

Wu et al., **Ectopic germline recombination activity of the widely used Foxp3-YFP-Cre knock-in mouse: a case report in the generation of Treg-specific InsR knockout mice**
Supporting information

Annotated sequence of a floxed allele near Exon 4 of *Insr* (WT intron, Cloning remnant, loxP, Exon4)

TCTCAAAAACAAGCTAAAAGGCTCCTGAAGAATGACACCCAAAGTTGACCTCTGATCTCCATAAAGGTGTGT
ATGTACCCCCAACGCTACACATCATATGCATATGAA
CATAAAGTTCTATCATGTGATCAATGATTCTATCAACCGTGCCTAGAGACTCCAAGACAAACTTTATAGGACT
AGTCGACCTGCAGCCAAGCTAGCCTGGCTGGACGTAACCTCCTCTTCAGACCTATAACTTCGTATAGCAT
ACATTATACGAAGTTATATTAAGGGTTATTGAATATGATCGGAATTCCTCGAGTCTAGTGAGGTGACAGGGTT
GGTAAATGTGGACTTCAGTTTGTCTTAGCACCCTGGGAAAGGTGGACATGGTGGTCTGTGCTTGTTA
TACCAGCACTGGAGAGGTGTGGTGTCTGTGATCTGCCCTGTGCAGGGAATGAAGGAAGTTTTTATACCA
GGAAAGGAATTTGTGTGTTTGTGTGTATGCAGCTATTTGTGAGTGGGTGTAGGGAGATCAGAGGACAACC
TCAGATATTATTCTCAGAATTTACCTACTTTCTTTGTGATAGGGTCTCCCAGTGACCCTGAGCCTGACCTAT
TAGGCTAGGCTACCTGATCAGCAAACCCAGGATTCTCTCTCCTTCCTCAGTACTACTCTTACCAATATCTAA
CAGTGTGTTGATATTTCCAAGAAAACCTTTCATTGCGTATGATTTATTTTTATGCGTGTGTGTGTGTGTGTGT
GTGTGTGTGTGTGTGCGTGCAAGCGCACGCGCGCACAGGGTTAGTATGTGGTGCTCAGTTCTCAGAACTT
GCTTTCCTTCTTTCACTTTGTTGGGCCAGGGATTGAAGTTCAGGTTAGCAGGCTTGGCTGCAAGCAGGTAC
CTTTACCCTATGAGCTGTCTTCCAGCCAATGCCTGACATTTTAGGTGGGCTATAGGGTTCAACTGAGGT
TCTCATATTTATGAGGTGAATATTCCATGGTCTGAACACTCCCTTAAACCCTGGGCAATGAATTTAAGGGGA
TAATTGGCCATTTAAAACCTGTAGGAGAAGGTTGACAATGCCTGGGAATAATTTTATTGTGGCAATTTAGAG
ATGAGATGGTGTGTTGGTACTGAGCACCAGTTGGGAATCTGGCTCTGCATCGTATACTGCCCAAGGTTGCCTC
ATGACCACTGTGAAGACATATTTGGTGTCAAATATTTGGAATACTATATCAGTGAACCTTCTCTTGCTGTGATA
AACACTCTGACCAAAGGCAGCTTAGGGGAGGACAAGGTTTGTTCAGCTTATACTGCTTGGTTACATTCT
GTCACAGAGTGAAGTCAAATAGAAACATAAAGCAGAAACCAAAACAGAGTTTCTCAACCTTCCTAGCAC
GAACCTTTTATACAGATCCTCATGTTGTAATGACCTATAACCATAAAATTATTTATCTGCTACTTCATAAGTGTA
GTTTTGCTACTGTTATGAGTCATAATGTTAATATCTGATAGGTAGGATCTCTGATATGTGACCAACAGAGGGG
TAGCAGTGTCTAGGCTGAAAACCACTGCCATAGAGCAGTGATGCTAGTTGACTCTGTTTCTAGCTCATGTG
CAGCTAGCTTTCTCAGATAGACCAGGCCACCTGCCTTGGAATGGCCACCCACAGTGGGCTGGACCCTC
CCCCATCAATCAACAATCGAGATAACTCCCCACAGTATAGTCATGGACCAATCTGATTTAGGCAATTCCTCAA
CTGAGATCCCCCTCATGATGACTCTAGGCTATATCCTGCTGACAGTTAAAAGTTAGCTAGCTCAACTGCTGA
GCTCAGCACATCCACTTTAGAGTAACAGACTGAGGTTGCCACTGGGTTCTGTGAAGAGTGTGTATTCCA
AATGACGGGGTGAGGGAACCCCTGTGCACCTCATTGATCTCTTTGCCTGTGCTCCACTCTCAGCTTGATGTG
CACCCATGTCTGGACCCTGCCCTAAGGTCTGCCAAATCCTCGAAGGTGAGAAGACCATTGATTCTGTG
ACATCTGCCAGGAGCTCCGAGGCTGCACTGTGATCAACGGTAGCCTGATCATCAACATCCGAGGGGGCA
GTGAGTATTTTGGAGCATCCCCACTTCTATGAGCTGTGAGCAGGGAAGCTCGAGATAACTTCGTATAGC
ATACATTATACGAAGTTATGCACAGCTCAGGGTATTCCACACATTCCCCTCTTAACCTTTCACGGCCAAATTT
ACTTTGTTCTGTGGTCTCAGCTATTGAGGCTGAGGCAGGAGGATTGCTTTAATCTAGCAAGTTCAGT
CTAGCCTAGATGACATAATCTTGTCCAAGAAAATAAAACAACATAACATGCAAAGTGACACATCCTTTAGTGG
CCCCAAATGAGAGACTTATACCAGGAGTTGCCCTTAAATCCAAATGAAAATTTTATGGCTCTACCTCTGCC
TGAAATTTCTATTTTGAATCTGCAGCAGGAATCTCTATTCTTTATTTTTATGTGTATGAGTGTGTTTGTCTGG
GTATATGTATATACATGGTGTATATGAGGTGTCCACAAAAAAGAGGGTATATAATACCCTGGAAGTGCAGTTA
GAGATGACTGTGAGTTACCATGTGGATGTGGGGACTGAACCCAGGCCCTCTGTGATAGCACCTGATTCTC
GTAATTAATTCT

PCR primers

The primers shown in Fig 1B. Designed by PMID 9844629

Forward primer **GATGTGCACCCCATGTCTG**

Reverse primer **CTGAATAGCTGAGACCACAG**

Product sizes

wt allele = 279 bp

floxed allele = 319 bp

The primers shown in Fig 3A. New PCR primer that give 3 distinct products

Forward primer **ACCGTGCCTAGAGACTCCAAGAC**

Reverse primer **CTGAATAGCTGAGACCACAG**

Product sizes

wt allele = 1983 bp

floxed allele = 2150 bp

recombined allele = 209 bp

qPCR Primers for estimating relative ectopic recombination (shown in Fig 4A)

Primers only amplify wt allele (product = 92 bp)

ACAAACTTTATAGGACTAGTGAGGT

ACCACCATGTCCACCTTTCCC

Primers only amplify floxed allele (product = 182 bp)

GCTTGGCTGGACGTAAACTCCT

ACCACCATGTCCACCTTTCCCA

Primers only amplify recombined allele (product = 209 bp)

ACCGTGCCTAGAGACTCCAAGAC

CTGAATAGCTGAGACCACAG

Housekeeping gene 1: *Ccr5*

CAGGCAACAGAGACTCTTGG, TCATGTTCTCCTGTGGATCG

Housekeeping gene 2: *Il10rb*

ATGATTCCACCCCTGAGA, GAGCTGTGAAAGTCAGGTTTCG

qPCR Primers for assessing *Insr* mRNA

Insr: TGTGACATCTGCCAGGAGCT, GCATAGGAGCGGCGGATCTTTAG

Sdha: CCTAAACATGCAGAAGTCGATGC, GCCTTCTTGCAATACACTCCCC

Rpl13a: CTCAAGGTTGTTCCGGCTGAA, CTGTCACTGCCTGGTACTTCCA

Antibodies for FACSsorting

Marker	Fluorochrome	Company	Catalogue	Clone
NK1.1	PE	BD	557391	PK136
CD3	BV605	BioLegend	100237	17A2
CD19	APC	BD	550992	1D3
CD4	eF450	eBioscience	48-0041-82	GK1.5
CD8a	PE-Cy7	eBioscience	25-0081-82	53-6.7
Fixable viability Dye	eF780	eBioscience	65-0865-14	