

# Maple Syrup Urine Disease and the DBT Gene

**Tessa Bachinski**



**WISCONSIN**  
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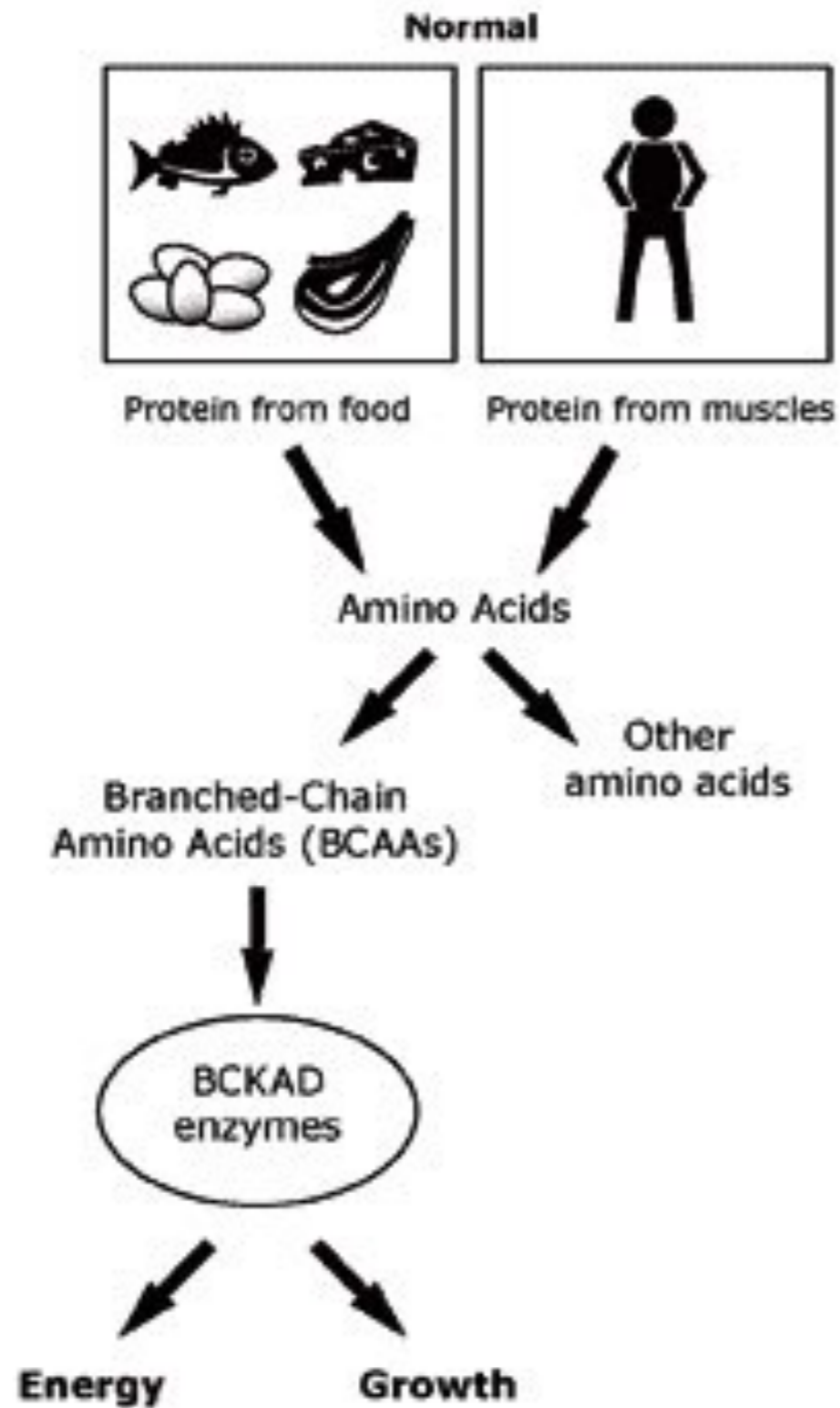
# What is Maple Syrup Urine Disease (MSUD)?

Recommended Uniform Screening Panel  
Core Conditions  
(As of July 2018)

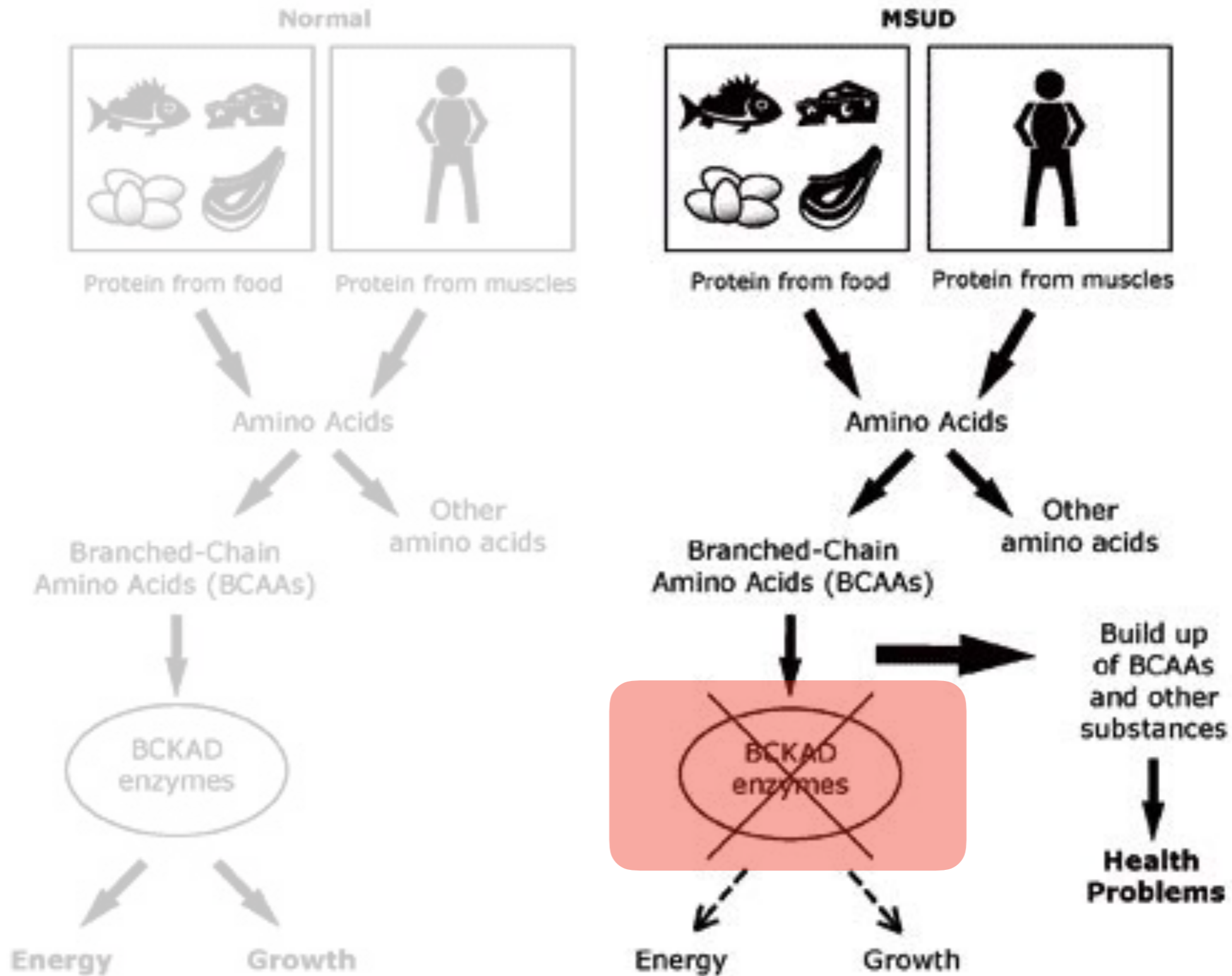


Core Condition	Metabolic Disorder			Endocrine Disorder
	Organic acid condition	Fatty acid oxidation disorder	Amino acid disorder	
Propionic Acidemia	X			
Methylmalonic Acidemia (methylmalonyl-CoA mutase)	X			
Methylmalonic Acidemia (Cobalamin disorders)	X			
Isovaleric Acidemia	X			
3-Methylcrotonyl-CoA Carboxylase Deficiency	X			
3-Hydroxy-3-Methylglutaric Aciduria	X			
Holocarboxylase Synthase Deficiency	X			
$\beta$ -Ketothiolase Deficiency	X			
Glutaric Acidemia Type I	X			
Carnitine Uptake Defect/Carnitine Transport Defect		X		
Medium-chain Acyl-CoA Dehydrogenase Deficiency		X		
Very Long-chain Acyl-CoA Dehydrogenase Deficiency		X		
Long-chain L-3 Hydroxyacyl-CoA Dehydrogenase Deficiency		X		
Trifunctional Protein Deficiency		X		
Argininosuccinic Aciduria			X	
Citrullinemia, Type I			X	
Maple Syrup Urine Disease			X	
Homocystinuria			X	
Classic Phenylketonuria			X	
Tyrosinemia, Type I			X	

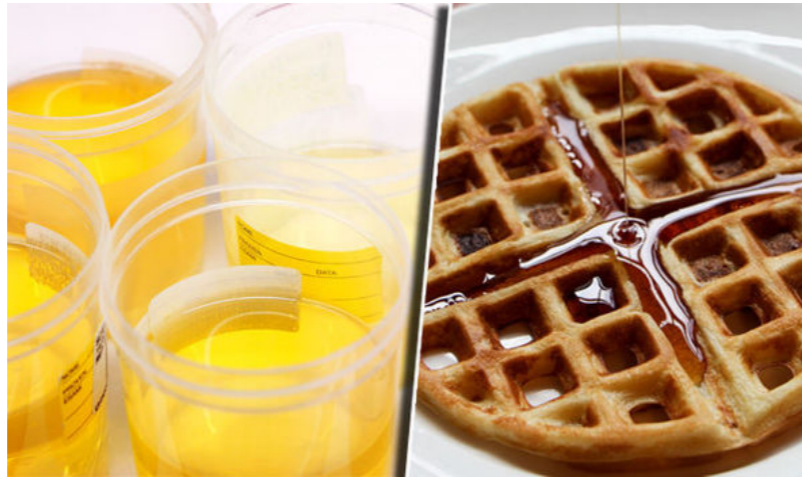
# What Causes Maple Syrup Urine Disease?



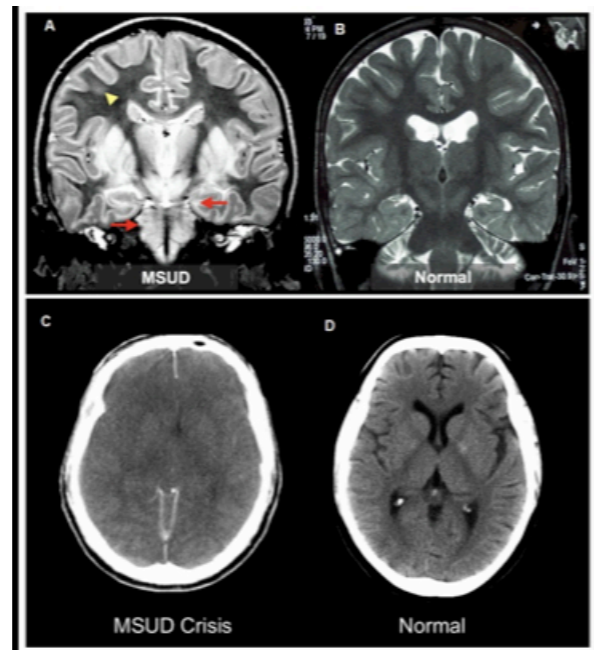
# What Causes Maple Syrup Urine Disease?



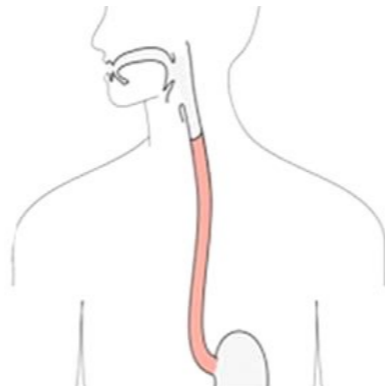
# What are the symptoms of Maple Syrup Urine Disease?



**Sweet Smelling Urine**



**Brain Damage, Seizures,  
and Other Neurological  
Symptoms**



**Poor Feeding, Lethargy**

# What is the **DBT** Gene?

Biotin lipoyl

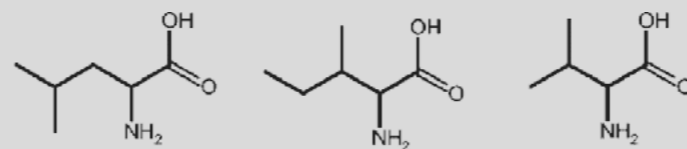
E3  
binding

2-oxoacid dehydrogenases  
acyltransferase (catalytic domain)

## Cellular Function



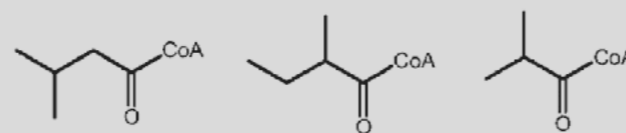
## Biological Function



Branched chain amino acid transaminase

$\alpha$ -keto-acid

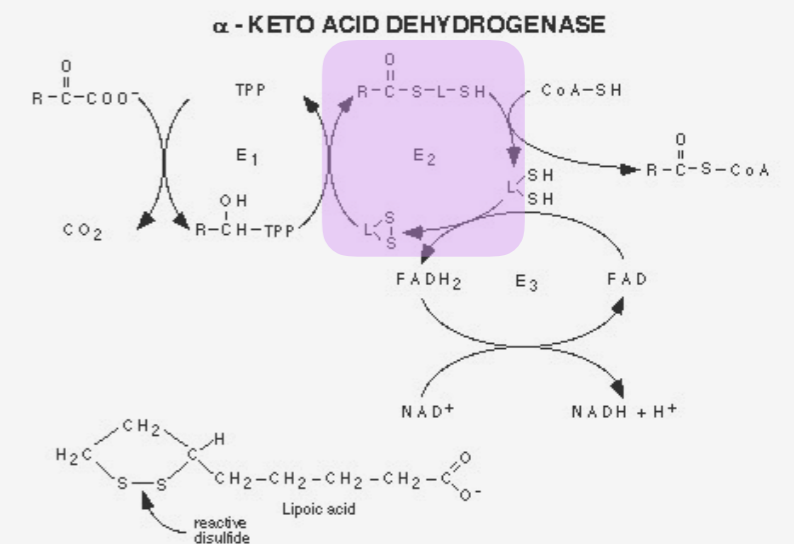
Branched chain keto acid dehydrogenase (Bkd or Esg)



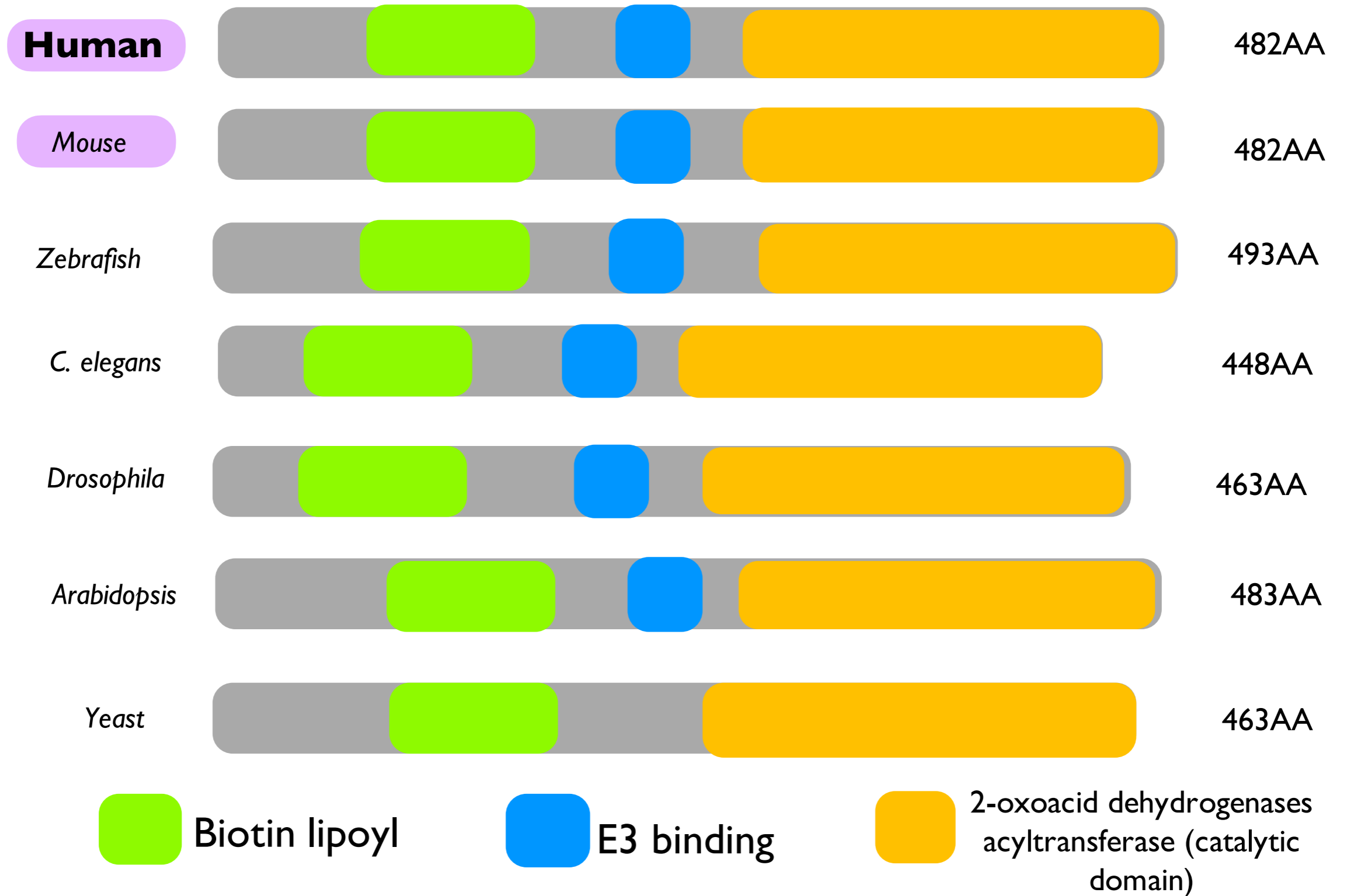
iso fatty acids  
ante-iso fatty acids

Secondary metabolites  
e.g. myxothiazol  
myxalamid, avermectin

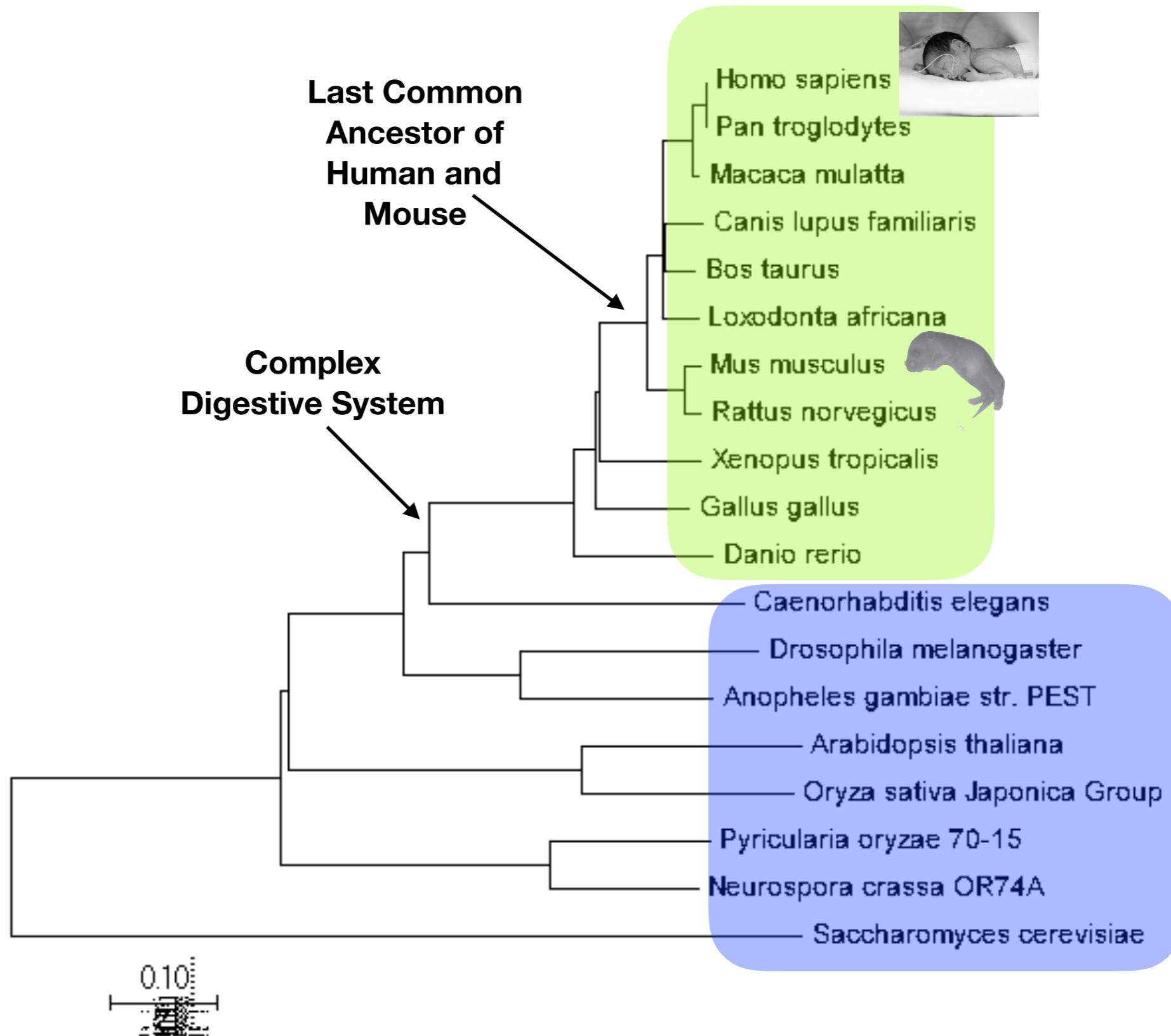
## Molecular Function



# How Well is **DBT** Conserved?

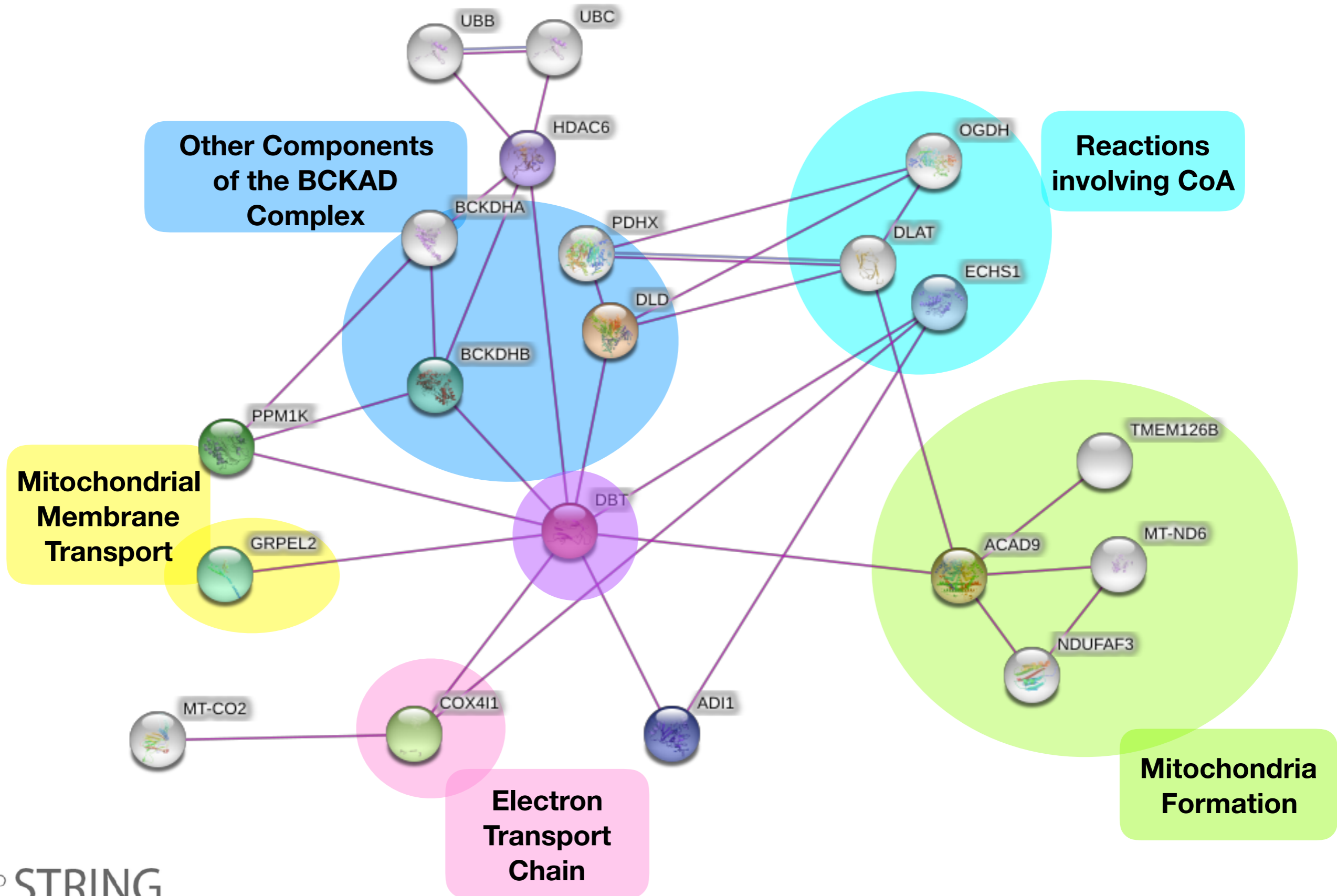


# What is the Phylogenetic Relationship?

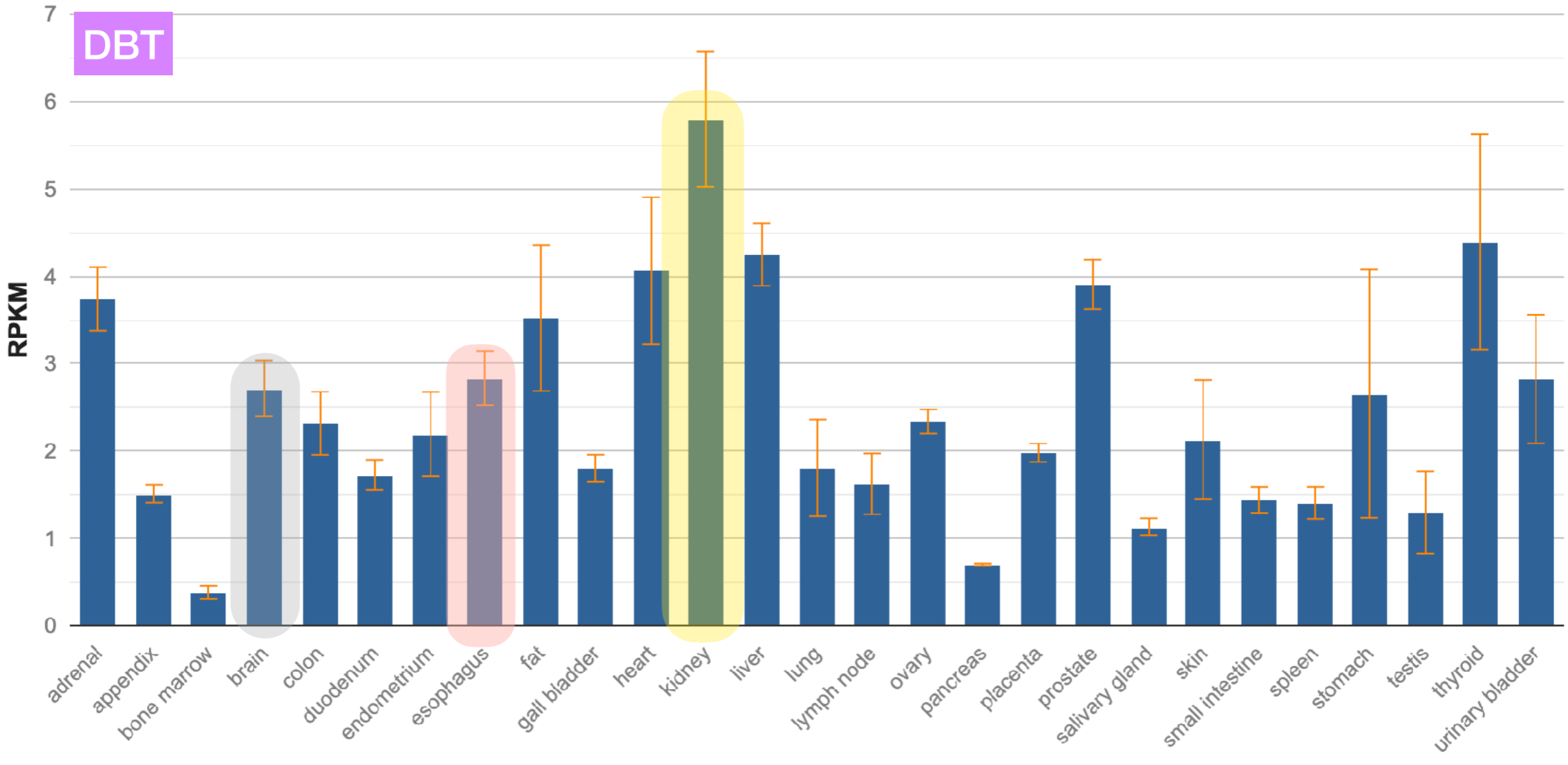




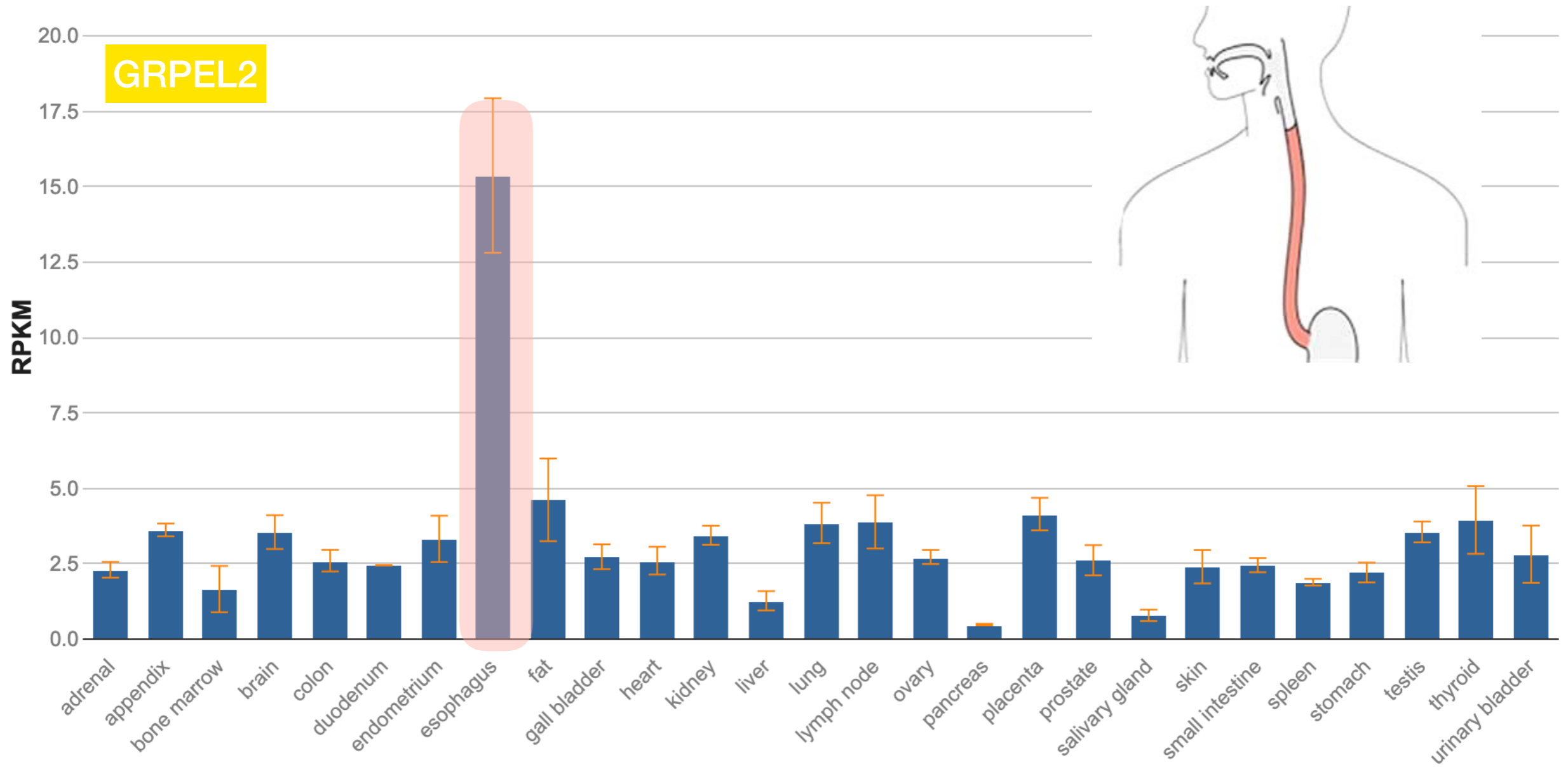
# DBT is associated with the mitochondria



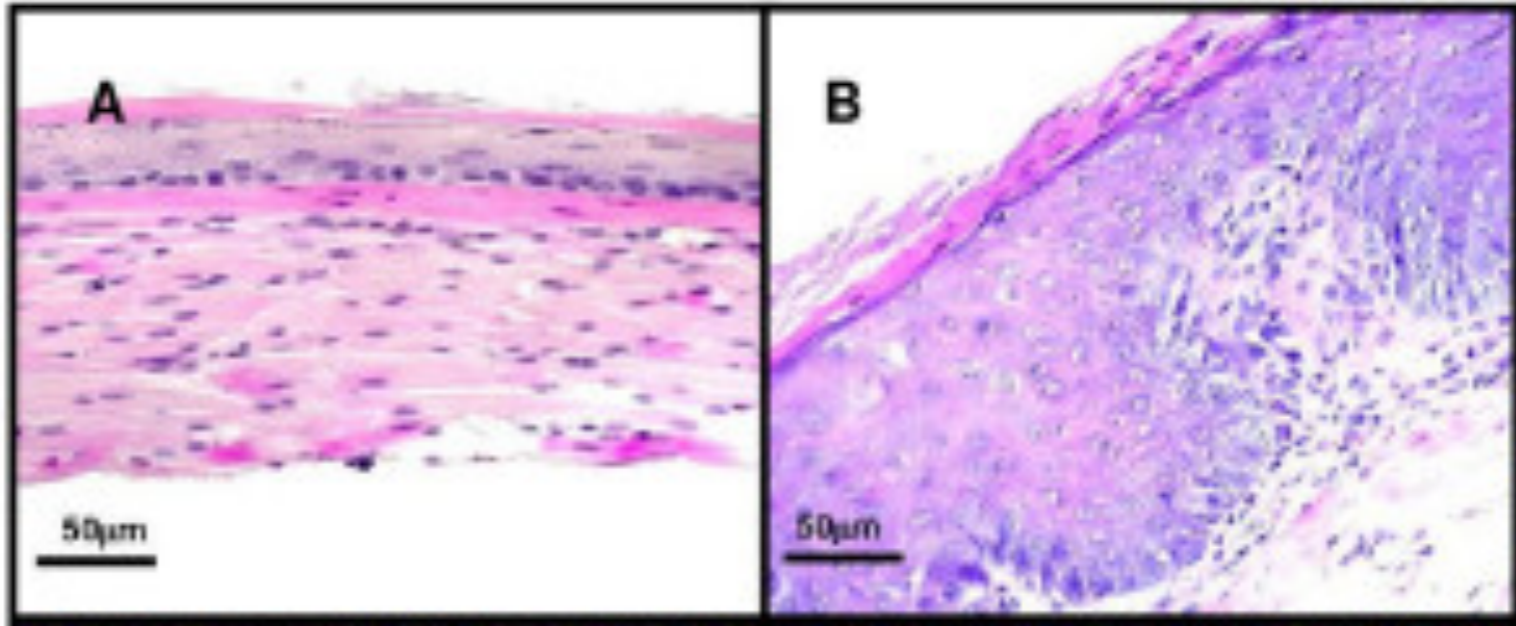
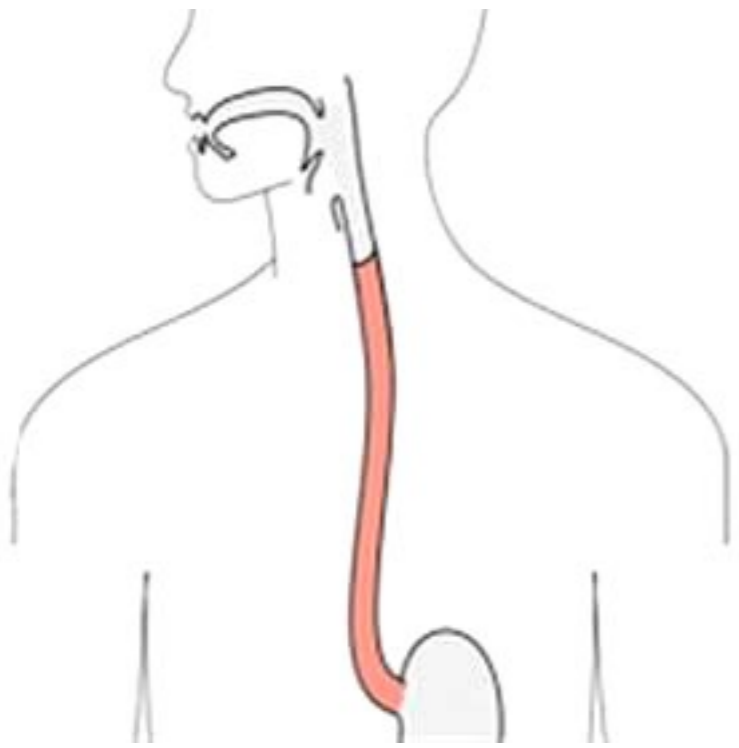
# DBT is highly expressed throughout the body



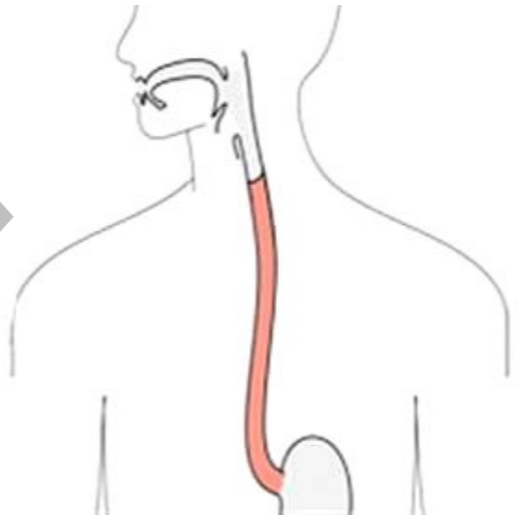
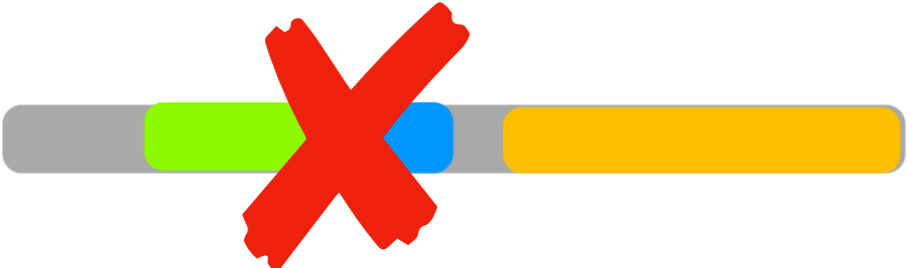
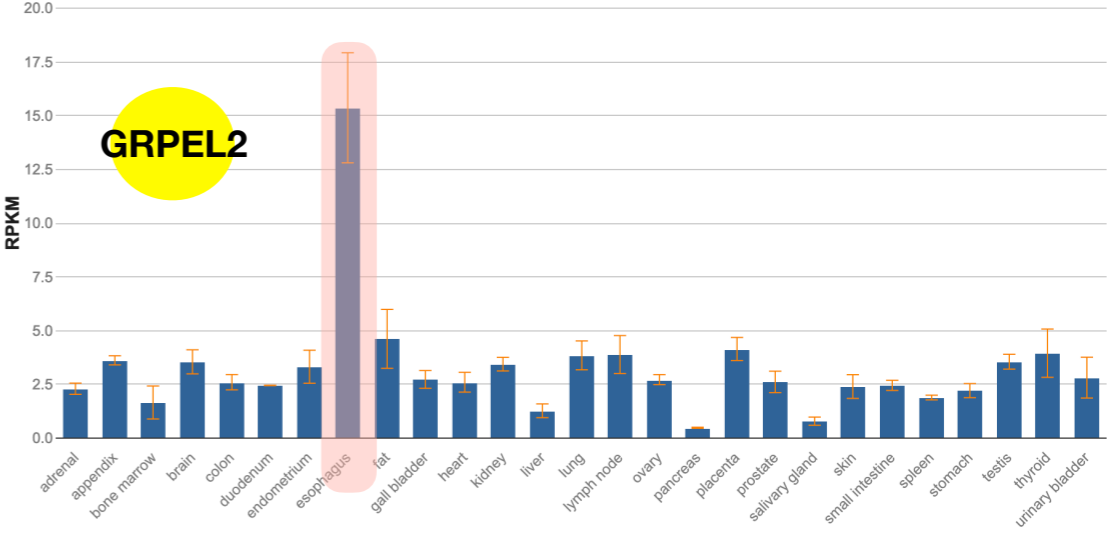
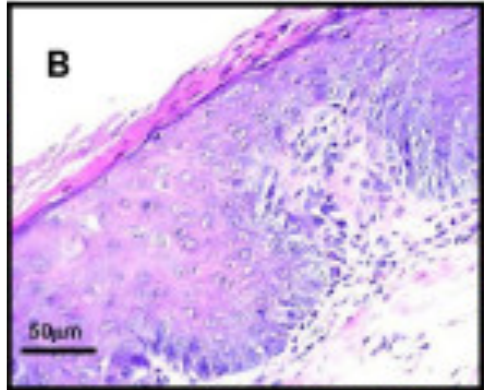
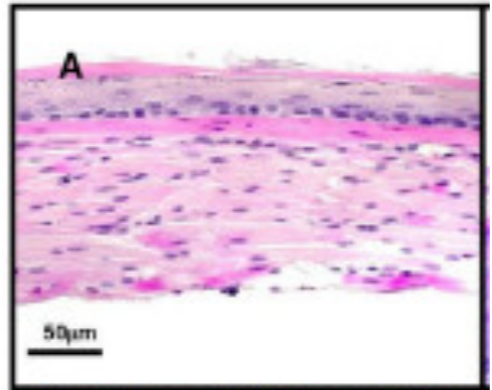
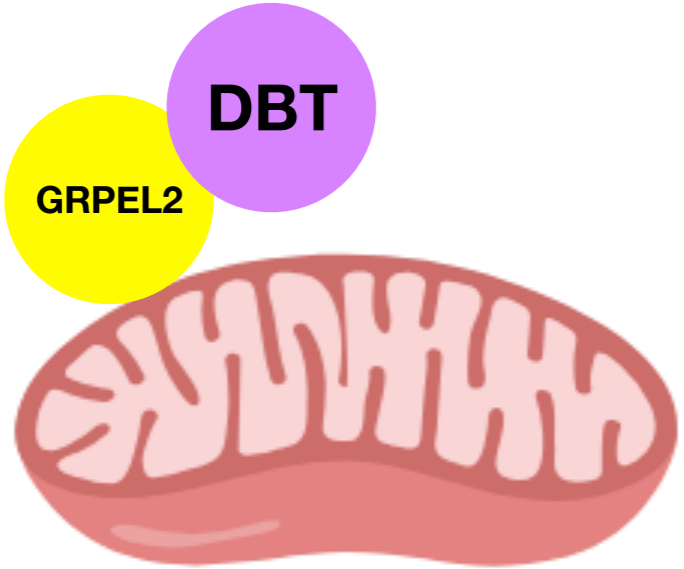
# Interacting protein **GRPEL2** highly expressed in the **esophagus**



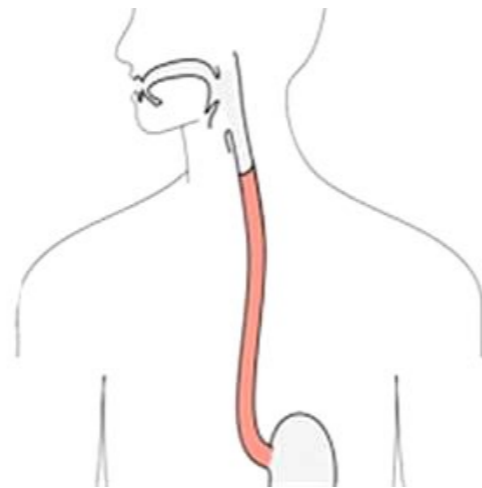
# Mitochondrial disorders have been known to cause GERD



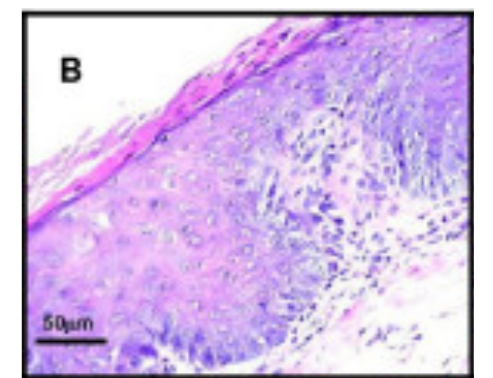
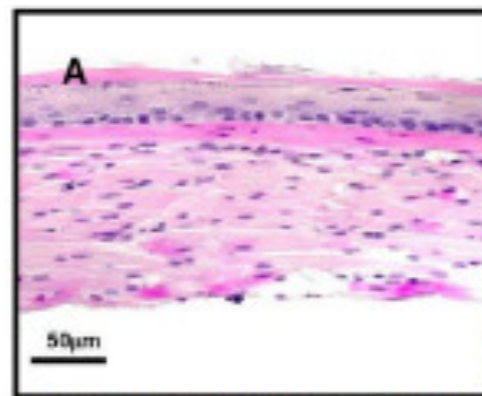
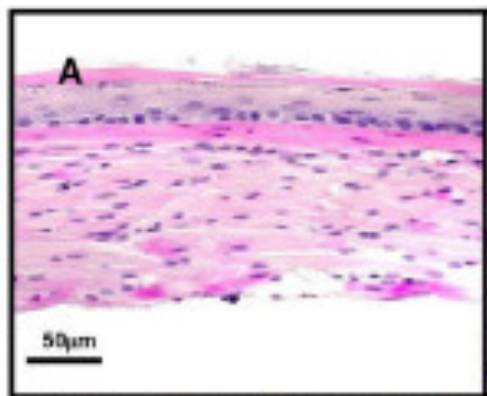
# What is the gap in knowledge?



# Primary Goal: Determine how **DBT** plays a role in esophagus function in the early postnatal period



# Model Organism: Mus Musculus



**Wild Type**

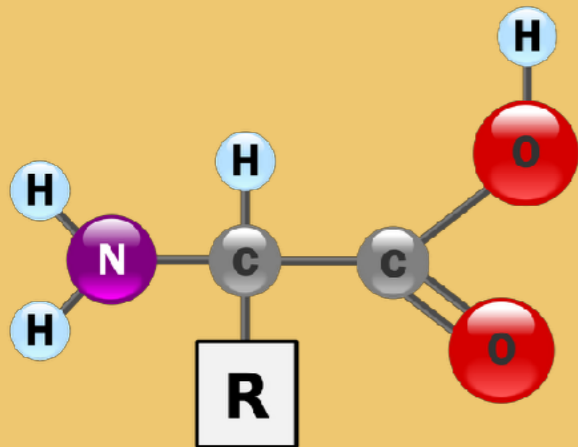
**DBT -/-**

**DBT -/-  
Poor Feeding**

# Determine the role of **DBT** and esophagus function

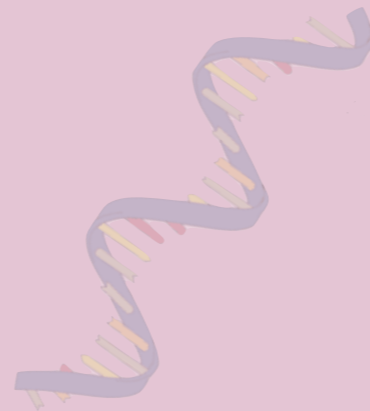
Aim 1:

Identify amino acids that are important for feeding



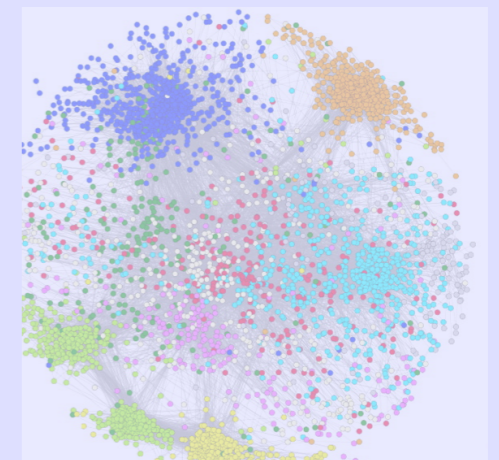
Aim 2:

Determine transcripts that impact feeding



Aim 3:

Identify proteins necessary for proper feeding



**Hypothesis: Conserved amino acids among complex digestive system organisms are important for feeding**



# Aim 1

Sequence Alignment

CRISPR Mutant Screen

Sequence Mutants

Simple



Complex

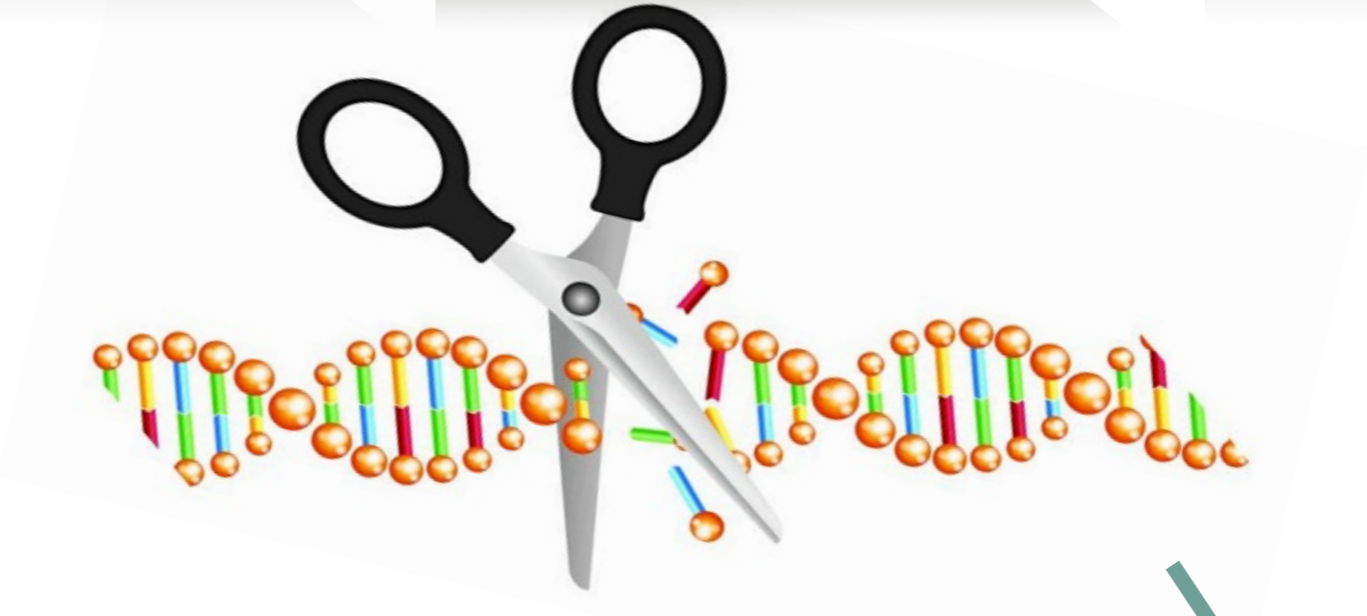
<i>Saccharomyces</i> /1-463	194	E - - - - -	- - - - -	- - -	AAPKKEVTEPKK-
[ <i>Pyricularia</i> /1-523	227	EDIYKFVQ	GKQSGSGV	APTIPA	- - - - -APA
[ <i>Neurospora</i> /1-562	260	EDVYKFLQ	ARDSAP	TLYP	SAATPTSPGGTA
[ <i>Arabidopsis</i> /1-483	213	EDVLRFS	QKGFVT	-DSV	SSEHAVIGG- - -
[ <i>Oryza</i> /1-523	248	EDVLSYAA	SKGLCK	-EPT	SALEENIDQVEL
[ <i>Drosophila</i> /1-462	191	GDI LEFLC	QVPPGT	-NVP	HPTLLAKTP- - -
[ <i>Anopheles</i> /1-470	198	GDVLEFL	EVIPKGT	-VKP	HPSLVAKEQ- - -
[ <i>Caenorhabditis</i> /1-448	176	EDVLKFLC	QVPADH	-TSG	STNIRTT- - - - -
[ <i>Danio</i> /1-493	203	EDILNFI	A KQTGAI	-LPP	APFQEIRPQPPA
[ <i>Xenopus</i> /1-492	203	EDILSFL	A KQTGAI	-LPP	SPQMEITPPPPK
[ <i>Mus</i> /1-482	202	EDILSFL	E KQTGAI	-LPP	SPKSEITPP- - -
[ <i>Rattus</i> /1-482	202	EDILNFL	E KQTGAI	-LPP	SPKSEITPP- - -
[ <i>Loxodonta</i> /1-482	202	EDILNYL	A KQTGAI	-LPP	SPKAEIMLP- - -
[ <i>Canis</i> /1-482	202	EDILNYL	E KQTGAI	-LPP	SPKAELVPP- - -
[ <i>Bos</i> /1-482	202	EDILNYL	E KQTGAI	-LPP	SPKAEIMPP- - -
[ <i>Macaca</i> /1-482	202	EDILNYL	E KQTGAI	-LPP	SPKAEIMPP- - -
[ <i>Homo</i> /1-482	202	EDILNYL	E KQTGAI	-LPP	SPKVEIMPP- - -
[ <i>Pan</i> /1-524	202	EDILNYL	E KQTGAI	-LPP	SPKVEIMPP- - -
[ <i>Gallus</i> /1-493	204	EDILSFL	A KQTGAI	-LPP	SPKAEIIAPLSK

# Aim 1

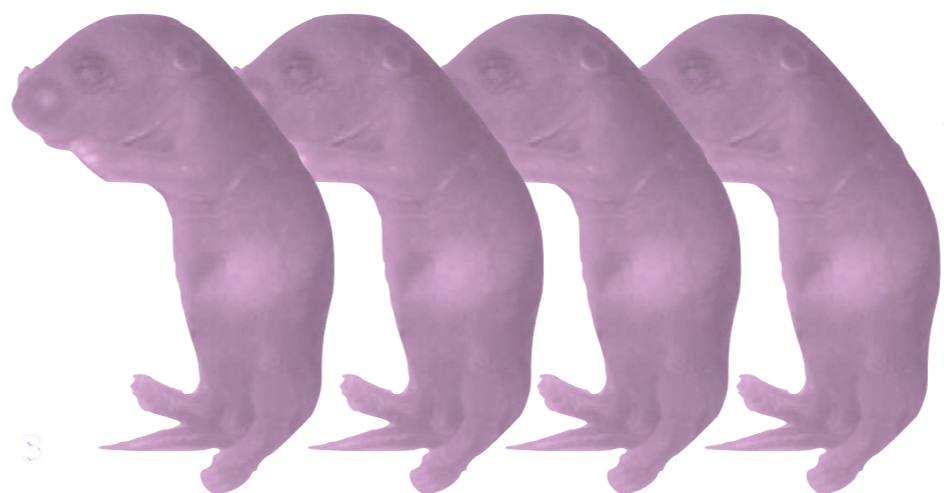
Sequence Alignment

CRISPR Mutant Screen

Sequence Mutants



**Wild Type**



**DBT -/-**



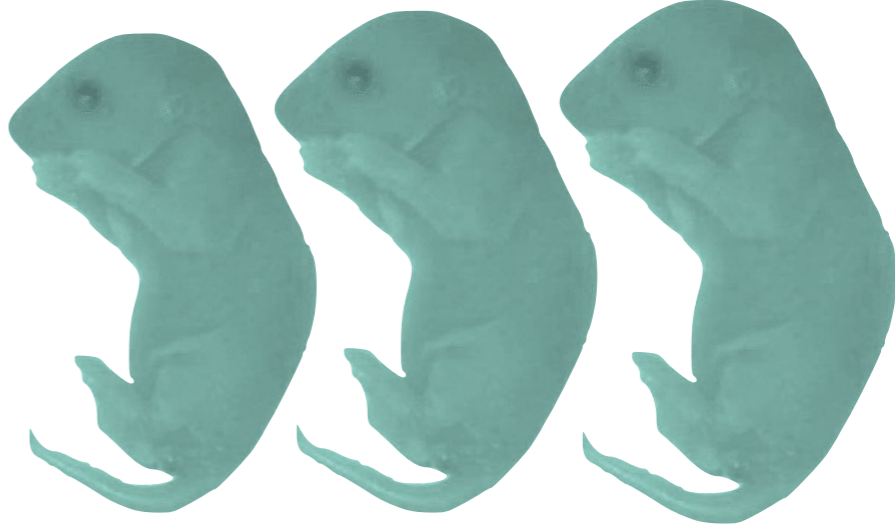
**DBT -/-  
Poor Feeding**

# Aim 1

Sequence Alignment

CRISPR Mutant Screen

Sequence Mutants



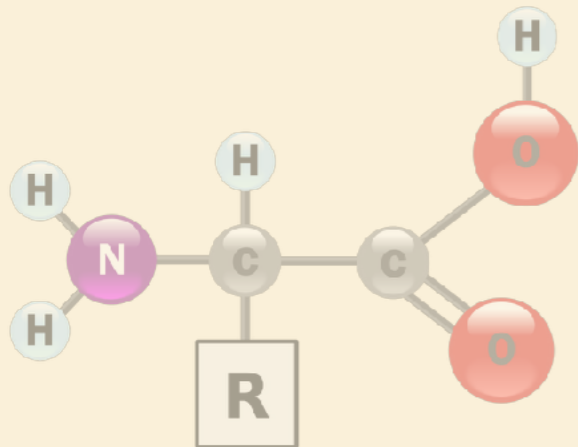
Arg301His  
Gly345Arg  
Tyr42Ter  
Lys313Asn

Lys211Gly  
Gln212Val  
Ile216Lys

# Determine the role of **DBT** and esophagus function

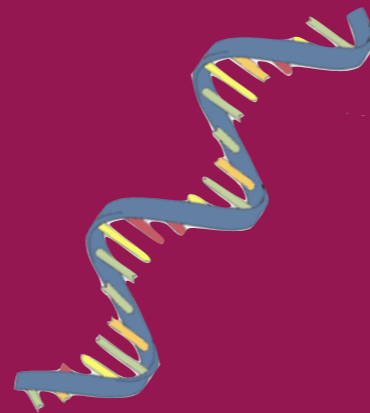
Aim 1:

Identify amino acids that are important for feeding



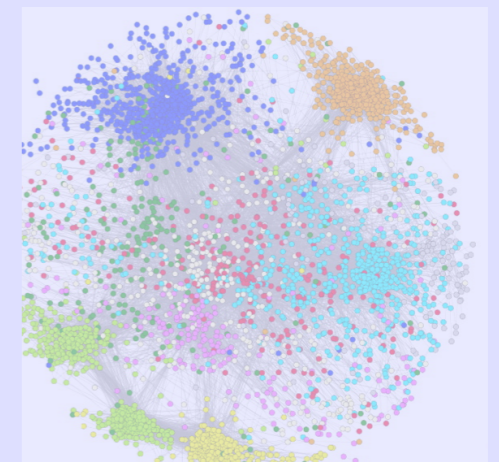
Aim 2:

Determine transcripts that impact feeding



Aim 3:

Identify proteins necessary for proper feeding



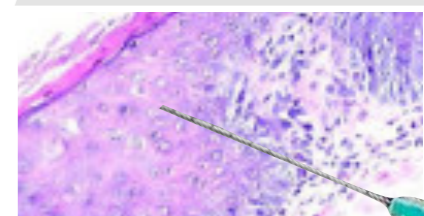
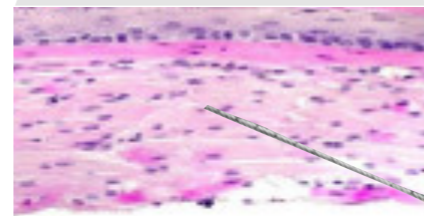
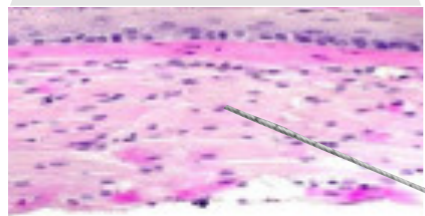
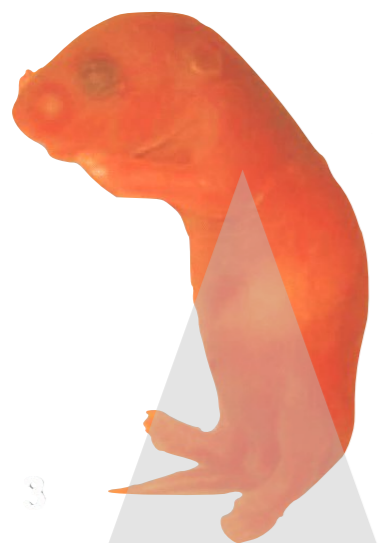
**Hypothesis: Transcripts of proteins involved in CoA reactions will be down regulated and transcripts involved in mitochondrial transport will be up regulated**

# Aim 2

## RNA Seq of Esophageal Tissue

## Expression Profiling

## CRISPR Knockout of Up and Down Regulated Genes

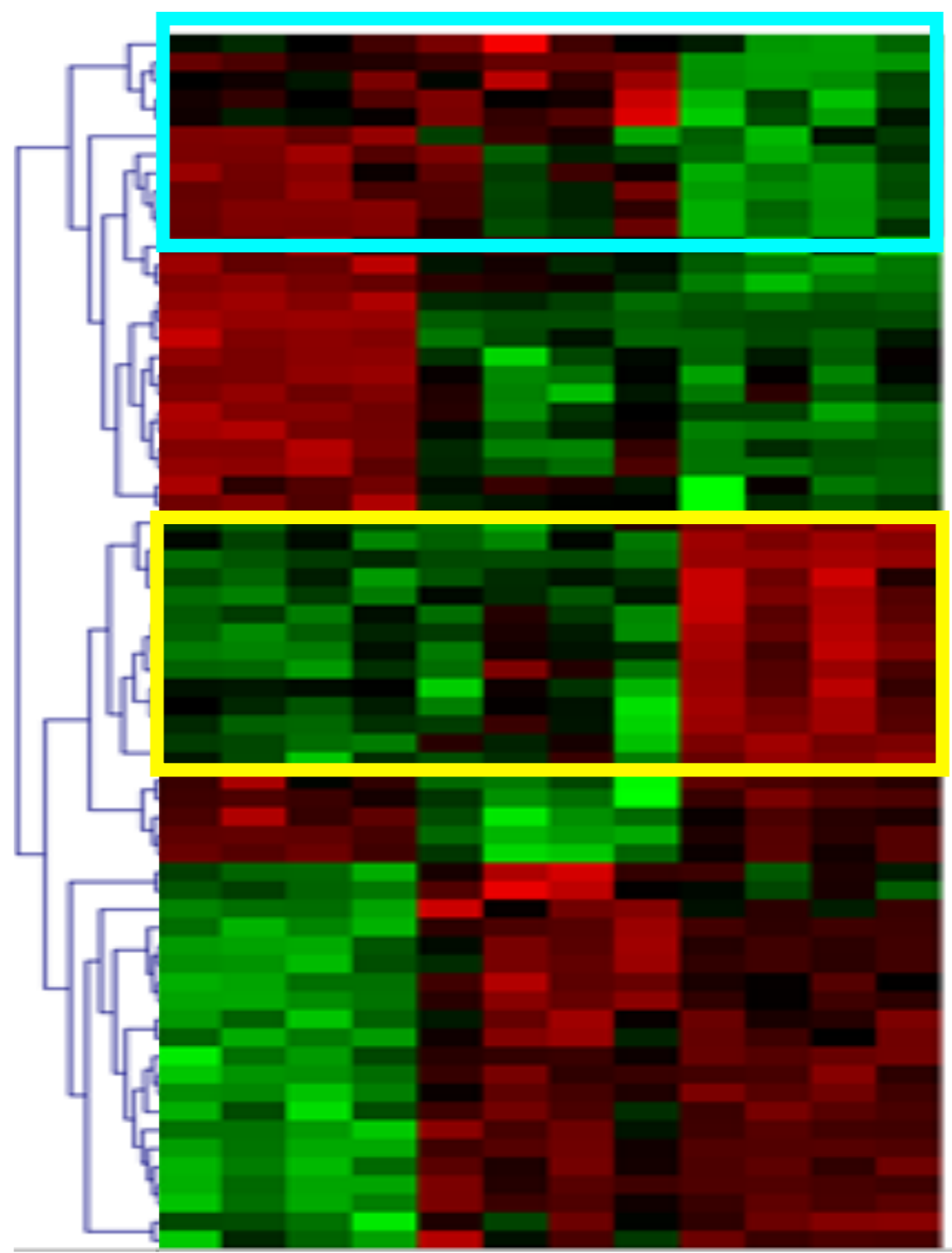


# Aim 2

RNA Seq of Esophageal Tissue

Expression Profiling

CRISPR Knockout of Up and Down Regulated Genes



Involved in Reactions with CoA

Mitochondrial Membrane Transport



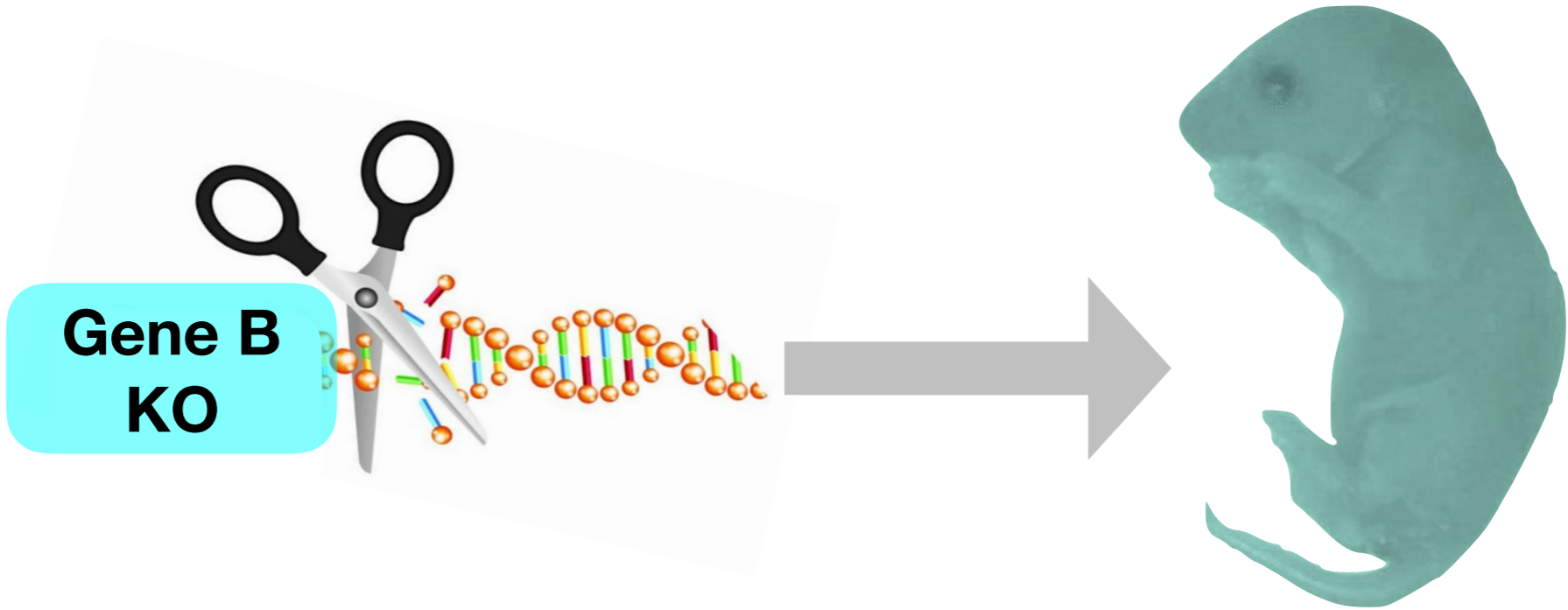
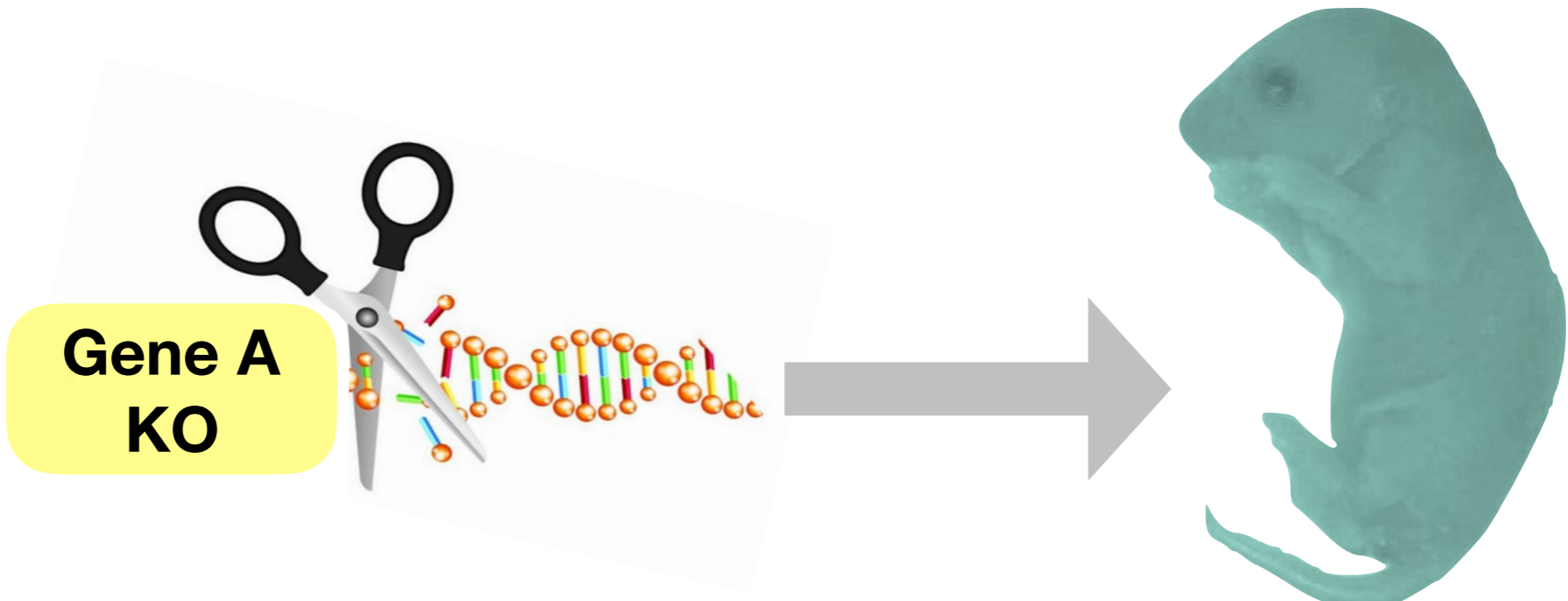
WT DBT -/- DBT -/- Poor Feeding

# Aim 2

RNA Seq of Esophageal Tissue

Expression Profiling

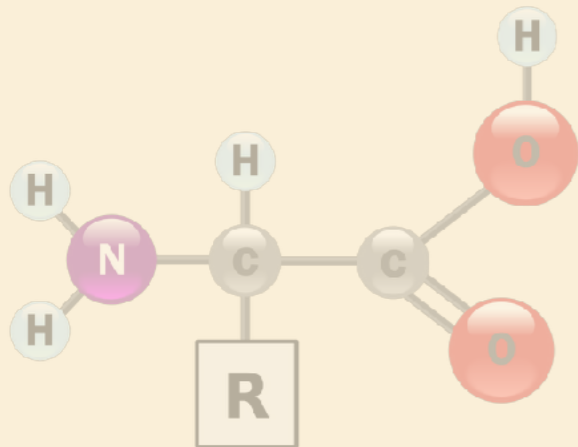
CRISPR Knockout of Up and Down Regulated Genes



# Determine the role of **DBT** and esophagus function

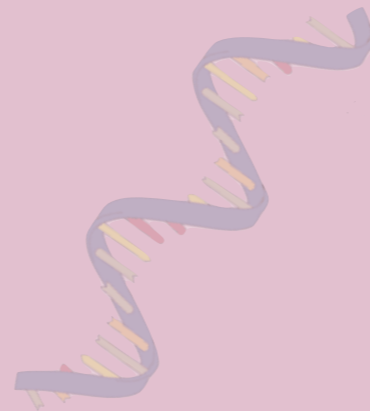
Aim 1:

Identify amino acids that are important for feeding



Aim 2:

Determine transcripts that impact feeding



Aim 3:

Identify proteins necessary for proper feeding



**Hypothesis: The protein interactions in the mutant DBT with poor feeding will have loss of protein interaction with mitochondrial transport proteins**

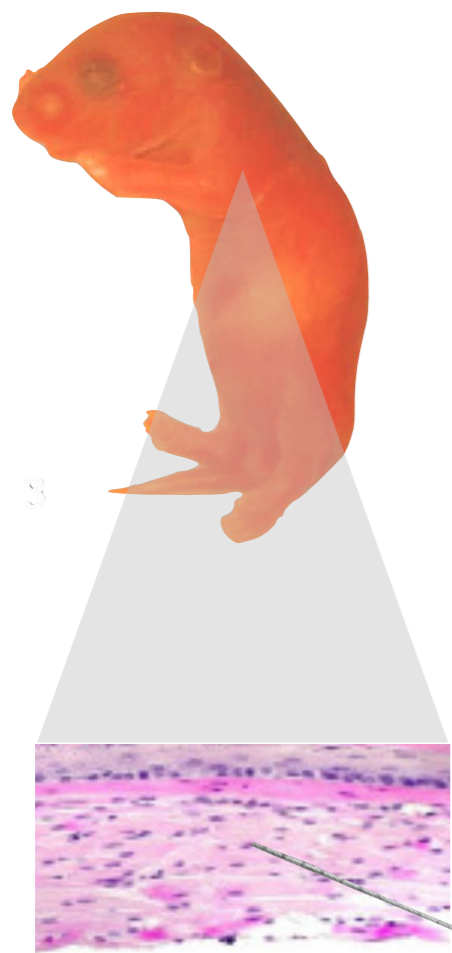


# Aim 3

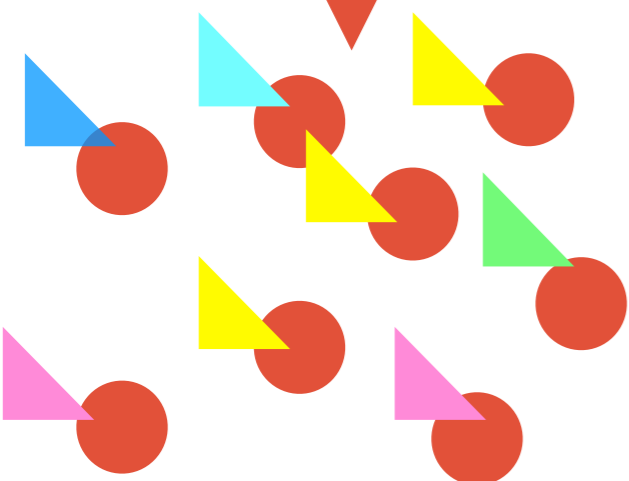
Identify Protein Interactions from Esophageal Tissue

Gene Ontology

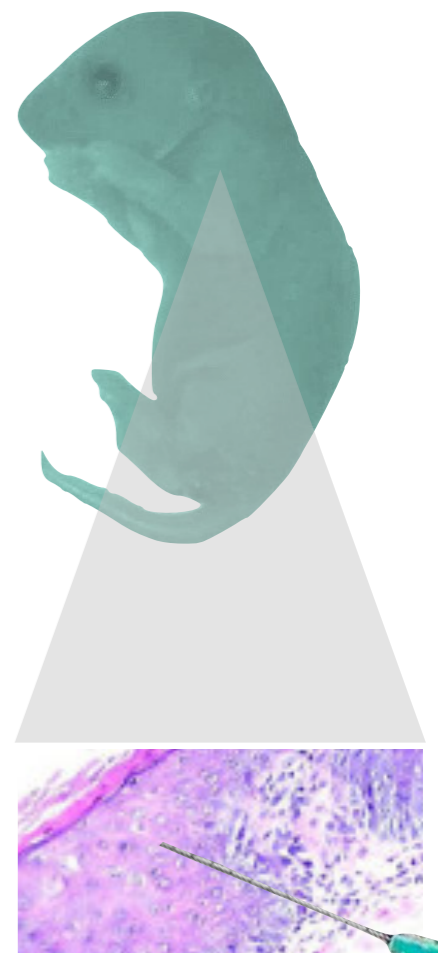
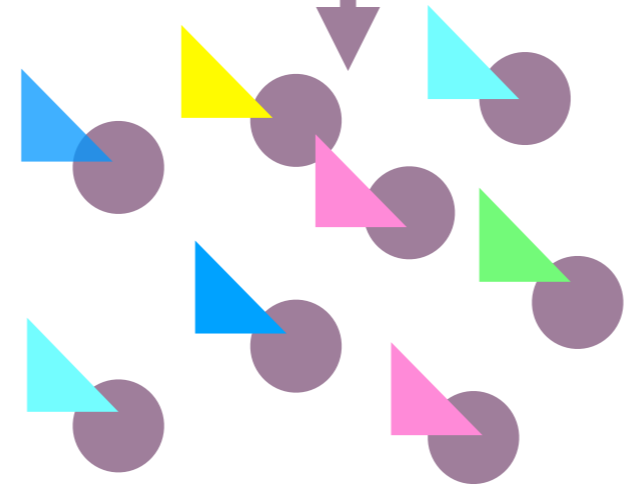
CRISPR Knockout of Proteins of Interest



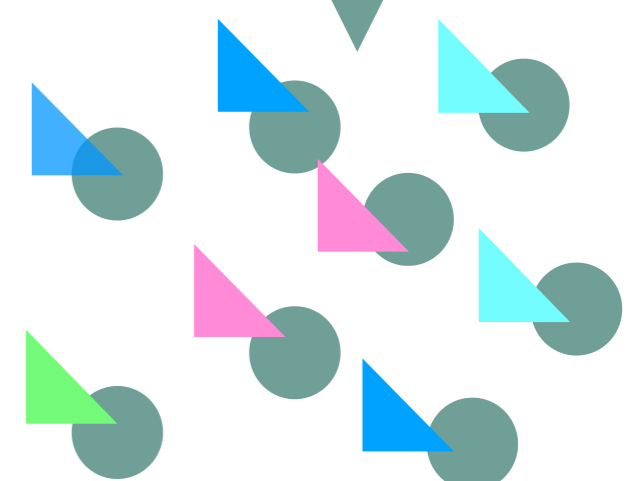
TAP



TAP



TAP



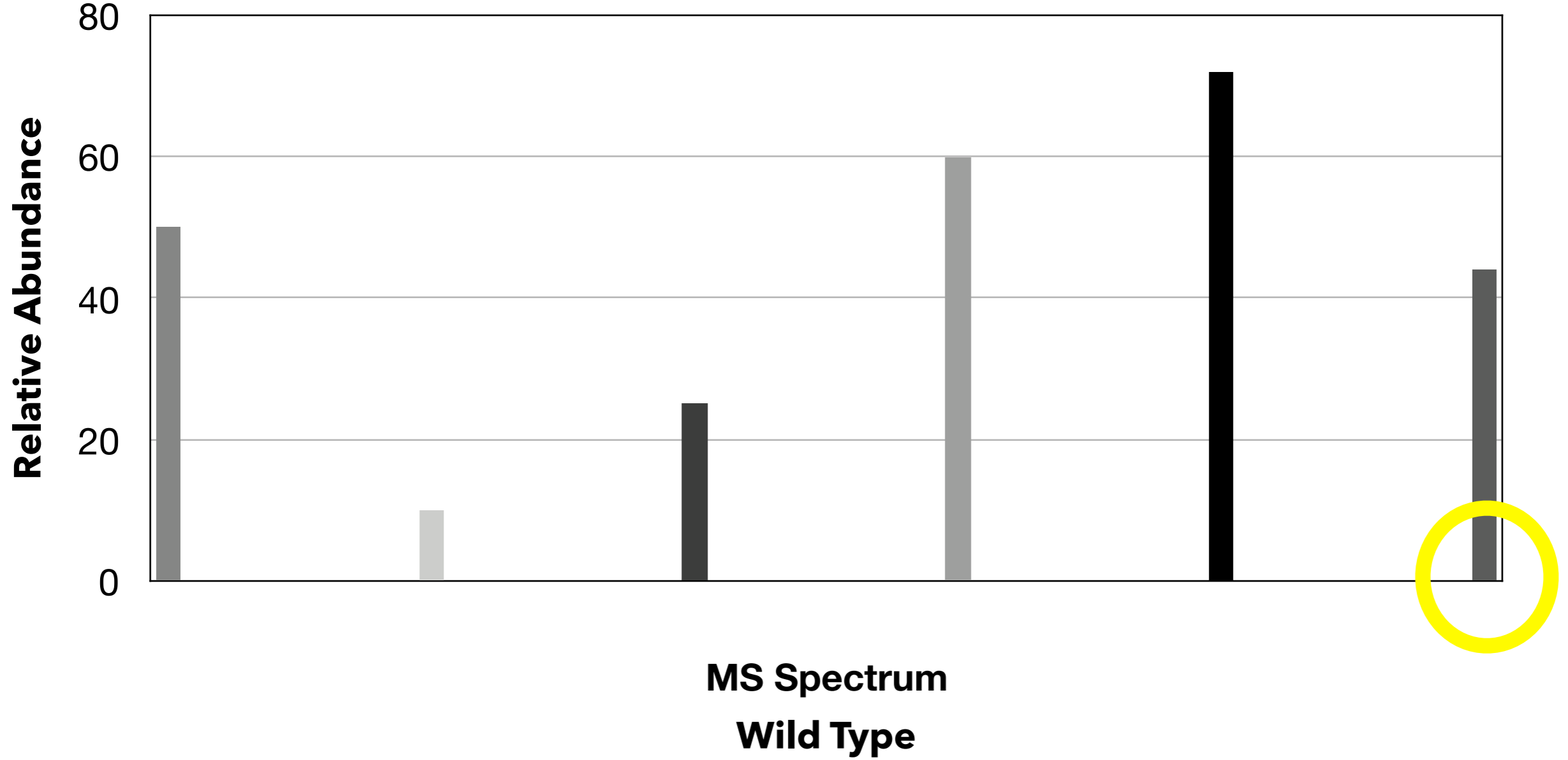
# Aim 3

Identify Protein Interactions from Esophageal Tissue

Gene Ontology

CRISPR Knockout of Proteins of Interest

## Mass Spectrometry

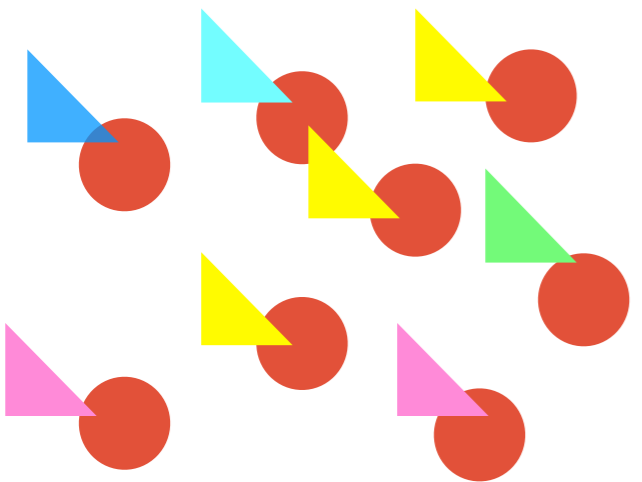


# Aim 3

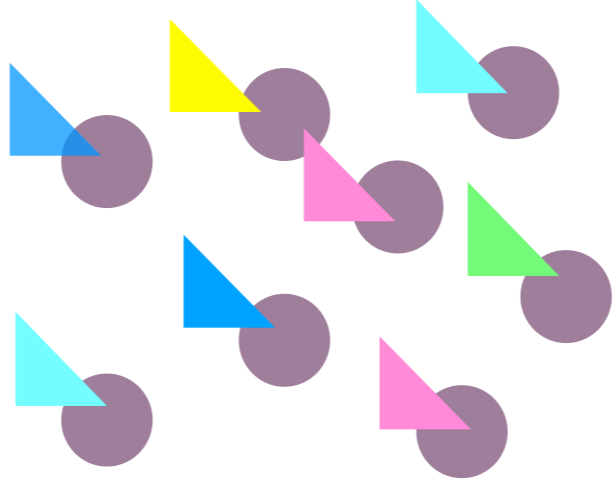
Identify Protein Interactions from Esophageal Tissue

Gene Ontology

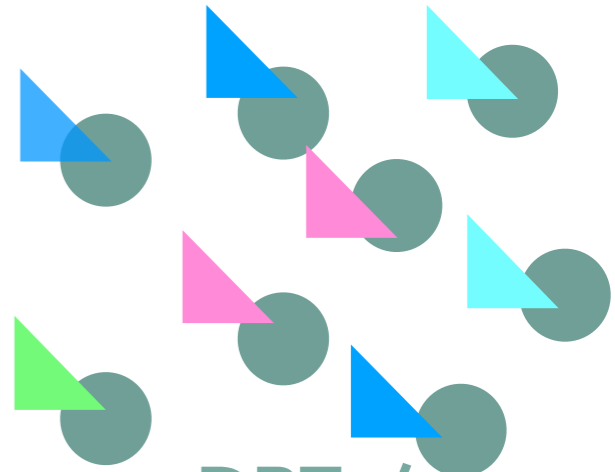
CRISPR Knockout of Proteins of Interest



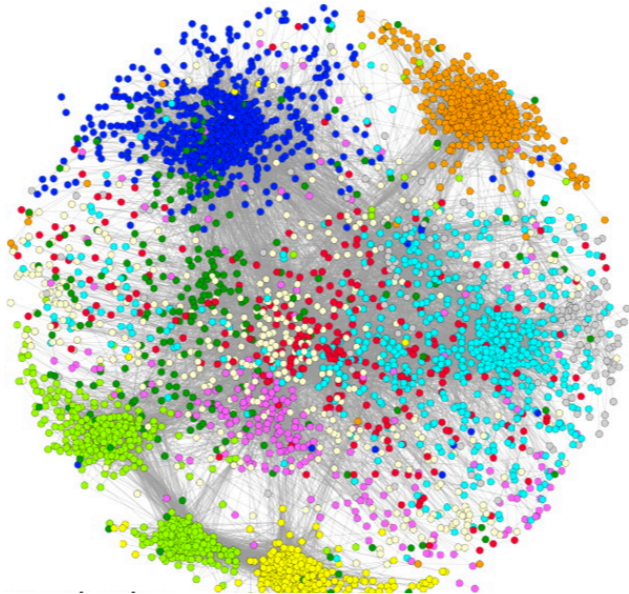
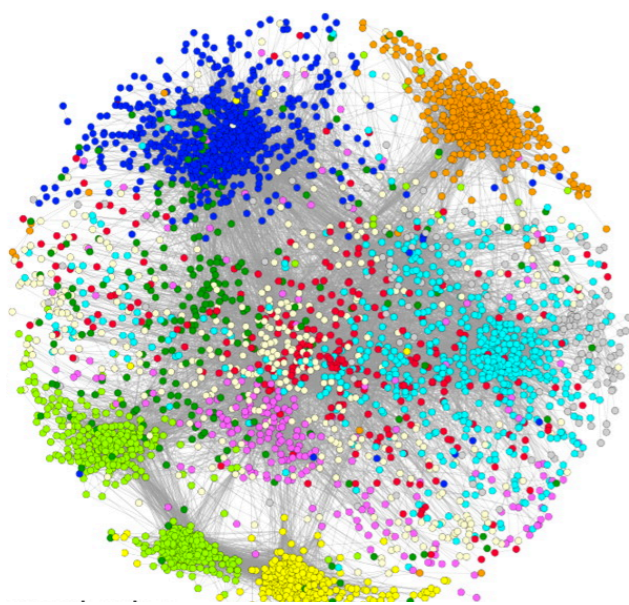
Wild Type



DBT -/-



DBT -/-  
Poor Feeding

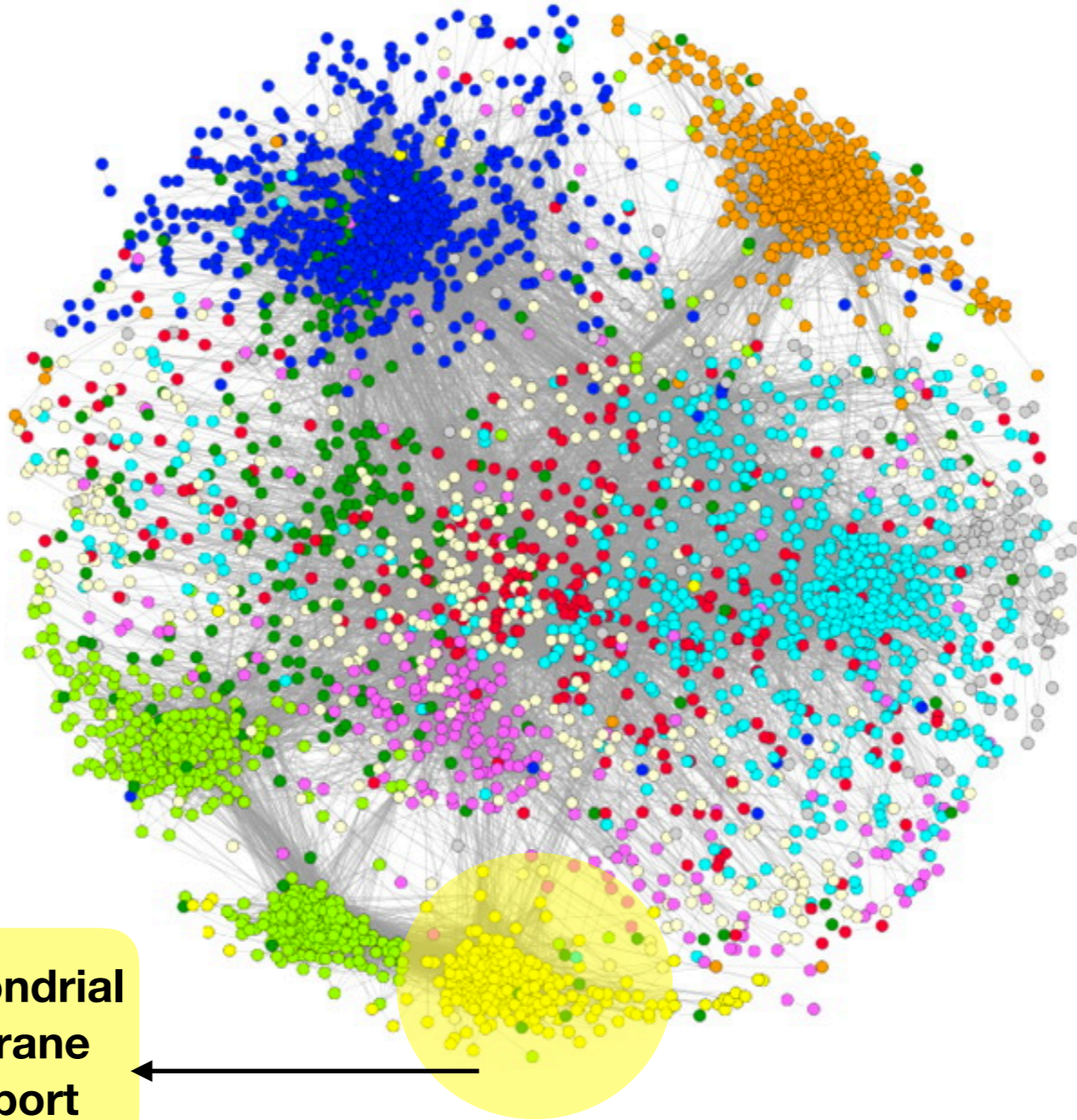


# Aim 3

Identify Protein Interactions from Esophageal Tissue

Gene Ontology

CRISPR Knockout of Proteins of Interest



Mitochondrial Membrane Transport

# Aim 3

Identify Protein Interactions from Esophageal Tissue

Gene Ontology

CRISPR Knockout of Proteins of Interest

GRPEL2  
KO

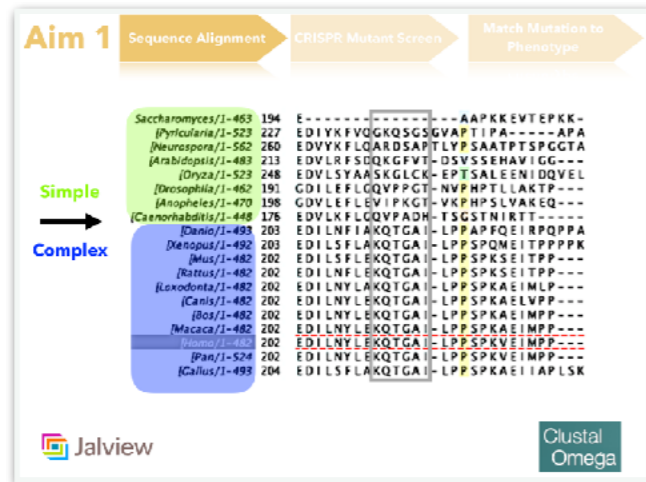


mtTransport  
GeneB KO

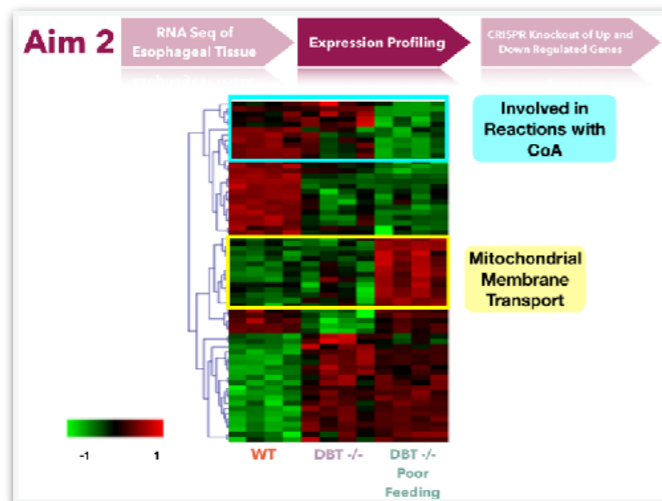


# Conclusions

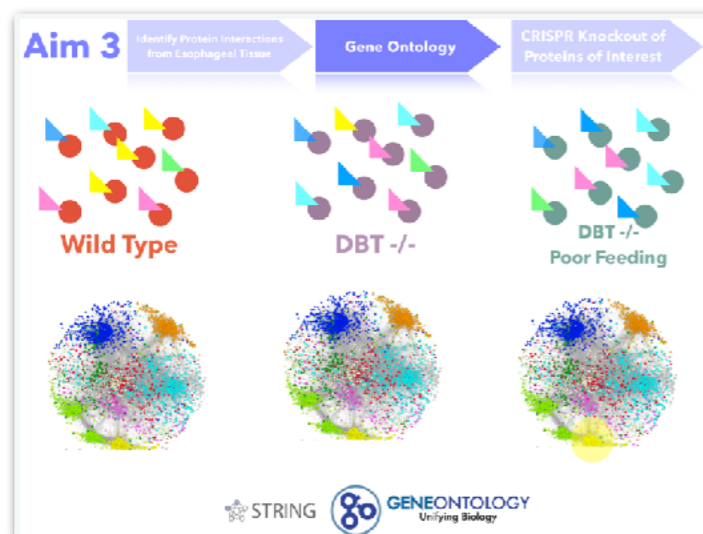
Sequence alignment of homologous proteins allows identification of conserved regions among organisms with complex digestive systems



RNAseq identifies transcript level changes in esophageal cells when mutant for DBT



Proteomic analysis allows for identification of protein interaction changes when mutant for DBT



# Future Directions



MEDICAL



HOSPITAL

DOCTOR

# Questions?





# Image References

Slide 1,32: <https://cbmaplefarm.com/shop/category/vermont-maple-syrup/>

Slide 2: <https://diyhealthacademy.com/newborn-screening-tests-state-requires/> <https://www.hrsa.gov/sites/default/files/hrsa/advisory-committees/heritable-disorders/rusp/rusp-uniform-screening-panel.pdf>

Slide 3,4: <https://www.newbornscreening.info/Parents/aminoaciddisorders/Images/MSUD.gif>

Slide 5: <https://cdn.images.express.co.uk/img/dynamic/11/590x/Urine-smell-841566.jpg> <https://dovemed-prod-k8s.s3.amazonaws.com/media/images/msud-Image004.width-750.png> [https://www.healthline.com/hlcmsresource/images/imce/feeding-tube-infants\\_thumb.jpg](https://www.healthline.com/hlcmsresource/images/imce/feeding-tube-infants_thumb.jpg) [http://clipart-library.com/image\\_gallery/496492.png](http://clipart-library.com/image_gallery/496492.png)

Slide 6: [http://education.med.nyu.edu/courses/molecular/AminoAcids06/questions/aa\\_answers/ans23a.html](http://education.med.nyu.edu/courses/molecular/AminoAcids06/questions/aa_answers/ans23a.html) [https://www.researchgate.net/profile/Rolf\\_Mueller4/publication/11290128/figure/fig1/AS:394580399804421@1471086706637/Degradation-of-leucine-isoleucine-and-valine-via-the-branched-chain-keto-acid.png](https://www.researchgate.net/profile/Rolf_Mueller4/publication/11290128/figure/fig1/AS:394580399804421@1471086706637/Degradation-of-leucine-isoleucine-and-valine-via-the-branched-chain-keto-acid.png) [biorender.com](http://biorender.com)

Slide 8: Generated by Mega Software

Slide 9: <https://string-db.org/cgi/network.pl?taskId=ldKnOD6d1YVA>

Slide 10: <https://www.ncbi.nlm.nih.gov/gene/1629>

Slide 11: <https://www.ncbi.nlm.nih.gov/gene/134266> [http://clipart-library.com/image\\_gallery/496492.png](http://clipart-library.com/image_gallery/496492.png)

Slide 12: [biorender.com](http://biorender.com) [http://clipart-library.com/image\\_gallery/496492.png](http://clipart-library.com/image_gallery/496492.png) [https://www.researchgate.net/figure/Histopathology-of-mouse-esophagus-after-reflux-surgery-A-In-the-non-operated-control\\_fig3\\_26690937](https://www.researchgate.net/figure/Histopathology-of-mouse-esophagus-after-reflux-surgery-A-In-the-non-operated-control_fig3_26690937)

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Slide 16, 20, 24: <https://upload.wikimedia.org/wikipedia/commons/thumb/c/ce/AminoAcidball.svg/1200px-AminoAcidball.svg.png> <https://www2.le.ac.uk/projects/vgec/diagrams/115-mrna.gif> <http://www.g3journal.org/content/ggg/2/4/453/F5.large.jpg>

Slide 17: Generated by Clustal Omega and Jalview

Slide 18: <https://gurumavin.com/wp-content/uploads/2017/12/image.jpg> [https://www.researchgate.net/profile/Brad\\_Bolon/publication/23679831/figure/fig3/AS:601637286580224@1520452915347/Neonatal-mice-postnatal-day-1-Relative-to-its-Clc-wild-type-littermate-left-the-Clc.png](https://www.researchgate.net/profile/Brad_Bolon/publication/23679831/figure/fig3/AS:601637286580224@1520452915347/Neonatal-mice-postnatal-day-1-Relative-to-its-Clc-wild-type-littermate-left-the-Clc.png)

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Slide 21, 25: [https://www.researchgate.net/profile/Brad\\_Bolon/publication/23679831/figure/fig3/AS:601637286580224@1520452915347/Neonatal-mice-postnatal-day-1-Relative-to-its-Clc-wild-type-littermate-left-the-Clc.png](https://www.researchgate.net/profile/Brad_Bolon/publication/23679831/figure/fig3/AS:601637286580224@1520452915347/Neonatal-mice-postnatal-day-1-Relative-to-its-Clc-wild-type-littermate-left-the-Clc.png) [https://www.researchgate.net/figure/Histopathology-of-mouse-esophagus-after-reflux-surgery-A-In-the-non-operated-control\\_fig3\\_26690937](https://www.researchgate.net/figure/Histopathology-of-mouse-esophagus-after-reflux-surgery-A-In-the-non-operated-control_fig3_26690937) [https://www.google.com/search?q=biopsy+needle&client=safari&rls=en&source=lnms&tbm=isch&sa=X&ved=0ahUKEwjzP36tuDhAhVGRK0KHZ70BAEQ\\_AUIDigB&biw=1248&bih=656&dpr=2#imgrc=5oYJTbJPc2lv5M:](https://www.google.com/search?q=biopsy+needle&client=safari&rls=en&source=lnms&tbm=isch&sa=X&ved=0ahUKEwjzP36tuDhAhVGRK0KHZ70BAEQ_AUIDigB&biw=1248&bih=656&dpr=2#imgrc=5oYJTbJPc2lv5M:)

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