GemPharmatech™

# Models to Accelerate Innovation

# CSS750-*Akt2* Cas9-KO Mouse Model Strategy

## CRISPR-Cas9 Technology

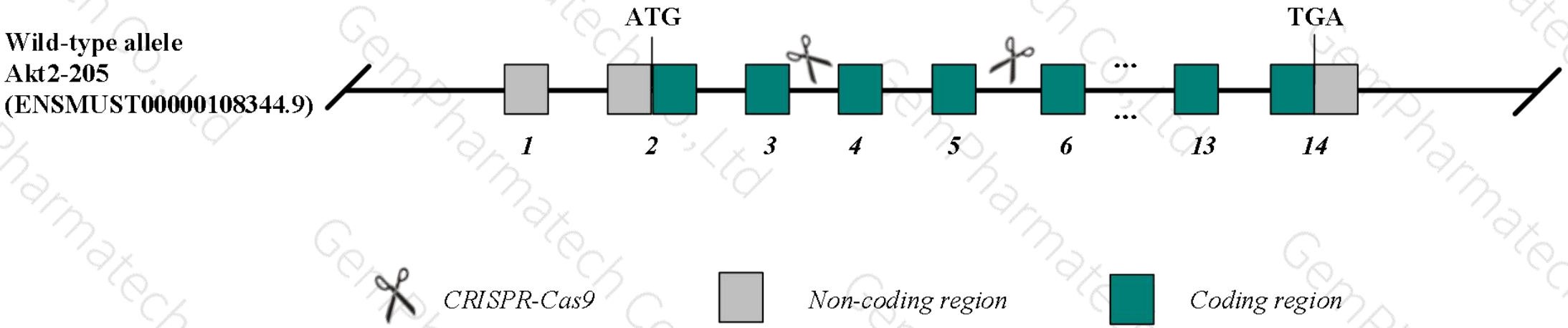
| | |
|---|---|
| **Designer** | **Qin Xia** |
| **Reviewer** | **Zihe Cui** |
| **Date** | **2022-10-14** |

# Project Overview

| | |
|---|---|
| **Project Name** | **CSS750-Akt2 Cas9-KO** |
| **Project Type** | **Cas9-KO** |
| **Background** | **D000750&CSS750** |
| **Timeline** | **4-6 Months** |

# Strategy

This model will use CRISPR-Cas9 technology to edit the mouse *Akt2* gene and the schematic diagram is as follows:



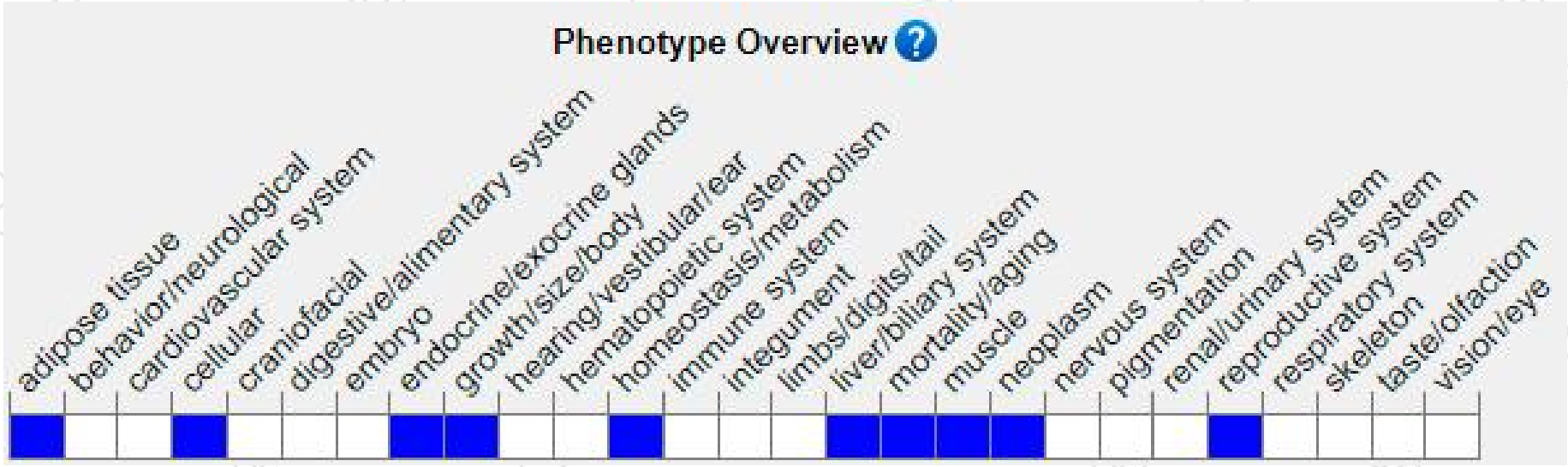
# Technical Description

- The Mouse *Akt2* gene has 14 transcripts. The transcript *Akt2*-205 (ENSMUST00000108344.9) is selected for this strategy. It contains 14 exons and codes 481 aa. The ATG is located in exon 2, and the TGA is located in exon 14.
- According to the structure of *Akt2* gene, exon 4-exon 5 of *Akt2*-205 (ENSMUST00000108344.9) transcript is recommended as the knockout region. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Akt2* gene. The brief process is as follows: CRISPR-Cas9 system were microinjected into the fertilized eggs of D000750&CSS750 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 D000750&CSS750 mice.

# Note

- According to MGI data, homozygotes for targeted null mutations exhibit insulin resistance and elevated plasma triglycerides. In males, the insulin resistance may progress to overt diabetes.
- The *Akt2* gene is located on the Chr 7. 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 the currently available information in the existing databases. Due to the complexity of gene expression regulation, the effect of this strategy on gene expression cannot be completely predicted at the present technology level.

# Phenotype Information (MGI)




Homozygotes for targeted null mutations exhibit insulin resistance and elevated plasma triglycerides. In males, the insulin resistance may progress to overt diabetes.

http://www.informatics.jax.org/marker/MGI:104874

# Basic Information of Mouse *Akt2* Gene

| | |
|---|---|
| **Gene name** | Mouse *Akt2* |
| **Gene ID (NCBI)** | 11652 |
| **Gene link (NCBI)** | https://www.ncbi.nlm.nih.gov/gene/11652 |
| **Gene link (Ensembl)** | http://asia.ensembl.org/Mus_musculus/Gene/Summary?g=ENSMUSG00000004056;r=7:27290977-27340251 |
| **Chromosome location** | Chr 7 |

# Mouse *Akt2* Gene Information (NCBI)

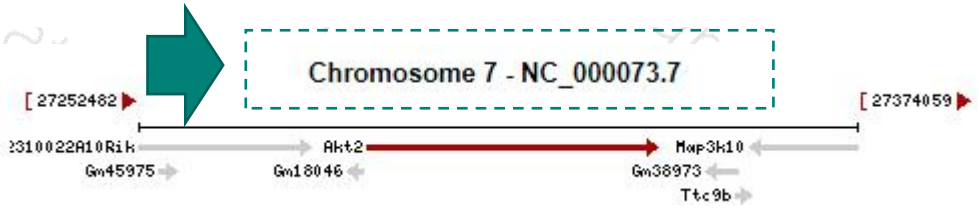
**Akt2 thymoma viral proto-oncogene 2 [ *Mus musculus* (house mouse) ]**

Download Datasets

Gene ID: 11652, updated on 2-Oct-2022

## Summary

| | |
|---|---|
| **Official Symbol** | Akt2 provided by MGI |
| **Official Full Name** | thymoma viral proto-oncogene 2 provided by MGI |
| **Primary source** | MGI:MGI:104874 |
| **See related** | Ensembl:ENSMUSG00000004056 AllianceGenome:MGI:104874 |
| **Gene type** | protein coding |
| **RefSeq status** | VALIDATED |
| **Organism** | Mus musculus |
| **Lineage** | Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus |
| **Also known as** | PKB; PKBbeta; 2410016A19Rik |
| **Summary** | Enables protein serine/threonine kinase activity. Involved in several processes, including cellular response to insulin stimulus; intracellular protein transmembrane transport; and positive regulation of transport. Acts upstream of or within with a negative effect on protein localization to nucleus. Acts upstream of or within several processes, including activation of GTPase activity; cellular response to high light intensity; and peripheral nervous system myelin maintenance. Located in cell cortex and ruffle membrane. Is expressed in several structures, including brown fat; central nervous system; genitourinary system; retina; and spleen. Used to study polycystic ovary syndrome and type 2 diabetes mellitus. Human ortholog(s) of this gene implicated in glucose metabolism disease (multiple); high grade glioma; hypoinsulinemic hypoglycemia with hemihypertrophy; and reproductive organ cancer (multiple). Orthologous to human AKT2 (AKT serine/threonine kinase 2). [provided by Alliance of Genome Resources, Apr 2022] |
| **Expression** | Ubiquitous expression in subcutaneous fat pad adult (RPKM 40.3), mammary gland adult (RPKM 35.7) and 28 other tissues See more |
| **Orthologs** | human all |



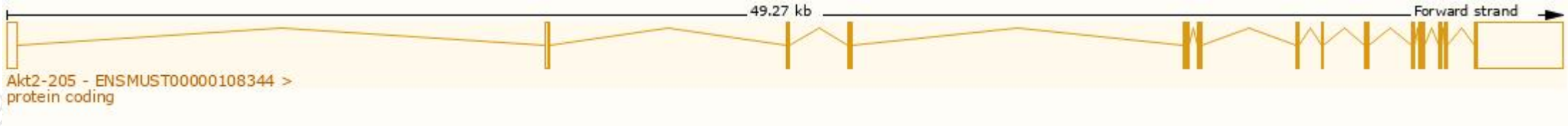
# Mouse *Akt2* Transcript Information (Ensembl)

The gene has 14 transcripts. All transcripts are shown below:

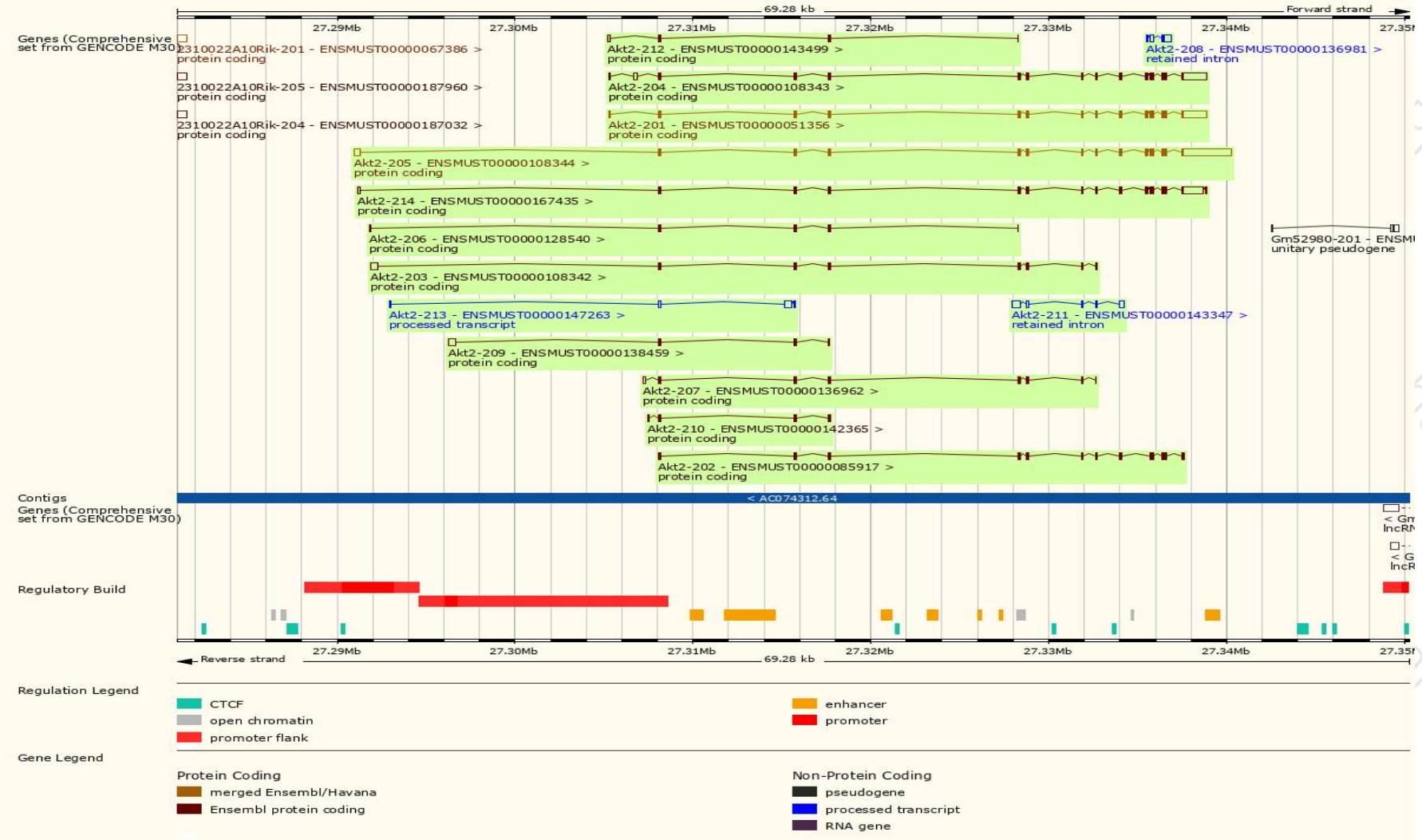
Show/hide columns (1 hidden) Filter

| Transcript ID | Name | bp | Protein ▲ | Biotype | CCDS | UniProt Match | Flags |
|---|---|---|---|---|---|---|---|
| ENSMUST00000143499.8 | Akt2-212 | 417 | 63aa | Protein coding | | D3Z5X2 | TSL:3 CDS 3' incomplete |
| ENSMUST00000138459.8 | Akt2-209 | 712 | 67aa | Protein coding | | D3Z0M3 | TSL:5 CDS 3' incomplete |
| ENSMUST00000142365.8 | Akt2-210 | 448 | 84aa | Protein coding | | D3YZJ5 | TSL:3 CDS 3' incomplete |
| ENSMUST00000128540.8 | Akt2-206 | 453 | 98aa | Protein coding | | D3Z490 | TSL:5 CDS 3' incomplete |
| ENSMUST00000108342.8 | Akt2-203 | 1125 | 222aa | Protein coding | | D3YXM7 | TSL:3 CDS 3' incomplete |
| ENSMUST00000136962.8 | Akt2-207 | 940 | 229aa | Protein coding | | D3Z3N2 | TSL:5 CDS 3' incomplete |
| ENSMUST00000085917.5 | Akt2-202 | 1392 | 438aa | Protein coding | | F8WHG5 | GENCODE basic TSL:5 |
| ENSMUST00000108344.9 | Akt2-205 | 4544 | 481aa | Protein coding | CCDS21027 | Q3TY95 Q60823 | Ensembl Canonical GENCODE basic APPRIS P1 TSL:1 |
| ENSMUST00000108343.8 | Akt2-204 | 3122 | 481aa | Protein coding | CCDS21027 | Q3TY95 Q60823 | GENCODE basic APPRIS P1 TSL:5 |
| ENSMUST00000051356.12 | Akt2-201 | 2923 | 481aa | Protein coding | CCDS21027 | Q3TY95 Q60823 | GENCODE basic APPRIS P1 TSL:1 |
| ENSMUST00000167435.8 | Akt2-214 | 2865 | 481aa | Protein coding | CCDS21027 | Q3TY95 Q60823 | GENCODE basic APPRIS P1 TSL:1 |
| ENSMUST00000147263.2 | Akt2-213 | 685 | No protein | Processed transcript | | - | TSL:3 |
| ENSMUST00000143347.2 | Akt2-211 | 983 | No protein | Retained intron | | - | TSL:3 |
| ENSMUST00000136981.2 | Akt2-208 | 770 | No protein | Retained intron | | - | TSL:2 |

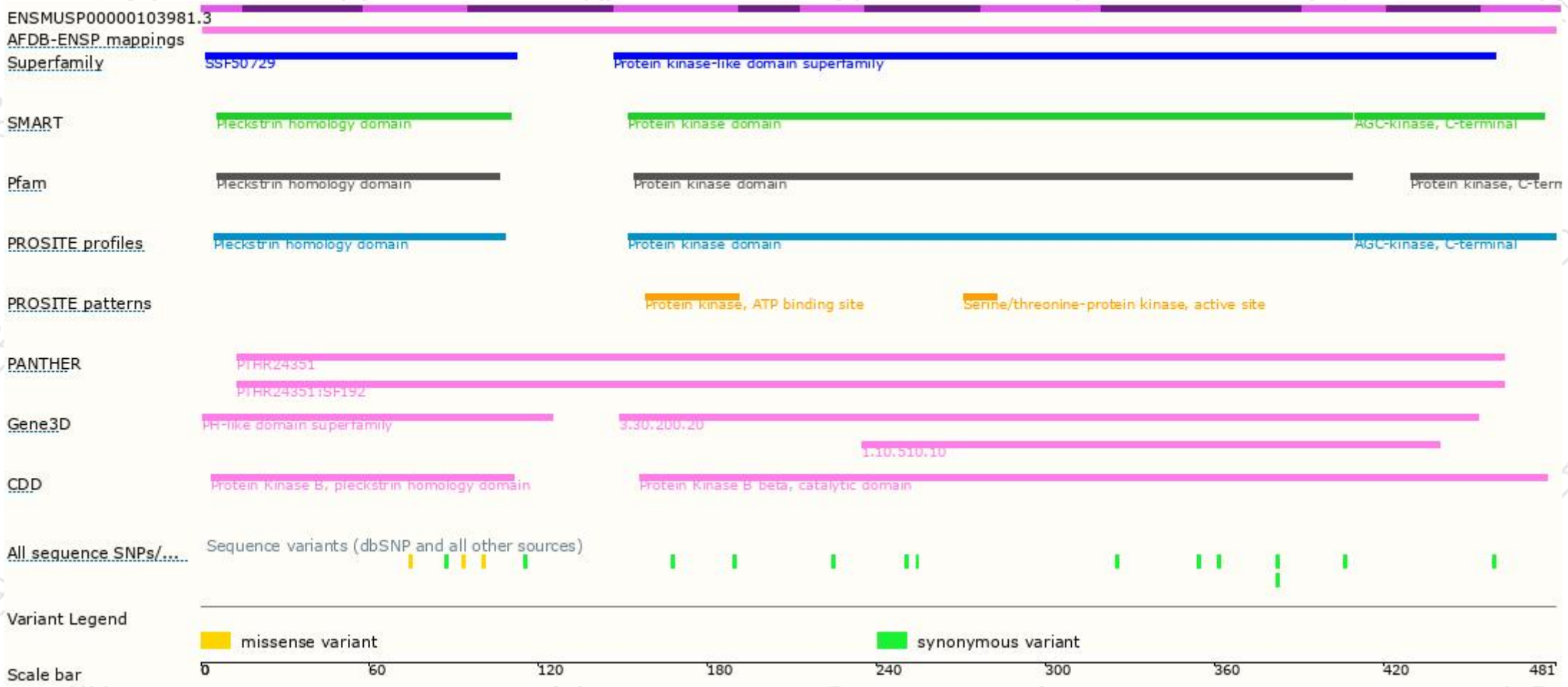
The strategy is based on *Akt2*-205, which contains 14 exons, is 4544 bps long, and encodes 481 amino acids.



# Mouse *Akt2* Genomic Information



# Mouse *Akt2* Protein Information



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**U.S. and E.U.**

1521 Concord Pike, Suite 301
Wilmington, DE 19803. USA
1.888.899.5899

**Asia and Other**

12 Xuefu Road, Jiangbei New Area District,
Nanjing, 210061 P.R., China
025-58641508

**Online**

globalservice@gempharmatech.com
http://gempharmatech.us/