. 4. Fractionation and coding responses of Sec tRNA<sup>[Ser]Sec</sup>. In A, total tRNA was loaded onto a RPC-5 column, the column was washed and tRNA fractionated in a linear 0.50–0.70 M NaCl gradient, aliquots of every other fraction were blotted on a filter and the filter was hybridized with probe (see Section 2). Fractions were pooled as shown by the hatched areas in A, amino-acylated with [<sup>3</sup>H]serine and the resulting [<sup>3</sup>H]seryl-tRNAs were fractionated on the column in a linear 0.6–0.825 M NaCl gradient without  $Mg^{2+}$  (see Section 2). In B, peak I from graph A is shown and in C, peak II from graph A is shown. The hatched peak in B and that in C were pooled, prepared for encoding studies and encoding studies were carried out as given in Section 2. The total number of cpm added to assays shown in B was 4500 and that shown in C was 8430. None designates the amount of binding of [<sup>3</sup>H]seryl-tRNA<sup>[Ser]Sec</sup> to ribosomes in the absence of trinucleoside diphosphate. Codon UGA was a gift of M.W. Nirenberg.

#### 4. Discussion

Zebrafish contain two distinct tRNA<sup>[Ser]Sec</sup> genes. Both have a TATA box, a PSE and an AE in the 5'-flanking region which are the three regulatory elements [16] that govern expression of all other eukaryotic Sec tRNA<sup>[Ser]Sec</sup> genes examined to date [8]. Both genes are transcribed following their microinjection into *Xenopus* oocytes, but the level of transcription was quite low (data not shown) and comparable to that observed for the chicken tRNA<sup>[Ser]Sec</sup> gene in this transcription system [17]. The genes map at different loci within the zebrafish genome, but at present, we cannot determine whether they are localized on the same or different chromosomes.

Two Sec tRNAs<sup>[Ser]Sec</sup> were found in the total tRNA population. The two isoacceptors most certainly differ by base modification as the primary transcripts of both genes would have identical sequences. The isoacceptor that elutes first from the RPC-5 column may likely lack a  $N^6$ -isopentenyladenosine (i<sup>6</sup>A) modification at position 37. This modification is characteristic for Sec tRNAs<sup>[Ser]Sec</sup> isolated from other animals [8] and the absence of i<sup>6</sup>A causes tRNA<sup>[Ser]Sec</sup> to elute much earlier from the RPC-5 column [18] as was observed in the elution of the initial peak of zebrafish tRNA<sup>[Ser]Sec</sup> (see Fig. 4A).

The present studies demonstrate for the first time that the Sec  $tRNA^{[Ser]Sec}$  gene occurs in two copies in the genome of an animal even though gene duplication appears to be a frequent occurrence in zebrafish [9–11]. The present study provides an

initial characterization of the machinery involved in the selenoprotein biosynthesis in zebrafish and provides a foundation on which to expand the use of this organism as a model for exploring mechanisms of selenium-mediated chemoprevention in cancer.

*Acknowledgements:* The authors thank Dr Robert Geilser for analyzing the data of the retention pattern of the radiation hybrid panel, Gopal Sarngadharan for technical assistance and Dr A.M. Diamond for helpful suggestions in writing the manuscript. This work was supported in part by the Molecular Medicine Research Group Program, MOST, Korea (98-MM-02-A-03) to BJL.

#### References

- [1] Zon, L.I. (1999) Genome Res. 9, 99-100.
- [2] Cheng, R., Bradford, S., Barnes, D., Williams, D., Hendricks, J. and Bailey, G. (1997) Mol. Mar. Biol. Biotech. 6, 40–47.
- [3] Marcos-Gutierrez, C., Wilson, S., Holder, N. and Pachnis, H. (1997) Oncogene 14, 879–889.
- [4] Cheng, R., Ford, B.L., O'Neal, P.E., Mathews, C.Z., Bradford, C., Thongtan, T., Barnes, D., Hendricks, J. and Bailey, G. (1997) Mol. Mar. Biol. Biotech. 6, 88–97.
- [5] Gladyshev, V.N., Factor, V.M., Housseau, F. and Hatfield, D.L. (1998) Biochem. Biophys. Res. Commun. 251, 488–493.
- [6] Gladyshev, V.N. and Hatfield, D.L. (1999) J. Biomed. Sci. (in press).
- [7] Hatfield, D., Choi, I., Ohama, T., Jung, J., Diamond, A. (1994) in: Selenium in Biology and Human Health (Burk, R.F., Ed.), pp. 25–44, Springer-Verlag, Berlin.
- [8] Hatfield, D.L., Gladyshev, V.N., Park, J., Park, S.I., Chittum, H.S., Baek, H.J., Carlson, B.A., Yang, E.S., Moustafa, M.E.,

Lee, B. (1999) in: Comprehensive Natural Products Chemistry (Kelly, J.W., Ed.), pp. 353–380, Elsevier Science, Oxford.

- [9] Amores, A., Force, A., Yan, Y.L., Joly, L., Amemiya, C., Fritz, A., Ho, R.K., Langeland, J., Prince, V. and Wang, Y.L. et al. (1998) Science 282, 1711–1714.
- [10] Nornes, S., Clarkson, M., Mikkola, I., Pedersen, M., Bardsley, A., Martinez, J.P., Krauss, S. and Johansen, T. (1998) Mech. Dev. 77, 185–196.
- [11] Postlethwait, J.H., Yan, Y.L., Gates, M.A., Horne, S., Amores, A., Brownlie, A., Donovan, A., Egan, E.S., Force, A. and Gong, Z. et al. (1998) Nat. Genet. 18, 345–349.
- [12] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Cold Spring Harbor Laboratory Press, New York.

- [13] McBride, O.W., Rajagopalan, M. and Hatfield, D. (1987) J. Biol. Chem. 262, 11163–11166.
- [14] Hatfield, D., Matthews, C.R. and Rice, M. (1979) Biochim. Biophys. Acta 564, 414–423.
- [15] Nirenberg, M. and Leder, P. (1964) Science 145, 1399-1407.
- [16] Carbon, P. and Krol, A. (1991) EMBO J. 10, 599-606.
- [17] Lee, B.J., Kang, S.G. and Hatfield, D. (1989) pp. 9696–9702.
  [18] Choi, I.S., Diamond, A.M., Crain, P.F., Kolker, J.D., McClos-
- key, J.A. and Hatfield, D.L. (1994) Biochemistry 33, 601–605.
  [19] Lee, B.J., Rajagopolan, M., Kim, Y.S., you, K.H., Jacobson, K.B. and Hatfield, D. (1990) Mol. Cell. Biol. 10, 1940–1949.