

GENIQUEST Guide Part III

Disease at Gandwar

Driving Question- When is it appropriate to use genetics to study disease?

This section introduces the scale problem facing dragons. Because the disease strikes some populations but not others and does not appear to be communicable, it is appropriate to turn to genetics. The smaller, prolific “drake” which has a much shorter lifespan than the dragon can serve as a model much like the mouse provides a model for the study of human disease.

It is important that students understand that the QTL techniques they will learn are suited for diseases where two populations vary in susceptibility to disease. They should also be able to identify some advantages to using model organisms.

Questions for students to ponder after completing this section:

- 1. Why is it necessary to have healthy animals in this study when you are trying to study disease?***
- 2. List some organisms commonly used to model human biology.***
- 3. What are some concerns about using a model organism?***
- 4. How might the environment affect the disease state?***

Disease at Gandwar- Disease strikes the dragons	1
Trouble has come to Gandwar. In recent years, dragons have begun to become sick. Usually an extremely rare event indeed, this sickness has begun to affect dragon populations in several parts of the island. Healthy dragons have begun to become lethargic. The disease progresses gradually, causing the dragons' beautiful, glossy scales to turn white and fall off, beginning at the extremities and then proceeding toward the face. The disease progresses rapidly and leads to infertility and death. As this terrible condition known as Scaleblanche has arrived, dragons across the island have begun to fear for the survival of populations that have been hearty and hale for eons.	

Disease at Gandwar - Disease strikes the dragons	2
The dragon populations across the island have decided to hold a council of their elders and geneticists in an attempt to learn enough about the problem to protect themselves from further harm. At the council, they compare stories and experiences and learn some important things:	
1. Only the dragons from the Swamp, Valley and Mountain populations are affected by this disease. Desert, Ice and Forest dragons have been strangely unaffected.	
2. This disease does not seem to be contagious from one dragon to another.	
Because of these clues, the council decrees that a genetic study should be undertaken to	

attempt learn more about this disease.

OTrunk Viewer

File

 GENIQUEST

Main Activity

Disease at Gandwar

QTL Analysis

QTL Pitfalls

Disease Detectives

Disease at Gandwar

Disease strikes the dragons 2

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Sad dragon
dragonmarkers
Male
Color: Yellow

Navigation icons: back, forward, search, and a 3D cube icon.

User Data Will Not Be Saved!

Disease at Gandwar - Genetic study

3

The dragons have spent much time studying genetics to date, and have stumbled upon small creatures ideal for these studies. Miniature dragon-like creatures known as drakes, populate much of the island. They reproduce easily and live much shorter lifespans than dragons. These qualities make them ideal for breeding and studying. Because the genetics of drakes are quite similar to those of the dragons, they have been studied extensively.

The dragon elders explain that by breeding strains of these drakes, they can perform analyses to determine where on chromosomes genes are located. Your ears perk up – this is precisely what you did to discover where the gene for plates was located! You share your experience with them, hoping it may help.

The elders are indeed very impressed. After hearing about what you have learned at the conference, they hold a vote, and decide that the island dragons will combine their resources. They decree that you should be trained in the process of QTL Analysis. Once you have been trained, they will hire you to perform an analysis to determine where the gene for scaleblanche is located.

Continue to the next section to begin your training.

Introduction to QTL

Driving Question: What information does a QTL graph provide and how is the importance of a QTL peak evaluated?

This section introduces the graph of a QTL (quantitative trait loci) analysis which is used to predict the location of one of several genetic regions sharing responsibility for a quantitative trait. Although QTL analysis can help to identify a location for a gene solely responsible for a trait, one strength in the analysis is that it can be used to predict what portion of the variation can be explained by each QTL region. In this exercise, the statistical analysis remains in a “black box” but the output is easily interpreted. The x-axis bears chromosome position and along it is plotted the probability that region along the chromosome(s) contains genetic info responsible for the variation in trait. If no portion of the chromosome is associated with the trait you would expect random probability and relatively horizontal line. Peaks point toward areas of interest and here students are introduced to two statistical measures, LOD scores and confidence intervals.

Logarithms of probabilities for association are plotted on the y-axis as likelihood of odds (LOD) scores. Students are introduced to the idea that not all QTL peaks are significant and that LOD score alone can not be used as a measure of significance of peak height. Instead, the data helps determine acceptable thresholds for 63%, 90%, and 95% probability. Students are also introduced to the idea that more than one peak may exceed the thresholds.

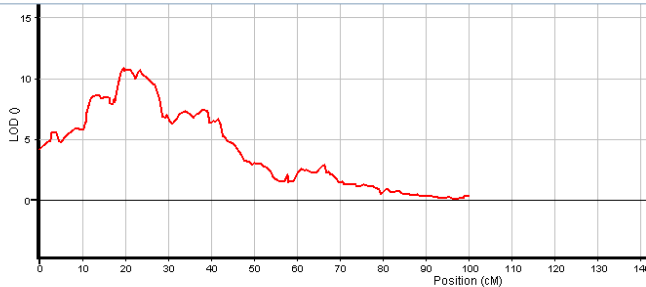
After identify and marking the highest peaks, students are introduced to the Confidence Intervals which flank the peaks. The physical area of the chromosome which falls within the 95% confidence interval describes the portion of the chromosome most likely to contain the genetic information responsible for the variation in observed phenotype. They are also introduced to one unit of measure of the chromosome, the CentiMorgan.

Students should understand that the red jagged line represents how well the pattern of genotype matches the pattern of phenotype. They should also understand that probability defines both the physical area(s) of the chromosomes most strongly associated with the phenotype under analysis and the thresholds for meaningful differences.

Questions for students to ponder after completing this section:

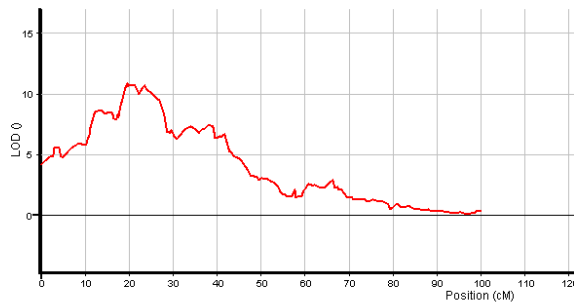
- 1. In which areas of the graph is there a strong relationship between genotype and phenotype?*
- 2. What would the graph look like if all sections of the chromosome had the same probability of explaining the trait?*
- 3. What would the graph look like if crossing over never occurred?*
- 4. Why is it important to include many animals in the analysis?*

5. *What is the probability that the gene(s) responsible for the peak lie outside the 95% confidence interval?*
6. *How do thresholds differ from confidence intervals?*
7. *What might be the purpose of multiple thresholds?*
8. *If an entire chromosome is scaled from 0-100 cM, can you estimate how many base pairs a centimorgan represents?*

Introduction to QTL- QTL Analysis		1
This is a plot of a QTL analysis. It was created by breeding two strains of dragons together, one that had a certain trait, and another that didn't, then breeding their children to make many offspring, as you did in a previous section. For example, some of the F2-generation children you made had plates, and others didn't. Because the children's genes were crossed over at many different points, we can use statistics to determine where the gene for plates is most likely to occur. This graph is similar to the graph that you created, but contains many more points. It shows how likely it is for the gene to occur at a certain place on a chromosome .		
 Main Activity Disease at Gandwar Introduction to QTL Disease Detectives	 What is the name for the variable on the graph's y-axis that shows probability? LOD Log of Odds What is plotted on the x axis of this graph? Location along chromosome in centi Morgans (cM) Which of these terms relates to physical distance? ☐ LOD ☒ cM Check Answer Correct! ✕ i That's right! Continue to the next page. OK	

Introduction to QTL- QTL Analysis

2



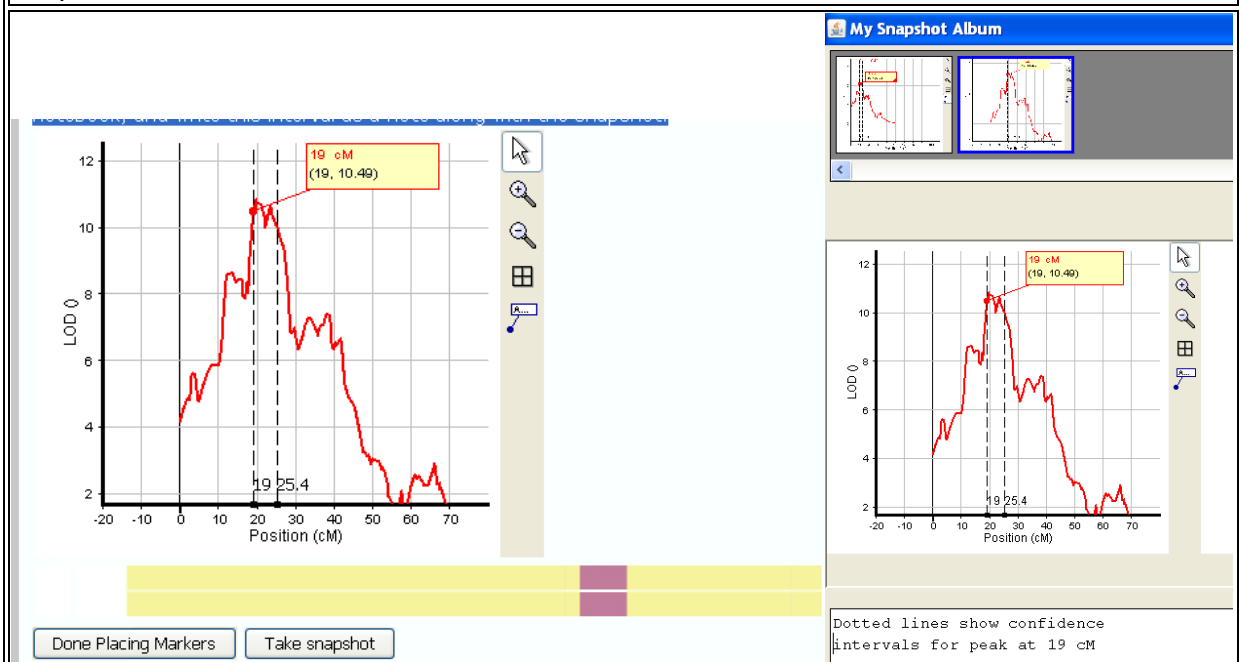
The LOD score is like the match score you saw earlier. It shows the likelihood that an area of the chromosome contains the gene that is causing your targeted phenotype.

The x-axis on the graph has units of centiMorgans, and relates to the relative distance along the chromosome. A chromosome is 100 centiMorgans long.

QTL Analysis - Pinpointing a Peak

3

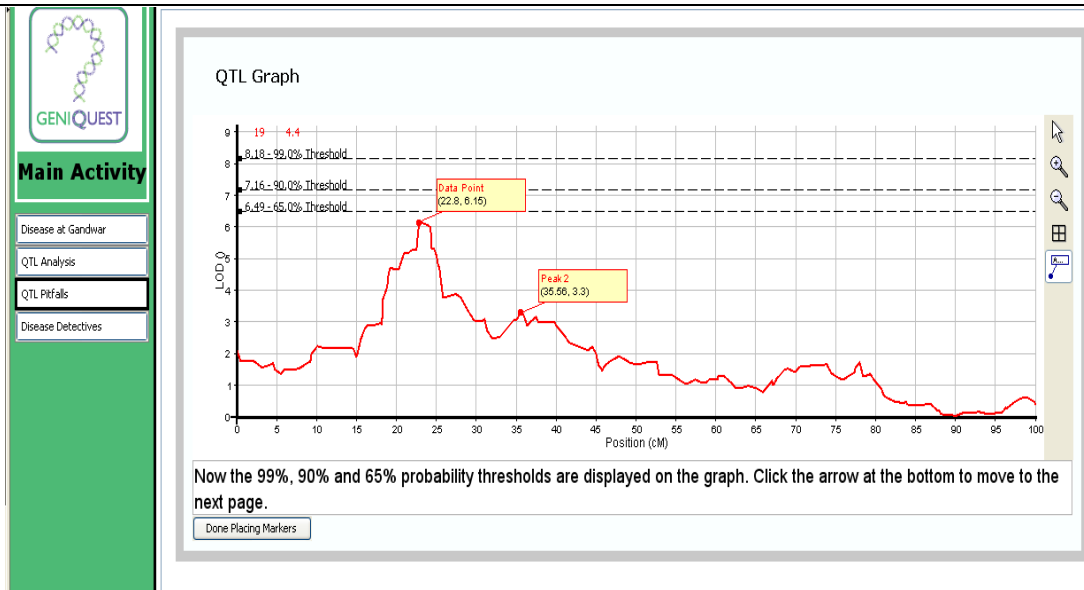
Place a marker on this graph where you think the gene for plates is most likely to be found. When you are done, click the button to show the 95% confidence interval for this peak. If you had to look somewhere along the chromosome and claim that you were 95% sure that the gene of finding the gene you were seeking, between which two locations would you have to look? Take a snapshot for your lab notebook, and write this interval as a note along with the snapshot.



Introduction to QTL – QTL Graph

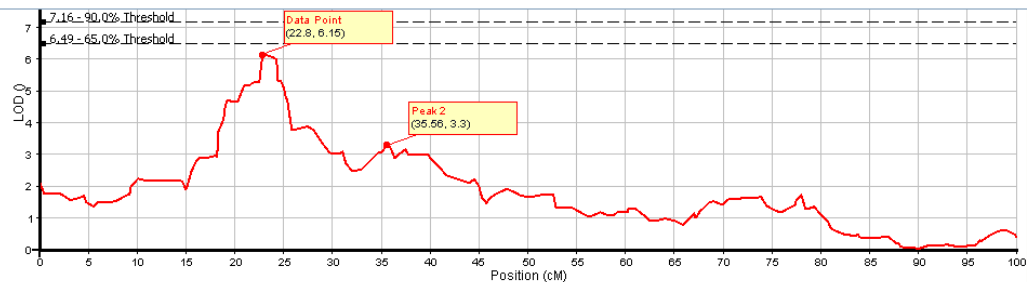
4

Here is a graph of a QTL main scan of Chromosome 1 for a cross between two strains of drakes. Place a label on any peaks you think might be worth exploring further. When you're finished placing markers, click on the button below.



QTL Pitfalls – QTL Graph

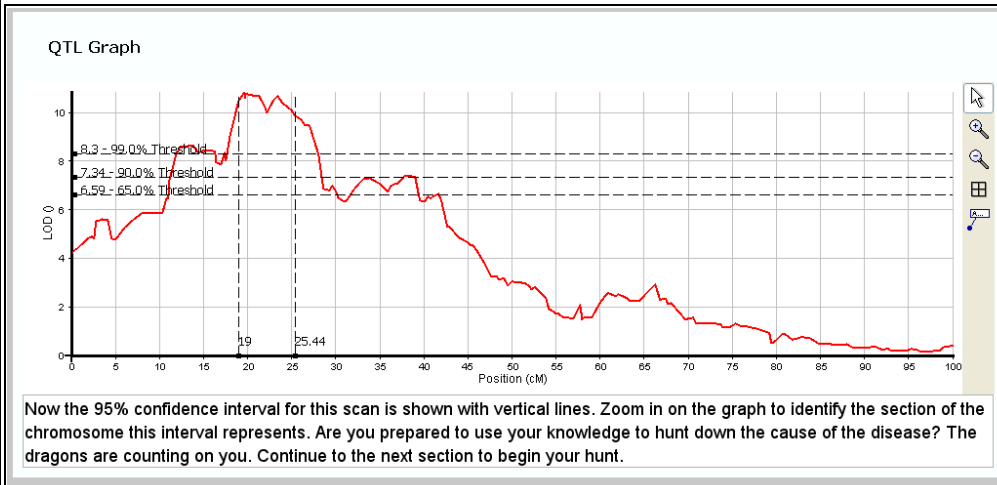
5



The horizontal threshold lines show the LOD score levels that would be expected when a peak on a QTL main scan graph has a 65%, 90% or 95% chance of not occurring entirely by chance. Based on the information from these threshold lines, do you still think your selected peaks are meaningful to investigate?

Introduction to QTL – QTL Graph

6



Now the 95% confidence interval for this scan is shown with vertical lines. Zoom in on the graph to identify the section of the chromosome this interval represents. Are you prepared to use your knowledge to hunt down the cause of the disease? The dragons are counting on you. Continue to the next section to begin your hunt.

Disease Detectives

Driving Question- How can QTL analysis lead to new connections between genes and disease?

Armed with a limited budget, students have the opportunity in this section to use QTL analysis to make pair-wise comparisons of six different drakes in order to identify:

- 1) The region of chromosome 1 containing a gene responsible for the disease seen in the dragons of the mountain, valley, and swamp.**
- 2) A possible gene within said region which might be involved.**

Selecting pairs with similar resistance will fail to be informative resulting in an exhaustion of resources before conclusions can be drawn. A fresh start will provide additional funds. Once a region is identified, input of the overlapping confidence intervals of the QTL outputs will send the user to a dragon browser where several drake genes such as *Arfp1*, *Mef2d*, *Tmprss6*, *Sprr2d*, *Mlt11*, and *Sv2a* which are shared with mice are found. Clicking on an individual gene directs the user to the Mouse Genome Informatics (MGI) query which will list any genes with that gene symbol and provide the full name of the gene along with a hyperlink to the MGI webpage for that gene. There, the reader can learn a plethora of information about the gene including it's unique MGI identifying number, on which mouse chromosome it is found, its mouse physical location defined by base pair, and whether it has been identified in other mammals. Most useful to study of dragon genetics is information about known phenotypes of alleles and any reported effects of mutations. Using this information, the student may conclude that a mutation on *Tmprss6* may be responsible for the disease.

Students must understand that QTL analysis depends on selecting strains that differ in the trait in question, and that different pair combinations of disease resistant and non-disease resistant strains results in different peaks and confidence intervals.

Questions for students to ponder after completing this section:

- 1. Which pairs of drakes were most/least informative?**
- 2. How many genes were found within the confidence intervals?**
- 3. Where within the MGI gene detail page did you find the information that implicated your gene was related to the disease?**

Disease Detectives - The Investigation

1

Your goal is to find out what is causing this debilitating disease among certain dragons. Remember that you have only Drakes on which to do experiments, and that the various strains of Drakes are matched to various groups of dragons.

Recall that the Mountain, Valley and Swamp dragons are the only strains that are affected with this disease.

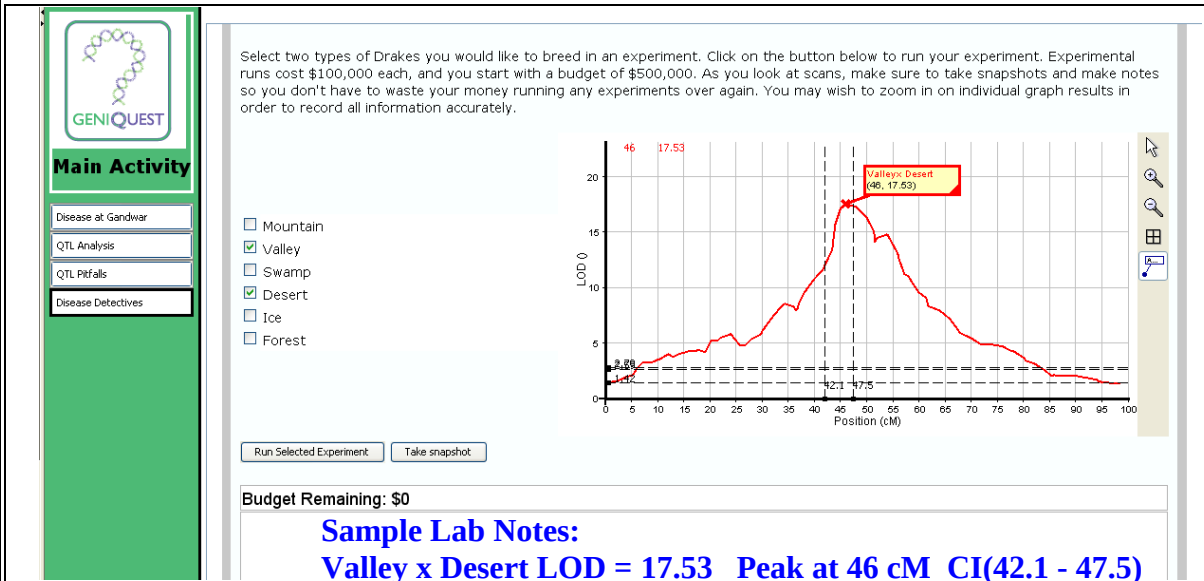
Experiments cost money and take time, so you will only be able to perform experiments on a few crosses. Use what you already know about Drakes to help you select which strains to breed in experiments and how many Drakes to use in each experiment. Make sure to keep watch on your budget so you don't run out!

When you find intervals you think you should explore, take notes on what the intervals are. You can explore them with a link on the next page.

Disease Detectives - Breeding

2

Select two types of Drakes you would like to breed in an experiment. Click on the button below to run your experiment. Experimental runs cost \$100,000 each, and you start with a budget of \$500,000. As you look at scans, make sure to take snapshots and make notes so you don't have to waste your money running any experiments over again. You may wish to zoom in on individual graph results in order to record all information accurately.



Gene Browsing Overview

Showing 64 kbp from 1, positions 190,000 to 254,000

Instructions
Search using a sequence name, gene name, locus, or other landmark. The wildcard character * is allowed. To center on a location, click the ruler. Use buttons to change magnification and position.

Examples: 1, 1:680000..780000, Gene:Sry.

[Hide banner] [Bookmark this] [Link to Image] [High-res Image] [Help] [Reset]

Search
Landmark or Region: Search

Data Source
Dragon

Overview
Scroll/Zoom: <<< Show 64 kbp >>> Flip

Details
These are individual genes. Click them to learn more.

The whole chromosome

The part being viewed below

Overview of 1
0.1M 0.2M 0.3M 0.4M 0.5M 0.6M 0.7M 0.8M 0.9M 1M 1.1M

Dragon Genes (Overview)

Brachyury
Prnd
Rabp9
Tnfrsf4
Acsc2
B230339V5R1A
Kcnk11

The image above shows an overview page of the gene browser. This page displays an overview of a specific chromosome opened to a certain interval, and shows the genes as black tick marks. Each gene displayed is named, and clicking on the gene name will display an overview page for that gene, as shown on the next page.

Gene Browsing Overview

MGH

Tour the MGH website

Home Genes Phenotypes Expression Function Pathways Strains / SNPs Orthology

Search Download More Resources Submit Data Find Mice (IMSR) Contact Us

Search Results

Search for: Tnfrsf4

Genes and Markers:

Searched Nomenclature (symbols, names, synonyms, alleles, and orthologs)

Chr: chr11 Genome Coordinates: 152987039-153036202 (+) Symbol Name: **Tnfrsf4** Matching Text: current symbol: Tnfrsf4

Accession IDs: No results found.

Click here for the gene info page

This image above shows the gene overview page. This page displays basic information about the selected gene. To show more detail, click on the gene name again. This will show the gene detail, as shown on the next page.

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Tm9sf4 Gene Detail Your Input Welcome

Symbol **Tm9sf4** *This is the detailed gene information page.*
 Name transmembrane 9 superfamily protein member 4
 ID MGI:2139220 [Nomenclature History](#)

Synonyms mKIA0255

Genetic Map Chromosome 2
 Syntide
If there is information available about how this gene appears, it will be here.
 Mapping data[...]

Sequence Map Chr2:132987039-133036202 bp, + strand
 (From VEGA annotation of NCBI Build 37)
[VEGA ContigView](#) | [Ensembl ContigView](#) | [UCSC Browser](#) | [NCBI Map Viewer](#)

Mammalian Homology human; chimpanzee; dog; domestic rat ([Mammalian Orthology](#))
 Protein Superfamily: transmembrane 9 superfamily protein, member 3 type
 Treefam: [TF354239](#)

Sequences

Representative Sequences	Length	Strain/Species	Flank
☐ genomic OTTMUSG00000015775 VEGA Gene Model MGI Sequence Detail	49164	C57BL/6J	a 0 Kb
☐ transcript NM_133317 RefSeq MGI Sequence Detail	3895	C57BL/6	
☐ polypeptide Q9B124 UniProt EBI MGI Sequence Detail	643	Not Applicable	

For the selected sequences [download in FASTA format](#) (Go)

Phenotypes (All phenotypic alleles[...]) : Gene trapped[...]
 Polygenic trait: SNPs within 245cM from 650Kp 800K 128K

Gene Ontology (Component) [integral to membrane](#), [membrane](#)...

This page shows detailed information about the gene you selected. Look in the Phenotypic section too see if there is useful information about inherited traits for the gene you are viewing. [Click here now to try out the genome browser](#)

Enter the beginning and end of an interval in units of cM, and click on the button to call up that interval in a genome browser. Your goal is to look through the genes you find there to find a candidate for causing the disease. Start: **45** End: **48**

Launch GBrowse

Dragon: 1:450000..480000 - Mozilla Firefox

http://cgd.jax.org/cgi-bin/genquest/dragon/?start=450000;stop=480000;ref=1;width=800;version=100;label=Genes%3Aoverview;Genes;grid=on

CGD GBrowse : Mus Musculus

CGD Home Back to Karyoview Other Genome Browsers: MGI UCSC Ensembl Perlegen NCBI VEGA

Draco familiaris

Disclaimer: This is a test site for the Dragon genome at [link pending]. The information on this site is for test purposes only.

Showing 30 kbp from 1, positions 450,000 to 480,000

Instructions
Search using a sequence name, gene name, locus, or other landmark. The wildcard character * is allowed. To center on a location, click the ruler. Use the Scroll/Zoom buttons to change magnification and position.

Examples: 1, 1:680000..780000, Gene.Sry.

[Hide banner] [Bookmark this] [Link to Image] [High-res Image] [Help] [Reset]

Search
Landmark or Region: 1:450000..480000 Search

Data Source
Dragon

Overview

Overview of 1

Dragon Genes (Overview)

Dragon Genes

Arfip1 Mef2d Tmprss6 Sprr2d M11411

460k 470k

Clear highlighting Update Image

Scroll/Zoom: <<< < < Show 30 kbp > >> >>> Flip

Overview of 1

0.1M 0.2M 0.3M 0.4M 0.5M 0.6M 0.7M 0.8M 0.9M 1M 1.1M

(Overview)

460k 470k

Arfip1 Mef2d Tmprss6 Sprr2d

Click on hyperlink to learn more about desired gene.

MGI Quick Search Results - Mozilla Firefox

http://www.informatics.jax.org/searchtool/Search.do?query=tmprss6

Quick Search Results for: [Search Again](#) [Reset](#) [Your Input Welcome](#)

Examples: embry* develop* NM_013627 Fas-clpr Pax* axial "skeletal dysplasia" Tg(ACTB-cre)2Mit

See [details](#) for this search.

Genome Features sorted by best match, showing 1-1 of 1

Score	Type	Symbol	Name	Chr	Best Match
★★★★	Gene	Tmprss6	transmembrane serine protease 6	15	SYMBOL: Tmprss6 and 8 more...

[Get more data](#) for genome features 1 through 1

Vocabulary Terms no results

Other Results By ID no results

Search MGI with Google

[Search](#)

Contributing Projects: Mouse Genome Database (MGD), Gene Expression Database (GXD), Mouse Tumor Biology (MTB), Gene Ontology (GO), MouseCyc

[Citing These Resources](#) [Funding Information](#) [Warranty Disclaimer & Copyright Notice](#)

last database update 03/20/2009 MGI_4.2

The Jackson Laboratory

Click on hyperlink to learn more about desired gene.

Tmprss6 MGI Mouse Gene Detail - MGI:1919003 - transmembrane serine protease 6 - Mozilla Firefox

http://www.informatics.jax.org/javam2/servelet/WiFetch?page=markerDetail&key=55733

Tmprss6 Gene Detail [Your Input Welcome](#)

Symbol Name ID	Tmprss6 transmembrane serine protease 6 MGI:1919003			Nomenclature History
Synonyms	1300008A22Rik, matriptase-2			
Genetic Map	Chromosome 15 cytoband E2 Mapping data(2)			
Sequence Map	Chr15:78270098-78299064 bp, - strand (From Ensembl annotation of NCBI Build 37) Ensembl ContigView UCSC Browser NCBI Map Viewer			
Mammalian homology	human; dog, domestic; rat (Mammalian Orthology) Protein SuperFamily: matriptase-2 TreeFam: TF330647			
Sequences	Representative Sequences	Length	Strain/Species	Flank
☐ genomic	ENSMUSG00000016942	28967	C57BL/6J	± 0 kb
☐ transcript	NM_027902	3206	C57BL/6	
☐ polypeptide	Q9DBI0	811	Not Applicable	
For the selected sequences download in FASTA format Go				
All sequences(19)				
Phenotypes	All phenotypic alleles(4) : Targeted, knock-out(1) Chemically induced(3) Homozygosity for an inactivating mutation of this gene results in hair loss over the entire body except the face, microcytic anemia and female infertility, all reversible by dietary iron supplementation.			

Homozygosity for an inactivating mutation of this gene results in hair loss over the entire body except the face, microcytic anemia and female infertility, all reversible by dietary iron supplementation.

Disease Detectives	4
QTL Analysis Which gene did you identify as a potential candidate gene? Tmprss6 transmembrane serine protease 6 What evidence did you find that caused you to identify this gene in particular? Mutation of this gene results in hair loss over the entire body except the face, microcytic anemia and female infertility, all reversible by dietary iron supplementation.	

Main Scans of Drake Chromosome 1 For Scale Disease

