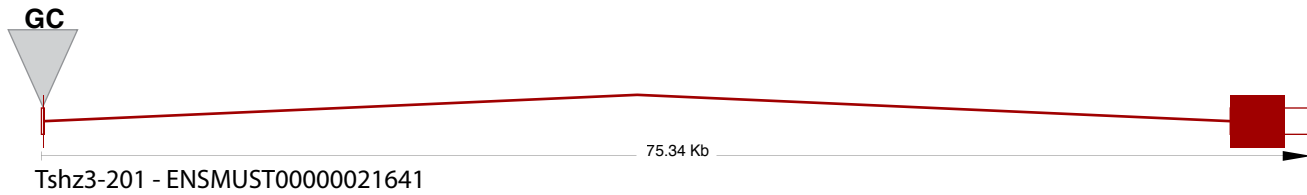


# Tshz3-GC Construct Overview

Created 8 June 2011  
Updated 22 July 2011

## Gene Overview



## Target site in cDNA

cDNA fpr Tshz3-201 (partial)

Transcript length: 5,002 bps Translation length: 1,081 residues

```

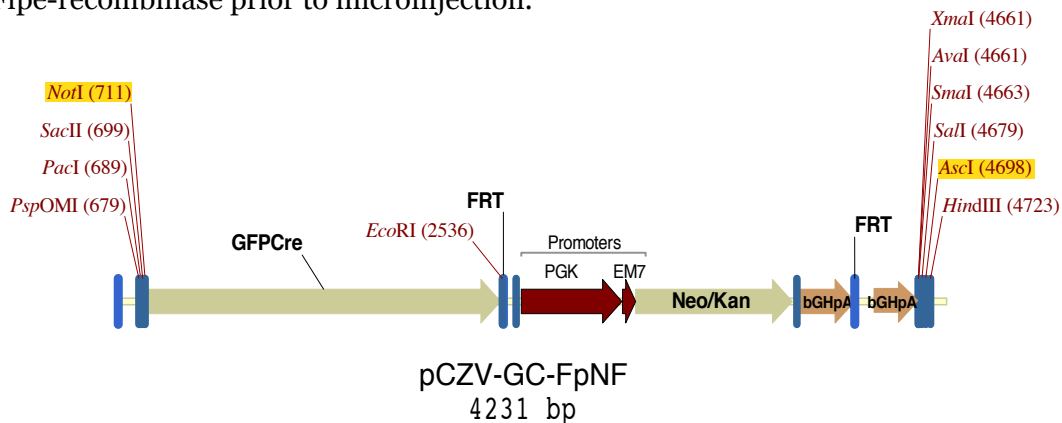
1  CCGGCCCTCTCCGGGGGAGGCTTCGAGCAGGCGCGCTGGCCGCCGCCCGCGCC
61  TTCCCTCCCTCCGGTCAAGCCGACGCGGGGGGGGGGGGGGGGGGGGGGGGGGGGG
121  GGGAGAAGCATATCCGAGGAGGAGGAGCAGCAGGCGCCCGCCGCGCAGCGCCTATGTT
181  TCCGATGAGTTAAAGGACGCTGGCTTGGTAGAAGATGACGTAGAGCCGAGGAAACAAGG
241  GCAGATGGAGACCCCTCAGCCAAGTACATGTCTCAGAGAAGAACTCAGCAAGCCCTGC
301  CCTAGCTACCAGAACTCACCAGCCGCTGAGTTCTCCAGCCATGAATGCACGCGAGTCT
361  CACTCAAGTGAAGCAGGTGACGGAGTGGCTACTTTGAAAGCAGTTCCTCAGAAATGAG
421  GAGGAGATCAGAGAGGCTCAGGGTCCCTCGAGGAGCAGCCAGTGTCTGATAGCTGGAG
481  CAGATGAAGCCCTGACAACTCCTCTGCAATTCCTATTCGTCAGCCCACTCAACCTG
541  AACCTGCACCAGCCCTCCTCCAGAACAAATGTTGGCAGCAGTAGCAGGACAGCAGTAGC
601  AGCAGCAGCTGTGGCAGCGGGAGCTTTGACTGGCACCAGAGTGCATGGCCGAGACCTGC
661  CAGCAGGTCTCCAGAACGAAATGCTGCGGAGCCAGGCTGTTCAGAGCCCTGCAGCTG
721  TACCCGAGAGCAGCTAGTCTAGGGTCCATTTCCACGGCCGAGAAAGTCCCTGTGG
781  AAAGACTGTAGGCCCTTACGACACCTCTGTAGAGCTGACCGTGCACATGAATGAGACA
841  GGTCATATCGGATGACAAACAGGAGCAGACAAACAACCCCAAGGCGCTGCCAAG
901  CCTCGCAACCGCTTTGTTGGAGATGGAGGGGAGGAGGATGCTCAGAAGGTGCTCAAG
961  TGTATGACTGTGGCCTCTTTGAGTCCCTCCAAAGCTTGAGCGTGCATATGATCAA
1021  ACTAAACACTACCAAAGTGGCTTCAAGGAAGCTCTTCAGACCCCTTGCAGCCAAATTC
1081  ATCCCTCTCTCCGGAAACCTCTCTGAGGTGAGCCCTCTAGTCTCCCTGATTCG
1141  ACAGGTGGAACCCCAAGCCACCTGCTGATGCCAGCAGCCCTGCAGAAAGACTCC
1201  AACCTTACATCAGCCAAATATCGTAGGGCCACCAAGAGGGGCGAGCTATGATGG
1261  CACTTCGAGGCCGAAAGTCTCAGATCCTCAAATGGATGGAGTGGAGCTCCCATGAC
1321  ACGTCCAGGAGCTCAGAGCTCAGATGATGTTCACTGGCCACTTTATGAGGTCAACAA
1381  TCGGCCATGAAAGGGGAGGAGCCATCATGAGACACCGGTGACGCCACCTCACCACC
1441  TTCTGGACGAGAAGTGCAGTGTGCTGCTGCGCTGACACCCACCACTTACGCTCCCTG
1501  AATAGCCCGCCGAGTGTCTCCCGAAGCTGGCGTGGAGATCAAAGAAAGTGGACRAAG
1561  GAGAAAGCAGTCCCGATGAGAAACCAAGAGAGAGAGAAAGCCAGTGAAGAGGAGGAG
1621  AAGTATGATATTTCTCAAGTACCACTATTTGACTGAAAAGGACCTAGAAGAGCCCT
1681  AAAGGTGGATGATATCTCAAATCCCTTGAGACACAGTAAAGCTTGCATCAACAAAG
1741  GCTGAGATGGACTCCAGTGGGCGGGTATCCGAGACACAGCTCCCTCCCTCAGCTAC
1801  CCCACATGATGAAAGTGTCTCCGCTGCTCCGGGAGAGGAGCCGCTGAAACCTATG
1861  TTTGGCAACAGGAAATCGTGTCTCCCAAAAACCCAGACCTGCTCTCCACCAGC
1921  AGCCAGACCTCCCAAGCCCAAGACAAATTTCAAGCCATGGAGAACTGGTGAAGAAA
1981  GTCAGGAGAAAGTTGCCAAAGTTGAGGAGAAATGAAAGAGCCAGAAAGGCAAGCTCTG
2041  CCACCCAGCGCCACCCCGTCCCGTGGAGCAGGAGCAGAGCGAAACCTCAGATG
2101  GAGGCTCCAGGAGCGAGTTCGAAAGTGAAGATAGCCGAGCCAGCCAGCAAGAT
2161  GCCTGCAAGGAGCGAGCCCTCCGAGAGCCCTGAGAAATGCCAAGAGCTGGTGAAG
2221  CCGCTGAGCGGGGTCTCAGCGGCGACAGCCATATCAGGGACACCGCCAGAAACAG
2281  CCTTTTGAACCCCTGAGTGCCTGAGTCCGATGATGATGATGATGATGATGATGATG
2341  GCCAAGCCCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
2401  CTGGCCGAGGAGGAGCTGTGGCCACCCACCCCTTCCAGCCAGAAAGGCGGAGCAT
2461  CTGAGCCGATTTCTACCACTGCAACATGACAGCCGATGACATGACAAAGGAGAG
2521  AGCCAGAAAGTGTCTCTTAGGTTCAAGGCTTTGTGCCCCACGCTCCAGATCCCGGCA
2581  ACTTCTCATCCAGGTGACAAAGGCAAGGACATCTGCCGTGTATCATTATGTCAAAC
2641  TCGCCGCTGCTGAGATGCTTGTGATGATATCCGATATGTTGAAGAACTTGACAGAG
2701  AGCCACAGTCAAATCTCCACTCTTCCAGCATCTCTGAGAGCTGACATGAGGGG
2761  GCCCCCTGAGAGGAGGAGGAGGAGTGGCTGCGGGCAGAGAGGAAAGGCCACCTGCA
2821  ACTGGAACTCTCAGCAGCTCTATCTCCAGAGCCGCTGCTGCTGCTGCTGCTGCTGCTG
2881  ACTTCAGAAAGGAGTACATCATGTCGATCTGAGCCCAAGGAGGAGTGCACATCTCC
2941  AGGTCACCGCTCTCCATGACCCATCAGCATTGGCTGCCAATGTGAAGTACCAA
3001  CTTCGAAGGAGGTTGGAAACAAATCTCAAAGCTTGGACACTGGAACCCAGTCTTC
3061  TTTTGAACGACTGTGCTCACAATCAGGACTCTTCCAGCTACATGACCCACTTAGAG
3121  TCGATCTGGGCTCCGGCTGAGACTTTCAAAATCTCAAAATCTCAAAATCTCAAAAT
3181  CAAATGACAAACCAAGTCCCGTCTGAAAAGTGTGACATCTCCCGGAGGAGGAT
3241  CTGGGACCACTACAGCTGCAAAATTTGTAATGGACCTTCCGCAAGCAATCTGCT
    
```

## Design comments

There is a single reported transcript from the Tshz3 locus (and reported as a Ensembl/Havana merged transcript).

## Reporter Cassette

A “GC” reporter cassette (eGFP fused to Cre-recombinase) was inserted at the consensus start ATG of the Tshz3 coding region. The Neo/Kan component is used for selection in bacteria and removed with transient expression of Flpe-recombinase prior to microinjection.



# Tshz3-GC Target Site Details

Created 17 July 2011  
Updated 23 July 2011

## Endogenous Targeting Site (includes homology arms)

PL

cgatttgtca aacttcccat tccgcgcgca ggcggtctct cctcctctct cctcctctcc tetgctctct cctccccctc gcgctctctc ttccccgcgc  
gctaaacagt ttgaaggta aggcggccgt cgcgccagga ggaggaggag gaggaggagg agacggagga ggagggggag cgcgaggagg aaggggcgcg

Tshz3 exon 1  
NotI

ctcccttgc cgcctcctt cctcctcac gttcgggat cgcgcccag cgcagcagg gggggacgc gagagccgg gcgcagcag atcctgcgg  
gagggaaacg gcggaggaa gggaggcgt caagccccta ggcgcctc gcgctctcc cccccgcgc ctctcggccc cgcgctctc taggacgcc

P5SHZOLRVXHBS (overlap)

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Tshz3 exon 1

NotI  
CGGCCGCTCT CCGGGGGGGA GGCTGCGAGC AGGCCCGCCT CGCCCCCCC CGCCGCCCCC TCCCTCCCTC GGCTCAGCCC GGCAGCGGCG GCGGCGCGCG  
GCCGCGGAGA GGGCCCCCT CCGACGCTCG TCCGGGCGGA GCGGGGGGG GCGGCGGGG AGGGAGGGAG CCGAGTCGGG CCGTCGCCG CGCCGCCCGC

P5SHZOLRVXHBS (overlap)

---

Tshz3 exon 1

cggcggcgt ggcagtcgc ggagaagcat cATGCCGAGG AGGAAGCAGC AGGCCGCCCG GCGCGCAGCA Ggtacgagc gttccccctt cctcctcgg  
gccgcgcgca cgcgtaagcgc cctctctgta gTACGGCTCC TCCTTCGTCG TCCGCGGGGC CGCGCGTCGT Ccatgctcgc cgaaggggaa ggaggaggcc

PM P3SHZOLRVXHBS (overlap)

PO

tctcctctct ctctctctgc tcgcgcgcgc cctctgggcc gccggcccc ggggtgctcg cgcactcct cgcctctctc tcattttctg tctgtctctg  
aggaggaggga gaaggagacg agcgcgcgcg ggagaccgg cggcggggg cgcacagcgc ggcgtgagga cgggagagag agtaaaagac agacagagac

XmaI  
SmaI

cgggcgctga gtaactcgc gagcccggc ggcctggggc tctcctgcc gggctgcgc gcccccagt tgccccgta tgttctctgc tcgaccggct  
gccccgcgact cattgagcc ctccggccga ccgagcccc agaggacggg cccacgcgc cggggggtca acgggggat acaagaagc agctggccga

PR

## Targeted Site - 5'

Left homology arm

CTCCCTTGCC CGCCCTCCTT CCCTCCGCAC GTTCGGGGAT CCGGCGGAG CGCAGCGAGG GGGGGACGCG GAGAGCCGGG GCGCAGCAGC ATCCTGCGGG  
GAGGAAACGG GCGGGAGGAA GGGAGCGCTG CAAGCCCCTA GCGCGCCTC GCGTCGCTCC CCCCCTGCGC CTCTCGGCC CGCGTCGTCG TAGGACGCC

NotI

NotI  
P5SHZOLRVXHBS (overlap)

Left homology arm

EagI  
CGGCCGCTCT CCGGGGGGGA GGCTGCGAGC AGGCCCGCCT CGCCCCCCC CGCCGCCCCC TCCCTCCCTC GGCTCAGCCC GGCAGCGGCG GCGGCGCGCG  
GCCGCGGAGA GGGCCCCCT CCGACGCTCG TCCGGGCGGA GCGGGGGGG GCGGCGGGG AGGGAGGGAG CCGAGTCGGG CCGTCGCCG CGCCGCCCGC

P5SHZOLRVXHBS (overlap)

Left homology arm

XhoI  
AvaI  
Kozak  
GFPcre

CGGCGCGGT GGCAGTCGCG GGAGAAGCAT Cgatatctcg aggcgcgca cATGGTGAGC AAGGGCGAGG AGCTGTTTAC CGGGTGGTG CCGATCCTGG  
GCCGCCGCA CCGTCAGCGC CCTCTTCGTA Gctatagag tccggcggtg gTACCACTCG TTCCCGCTCC TCGACAAGTG GCCCCACCAC GGTAGGACC

PM

## Targeted Site - 3'

BGH polyadenylation sequence

GGAAATTGCA TCGCATTGTC TGAGTAGGTG TCATTCTATT CTGGGGGGTG GGGTGGGCA GGACAGCAAG GGGGAGGATT GGAAGACAA TAGCAGGCAT  
CCTTTAACGT AGCGTAACAG ACTCATCCAC AGTAAGATAA GACCCCCAC CCCACCCGT CTGTCTTTC CCCCTCTAA CCCTTCTGT ATCGTCCGTA

BGH polyadenylation sequence

XbaI  
Sall

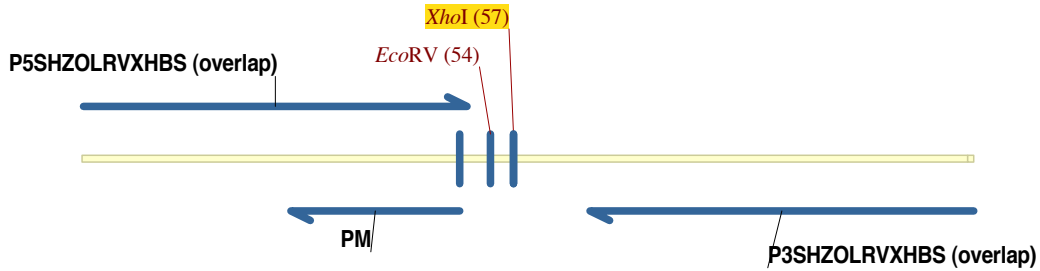
GCTGGGGATG CCGTGGGCTC TATGGCCCG GTGATCCTCT AGAGTCGACC TCTAGTGAGA TGGCGGCTCG AGCGCGCCC AGGAGGAAGC AGCAGGCGCC  
CGACCCCTAC GCCACCCGAG ATACCCGGCC CACTAGGAGA TCTCAGCTGG AGATCACTCT ACCGCGCAGC TCGCGCGGC TCCTCCTTCG TCGTCCGCGG

Right homology arm  
P3SHZOLRVXHBS (overlap)

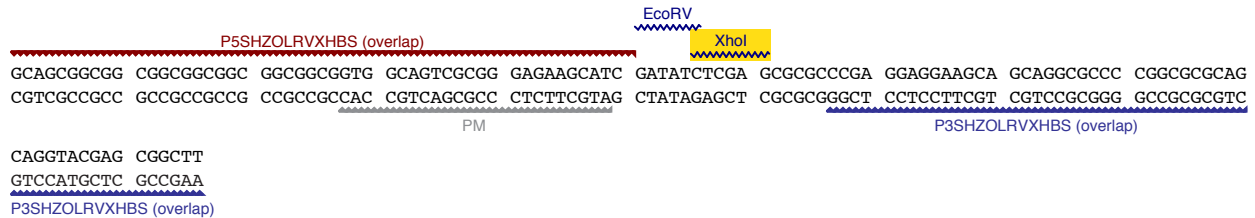
# BAC targeting cassette for Tshz3-GC

Created 19 July 2011  
 Updated 22 July 2011

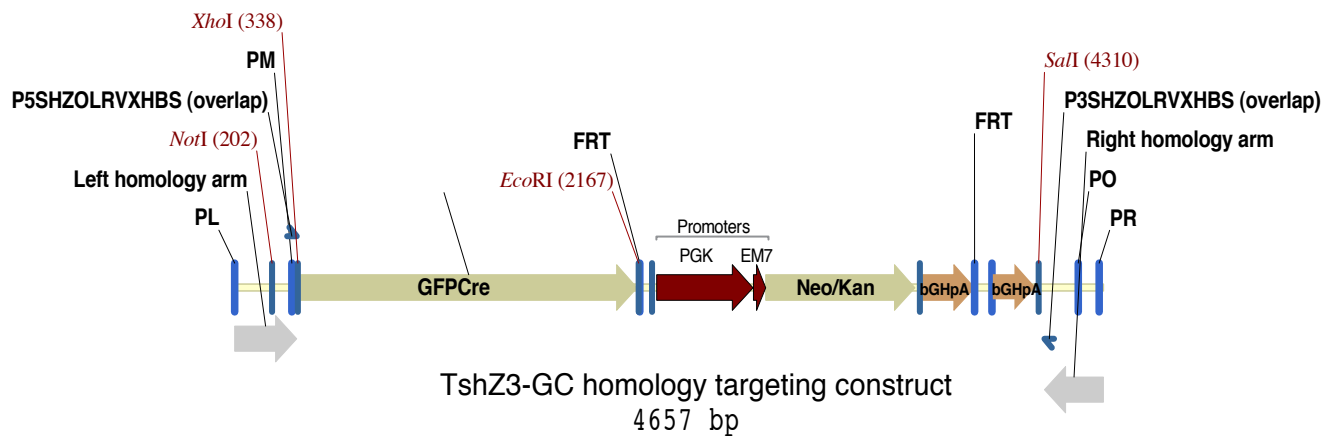
The homologous arms for recombineering were created by overlap-PCR using 50mer oligos. The resulting product, cloned into a shuttle vector (not shown), contained the short Left and Right homology arms joined by a polylinker sequence introduced into the overlap primers. This polylinker sequence included an XhoI restriction site for subsequent cloning of the GC reporter cassette into the center location using NotI and AscI (both then blunted by fill-in). This short-armed construct was then targeted to a long-armed fragment using recombineering. This resulted in the final BAC targeting construct list at the bottom of this figure with full length Left and Right homology arms. This final construct was used to target the Tshz3 BAC to make the transgene construct for injection.



Tshz3-Homology arm overlap PCR - primary targeting  
 116 bp



## Reporter + Arms - final



# Tshz3-GC BAC Transgene

Created 20 July 2011  
 Updated 23 July 2011

BAC clone RP23-382O3 was targeted by sequential recombineering. The genomic context of the GC reporter is shown below. The BAC and the target gene are highlighted in yellow.

