Appendix

Table 1. Sequences of indel primers used in the positional cloning of the *sne* mutant

Indel primer name	Sequence: Forward primer	Reverse primer
20.83	ATGGACGCAGTGGAGAAAAC	CAGCAGAAGCCCCTAATTCA
20.87	TGCTACGCCACTGCATAATC	GTGGCGACCCCTGATAAAT
20.97	TTTTCTCTGTGGCGATGACA	CCACTGATCAGGAAAATCTGC
21.01	TGCTACGCCACTGCATAATC	GTGGCGACCCCTGATAAAT
21.161	CAATTCGGTAATGGTTGTAGGC	TTTTGTTTTCGTATTGCATCTC
21.163	CACAAACTTTGGGATATTCAAGC	AAATCAAAGAGCATCAAACAAAA
21.18	TGAAGGCATTAAAATAAAAGACG	CCCAGGCAGGATGCTAAGT
21.196	TTTGAGTGAAAACTCTAATGAGCA	TTGGTGAGTTATGACAGGTTGTG
21.2	AGCAGGCATGCATGTTACAG	TTCAGCAACCTCACAAGTCA
21.22a	TGATCTATCCGCCCGTTAAG	CTGGCCATGGAATATTAGGC
21.22b	GGCTTGGACCTGAAAACAAG	ATGTCCTTCCATCTGCAACC
21.25	GGCATTAAAATAAAAGACGCACA	CCCAGGCAGGATGCTAAGTA
21.38	CCCATTCATCTCTGCATGTC	TGCAGAGTAAATCACACTCAATCA
21.46	GGCATAAAACACTGGTAAAAGCA	CAAATTTCTGGCCAAAAGAAA
21.63	TGGAATTATTCTGTATGGATGCAA	AATGGCTACATGCTGAACCAG
21.716	CAAGCCATCACATAGTCAAAAAG	TGACTTCTCATTCTTTTCTTGTTTT
21.76	CACGCAACACAAATAATTACCA	CTTCATTCGCCTGAGCAAA
21.762	TACCTCCCATTCGCTTCATT	GCCAGTCCGTTCTGTTATGG
21.765	AACAATGATGATGATAATGGTGATAA	TATTTCACACGCCTTCAACA
21.766	AGACTCCTTCGGCATAGCAA	TCGTTTCGGTTGAATTGACA
21.768	AGCAATGGCGGTAATCTGAC	CTGACCCAGGACCACAAAAT
21.77	GCTGTAAAGCAGCAGAAATGG	GGCTTTTTGTGAGGGAATGA
21.78	TTTGTAACATATCCGTCTCTCAAG	TGGAAAACAAATGCAAATGAA
21.79	CAGCATGCAAGAAGAGGAAA	TCCAGTGAATCTGTGTTTTATGC
21.86	TCCACCTTCTCTTCCGACAT	TTTGTTTCTGTCTTGCCTGAAC
22.09	TGCATGTGAAAACAAGATAATCA	CAATTTACCACAATAACCATCAAAA
22.16	TTTACACAGCGGATGCCTTT	GTCCAGCTGGGGTGAATATG
22.21	TGTTCAGCTAACAAAAAGTGGTG	TGTGATATTGCATGGCCCTA
22.5	GAAAAAGCTGCTCACCAACC	TCCAACCCTAATCAAACATGC
22.54	TGAAAGGAAGGAAGGACCAA	TCCTTTCATTTGAACGCTTCT
22.55	TGGATGGATGGATTAATAGATGG	TTAAAAATAGTGAGTGAAAAACTGACA
23	TGTGTTGATAGCAAGGCCAAT	GCATTTCAAAGCCTCATGAAT
23.17	GCAATGTGACCCAAACAGGT	GGTGTGAGGAATTGCAGGTT
23.3	TGGGCAGTTAAAATGTAAAGCA	ATCGGCCAGACGTAAAGAGA
23.34	TCTGTCAAAAGGACGTAGGC	AACGCCCTTTCAGCAATAGA
23.49	TGCCTTTTTATTTTTGGAAGGA	TTTTTGTGGCTGAATGATTTACA
24.11	CACACACACGAACCTAAGCA	AACTCCAGCAGAAGCACCAG
24.28	TGAAATTTTGTCAATTTCCACAT	AGGAAGTGCATCCACCAAAC
24.52	TGGGTTCACATCTTGAAATTCT	CAATTTGTTATTCTCAAGTCAAAATTC
25.01	TTCAGACTTCAACTGTATGTGTGTATG	GCGTTTTTAGACTCTGTGTTTGA
25.16	TCCGATATAGAGGGGGTTCA	ATGCTCTCAACTGGCTGGAT
25.67	AAAAGATTTGGGAGGGGTGT	CAGCCTTGAATAAATCTATTGTTGA
26.58	ACCGCGTCAGCTAAACTCTC	ACCAAACGAAAAAGCAATGG
27.36	AATGTAGCCGTGAAGGGATG	AGGGGCATCTTTTGAATGGT
28.14	TCAGAAGCAATCCATATGACAAA	CTGCAACAGCCAAATTTCAG
28.18	GGATGAGCATGCACAGAATG	TGCTTCACTGCTGTCTCTTG
28.37	ATTGTCGGTTTTGGGTGAAC	TGAACTGGGATGCAGAATGA
28.49	GCTGTCCTTTGCGAGTTTGT	CCGAGGGGGAAAACACTACT
28.68	TCATGCAACGTTACAGCAAA	CACACAAGTGCACTGTTAAATCT
28.73	GCACTGCTGTGTTCAGGATCT	TGAAGGGCAGGACAGAAAGT
28.98	TGATCTGATGAGGTCTGAAAACA	GCTGGAGGAAGTGTCTGGAG
29.15	TGACACAACTGGTTTTGCTTG	TAGGCTTTGAGCCAGCACTT
29.32	GAGAAGGTTGCTGGTTCGAG	ATGGATGCCCTTTCAGTCAG
29.51	TTGTGCTGTTTTCTCCACCA	TGGCTAATGCCTGTCTATGC
29.64	ATCCCAGGCTCTGTGTGAAT	GTGAAGAAAGCAGCTCACCA

Indel primer name	Sequence: Forward primer	Reverse primer
bx3.1	TCGCACAGGAATAGAATGAGC	AGACCTCCAGAGAGTTTAGAGACA
bx3.2	TCCAATCAGGAAGGGGTTATT	GCAGTGTTTGTGTTGTCAGGA
bx3.3	TTGTGATGGTATGATAACCTTGG	CAAGAGCAGAAAAGATATCCAAAGA
bx3.4	TTATCGTCCCATACCTAAGTGC	AATGGTGACAATTTGTATAAAGCA
bx3.5	CACAACATTTAAAGTTAATTCACACCA	TAAGGAAACCCCTGCCTGAT
bx3.6	ACCTGGCCTTCAGCAGAG	GATTTCCAGCAGCACAGTGA
bx3.7	TGAACTGTGCCTCAGTCGAG	TGGTGACCTCTGAAACAGAGAC
bx3.8	GCAATGACATGACCACATTGA	TGAACCTTAAAATAATGTGTGACCA
bx3.9	AAAAGCAGCCAATATCTCAAAAA	CCATTATGTTTATAACAAATTGCATTA
bx3.10	ACAGTCTGCAAGGTCCGATT	TTGGTGGTTTTCAGGGACTC
bx3.11	TGCATTCGATTAAGCAGCAG	GAGGGGCTAATAATTCAGACTTCA
bx3.12	AAGCTGTGTAGAATTGTCTTGAAAAA	AAAACAGGGGTGTCCAAACTT
bx3.13	TTGAGCTGAATTGGTTGCTG	CGTGCATCCATTGGAAATAA
bx3.14	ACAGAACACCCCAAAACTGC	TGACTACTTTTGGAAAATTGTAATTGA
bx3.15	CCGAAATTGGGAAGTGACAT	TTTGTTATGGTGAAGCTGCATT
bx3.16	CACTGATTTACAAACACCACAAGA	TTGTGTCGTGACAGTGCAGA
bx3.17	TCCCAAAAATCACATTTTCCA	TCTCTGTCCATCCGTCCTGT
bx3.18	GGACAGAGACAAACACACTGGAT	CCAACCATTCAACCATCAAC
bx3.19	GATGGTTGGATGGTCCAGTT	CCATCCATCCCTTTTTACCA
bx3.20	ATGGATGAAATGATGCATGG	TCTATCTGTCTACCTAGTGTGTCTGTC
cr38.1	GTTGAACACACGTCCACCAC	TTGTGAAAATTTCTTTGCTCTGTT
cr38.2	CGCTTTTATCCAAAGCGACT	CCTAATCTGCAATTGCCTCTC
cr38.3	TCTGTCTGAGCCAGCCTTCT	TTGCATACAAACCATTTTCCA
cr38.4	AGCAGTTTTCGGAGCCTACC	CTGGCAAAATCTCAGCCAAT

Table2. Sequences of primers used in amplification of *capzα1*and *capzβ* genomic and cDNA. The primer sequences used to amplify the actin control in the RT-PCR are also provided.

	Primer	Forward 5′′-3′	Reverse 5′-3′
capzα1	2	CAGCCAAGATGACCGACTTT	GGCTCAAACTTATGACCCTCA
	4	AGCATGGAGATCTGGGTCAG	AGGTTGAATGGAATCGCATC
	Fact3	TGTGCTCATTTTCCATTGATTT	AGGAAGAGGAAGTGGGTCGT
	Fact2	TGTAAAACGACGGCCAGTTGGT CAATCATTTCAATAACACC	AGGAAACAGCTATGACCATGTCG ATCTTGGTACGGGTGA
capzβ	capZb1 (RT-PCR)	CAGAATGAGCAGCAGTTGGA	GTGTGACCTGTCGGGTGAG
	capZb3 (genomic)	GCTCTGGAACCATGAACCTT	GAAAGAGAGACGGGTGGTGA
control	actin	GATCTTCACTCCCCTTGTTC	ACAGAGCAGAAGCCATGCTG

Table 3. Sequences of primers used to generate the template for the *capzα1, capzα2* and *capzβ in situ* probes.

Primer	Forward 5′-3′	Reverse 5′-3′
capZα1	TCTCCCCAAAAATGAAATTAAGA	TTATTCTGCCTTTTTGGTTATTAAA
capZα2	ATGAGGATGGAAACGTGCAG	AAAGGAGGCAGTCTCTTCAGC
capZβ	CAGAATGAGCAGCAGTTGGA	GTGTGACCTGTCGGGTGAG

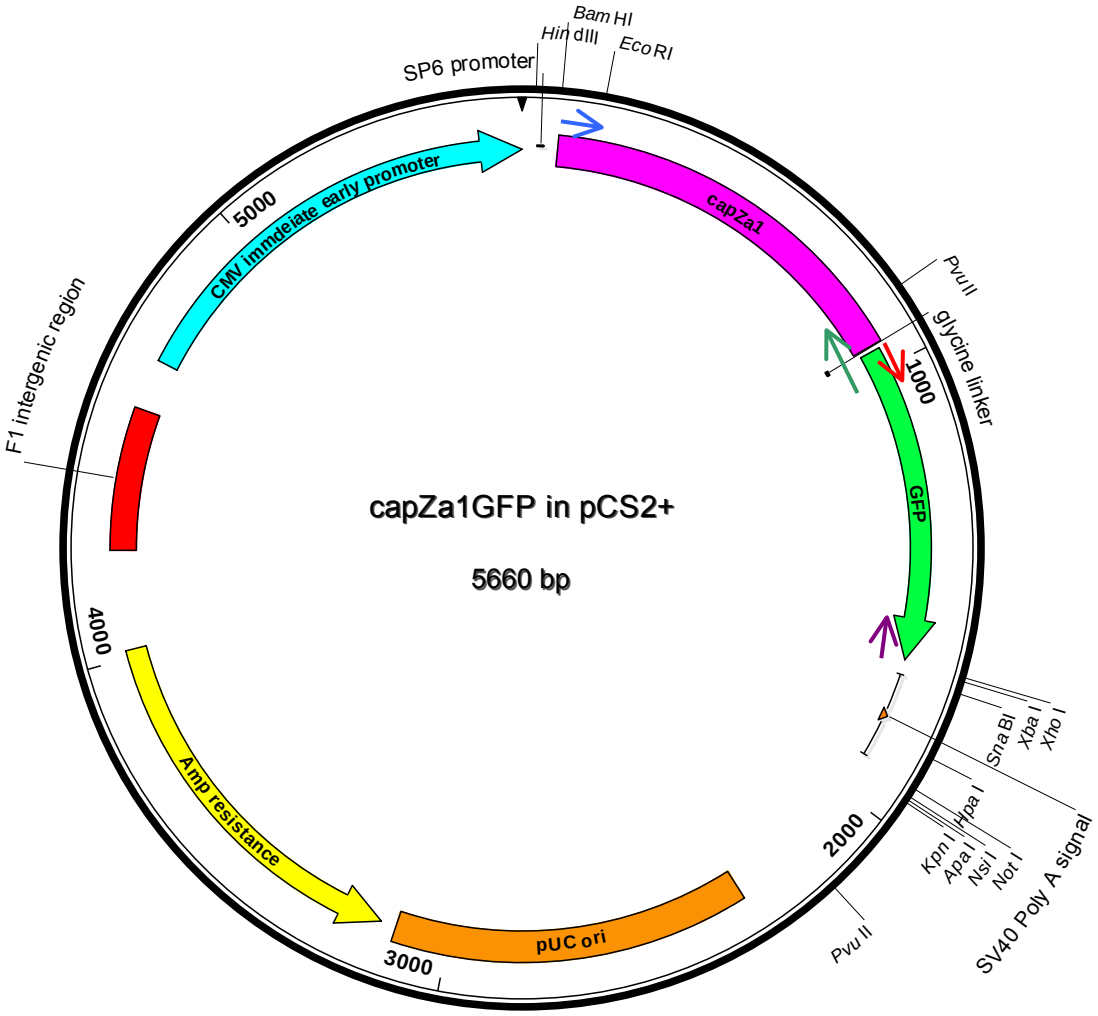

Fig 1. Plasmid map of capZα1-GFP pCS2+. The position of the capZORFF, GFPR1, GFPF2 and GFPR2 primers are represented by blue, red, green and purple arrows respectively.

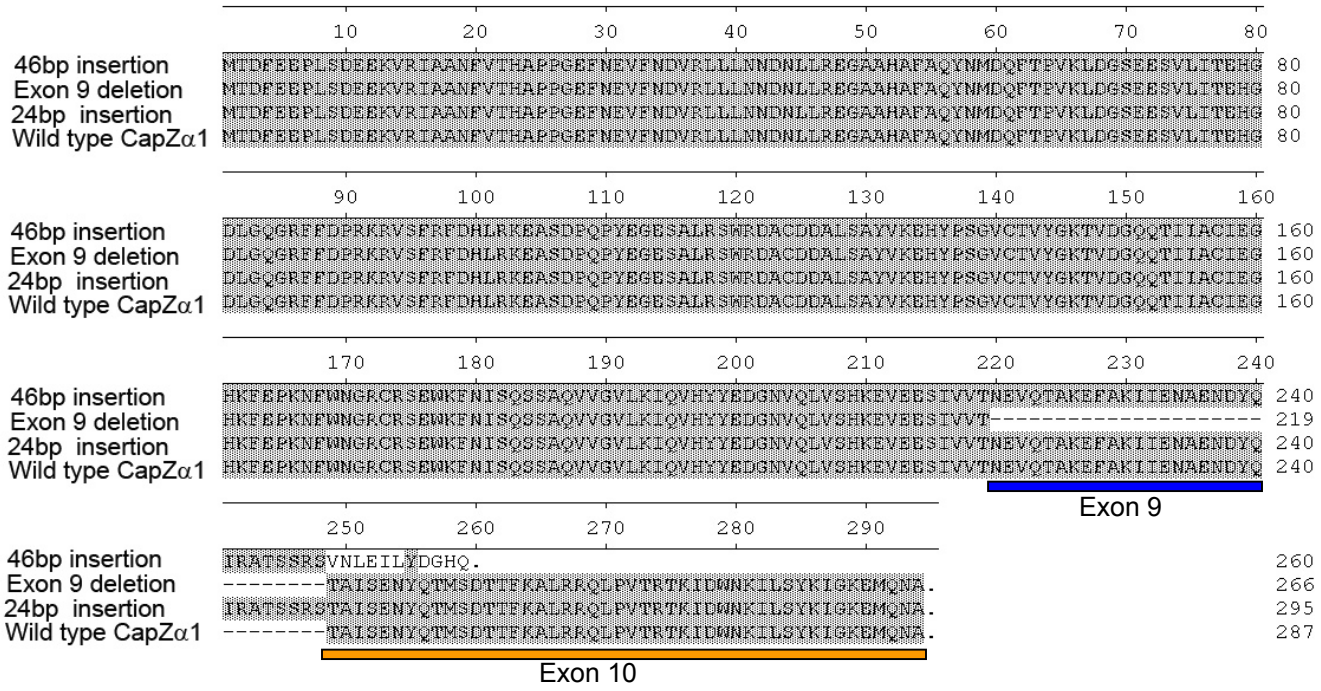

Fig. 2. Comparison of predicted CapZα1 *sne* mutant protein products with wild type CapZα1. The regions encoded by exon 9 and 10 are underlined in blue and orange respectively. The 46bp insertion of intron 9 produces a frame shift and exon 10 is not translated. The exon 9 deletion produces an in frame product where exon 10 is still translated. The 24bp insertion of intron 9 also produces an in frame product where exon 10 is translated, however, an additional eight amino acids are translated between exon 9 and 10.

Table 4. Number of experiments performed for all MOs used in this thesis. For each experiment approximately 50 embryos were injected. If over 90% of the embryos per MO experiment displayed similar phenotypes they were not scored on the basis of individual phenotype. All MOs were titrated to determine their optimum concentration prior to use.

Morpholino	Amount injected per embryo	Number of experiments performed
capZα1 ATG	5ng	4
capZα1 splice 1	4ng	3
capZα1 splice 2	10ng	5
capZα2 ATG	1.5ng	2
capZα2 splice	6ng	1
capZβ ATG	6ng	2*
capZβ splice	3ng	3*
Desmin ATG	3ng	2
Desmin splice	8ng	3
SK Tmod 4 and capZα1splice 2	3ng and 10ng	2
capZα1splice 2 injected into *buf* mutants	10ng	2
SK Tmod 4 injected into *buf* mutants	3ng	2

* Variable phenotypes were observed with these MOs. See appendix, Table 5 for specific descriptions

Table 5. List of phenotypes observed from capZβ ATG and splice MOs. All phenotypes were observed at 5dpf apart from capZβ splice 3ng on the 22/09/07 where phenotypes were observed at 48hpf.

	capZβ ATG 6ng 10/05/07	*capZβ ATG 6ng 20/05/07*	*capZβ splice 3ng (48 hpf) 22/09/06*	*capZβ splice 3ng 24/04/07*	*capZβ splice 3ng 20/05/07*
Severely truncated, heart edema and small brain	3	10	40		11
Truncated, curved axis, no heart edema		8		20	8
Straight axis, no swim bladder, reduced motility		1			
Straight axis, no swim bladder, frayed caudal fin, reduced motility		20			
Straight axis, no swim bladder, no heart edema, swim normally	32 (did not check for fin phenotype)			20	20 (12 of these look thinner than wild type)