

# Guiding Targeted Proteomics to detect Human Missing Proteins by Protein Identification in Dissected Mouse

Yassene Mohammed, Sarah Michaud, Christoph Borchers

University of Victoria  
Leiden University Medical Center

- Using human tissues for discovery proteomics analyses is ethically challenging and questionable
- It is difficult to predict in which tissue a missing protein might have an expression level high enough to be detected with current instrumentation
- It is possible to use animal model to collect information on protein expression in different tissues

# Approach

- Approximately 99% of mouse genes have counterparts in the human
- Step 1: use bottom-up proteomics for the analysis of mouse tissues
- Step 2: determine which tissues are the most likely candidates for identifying human missing proteins based on the identification of homologs in mouse tissues
- Step 3: use targeted proteomics with internal standards to verify the existence of the protein in the specific human tissue

# Mouse tissue used

- Samples for 41 tissues were dissected from 3 male and 3 female 12 week old C57BL/6NCrl mice

Salivary glands	Lung (left and right lobes)	White blood cells
Kidney	Ovaries	Pancreas
Thymus	Trachea	Sciatic nerve
Liver (Left + Right lateral lobes)	Uterus	Stomach
Testes	Seminal Vesicles	Tongue
Bone marrow	Small intestine	White fat
Cecum	Adrenal glands	Prostate
Epididymis	Brown fat	Lens (right)
Mesenteric Lymph Node	Brain	Plasma
Colon	Eye (left)	Red blood cells
Femur	Heart	Spinal cord
Liver (Caudate and right lobe)	Optical nerve	Spleen
Gallbladder	Urinary bladder	Mammary gland
Skin	Vagina	

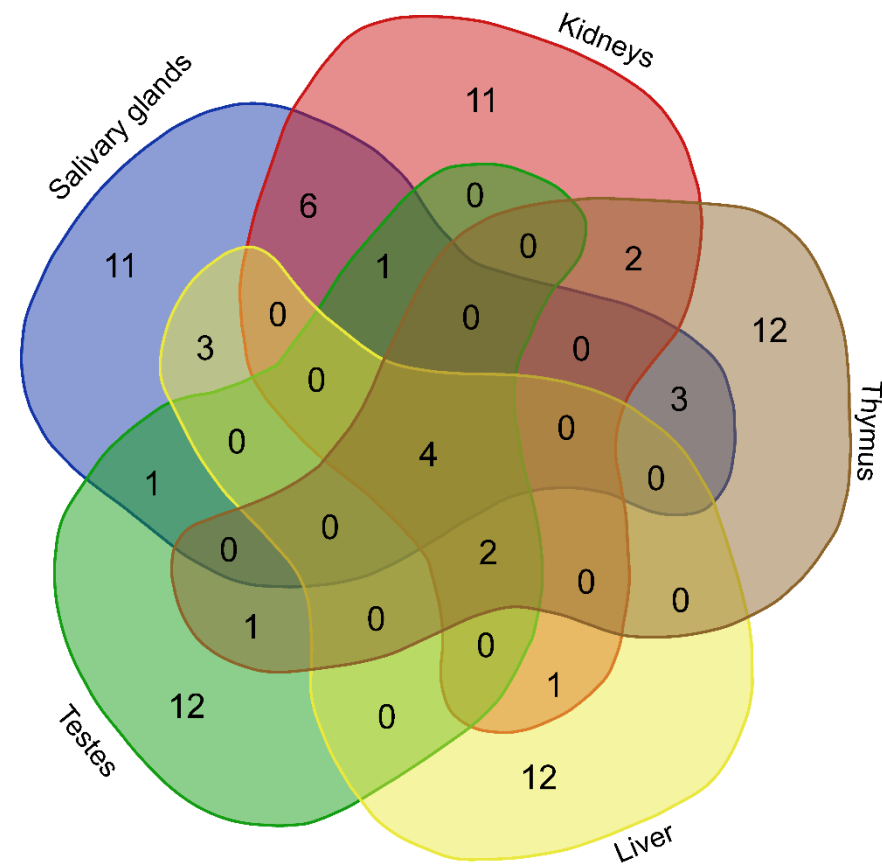
- We used Thermo Orbitrap Fusion with a gradient of 140 minutes
- Raw data was preprocessed in ProteoWizard, searched using Comet/TPP, followed by PeptideProphet, ProteinProphet and selection at FDR value of 1% with a minimum of two peptides per protein
- All identifications were filtered for uniqueness

- We found 195 mouse proteins in various tissues that correspond to human missing proteins from several chromosomes
  - 9 from chromosome 6
- The majority of these proteins were identified in 5 or fewer mouse tissues
- More than half were found in only a single tissue

# Results

Human Chromosome	Number of corresponding mouse protein homologs detected	Tissues with largest number of expressed gene products (number of expressed proteins is mentioned after the colon next to each tissue)
1	19	testes:4, bone marrow:3, colon:3, skin:3, thymus:3
2	18	adrenals glands:1, gall bladder:1, liver:1, liver:1, skin:1
3	10	thymus:3, femur:2, kidneys:2, ovaries:2, trachea:2
4	8	cecum:2, salivary glands:2, adrenals glands:1, brain:1, brown fat:1
5	8	tongue:2, uterus:2, brain:1, heart:1, kidneys:1
6	9	prostate:3, spleen:2, bone marrow:1, cecum:1, colon:1
7	10	brownfat:2, thymus:2, adrenals glands:1, epididymis:1, femur:1
8	6	kidneys:6, salivary glands:6, seminal vesicles:5, epididymis:2, adrenals glands:1
9	10	colon:1, epididymis:1, lung:1, tongue:1, uterus:1
10	6	mln:4, femur:3, lens right:2, skin:2, small intestine:2
11	7	liver:7, liver c-r:6, salivary glands:5, adrenals glands:4, bone marrow:4
12	14	eye left:3, bone marrow:2, kidneys:2, lens right:2, liver:2
13	2	epididymis:3, salivary glands:2, thymus:2, adrenals glands:1, brain:1
14	9	bone marrow:2, eye left:1, lung:1, ovaries:1, plasma:1
15	5	eye left:2, plasma:2, brain:1, cecum:1, liver c-r:1
16	5	bone marrow:2, cecum:2, liver l r lateral:2, testes:2, thymus:2
17	9	kidneys:2, bone marrow:1, brain:1, brownfat:1, cecum:1
18	4	salivary glands:4, colon:3, mln:3, ovaries:3, vagina:3
19	14	femur:2, gall bladder:1, heart:1, kidneys:1, liver l r lateral:1
20	3	trachea:2, urinary bladder:2, brownfat:1, colon:1, femur:1
21	2	cecum:2, colon:2, testes:2, thymus:2, bone marrow:1
22	5	kidneys:1, liver c-r:1, liver l r lateral:1
X	12	pancreas:3, bone marrow:2, cecum:1, epididymis:1, gallbladder:1

- Salivary glands were the tissue with the highest number of missing proteins homologs, 29 proteins
- 15 out of the 29 are annotated as membrane proteins
- High number of membrane proteins is also the case for other tissues with high numbers of homologs of human missing proteins





# Current and future analyses

- Detect homologs of identified proteins in corresponding human tissues by targeted proteomics
- We synthesized 90 heavy labeled peptides, proteotypic to 47 found homologs in the top 3 tissues with the highest number of identifications
- We are waiting for 3 sample of each of the 3 tissues with highest identifications
  - From two different sources (cadaver and fresh)

# Summary

- We used mouse tissue to determine which tissue has a homolog of a human missing protein that is expressed to a level of detection
- 195 mouse proteins were detected in various tissues that correspond to human missing proteins from several chromosomes
- With 29 proteins, the salivary glands had the highest number of homologs of human missing proteins
- Validation in human tissue will follow soon

# Questions?