E4f1 Cas9-CKO Strategy

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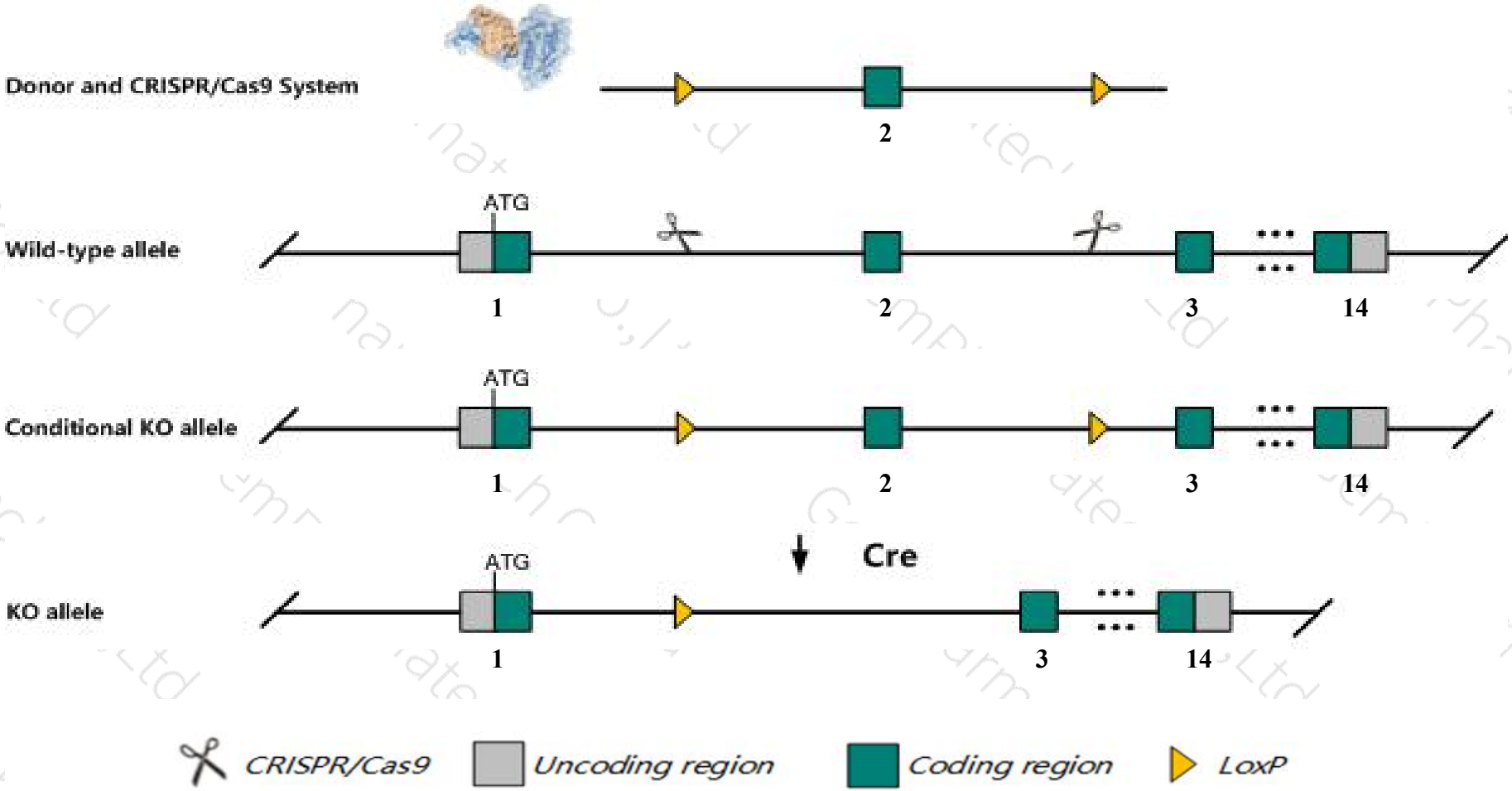
Project Overview

Project Name	***E4f1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *E4f1* gene. The schematic diagram is as follows:



Technical routes

➢ The *E4f1* gene has 8 transcripts. According to the structure of *E4f1* gene, exon2 of *E4f1-201*(ENSMUST00000056032.8) transcript is recommended as the knockout region.The region contains 152bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *E4f1* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA and Donor were microinjected into the fertilized eggs of C57BL/6JGpt 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 C57BL/6JGpt mice.

➢ The flox mice will be knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.

Notice

➢ According to the existing MGI data,homozygous null mice display early embryonic lethality with mitotic progression failure and increased apoptosis.

➢ The *E4f1* gene is located on the Chr17. 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information （NCBI）

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E4f1 E4F transcription factor 1 [Mus musculus (house mouse)]

Gene ID: 13560, updated on 13-Mar-2020

Summary

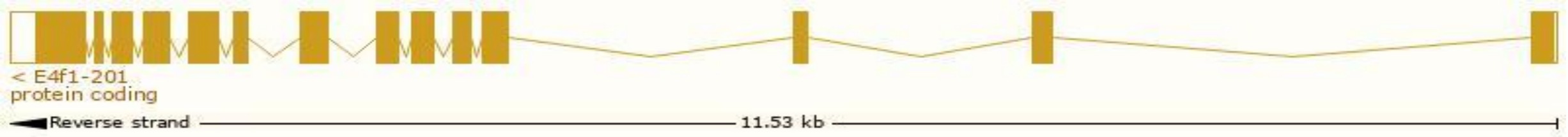
Official Symbol	E4f1 provided by MGI
Official Full Name	E4F transcription factor 1 provided by MGI
Primary source	MGI:MGI:109530
See related	Ensembl:ENSMUSG00000024137
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	p120E4F, phi-AP3
Summary	This gene encodes a member of the GLI-Kruppel zinc finger family. The encoded protein is likely to be multi-functional, with both adenovirus E1A-regulated transcription factor and ubiquitin E3 ligase activities, including roles in cell cycle regulation and the ubiquitination of p53. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Sep 2014]
Expression	Ubiquitous expression in ovary adult (RPKM 25.6), thymus adult (RPKM 24.6) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

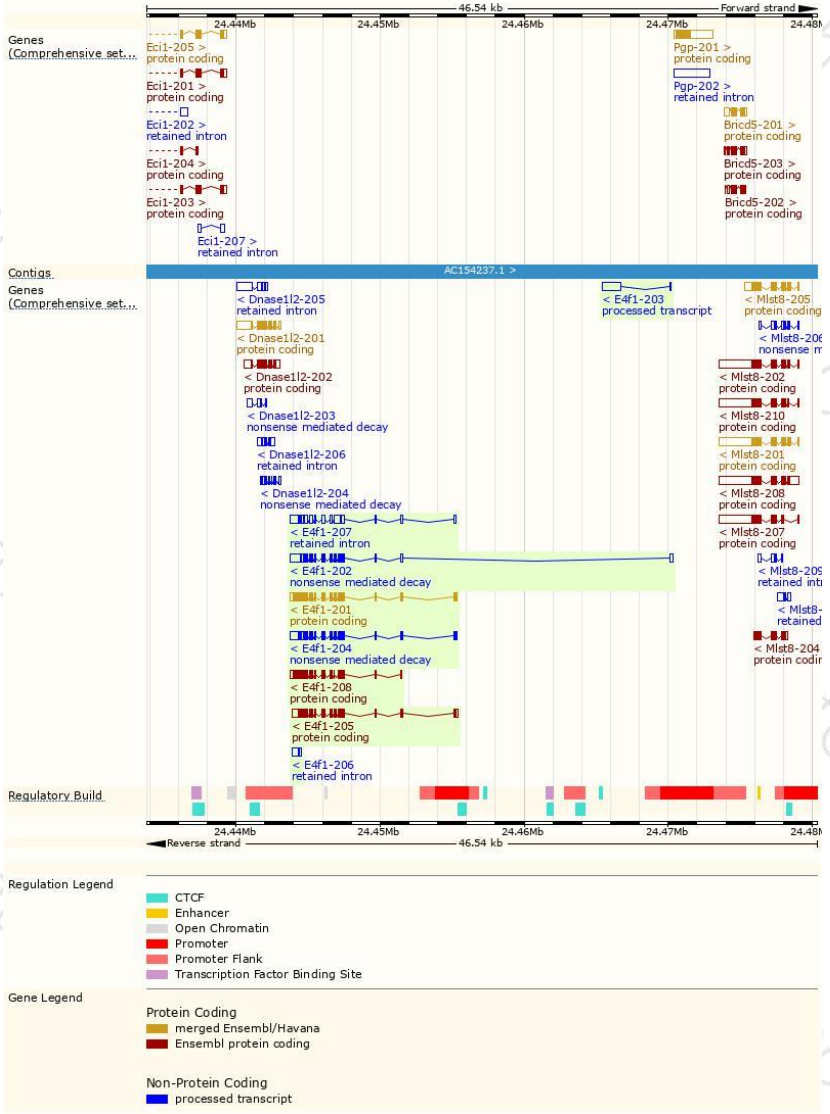
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
E4f1-201	ENSMUST00000056032.8	2574	782aa	Protein coding	CCDS28481	Q8CCE9	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P2
E4f1-205	ENSMUST00000226941.1	2642	684aa	Protein coding	-	Q8CCE9	GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT2
E4f1-208	ENSMUST00000228882.1	2226	675aa	Protein coding	-	A0A2I3BR50	CDS 5' incomplete
E4f1-202	ENSMUST00000226654.1	2674	427aa	Nonsense mediated decay	-	A0A2I3BRE9	
E4f1-204	ENSMUST00000226754.1	2552	586aa	Nonsense mediated decay	-	A0A2I3BPE3	
E4f1-203	ENSMUST00000226743.1	1377	No protein	Processed transcript	-	-	
E4f1-207	ENSMUST00000227293.1	2708	No protein	Retained intron	-	-	
E4f1-206	ENSMUST00000227241.1	556	No protein	Retained intron	-	-	

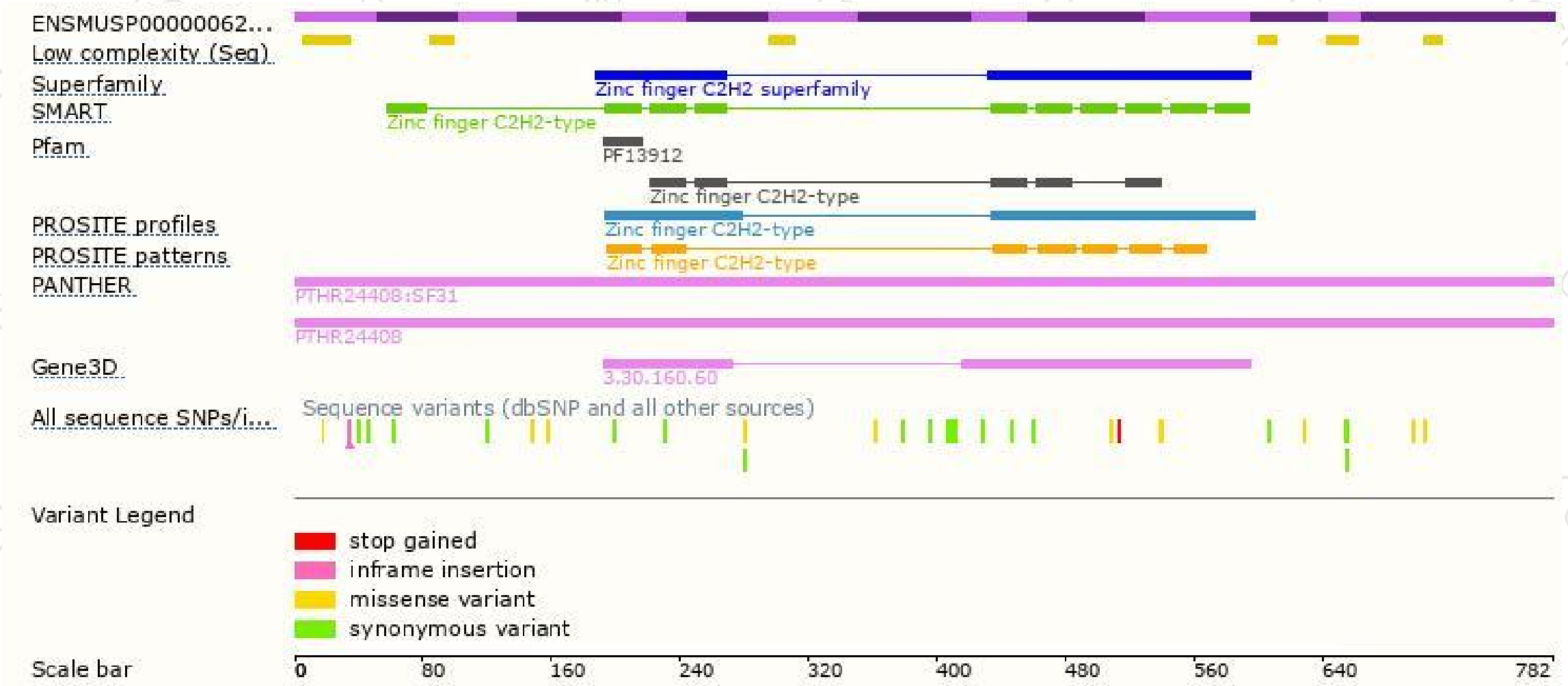
The strategy is based on the design of *E4f1-201* transcript,the transcription is shown below:



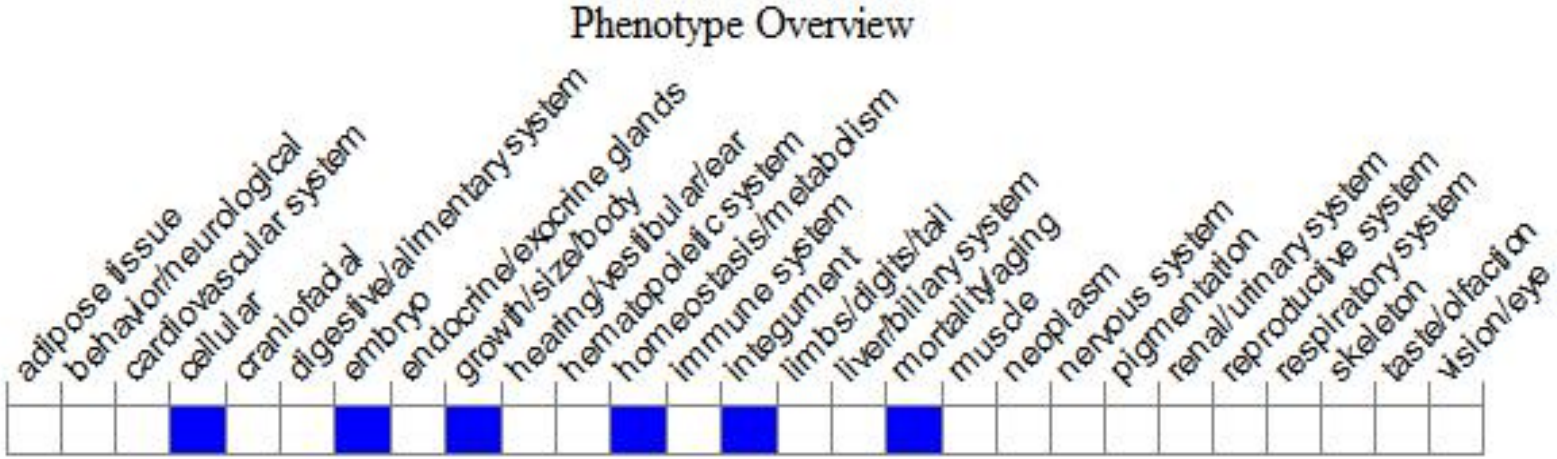
Genomic location distribution



Protein domain



Mouse phenotype description(MGI)



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,homozygous null mice display early embryonic lethality with mitotic progression failure and increased apoptosis.

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If you have any questions, you are welcome to inquire.

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