식품의약품안전처
식품의약품안전평가원

질환모델동물 정보서

관리번호	

질환모델동물 정보

질환모델동물명		C57BL/6-Rag2^{em1hwl}/Korl
유전자 조작 방법		KO created by RGEN-induced NHEJ
Background Strain		C57BL/6JBomTac
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개발일		2015-08-19

이관동물 세대수

Live animal	F3
수정란/정자	

Genetic Engineering 정보

유전자명	Rag2, Recombination activating gene 2
유전자 기능	B cell 과 T cell의 발달 시 V(D)J 재조합을 일으키는 유전자로서 KO 되었을 때 면역결핍

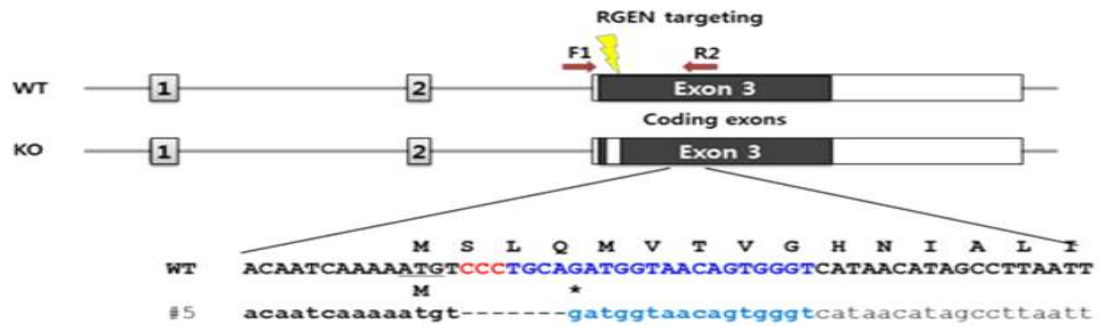
유전자 조작 위치(Chr/gene/exon no. 포함)

☐ Chromosome Location: Chr 2

☐ NCBI Gene ID: 19374

☐ Targeting Region: Exon 3

Construct/Mutation Map



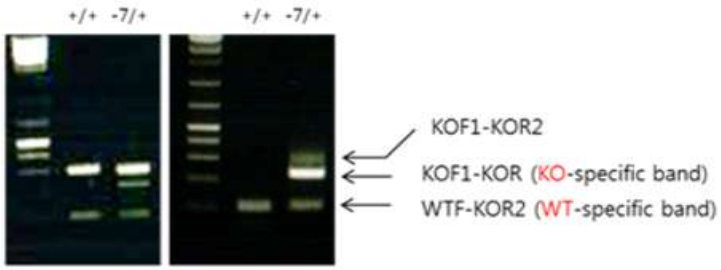
○ The sequence in red denotes PAM and that in blue indicate RGEN target sequence just downstream of the start codon of mouse Rag2 gene. 7-bp deletion induces a premature stop codon(*)

Genotyping Strategy

Genotyping Primer 정보 및 결과

Primer Sequence	R2Ba WT F (WT-specific)	5′-ACAATCAAAAATGTCCCTGCA-3′
	Rag2 KO R2 (WT-specific)	5′-AGCCTGGTTGAATTAAGGCTATG-3′
	Rag2 KO F1 (KO-specific)	5′-GCTGCTGCCACAATAAAGTAGTG-3′
	R2Ba KO R (KO-specific)	5′-CCACTGTTACCATCACATTT-3′

Loading: 2% agarose gel



PCR result: WT HET WT HET

*** Product size**
WT : 65 bp
KO : 230 bp

○ Common primer에 의한 band가 나타날 수 있지만, KO specific primer에 의한 band size와 구별됨

Genotyping Protocol

PCR 반응액 조제	구성물	농도		양	
	Sterile water		-	13.6	μℓ
	Genomic DNA	100	ng/μℓ	1	μℓ
	dNTP	10	mM	0.4	μℓ
	PCR buffer	10	×	2	μℓ
	Forward primer	10	pM	1	μℓ
	Reverse primer	10	pM	1	μℓ
	Taq polymerase	1	U/μℓ	1	μℓ
PCR 반응 조건	단계	온도		시간	
	Pre-denaturation	95	℃	3 min	sec
	Denaturation	95	℃	min 30	sec
	Annealing	62	℃	min 15	sec
	Elongation	72	℃	2 min 30	sec
	cycle 수	33 Cycles			
	Post-elongation	72	℃	5 min	sec
	Hold	12	℃		
증폭 산물 크기	WT	65			bp
	KO	230			bp
특이사항	○ PCR product는 2% agarose gel에서 구별됨				

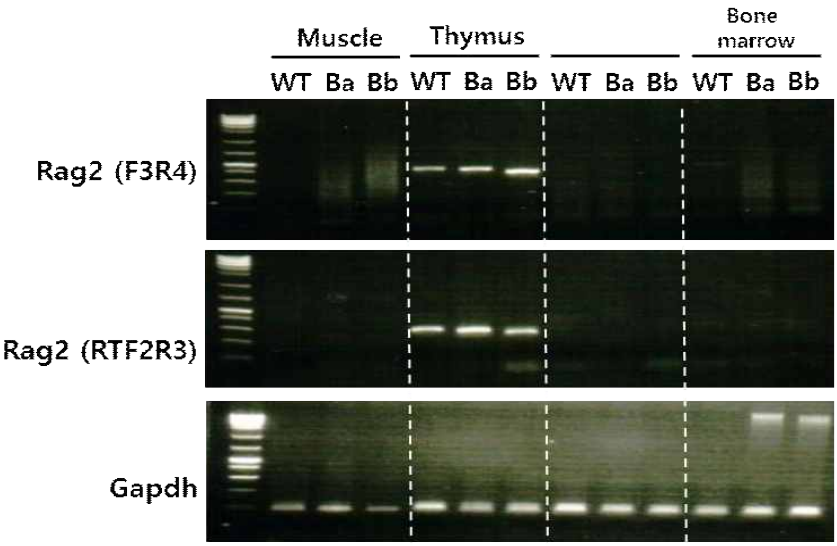
Expression test 정보 및 결과

RT-PCR 정보 및 결과

Primer Sequence (Set1)	F	'5-TTCACATCCACAAGCAGGAAGT-3' (F3R4)
	R	'5-GAGGTGGGAGGTAGCAGGAA-3' (F3R4)
Primer Sequence (Set2)	F	'5-CCCCTCTGGCCTTCAGTGC-3' (F2R3)
	R	'5-TGGTGTTTTCCCTCCGTGAA-3' (F2R3)

PCR 반응 조건

단계	온도		시간
Pre-denaturation	94	℃	3 min sec
Denaturation	94	℃	min 30 sec
Annealing	62	℃	min 30 sec
Elongation	72	℃	min 20 sec
Post-elongation	72	℃	5 min sec
cycle 수	30 cycles		



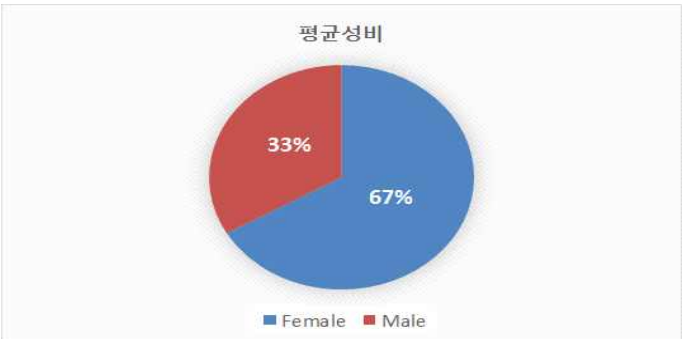
Western blot 정보 및 결과

antibody	Antibody is not available for WB

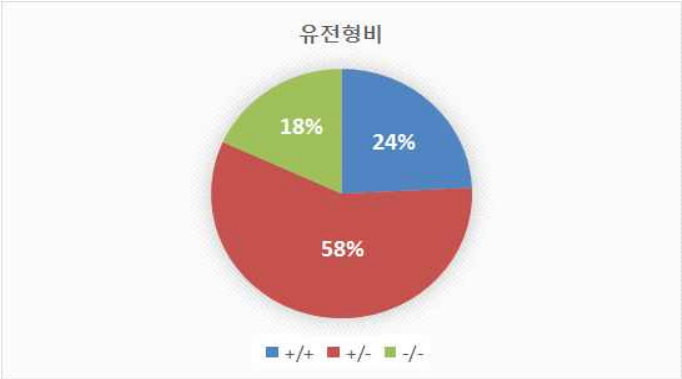
Antibody is not available for WB

Phenotype 정보 (hetero x hetero 기준)

○ 평균산자수 및 성비(n=33)



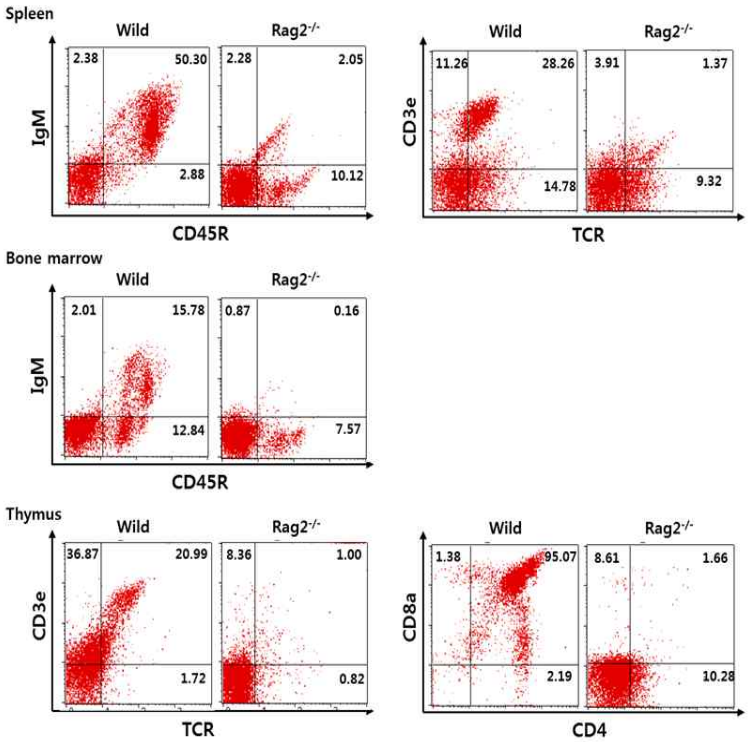
○ 유전형비(n=33)



○ 면역세포 분포 분석(Flow cytometry)

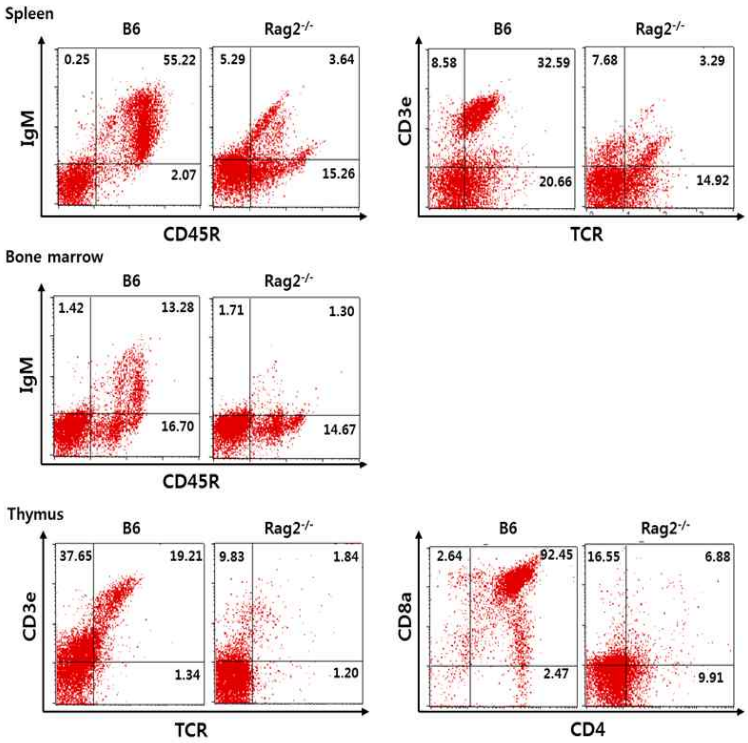
(1) WT n=2, KO n=3, male, 8w

Tissue	Phenotype	B6-*Rag2*$^{-/-}$	Wild
Spleen	CD45R$^+$/IgM$^+$	1.03±1.03	49.83±0.67
	TCR$^+$/CD3e$^+$	1.62±0.87	31.88±5.12
Bone Marrow	CD45R$^+$/IgM$^+$	1.83±3.01	16.46±0.96
Thymus	TCR$^+$/CD3e$^+$	1.64±0.64	19.01±2.81
	CD4$^+$/CD8a$^+$	2.30±2.35	95.48±0.57



(2) WT n=2, KO n=3, female, 8w

Tissue	Phenotype	B6-*Rag2*$^{-/-}$	Wild
Spleen	CD45R$^+$/IgM$^+$	4.80±2.19	54.52±1.00
	TCR$^+$/CD3e$^+$	2.50±0.93	32.88±0.41
Bone Marrow	CD45R$^+$/IgM$^+$	1.94±0.58	13.63±0.49
Thymus	TCR$^+$/CD3e$^+$	2.12±0.24	18.31±1.27
	CD4$^+$/CD8a$^+$	6.08±0.69	92.87±0.59



○ 혈중 면역글로불린 측정(ELISA)

- KO male=2, female=2, 8w

항목	IgM	IgG1	IgG2a	IgG2b	IgG3	IgA
B6-*Rag2*$^{-/-}$	5.66μg/mℓ	0.5μg/mℓ 이하	0.1μg/mℓ 이하	0.5μg/mℓ 이하	2.72μg/mℓ	0.1μg/mℓ 이하
B6	100μg/mℓ 이상	30μg/mℓ 이상	10μg/mℓ 이상	100μg/mℓ 이상	100μg/mℓ 이상	10μg/mℓ 이상

○ 조직 및 면역염색

(1) 조직염색(H&E 염색)

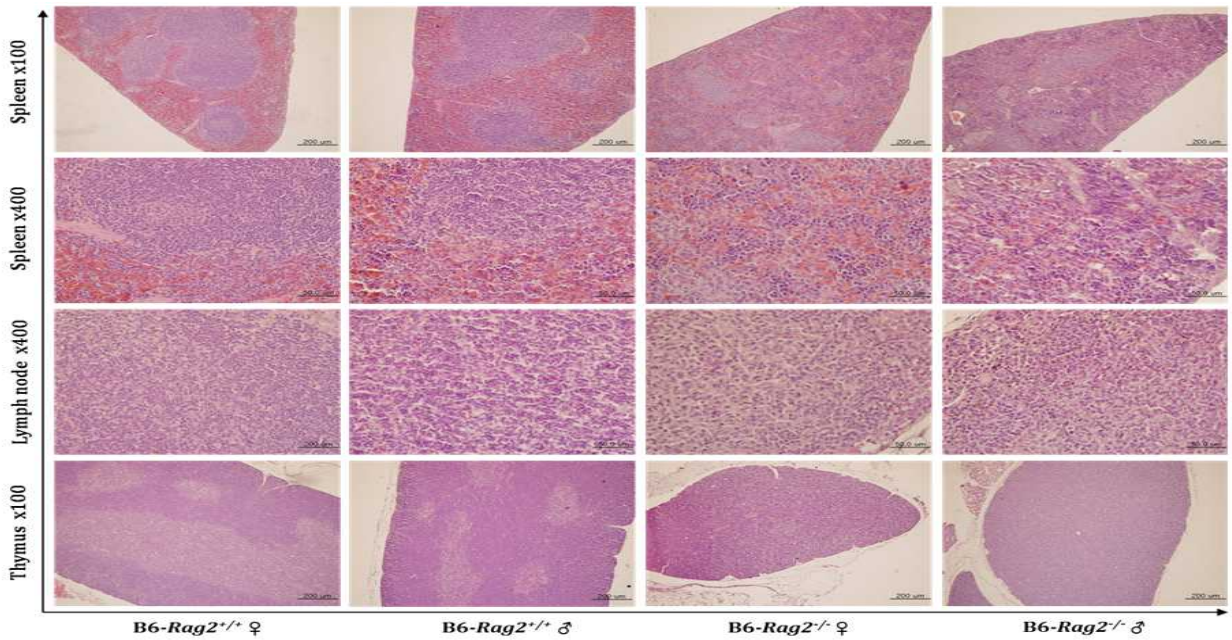


Fig. 1. H&E stained immune organs of B6-*Rag2* knockout mouse. B6-*Rag2* knockout mouse has smaller thymus than B6 control mouse. Spleen of the knockout mouse has

slightly smaller than control B6 mouse, which is different from FVB-*Rag2* knockout mouse.

(2) B cell 염색(CD45 antibody)

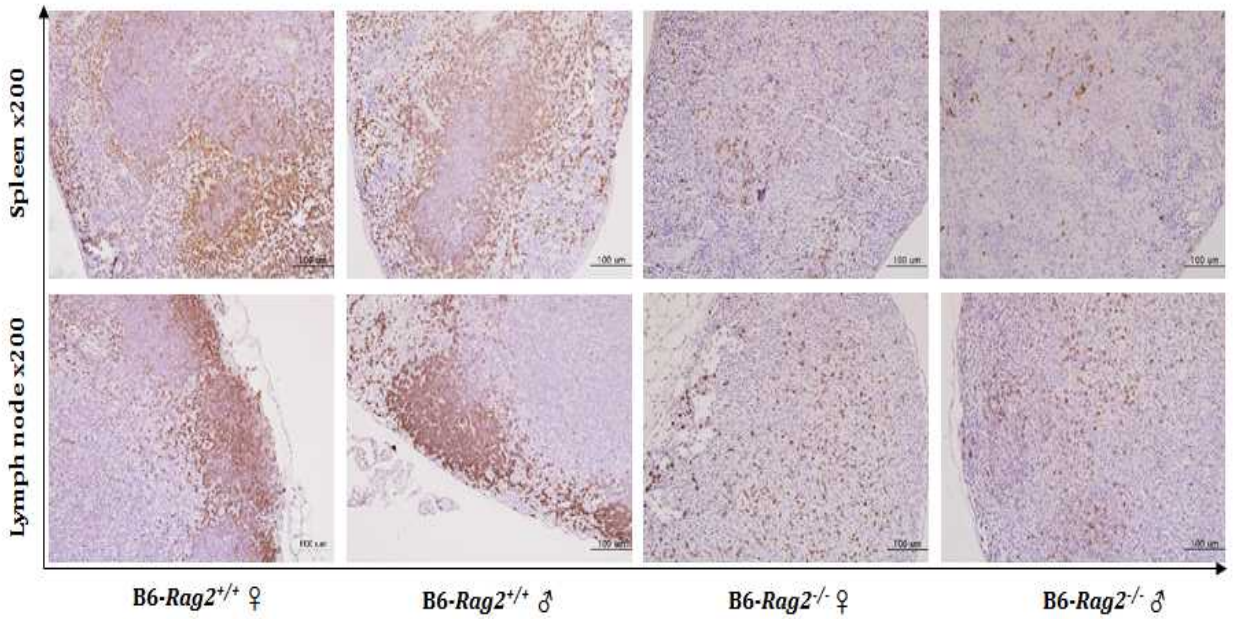


Fig. 2. IHC staining for CD45R antibody in spleen and lymph node of B6-Rag2 knockout mouse. Significantly d ecreased CD45R+ cells in B6-Rag2 knockout mouse.

(3) T cell 염색(CD4 antibody)

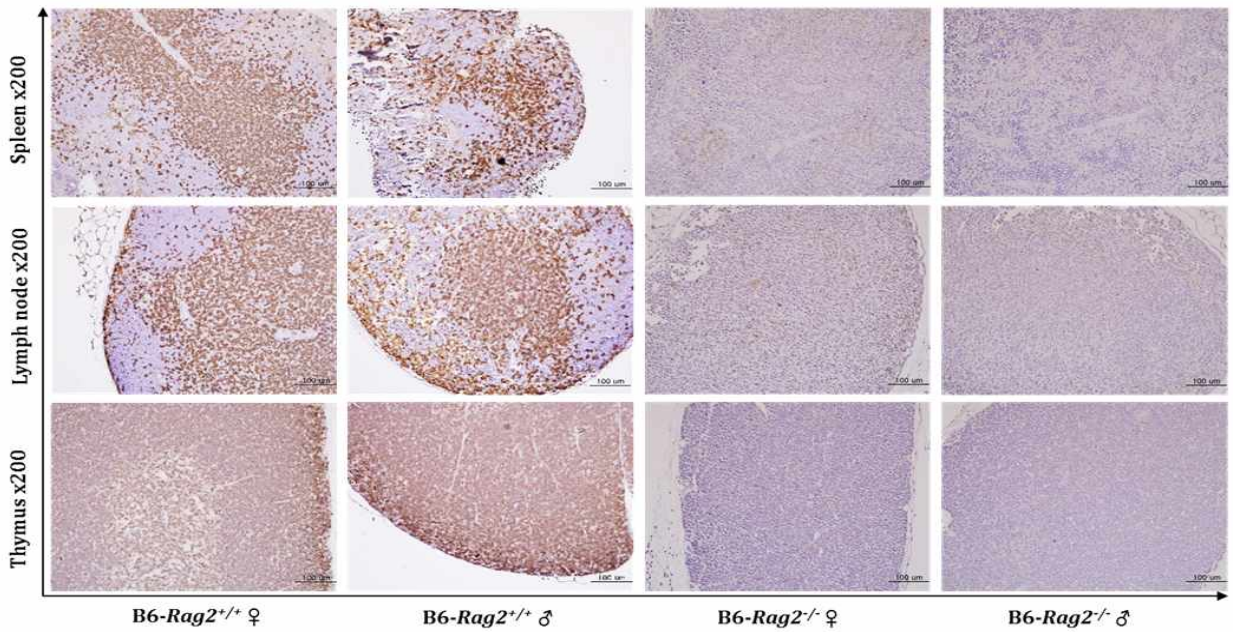


Fig. 3. IHC staining for CD4 antibody in spleen, lymph node and thymus of B6-Rag2 knockout mouse. Significantly decreased CD4+ cells in immune organs of B6-Rag2 knockout mouse.

사육관리 정보

번식능력 및 번식방법(Breeding Scheme) (HT×WT 기준)

○ 평균 한배 새끼 수 : 6~9마리

* Breeding을 위해 HT×WT mating 실시

○ C57BL/6J strain으로 생산되었으며, genetic background를 유지하기 위해서 C57BL/6J strain의 마우스로만 mating을 실시하여 strain을 유지 ○ homozygote mouse is also viable and fertile
사육관리에서 특별히 요구되는 사항
○ Homozygote일 때, 면역결핍 증상을 보이므로 감염에 주의
Reference
해당없음