

Organism	ZRS Forward (5') Primer	ZRS Reverse (3') Primer
<i>M. musculus</i>	AGTTCAAGAAACCGCACACC	GACAGCAACATCCTGACCAA
<i>A. carolinensis</i>	GGCAGAGACAGACGTGATGA	TGGCATGCTTTTCCTAGCTT
<i>L. menadoensis</i>	CAGCAAAGACCCCCAAATAG	GGAAAAAGACATTTTGTAGTGTCTCC
<i>O. latipes</i>	ACTTTCTTCTGGTGTGCGTC	TCACCGCAGAAAGCTCATTA
<i>D. rerio</i>	AACACGGGACAATTTTGAGC	TTTCTGCTGCTGCATACAAAT
<i>L. oculatus</i>	GGTGCTTTCCTCATCTCTGC	AGTGAAACAGGTGCCGTCTT
<i>L. erinacea</i>	ATGGATCATCAATGGGGAGA	TTTGAGTTGACCATGCAAGC
<i>P. marinus</i> (1)	GTGATGTTGGTTGTCGTTGTG	CTAAGTCACGTGGGAAAGCTG
<i>P. marinus</i> (2)	ACTTGGTCTTCTTGTGCTCCA	CGAGGTCAAGTAGTGCCCTCAC
<i>B. lanceolatum</i>	ATCGTGCTGAGGAAGGTGAC	TGTGCCTCTACGCATTCAAG

	sZRS Forward (5') Primer	sZRS Reverse (3') Primer
<i>D. rerio</i>	TTTTCATCAGCCTTCTGAACC	CAAACCTGCAACCAACACTG
<i>H. sapiens</i>	TATGGTTTGCCTGTGTCCCC	CCCCTTGGCCTCCTAAAGTG
<i>O. latipes</i>	AGACAAGGTCGCTAGAACTGC	GTGTTTCAGCAGCACATCCAG

	Other regions tested	
	Forward (5') Primer	Reverse (3') Primer
<i>O. latipes</i>	TGTTTTACCCGCAGATGCAG	TCACATTTCGCAGCGATTGG
<i>O. latipes</i>	GGACATCACAGGTAGGGCTG	ATGCTGTACGCCTGTACACC

	4C-seq primers	
	Enzyme: DpnII	Enzyme: Csp6I
<i>M. musculus</i>	<i>Shh</i> CCAGTCTGGGTGGGGATC	<i>Shh</i> CCTTATTTACACCCCATCCCC
<i>O. latipes</i>	<i>Shh</i> CATCAAGACTTCAGGACTGGATC	<i>Shh</i> TTTTGCCTTCGTATCTGCCGC
<i>O. latipes</i>	ZRS CTGGGAAAGGAGAGGAGGATC	ZRS ACAATCCTTGAAACGGCGAG

	CRISPR target sites	
<i>O. latipes</i> Δ401	GGTGAGTAATCAATTAAACG AGG	GTCCAGCTGTATTACCATGC AGG
<i>O. latipes</i> Δ948	GGCTTCAACATTAGCCAG AGG	AGCTGTATTACCATGCAGG AGG
<i>O. latipes</i> Δ3.4kb	GTCCAGCTGTATTACCATGC AGG	TGGCCTCTAACGAGCCACAC GGG

	Deletion screening primers	
	Forward (5') Primer	Reverse (3') Primer
<i>O. latipes</i> Δ401/Δ948	ATTTTAAAAAGCATCCACATTTCC3	AAATGAAGTGTCTGGGTGTATCCT3
<i>O. latipes</i> Δ3.4kb	CACATCATCTGTGATTGGAATTTTA	TTAAAAGCATGAATGAGTGTGAAAC

	Lamprey cloning primers	
	Forward (5') Primer	Reverse (3') Primer
<i>L. camtschaticum</i> MyHC1	ACGTGCAGGGACAGATCAAG	TCTCCAGCCTTGACAACACC
<i>L. camtschaticum</i> ColA	CGATTCTACCGTGGCGATCA	CCGAACTCCTGATCCACACC

Supplementary Table 1

Oligos used for PCR amplification of the ZRS and sZRS enhancer from different species. Also listed are primers used for the 4C-seq analyses, genomic deletion target sites (PAM sequence in bold), genomic deletions screening and lamprey cloning.