# Maple Syrup Urine Disease and the DBT Gene

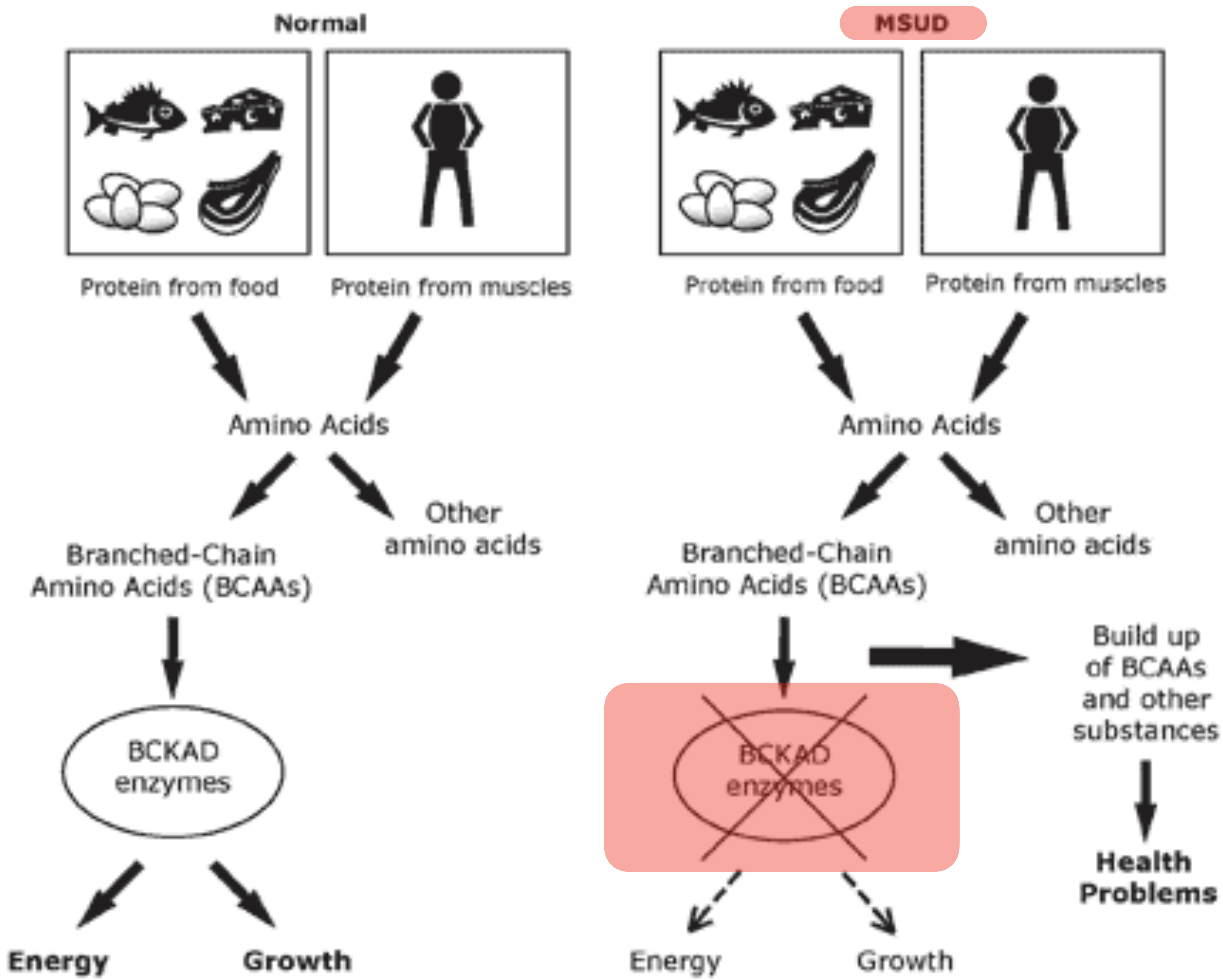
Tessa Bachinski

# What is Maple Syrup Urine Disease (MSUD)?

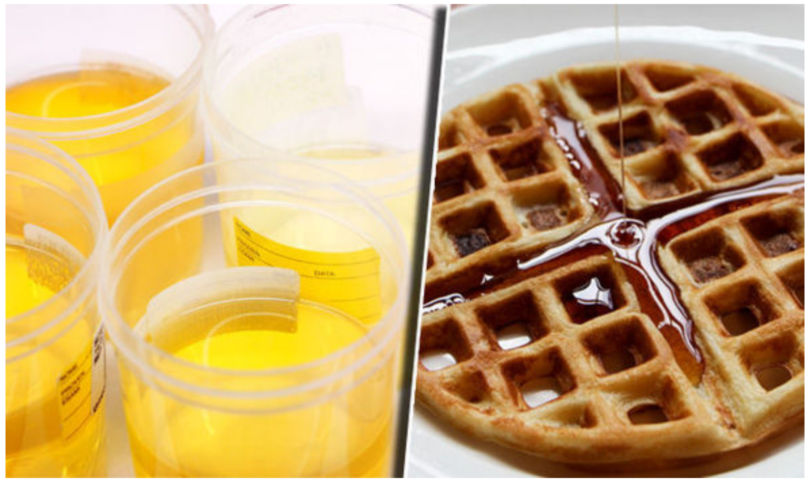

Recommended Uniform Screening Panel
Core Conditions
(As of July 2018)

| Core Condition | Metabolic Disorder | | | Endocrine Disorder | Hemoglobin Disorder | Other 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-Methyglutaric Aciduria | X | | | | | |
| Holocarboxylase Synthase Deficiency | X | | | | | |
| ß-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 | | | |
| Primary Congenital Hypothyroidism | | | | X | | |
| Congenital adrenal hyperplasia | | | | X | | |
| S,S Disease (Sickle Cell Anemia) | | | | | X | |
| S, βeta-Thalassemia | | | | | X | |
| S,C Disease | | | | | X | |
| Biotinidase Deficiency | | | | | | X |
| Critical Congenital Heart Disease | | | | | | X |
| Cystic Fibrosis | | | | | | X |
| Classic Galactosemia | | | | | | X |
| Glycogen Storage Disease Type II (Pompe) | | | | | | X |
| Hearing Loss | | | | | | X |
| Severe Combined Immunodeficiencies | | | | | | X |
| Mucopolysaccharidosis Type 1 | | | | | | X |
| X-linked Adrenoleukodystrophy | | | | | | X |
| Spinal Muscular Atrophy due to homozygous deletion of exon 7 in SMN1 | | | | | | X |

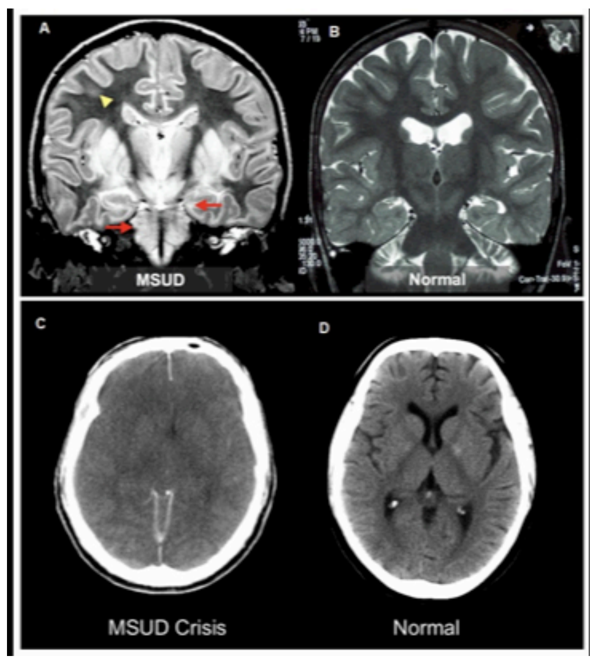
# What Causes Maple Syrup Urine Disease?



# What are the symptoms of Maple Syrup Urine Disease?



**Sweet Smelling Urine**

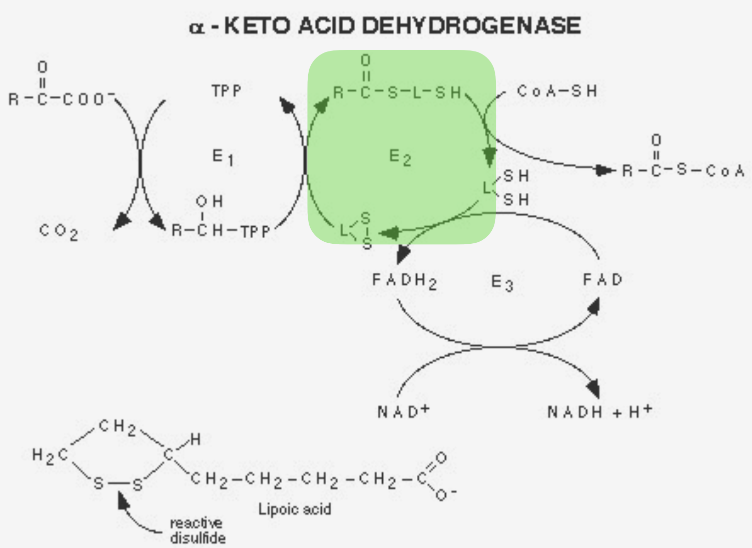



**Brain Damage, Seizures, and Other Neurological Symptoms**

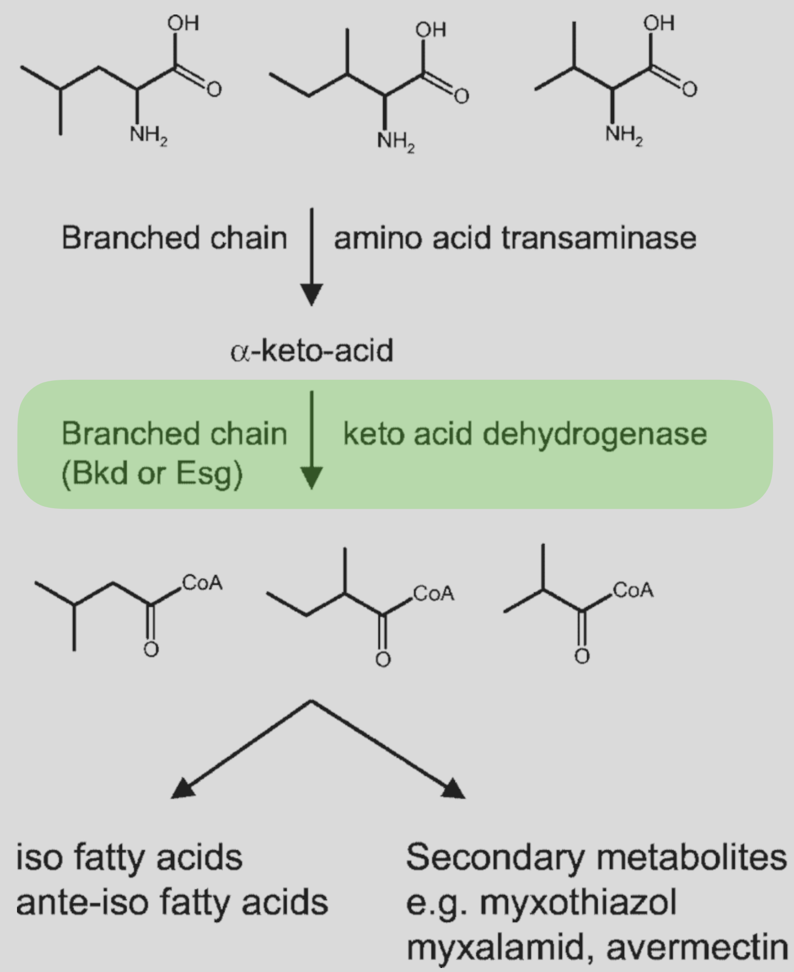
**Poor Feeding, Lethargy**

# What is the DBT Gene?

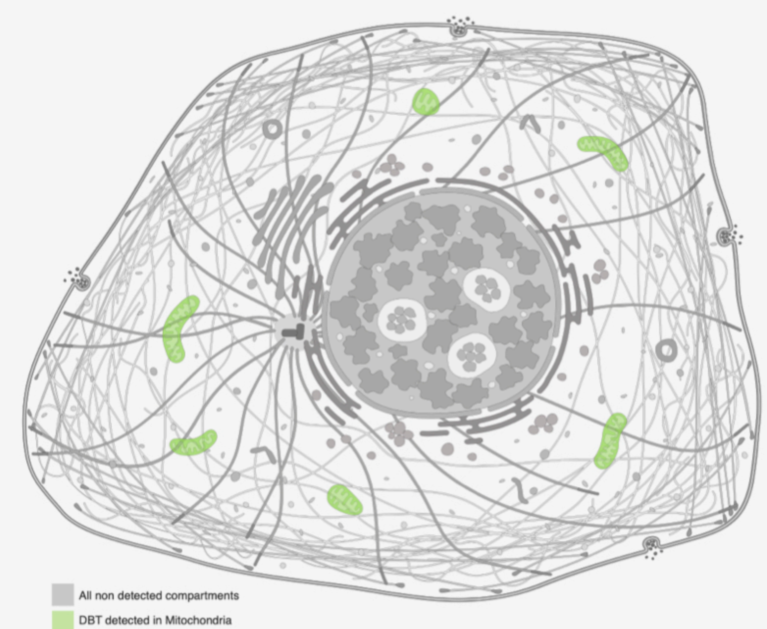

## Molecular Function



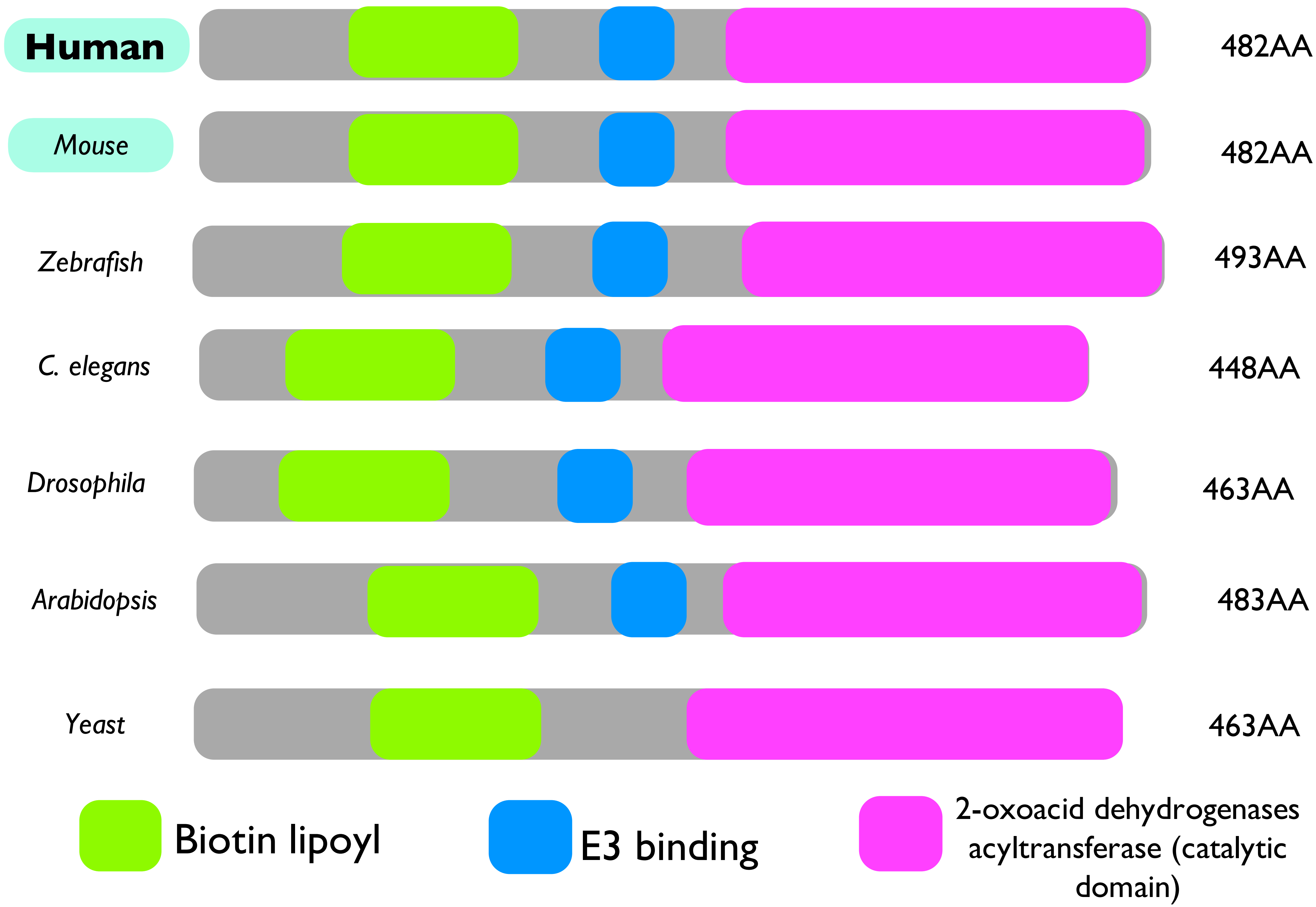

## Biological Function



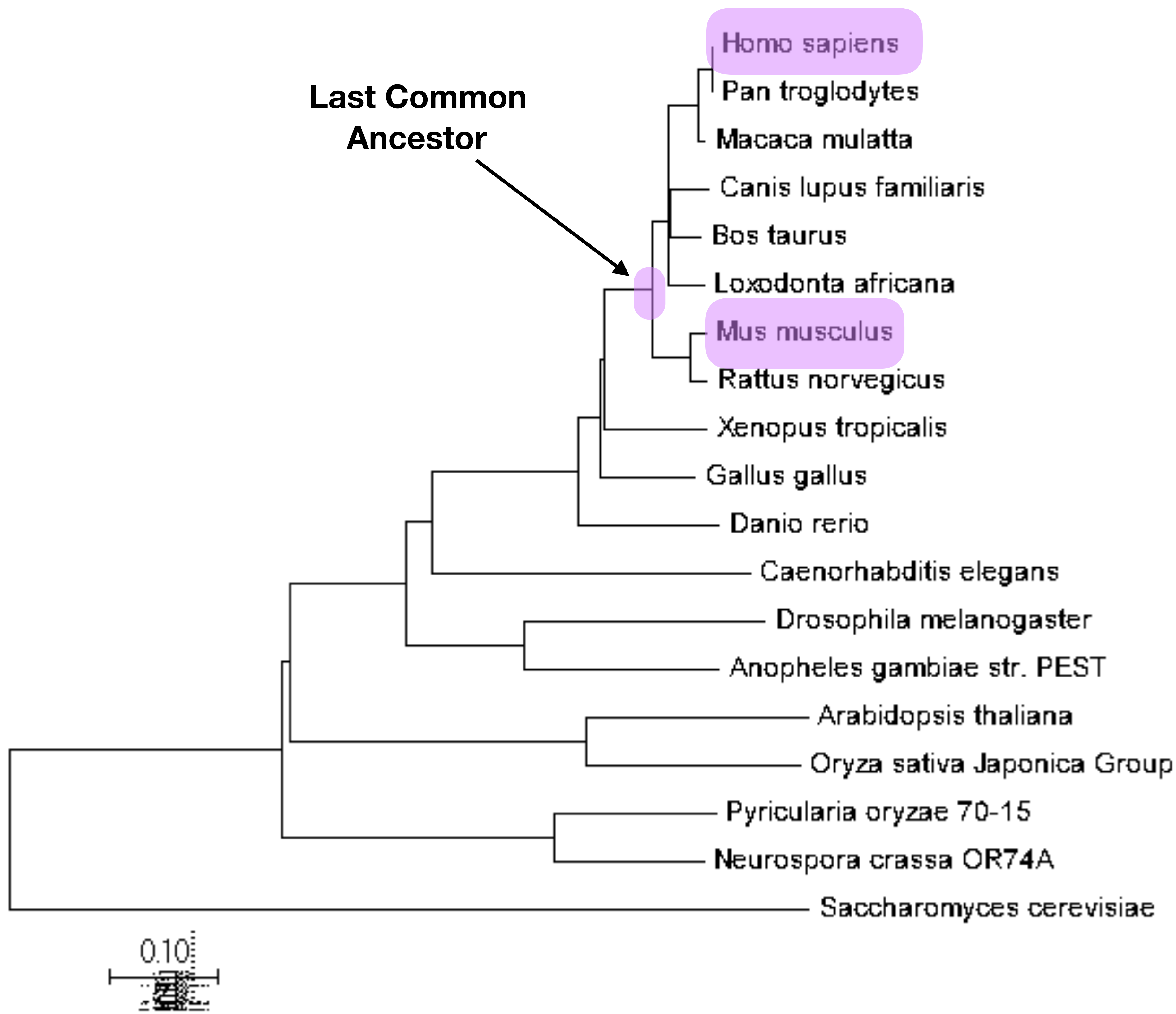

## Cellular Function



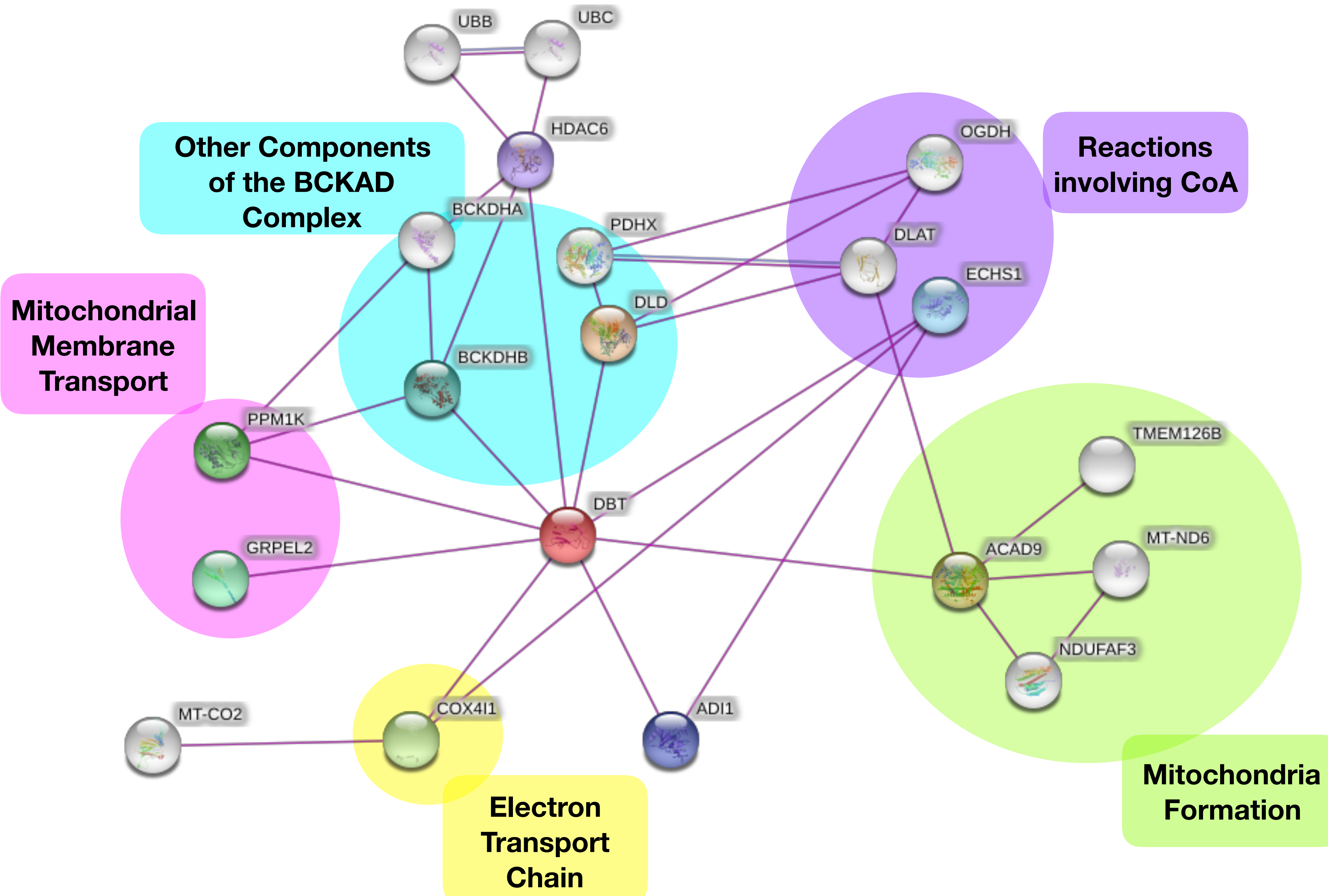
# How Well is DBT Conserved?



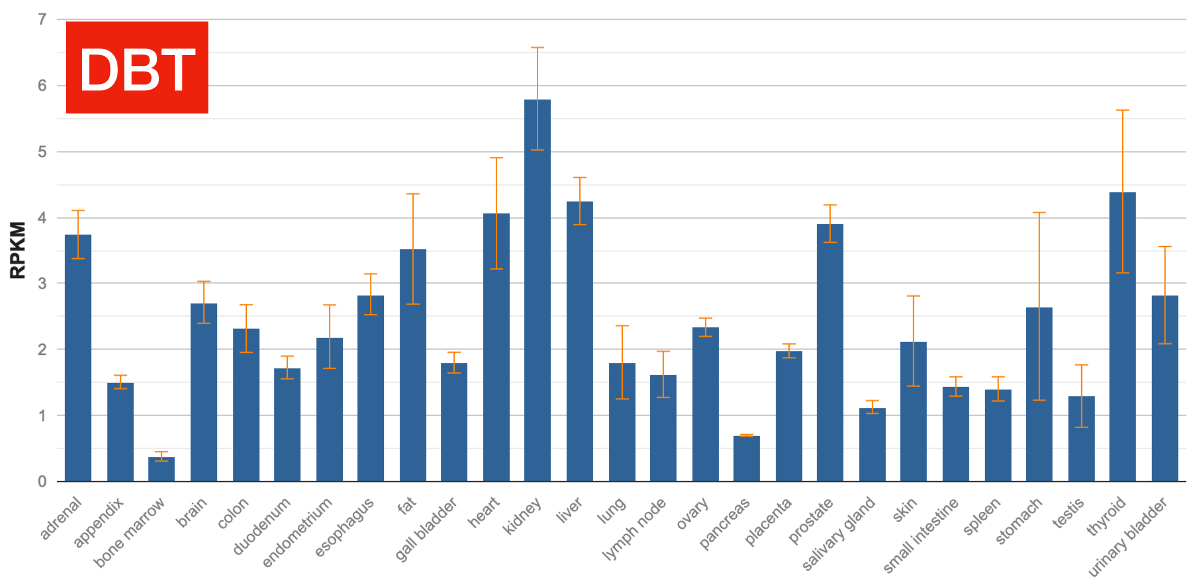
# What is the Phylogenetic Relationship?

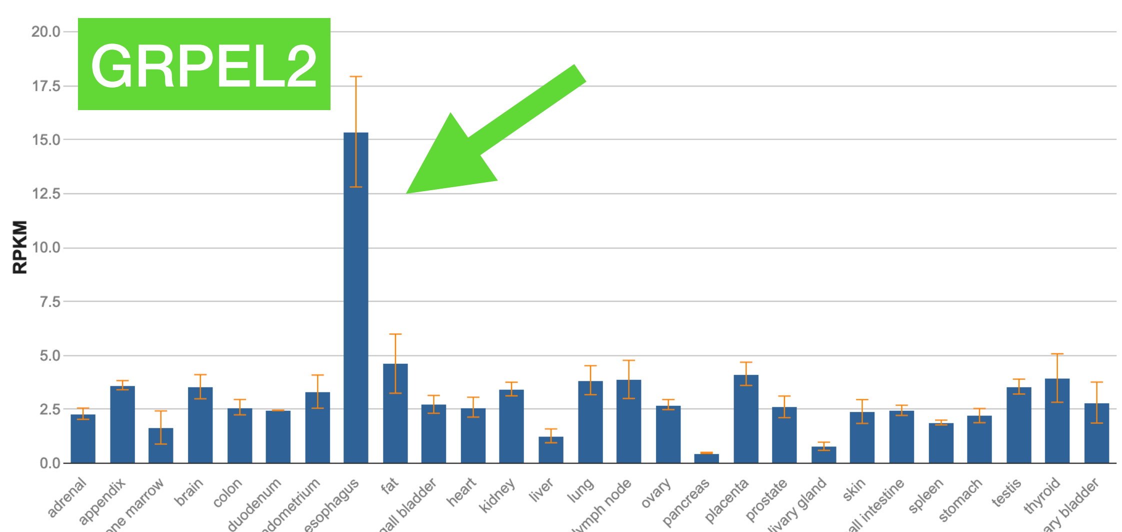


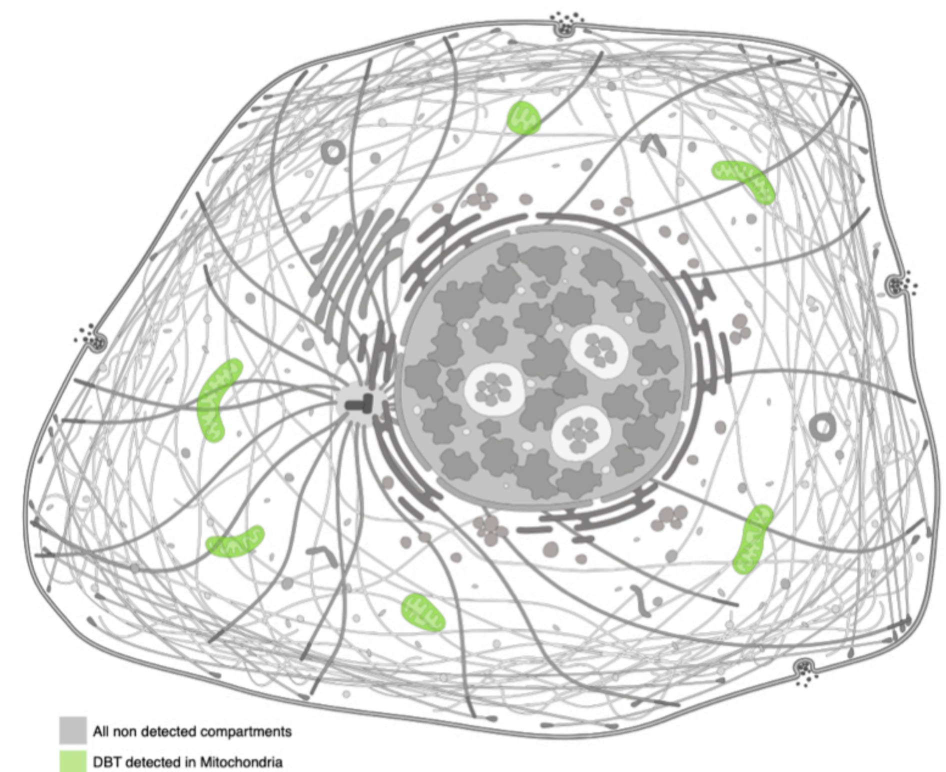
# What are the DBT protein interactions?



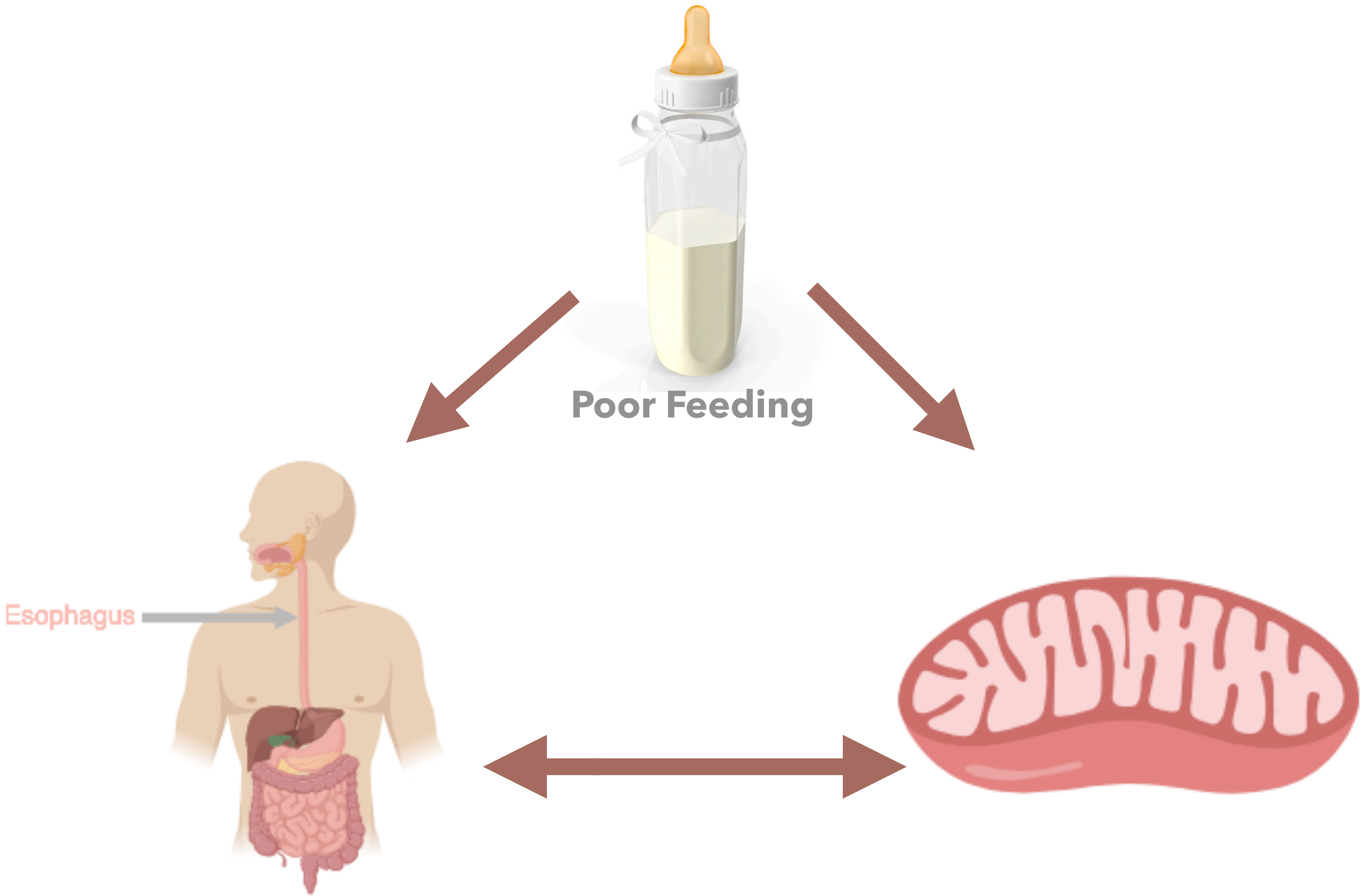
# Where do DBT and its interacting proteins localize?



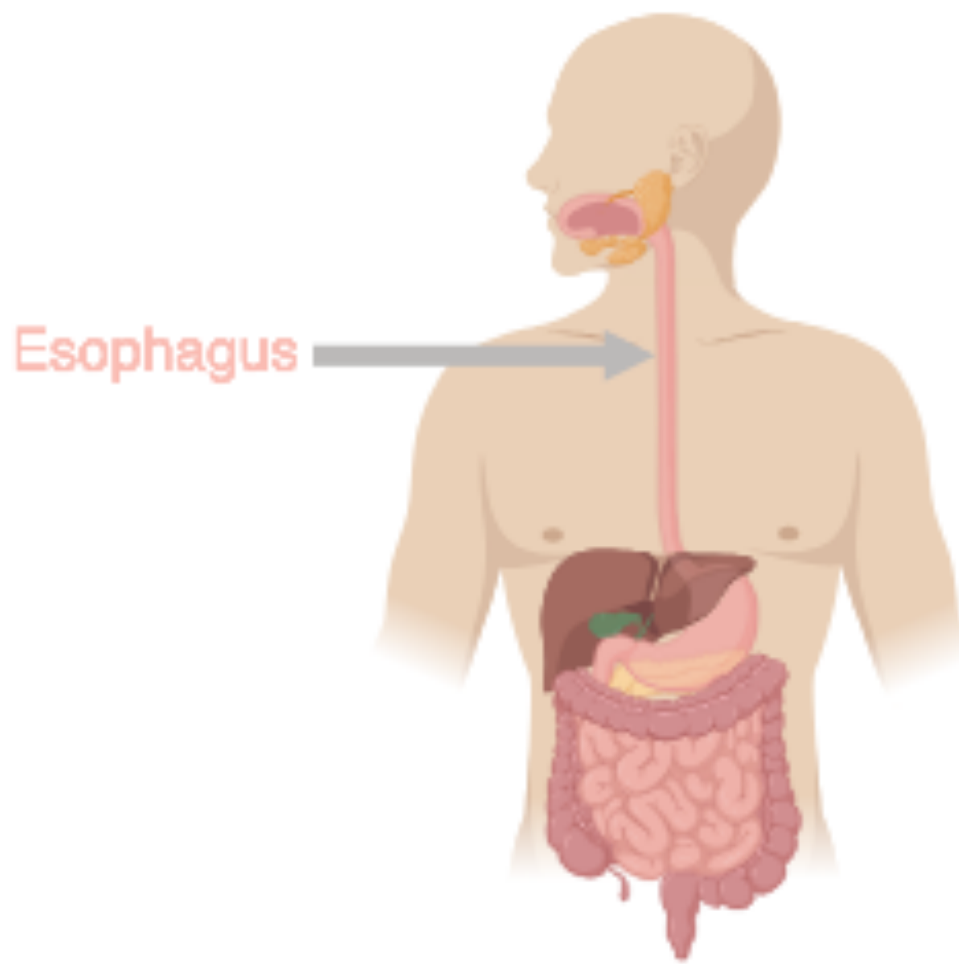



# What is the gap in knowledge?



# What is the primary goal?


**Determine how DBT plays a role in esophagus development**

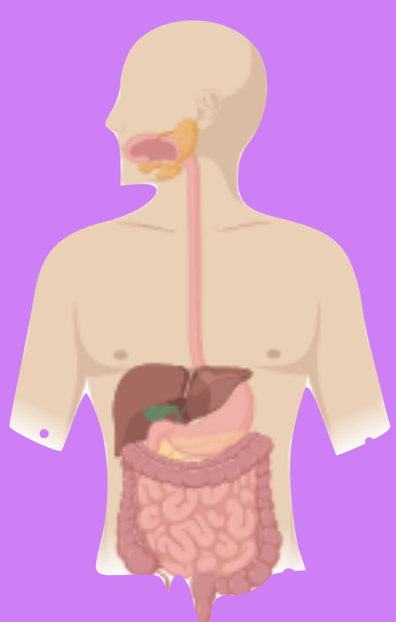
# What model organism will be used?

**Mus Musculus**

# How will the primary goal be reached?

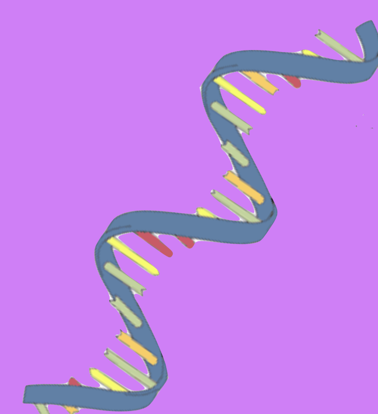
**Aim 1:**

Identify amino acids that are important for feeding behavior.



**Aim 2:**

Determine if mitochondrial genes are highly or lowly expressed between wild type and DBT mutant that are important for feeding



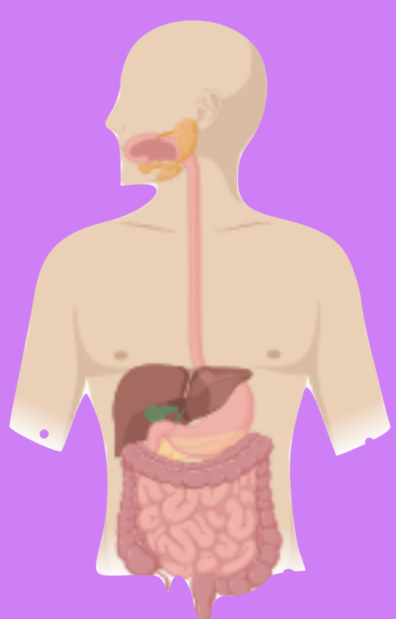
**Aim 3:**

Identify proteins necessary for mitochondrial function and feeding

# How will the primary goal be reached?

**Aim 1:**

Identify amino acids that are important for feeding behavior.



**Aim 2:**

Determine if mitochondrial genes are highly or lowly expressed between wild type and DBT mutant that are important for feeding

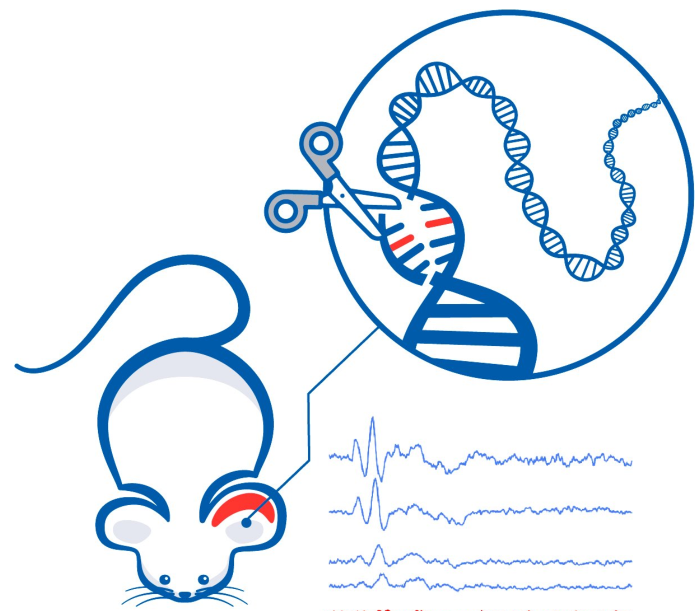
**Aim 3:**

Identify proteins necessary for mitochondrial function and feeding

# Aim 1: Identify mutations in the DBT protein that result in poor feeding phenotype

Clustal Omega

Jalview



CRISPR/Cas9 Editing

Phenotype Assay

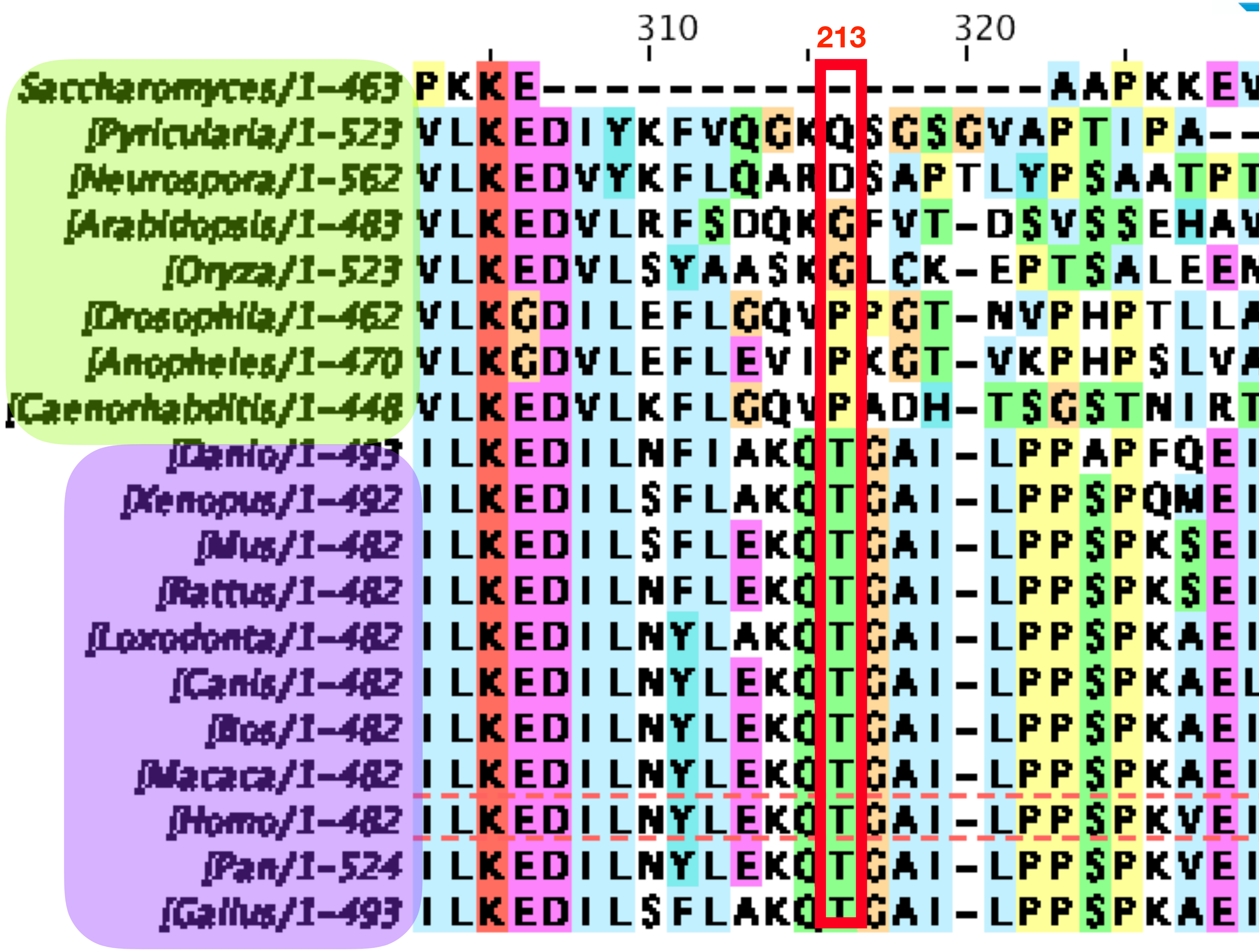
**Hypothesis: Amino acids that are conserved in complex digestive organisms, but not simple digestive organisms and are known to be phosphorylated, when mutated will result in a phenotype that exhibits poor feeding and high BCAA levels in urine.**

# Aim 1: Identify mutations in the DBT protein that result in poor feeding phenotype

Clustal Omega

Jalview

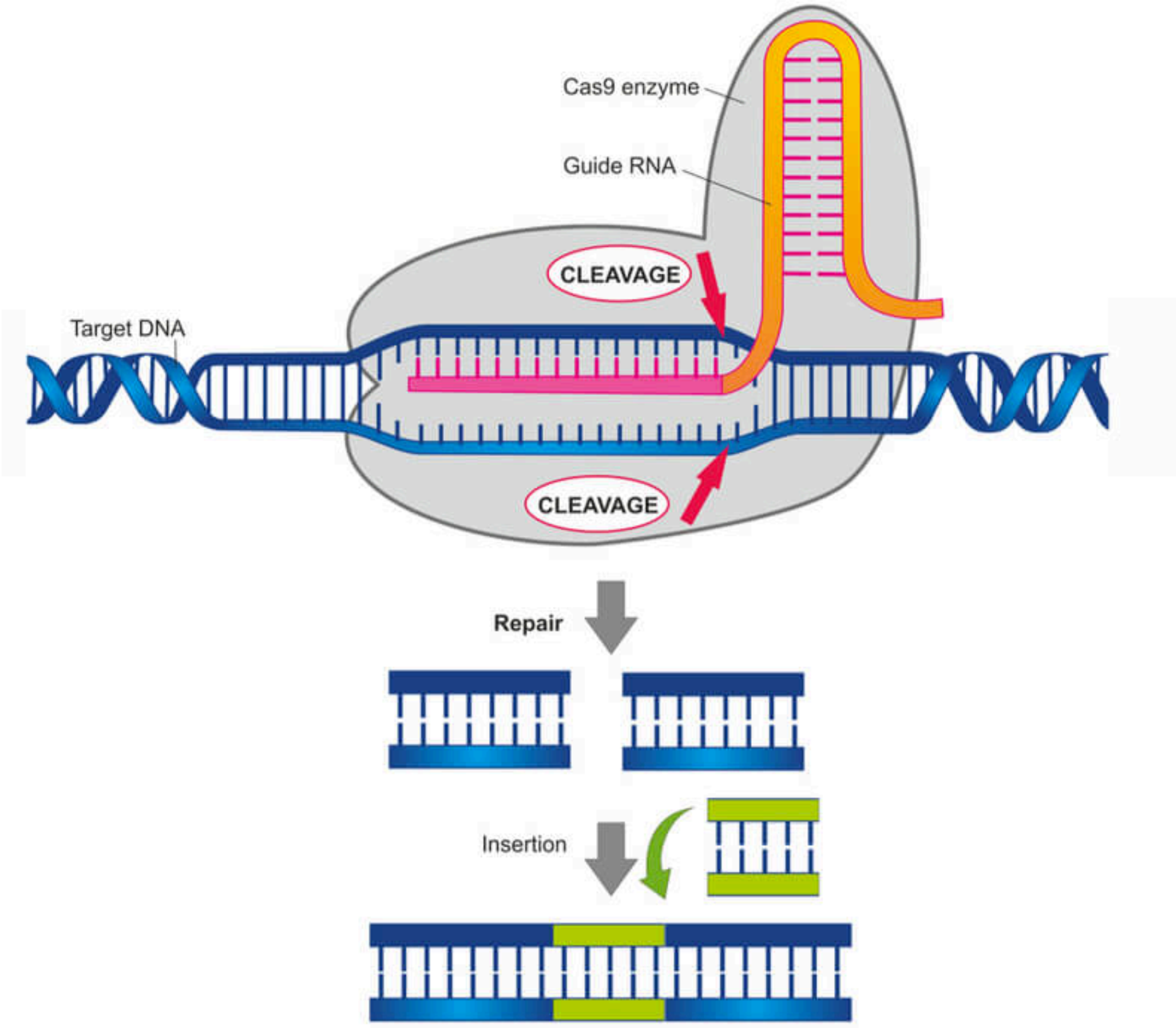
Simple → Complex




```
# Sequence                213 T   LEKQTGAIL   0.540   PKA        YES
```

**Align the sequences to identify amino acids that are different between organisms with simple and complex digestive system**

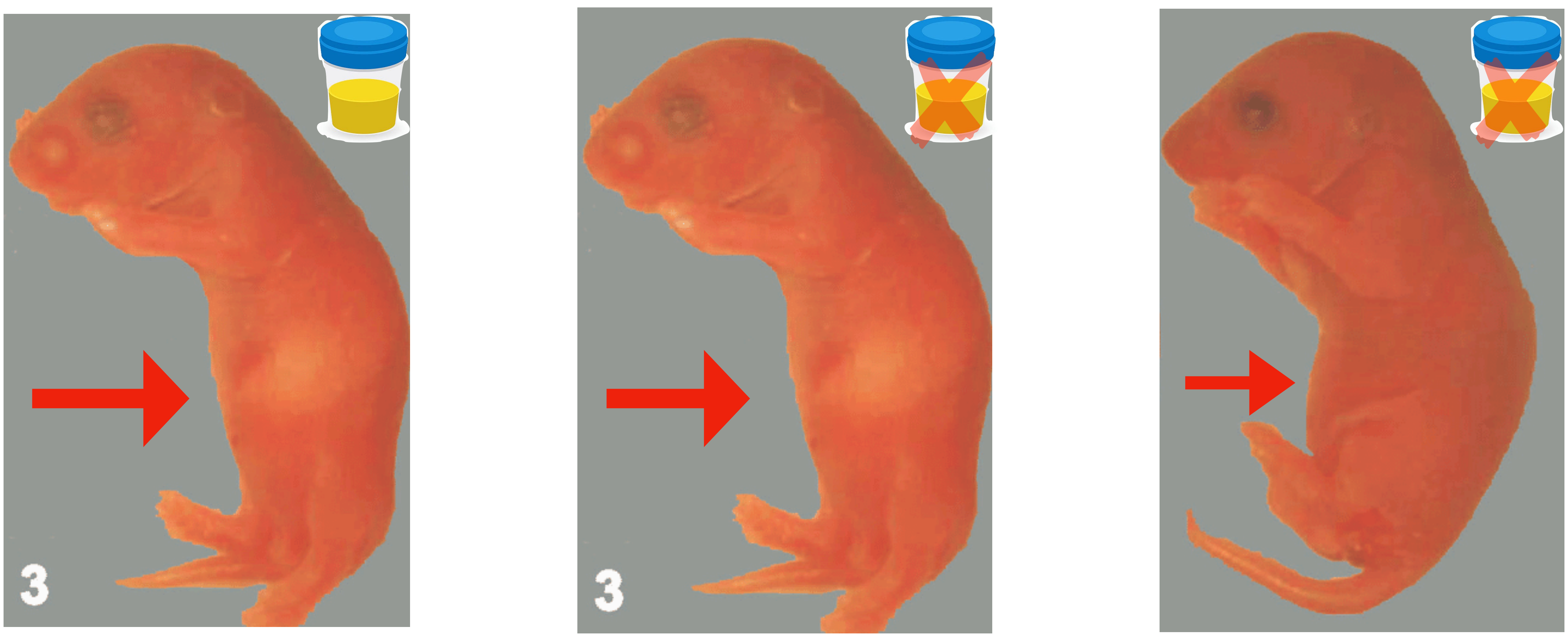
# Aim 1: Identify mutations in the DBT protein that result in poor feeding phenotype




**Use CRISPR/Cas9 technology to change amino acids at those sites to an amino acid found in lower level organisms**

# Aim 1: Identify mutations in the DBT protein that result in poor feeding phenotype

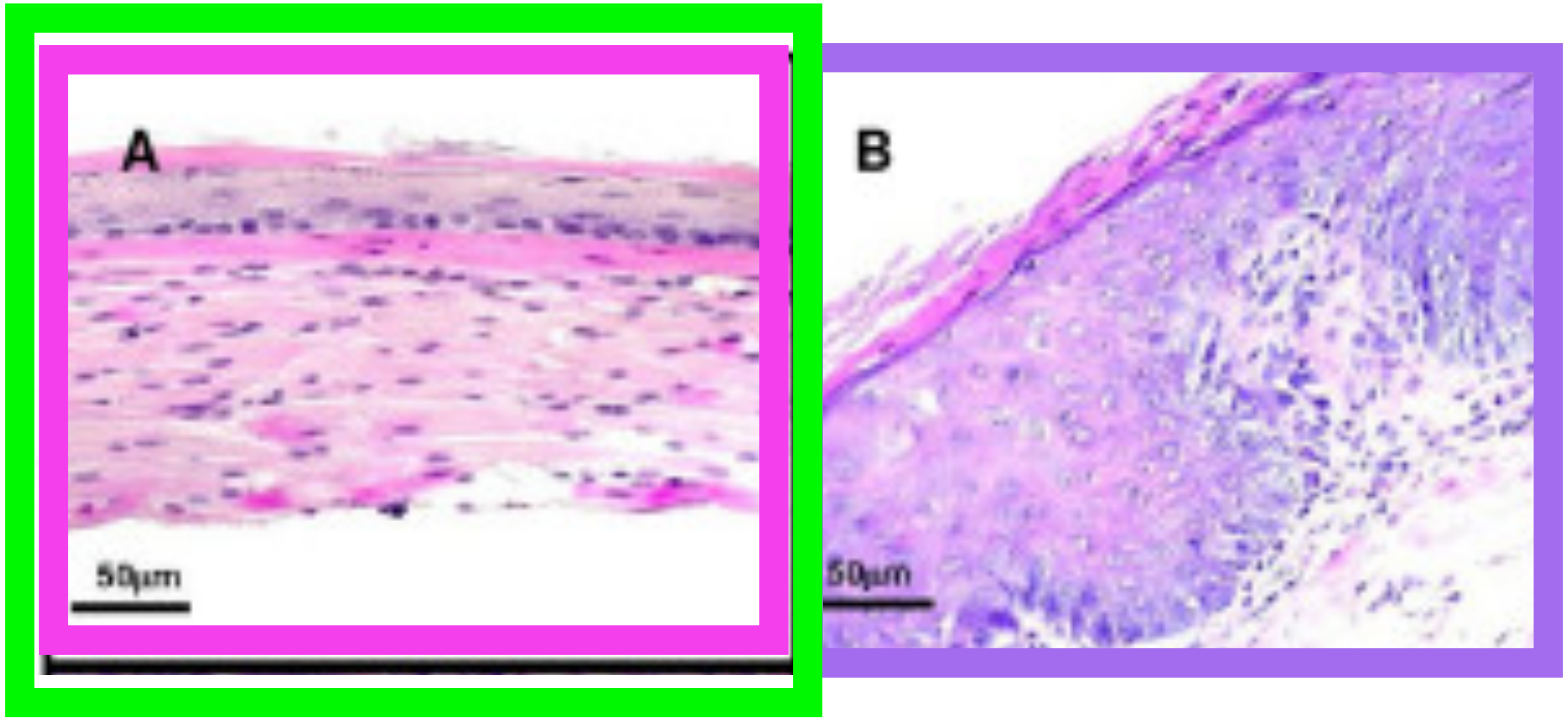
**Negative Control** | **MSUD Mutant** | **MSUD Mutant + Poor Feeding**




**Screen for mice that both exhibit poor feeding and have the urine phenotype**

# Aim 1: Identify mutations in the DBT protein that result in poor feeding phenotype

## Extraction of Esophageal Tissue:




**Hypothesis: Esophageal tissue will be distorted in mice who display the poor feeding phenotype (B) and will not be distorted in mice that do not (A)**

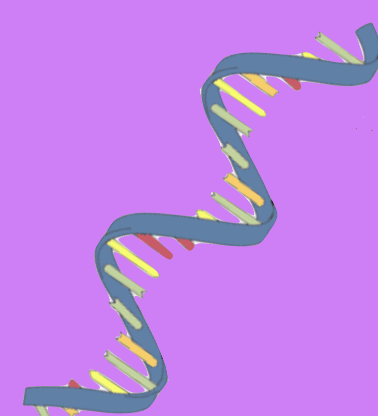
# How will the primary goal be reached?

**Aim 1:**

Identify amino acids that are important for feeding behavior.

**Aim 2:**

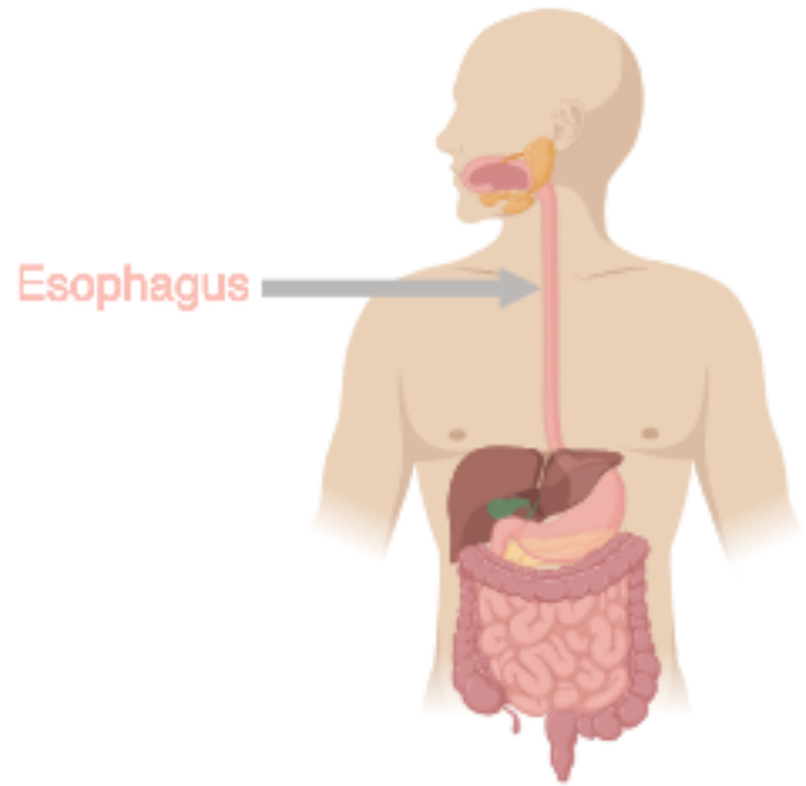
Determine if mitochondrial genes are highly or lowly expressed between wild type and DBT mutant that are important for feeding



**Aim 3:**

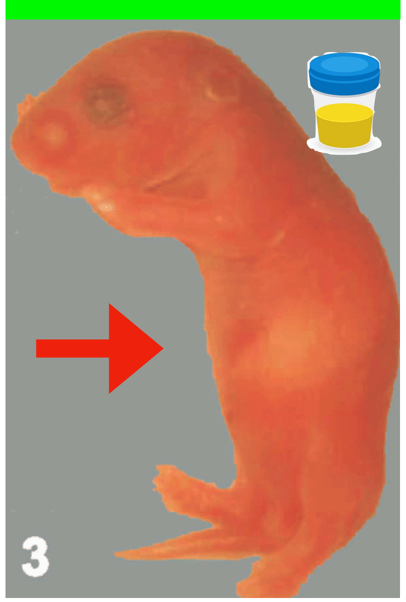
Identify proteins necessary for mitochondrial function and feeding

# Aim 2: Determine if mitochondrial genes are highly or lowly expressed between wild type and DBT mutant that are important for feeding

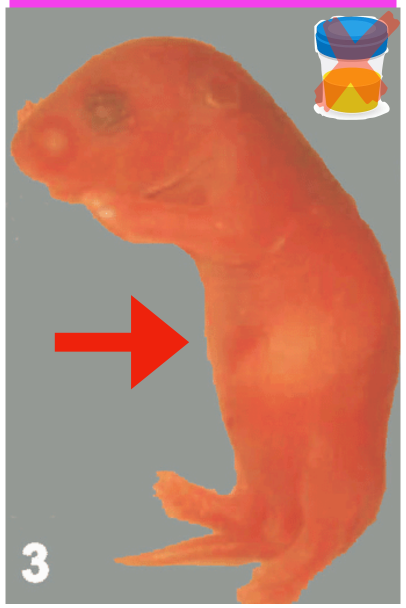



**Extract esophageal tissue from all three test groups**

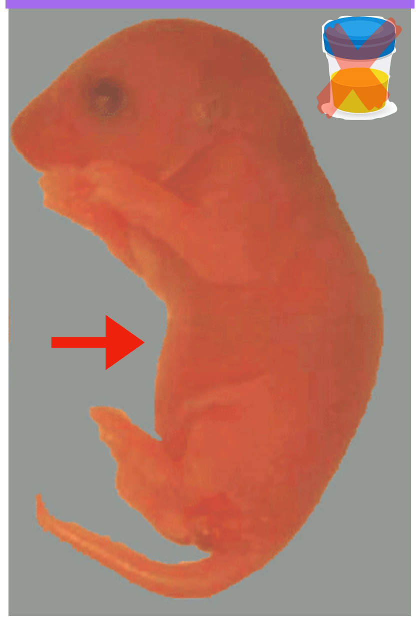
Negative Control



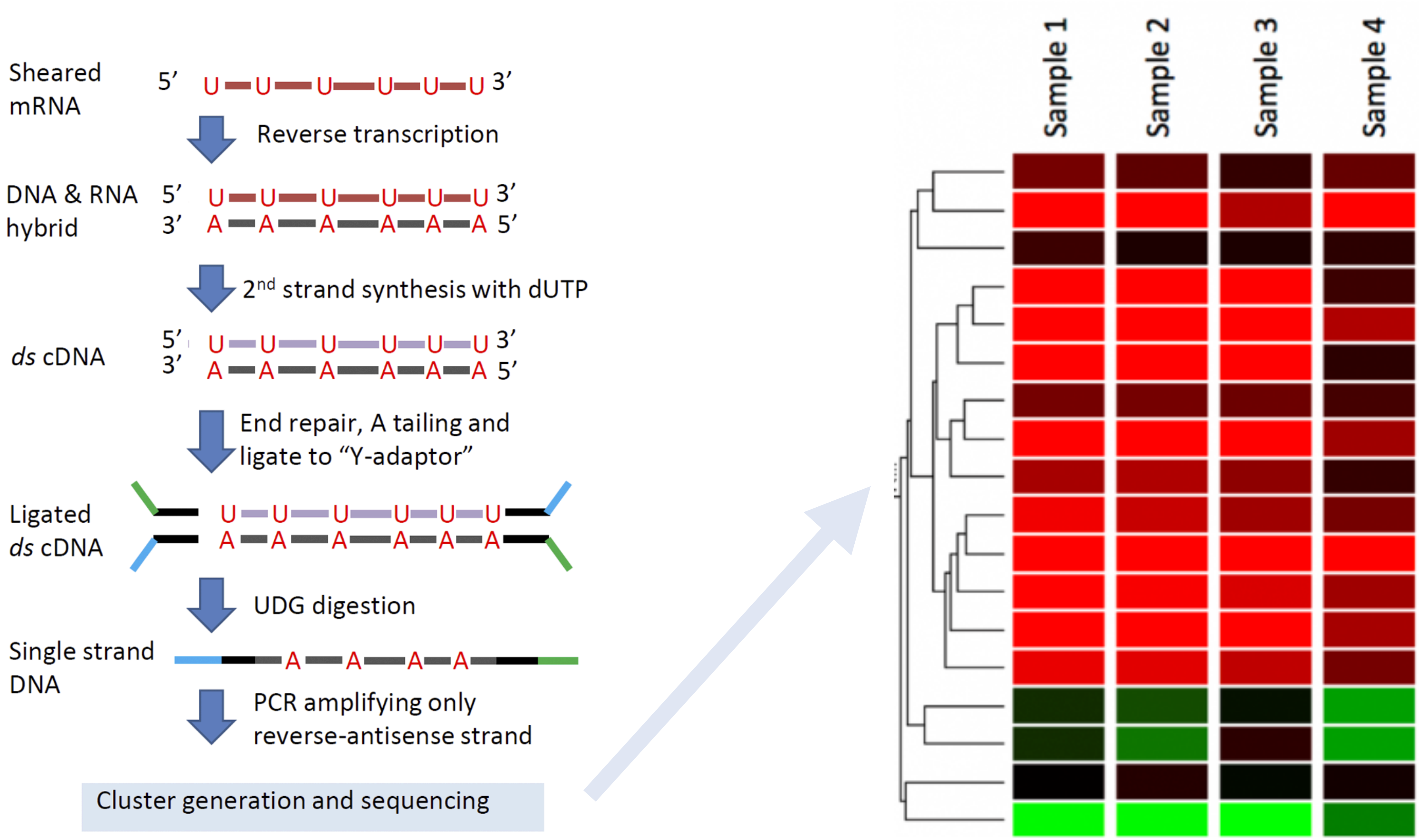

MSUD Mutant




MSUD Mutant + Poor Feeding

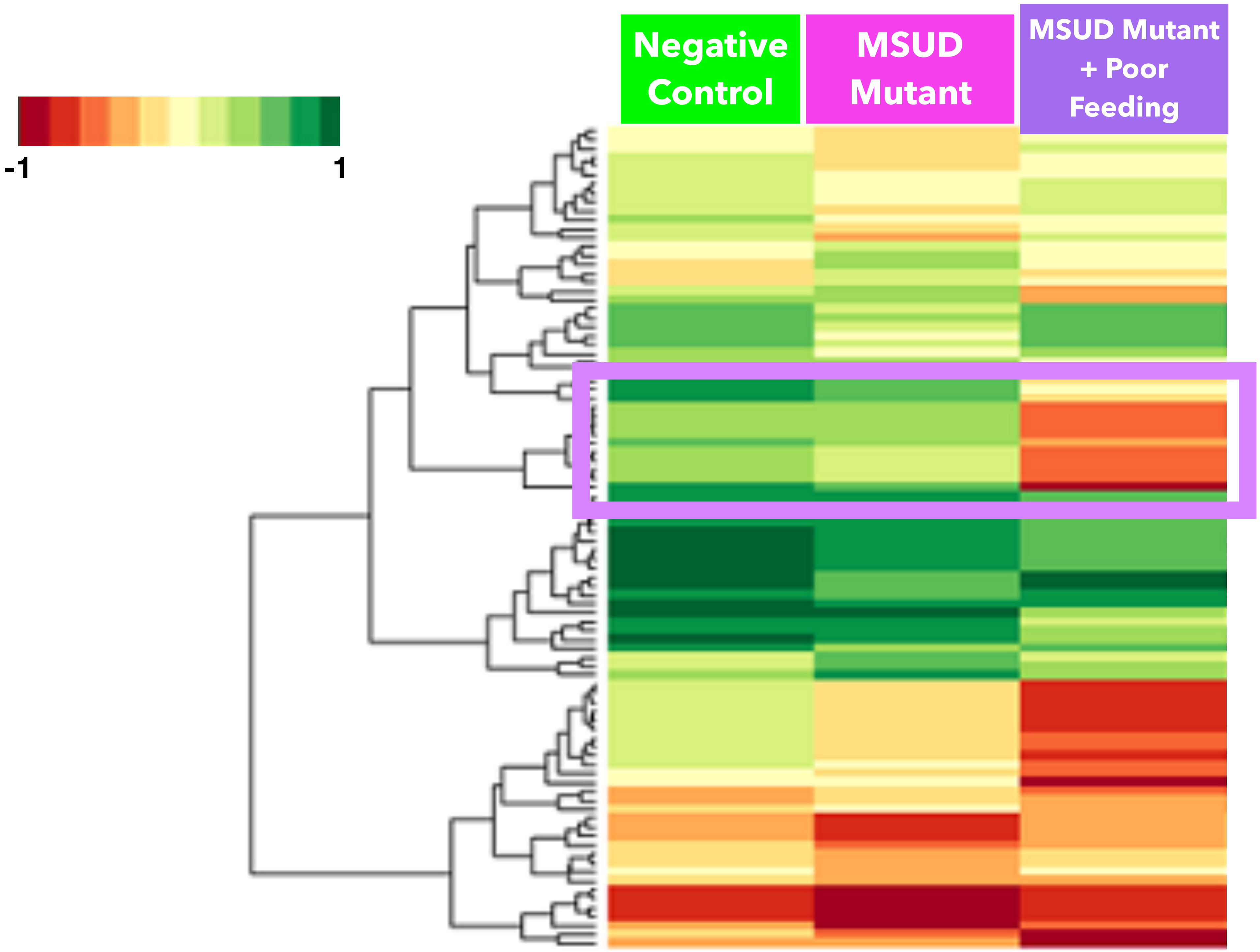


# Aim 2: Determine if mitochondrial genes are highly or lowly expressed between wild type and DBT mutant that are important for feeding




# RNA-Seq

# Aim 2: Determine if mitochondrial genes are highly or lowly expressed between wild type and DBT mutant that are important for feeding




**Hypothesis: Transcripts that interact with DBT and are highly localized in the esophagus will be under expressed in MSUD mutants with poor feeding**

# How will the primary goal be reached?

**Aim 1:**

Identify amino acids that are important for feeding behavior.

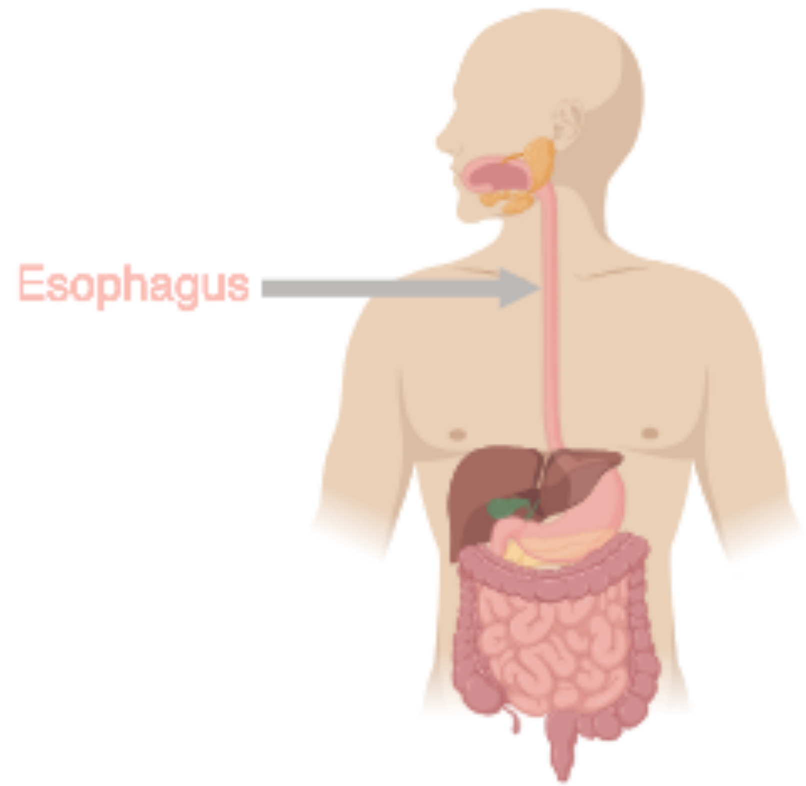
**Aim 2:**

Determine if mitochondrial genes are highly or lowly expressed between wild type and DBT mutant that are important for feeding

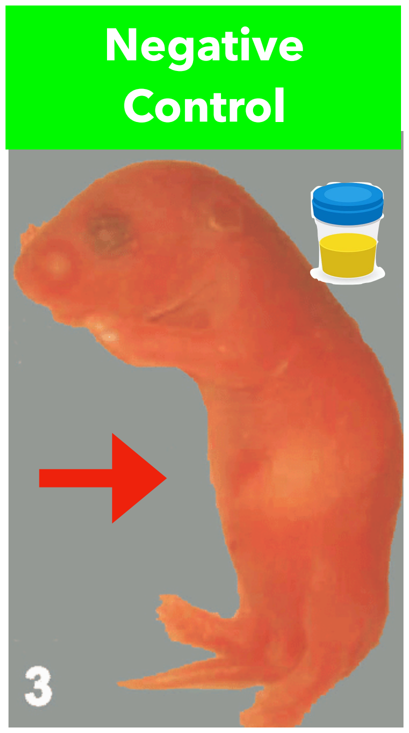
**Aim 3:**

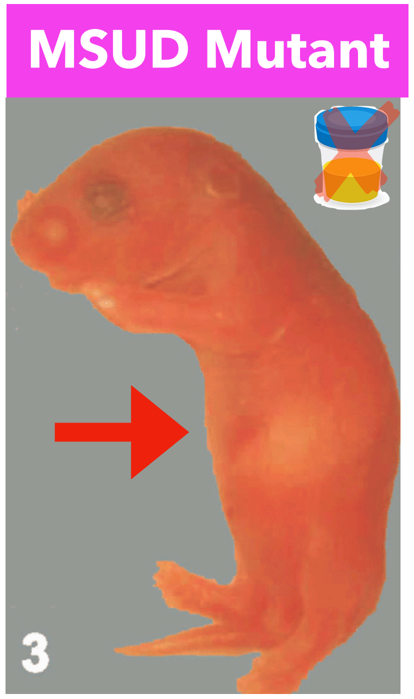
Identify proteins necessary for mitochondrial function and feeding

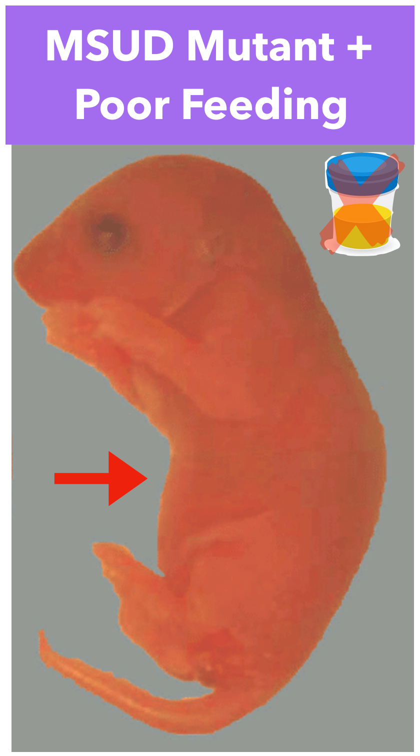
# Aim 3: Identify proteins necessary for mitochondrial function and feeding



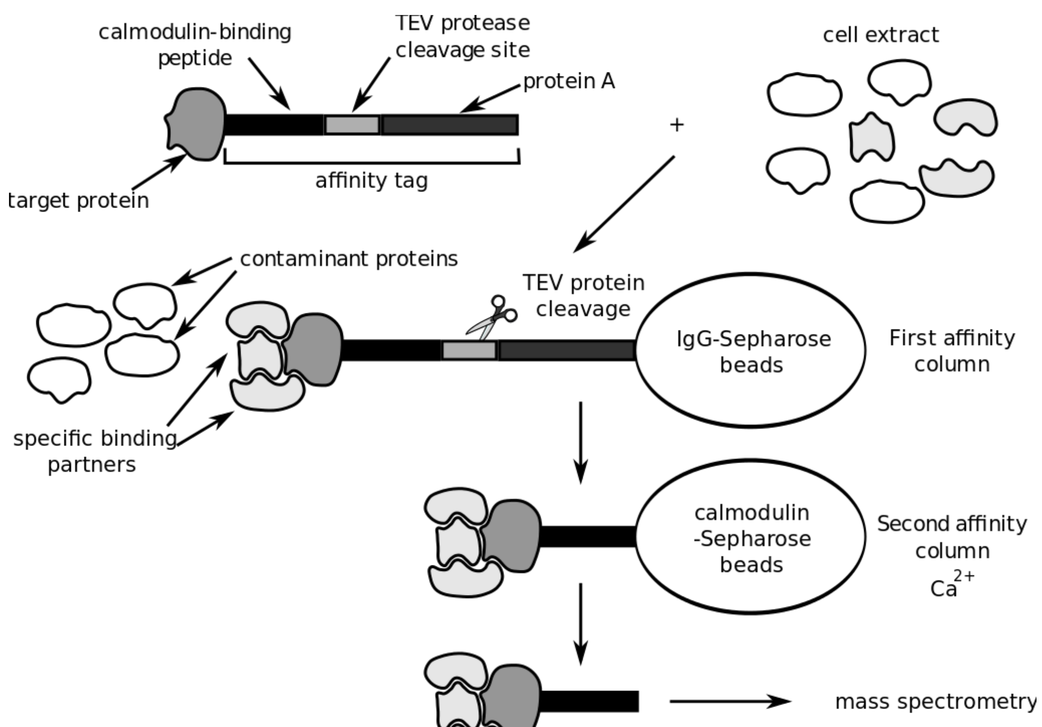

**Extract esophageal tissue from all three test groups**



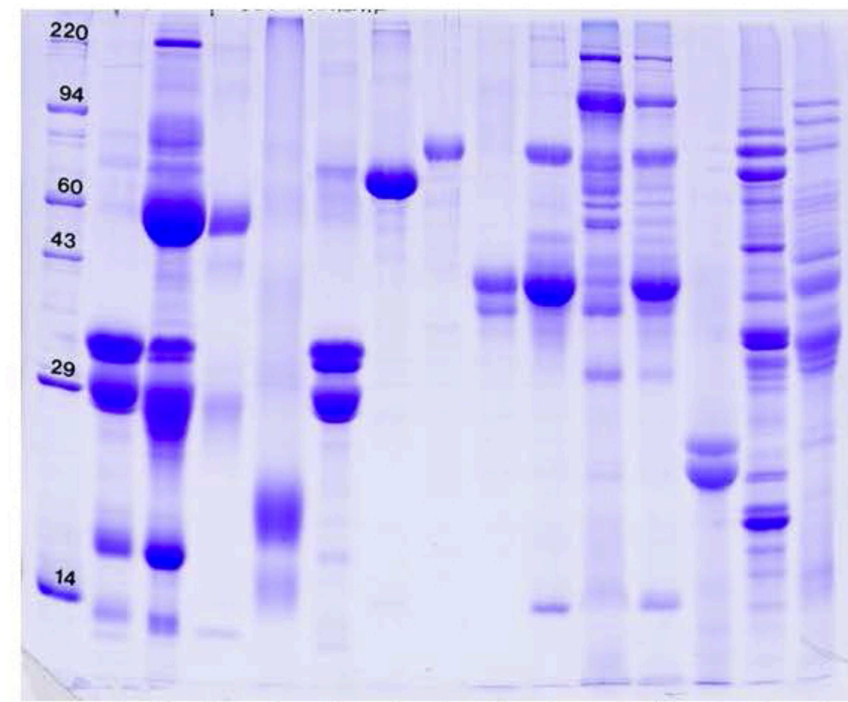



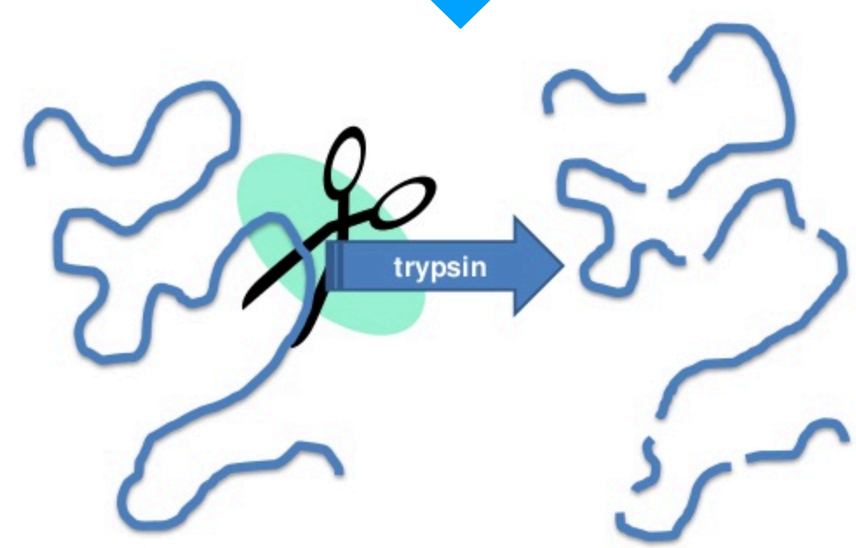
# Aim 3: Identify proteins necessary for mitochondrial function and feeding



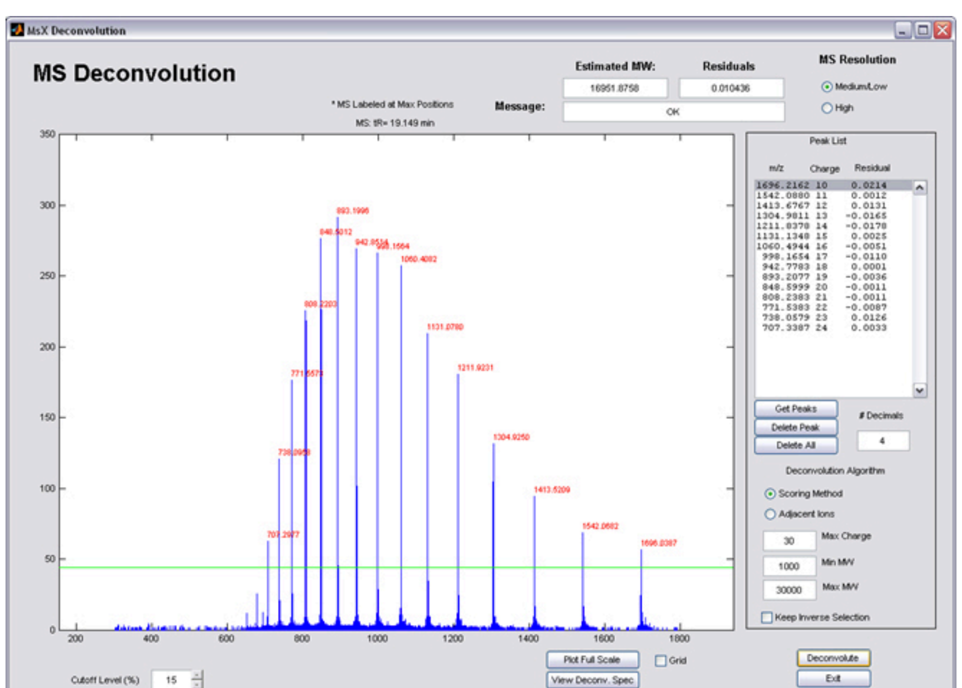

**Tandem Affinity Purification**




**SDS Page Separation**




**Trypsin Digestion**



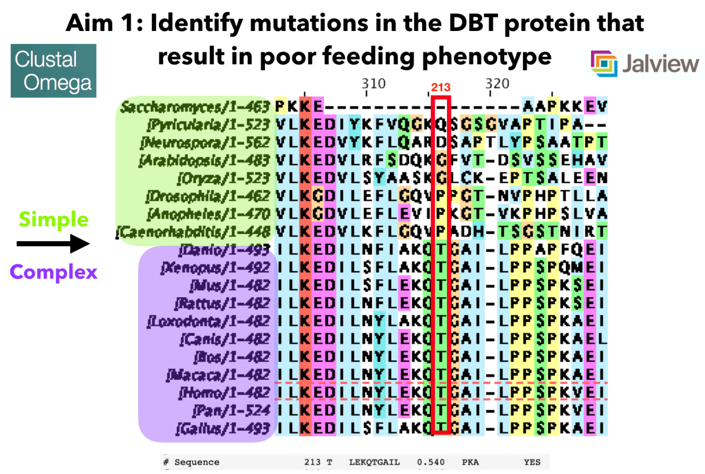

**Mass Spectrometry**

# Aim 3: Identify proteins necessary for mitochondrial function and feeding

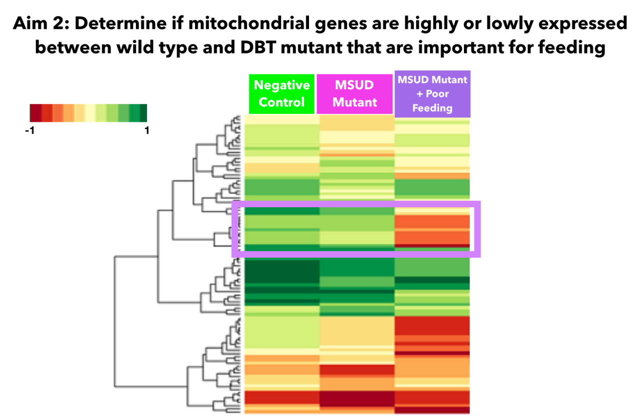
**Organize protein interaction network based on pathways and function**

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

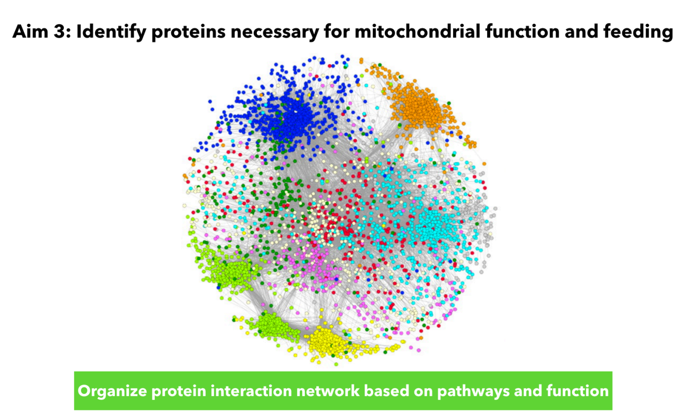
# Conclusions




**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**

# Image References


Title Slide: https://cbmaplefarm.com/shop/category/vermont-maple-syrup/
Slide 1: 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 2: https://www.newbornscreening.info/Parents/aminoaciddisorders/Images/MSUD.gif
Slide 3: 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
Slide 4: 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.proteinatlas.org/ENSG00000137992-DBT/cell
Slide 6: Generated by Mega Software
Slide 7: https://string-db.org/cgi/network.pl?taskId=IdKnOD6d1YVA
Slide 8: https://www.ncbi.nlm.nih.gov/gene/1629 https://www.ncbi.nlm.nih.gov/gene/134266 https://www.proteinatlas.org/ENSG00000137992-DBT/cell
Slide 9: http://atlas-content-cdn.pixelsquid.com/stock-images/white-baby-bottle-half-full-ENmmZkB-600.jpg biorender.com
Slide 10: biorender.com
Slide 11: https://encrypted-tbn0.gstatic.com/images?q=tbn:ANd9GcTQt1xRRpXmWYootdNbnWVYW17Qnde1hSG0vTFqxbRHTr5TnPGs
Slide 12,13,19, 23: biorender.com https://www2.le.ac.uk/projects/vgec/diagrams/115-mrna.gif
Slide 14: https://lw-static-files.s3.amazonaws.com/public/logos/2997.png http://www.jalview.org/Web_Installers/InstData/com/zerog/ia/installer/images/Splash.gif https://pbs.twimg.com/media/DWGv7eCU8AAdct9.jpg https://cdn.the-scientist.com/assets/articleNo/32610/iImg/6309/663848b7-98da-4684-a5ac-5d07d1464c84-lt1.jpg
Slide 15: https://lw-static-files.s3.amazonaws.com/public/logos/2997.png http://www.jalview.org/Web_Installers/InstData/com/zerog/ia/installer/images/Splash.gif http://www.cbs.dtu.dk/cgi-bin/webface2.fcgi?jobid=5CA66549000010A4B31756DC&wait=20
Slide 16: https://294305267s7hqfks2cfh08ip-wpengine.netdna-ssl.com/wp-content/uploads/2017/05/crispr-cas9-drug-discovery2-1.jpg
Slide 17, 20, 24: https://media.istockphoto.com/vectors/urine-test-vector-id495099954?k=6&m=495099954&s=612x612&w=0&h=UCor4LAlkUsFlpNcLpQDL1gB1r46H8u3IPt3pHaJ8Zl= 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 biorender.com
Slide 18: https://www.researchgate.net/figure/Histopathology-of-mouse-esophagus-after-reflux-surgery-A-In-the-non-operated-control_fig3_26690937
Slide 21: https://www.google.com/search?q=transcriptomics+heat+map&source=lnms&tbm=isch&sa=X&ved=0ahUKEwi3zoWr4bfhAhUMw4MKHc7KBbQQ_AUIDigB&biw=1269&bih=623#imgrc=NkG4AM3I7huskM: https://journals.plos.org/plosone/article/file?id=10.1371/journal.pone.0026426.g002&type=large
Slide 22: https://newonlinecourses.science.psu.edu/stat555/sites/onlinecourses.science.psu.edu.stat555/files/cluster/heatmap_01/index.png
Slide 25: https://upload.wikimedia.org/wikipedia/commons/thumb/9/96/Principle_of_Tandem_Affinity_Purification.svg/1024px-Principle_of_Tandem_Affinity_Purification.svg.png https://kendricklabs.com/wp-content/uploads/2017/03/bigger-size-gel-image-nutrition.jpg https://image.slidesharecdn.com/1-141219000811-conversion-gate02/95/1proteomics-coursework3-dec2012aky-19-638.jpg?cb=1418949297 http://www.msmetrix.com/wp-content/uploads/2013/05/ProteinDeconvolution.jpg
Slide 26: http://www.g3journal.org/content/ggg/2/4/453/F5.large.jpg

# References


Strauss, K., Puffenberger, E., Morton, H. (May 2013). Maple Syrup Urine Disease. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK1319/#msud.molgen.TA

National Organization for Rare Disorders. (2017). Rare Disease Data Base: Maple Syrup Urine Disease. Retrieved from https://rarediseases.org/rare-diseases/maple-syrup-urine-disease/#supporting-organizations

Abiri, M., Karamzadeh, R., Mojbafan, M., et al. (August 2016). In silico analysis of novel mutations in maple syrup urine disease patients from Iran. Retrieved from https://link.springer.com/article/10.1007%2Fs11011-016-9867-1

Menkes, J. (February 1959). Maple Syrup Disease Isolation and Identification of Organic Acids in the Urine. Retrieved from http://pediatrics.aappublications.org.ezproxy.library.wisc.edu/content/pediatrics/23/2/348.full.pdf

Evarsson, A., Chuang, J., Wynn, R., et al. (March 2000). Crystal structure of human branched-chain α-ketoacid dehydrogenase and the molecular basis of multienzyme complex deficiency in maple syrup urine disease. Retrieved from https://www.sciencedirect.com/science/article/pii/S0969212600001052

ClinVar. NCBI. (2019). Retrieved from https://www.ncbi.nlm.nih.gov/clinvar

Homanics GE, Skvorak K, Ferguson C, Watkins S, Paul HS. (March 2006). Production and characterization of murine models of classic and intermediate maple syrup urine disease. Retreived from https://www.ncbi.nlm.nih.gov/pubmed/16579849?dopt=Abstract

Peinemann, F., Danner, D. (1994). Maple Syrup Urine Disease 1954 to 1993. Retrieved from https://link-springer-com.ezproxy.library.wisc.edu/content/pdf/10.1007%2FBF00735389.pdf

Danner, D., Armstrong, S., Heffelfiner, E., et al. (March 1985). Absence of Branched Chain Acyl-Transferase as a Cause of Maple Syrup Urine Disease. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC423615/pdf/jcinvest00120-0092.pdf

Danner, D., Doering, CB (June 1998). Human mutations affecting branched chain alpha ketoacid dehydrogenase. Retrieved from https://www.bioscience.org/1998/v3/d/danner/3.htm

Ali, E., Ngu, L. (September 2018). Fourteen new mutations of BCKDHA, BCKDHB and DBT genes associated with maple syrup urine disease (MSUD) in Malaysian population. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6140420/#bb0155

Imtiaz, F., Al-Mostafa, A., Allam, R., et al. (June 2017). Twenty novel mutations in BCKDHA, BCKDHB and DBT genes in a cohort of 52 Saudi Arabian patients with maple syrup urine disease. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5388912/

Blackburn, P. R., Gass, J. M., Vairo, F., et al. (September 2017). Maple syrup urine disease: mechanisms and management. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5593394/

Griffiths AJF, Miller JH, Suzuki DT, et al. (2000). An Introduction to Genetic Analysis. 7th edition. Recombinant DNA technology in eukaryotes. Retrieved from: https://www.ncbi.nlm.nih.gov/books/NBK22002/

Kohlmeir, M. (2013). Nutrigenetics. Chapter 4 – How Nutrients are affected by Genetics. Retrieved from https://www.sciencedirect.com/science/article/pii/B9780123859006000046

String. https://string-db.org/cgi/network.pl?taskId=IdKnOD6d1YVA

Oscar P., Friederike C., Guillaume R., et al. (2001) The Tandem Affinity Purification (TAP) Method: A General Procedure of Protein Complex Purification. Retrieved from: http://www.tulane.edu/~biochem/lecture/723/fig/TAPtag.pdf

Gilles, Hepple, & T., R. (2016, September 20). Editorial: Mitochondria in Skeletal Muscle Health, Aging and Diseases. Retrieved from https://www.frontiersin.org/articles/10.3389/fphys.2016.00446/full

Finsterer, J., & Frank, M. (2016). Gastrointestinal manifestations of mitochondrial disorders: a systematic review. *Therapeutic advances in gastroenterology.* Retrieved from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5330602/