

Rag1&Il2rg Cas9-KO Strategy

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Project Overview





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Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Rag1 and Il2rg gene. The schematic diagram is as follows:





> The *Rag1* gene has 1 transcript. According to the structure of *Rag1* gene, partial coding sequence of exon1 of MGP_NODShiLtJ_T0056045.1 transcript is recommended as the knockout region. Knock out the region will result in disruption of protein function.

> The *Il2rg* gene has 4 transcripts. According to the structure of *Il2rg* gene, partial coding sequence of exon3 of MGP_NODShiLtJ_T0096480.1 transcript is recommended as the knockout region. The region contains 185bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Rag1* and *Il2rg* gene. The brief process is as follows: CRISPR/Cas9 system were microinjected into the fertilized eggs of NOD/ShiLtJGpt mice. Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with NOD/ShiLtJGpt mice.

Notice-Rag1



- According to the existing MGI data, homozygotes for targeted null mutations exhibit arrested development of T and B cell maturation at the CD4-8- thymocyte or B220+/CD43+pro-B cell stage due to inability to undergo V(D)J recombination.
 Because *B230118H07Rik* gene overlap with *Rag1* gene, the deletion of *Rag1* gene may also disrupt *B230118H07Rik* gene.
- > The *Rag1* gene is located on the Chr2. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.
- > This strategy is designed based on genetic information in existing databases.Due to the complexity of biological processes,all risk of the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Notice-Il2rg



- According to the existing MGI data,males hemizygous for targeted null mutations exhibit markedly reduced numbers of T and B cells, lack NK cells and Peyer's patches, and have severely reduced numbers of gut-associated intraepithelial lymphocytes.
 The deleted region of *Il2rg* gene is near to the 5'UTR of *Med12* gene, and the deletion of *Il2rg* gene may influence the normal expression of the *Med12* gene.
- Because *Gm20489* gene overlap with *Il2rg* gene, the deletion of *Il2rg* gene can also disrupt *Gm20489* gene.
 The *Il2rg* gene is located on the ChrX. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.
- > This strategy is designed based on genetic information in existing databases.Due to the complexity of biological processes,all risk of the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information (NCBI)-Rag1

Rag1 recombination activating 1 [Mus musculus (house mouse)]

Gene ID: 19373, updated on 13-Mar-2020

Summary

 Official Symbol
 Rag1 provided by MGI

 Official Full Name
 recombination activating 1 provided by MGI

 Primary source
 MGI:MGI:97848

 See related
 Ensembl:ENSMUSG0000061311

 Gene type
 protein coding

 RefSeq status
 VALIDATED

 Organism
 Mus musculus

 Lineage
 Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muriade; Murinae; Mus; Mus

 Also known as
 Rag-1

 Expression
 Restricted expression toward thymus adult (RPKM 117.3)See more

 Orthologs
 human all

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Transcript information(Ensembl)-Rag1

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The gene has 1 transcript, and the transcript is shown below:

Name 🖕	Transcript ID 🍦	bp 🍦	Protein 🖕	Biotype 🍦	CCDS	UniProt Match 🖕	Flags 🖕
-	MGP_NODShiLtJ_T0056045.1	6608	<u>1040aa</u>	Protein coding	<u>CCDS16463</u> &	<u>P15919</u> &	-

The strategy is based on the design of MGP_NODShiLtJ_T0056045.1 transcript, the transcription is shown below:

]
< MGP_NODShiLtJ_T0056045 protein coding		
Reverse strand	11.24 kb	

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Genomic location distribution-Rag1



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Protein domain-Rag1



MGP_NODShiLtJ_P0 MobiDB lite Low complexity (Seg) Coiled-coils (Ncoils)	—	-	_						
Superfamily		SSF578	50 Zinc finger C2H2 s	superfamily					
SMART		Zind	finger, RING-type						
Pfam	RAG1 importin-binding		V(D)J recom	bination-activatin	a protein 1				
			V(D)1 recombinati	on-activating prot	ein 1. Zinc finge	ur.			
		Zin	finger C3HC4 RING-tur	a a a a a a a a a a a a a a a a a a a		•			
PROSITE profiles		200	V(D)) recombinati	on-activating prot	ein 1, Zinc finge				5
	RAG papamen-hinding domain								
1		71.00	finger PING-hung	er binding domai					
PROSITE patterns		200	inc finger, RING-type	inserved site					
PANTHER	V(D)1 recombination-activation	protein 1							
Gene3D	v(b) reconcilent accreating	processia	2 20 100 00						
			3,30,160,60						
CDD		Zinc fin	ger, RING/FYVE/PHD-type	e					
COD		cd16	530						
Scale bar	0 100	200 30	400	500	600	700	800	900	1040

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Mouse phenotype description(MGI)-Rag1



Phenotypes affected by the gene are marked in blue. Data quoted from MGI database(http://www.informatics.jax.org/).

According to the existing MGI data,homozygotes for targeted null mutations exhibit arrested development of T and B cell maturation at the CD4-8- thymocyte or B220+/CD43+pro-B cell stage due to inability to undergo V(D)J recombination.

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Gene information (NCBI)-Il2rg



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Rag1 recombination activating 1 [Mus musculus (house mouse)]

Gene ID: 19373, updated on 13-Mar-2020

Summary

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Transcript information(Ensembl)-Il2rg

The gene has 4 transcripts, and all the transcripts are shown below:

Name 🖕	Transcript ID 💧	bp 🍦	Protein 🖕	Biotype 🖕	CCDS 🍦	UniProt Match	Flags 🖕
-	MGP_NODShiLtJ_T0096480.1	1670	<u>369aa</u>	Protein coding	<u>CCDS30312</u> &	<u>B0QZX1</u> & <u>P34902</u> & <u>Q3UPA9</u> &	-
-	MGP_NODShiLtJ_T0096481.1	597	<u>153aa</u>	Protein coding	-	-	
-	MGP_NODShiLtJ_T0096483.1	558	No protein	Processed transcript	_		8 - 9
-	MGP_NODShiLtJ_T0096482.1	1335	No protein	Retained intron	<u>-</u>	1 <u>-</u>	12

The strategy is based on the design of MGP_NODShiLtJ_T0096480.1 transcript, the transcription is shown below:



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Genomic location distribution-Il2rg



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Protein domain-Il2rg

	CAN SIDE							and the second second
MGP_NODShiLtJ_P0 Transmembrane heli Low complexity (Seg) Cleavage site (Sign Superfamily SMART Pfam	_	Fibronectin type III superfi	amily	Fibronectin type III				
		type I cytokine receptor,	cytokine-bindin	ig domain				
				Fibronectin type III				
PROSITE profiles				Fibronectin type III				
PROSITE patterns					Short hemat	opoietin receptor, fami	ly 1, conserved site	
PANTHER	PTHR23036:SF172							
	PTHR23036							
Gene3D CDD		Immunoglobulin-like fold		Fibronectin type III		-		
Scale bar	0 40	80	120	160	200	240 280	320	369

Mouse phenotype description(MGI)-Il2rg





Phenotypes affected by the gene are marked in blue. Data quoted from MGI database(http://www.informatics.jax.org/).

According to the existing MGI data,males hemizygous for targeted null mutations exhibit markedly reduced numbers of T and B cells, lack NK cells and Peyer's patches, and have severely reduced numbers of gut-associated intraepithelial lymphocytes.

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If you have any questions, you are welcome to inquire. Tel: 400-9660890



