

# Proteomic analysis of *Taenia solium* excretion- secretion proteins

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# *T. solium* proteomics

- The project.
- Objectives of the study.
- Global methodology.
- What are the results so far?
- Discussion.
- What is next?





# PhD project

- An FWO - ICONZ funded project

High throughput analysis of *Taenia* excretion-secretion proteins (ESPs) for improved understanding of host-parasite interactions and optimization of immunodiagnostic tools in *Taenia solium* cysticercosis.



# Objectives

2 major objectives:

1. Analyze the excretion-secretion proteome of *T. solium* to improve current knowledge of host-parasite interaction.

So far, no studies of the complete ES proteome.



# Objectives

2. Optimization of immunodiagnostic tools in *T. solium* cysticercosis.

**PROBLEM:** antigen detection ELISA detects viable cysticerci, but is not species specific.

**SOLUTION:** find proteins unique for *T. solium* (or *T. hydatigena*) and make the ELISA more specific.



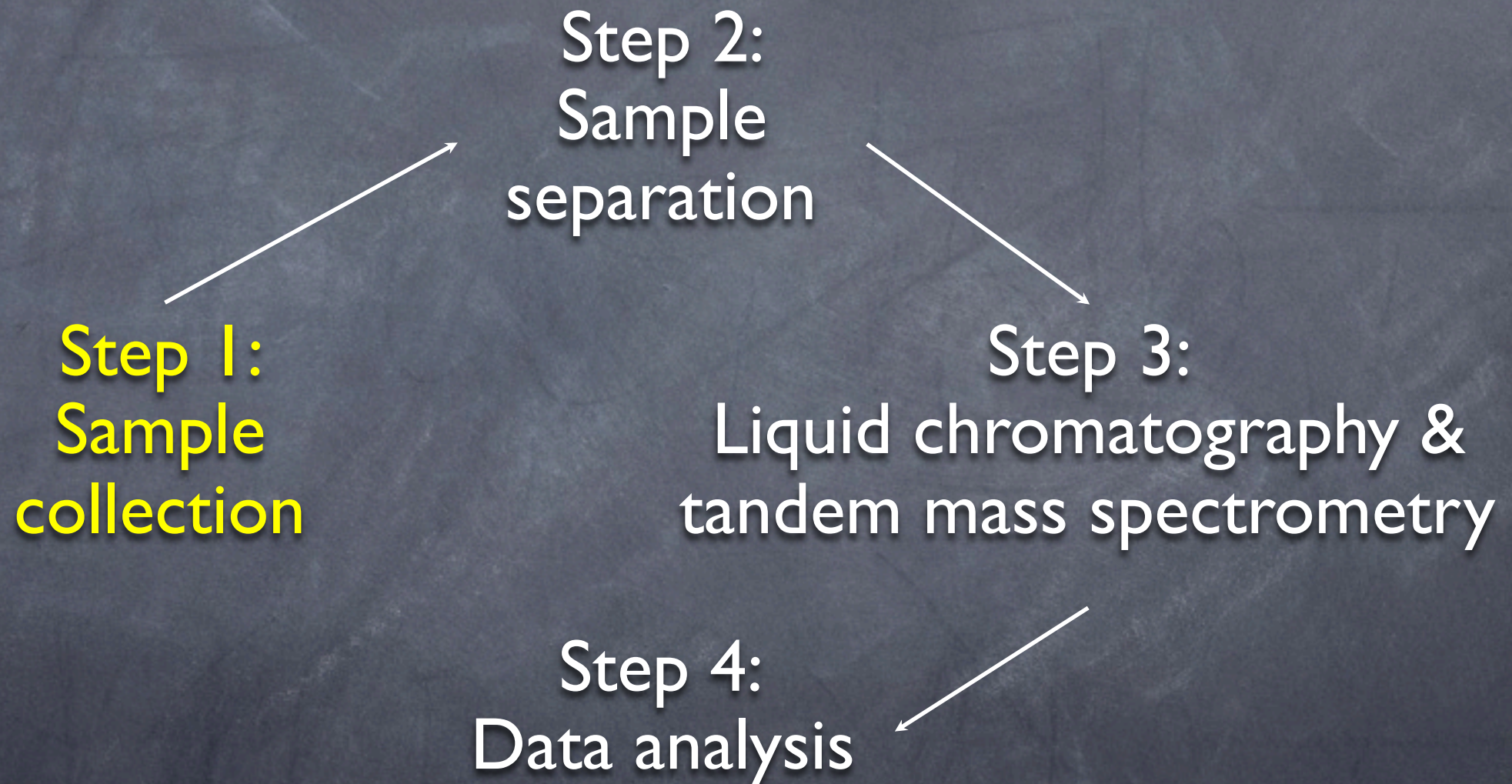
# Methodology

- Starting point is the same for both objectives:

A complete analysis of *T. solium* excretion-secretion proteins.



# Methodology





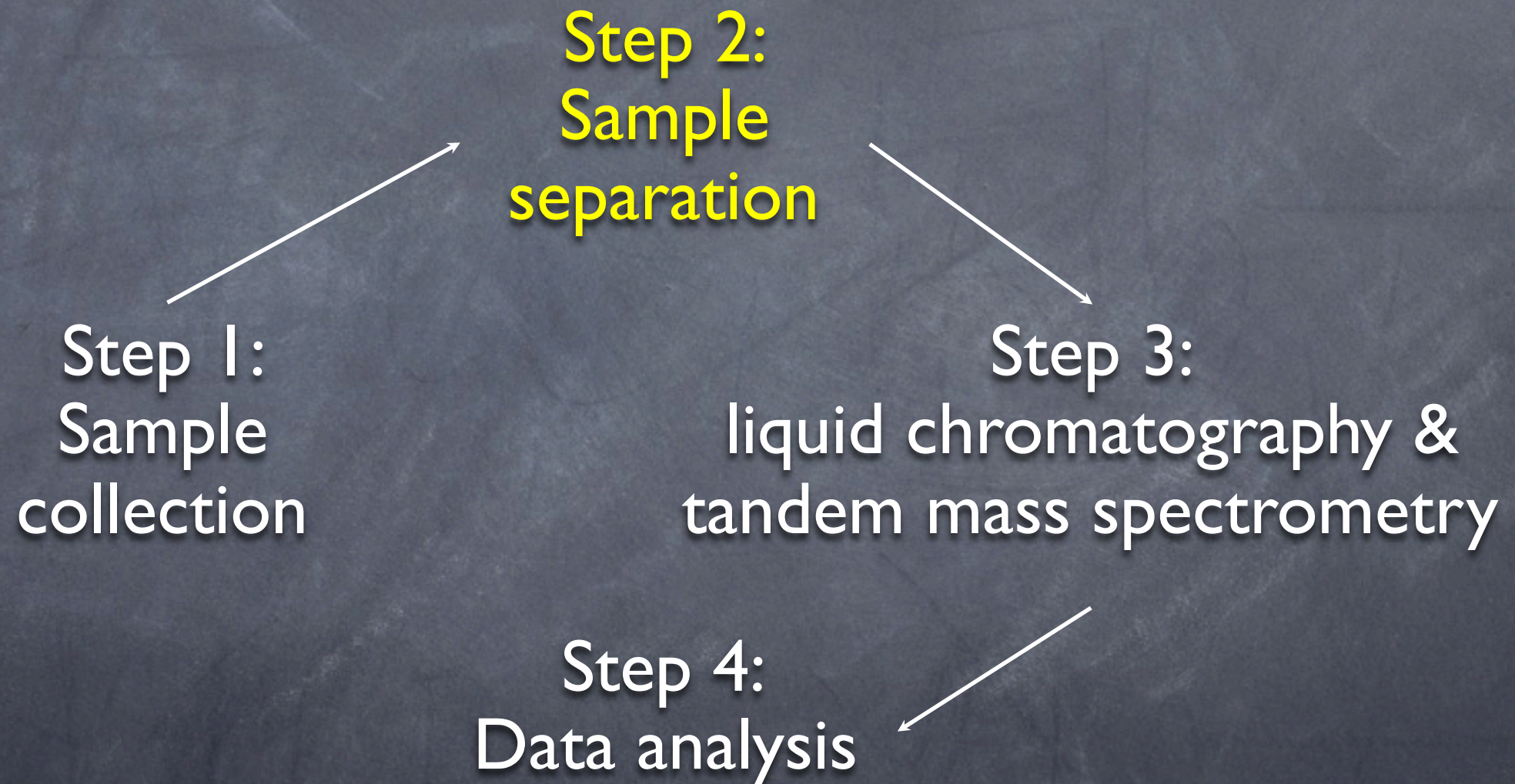
# Methodology

## Step I

- 5 pigs from Peru and Zambia.
- *In vitro* culture of cysticerci.
- Collect culture medium (= ESPs) after 24h and 48h.
- Concentrate ESPs (MWCO = 1kDa).



# Methodology

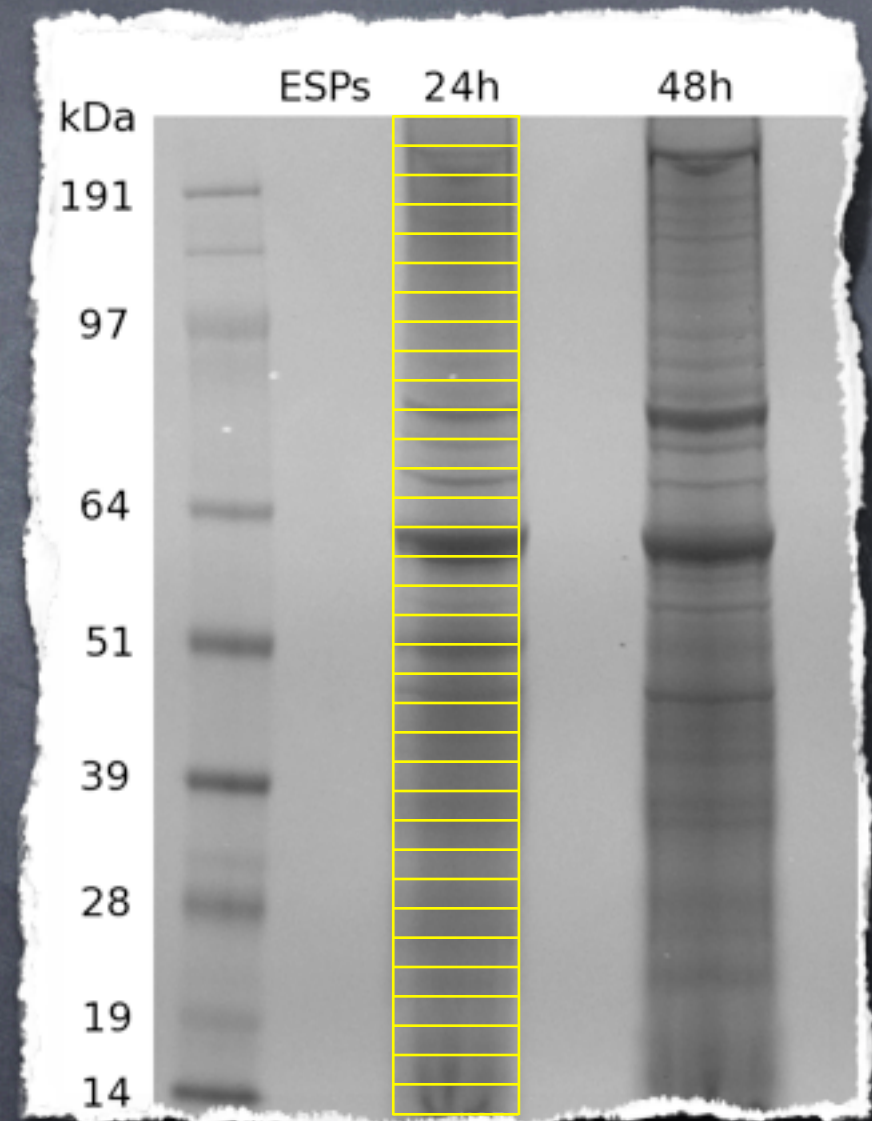




# Methodology

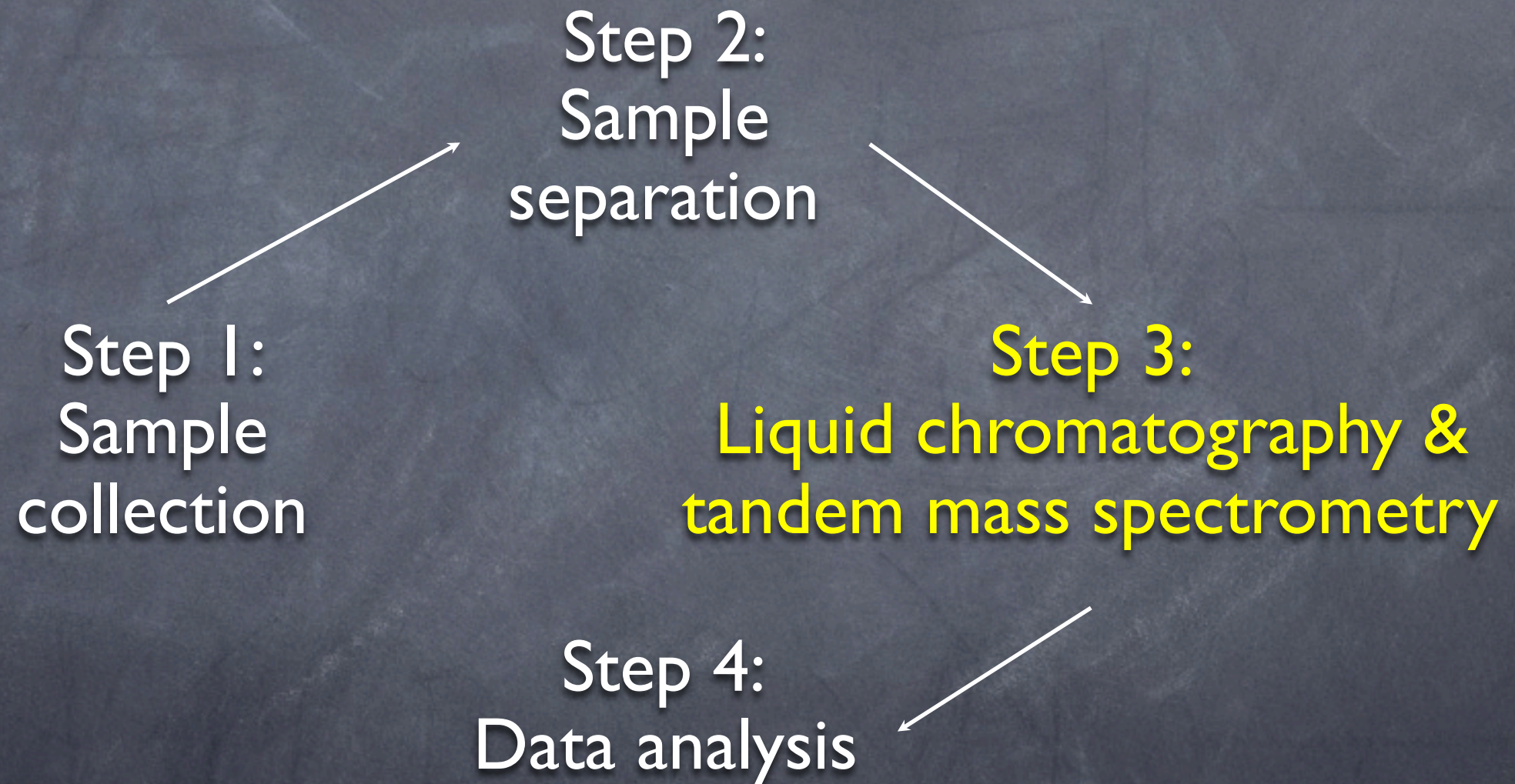
## Step 2

- Separate ESPs using I-D PAGE.
- Cut lane in 48 slices.
- Tryptic digest: proteins are cut into peptides.





# Methodology

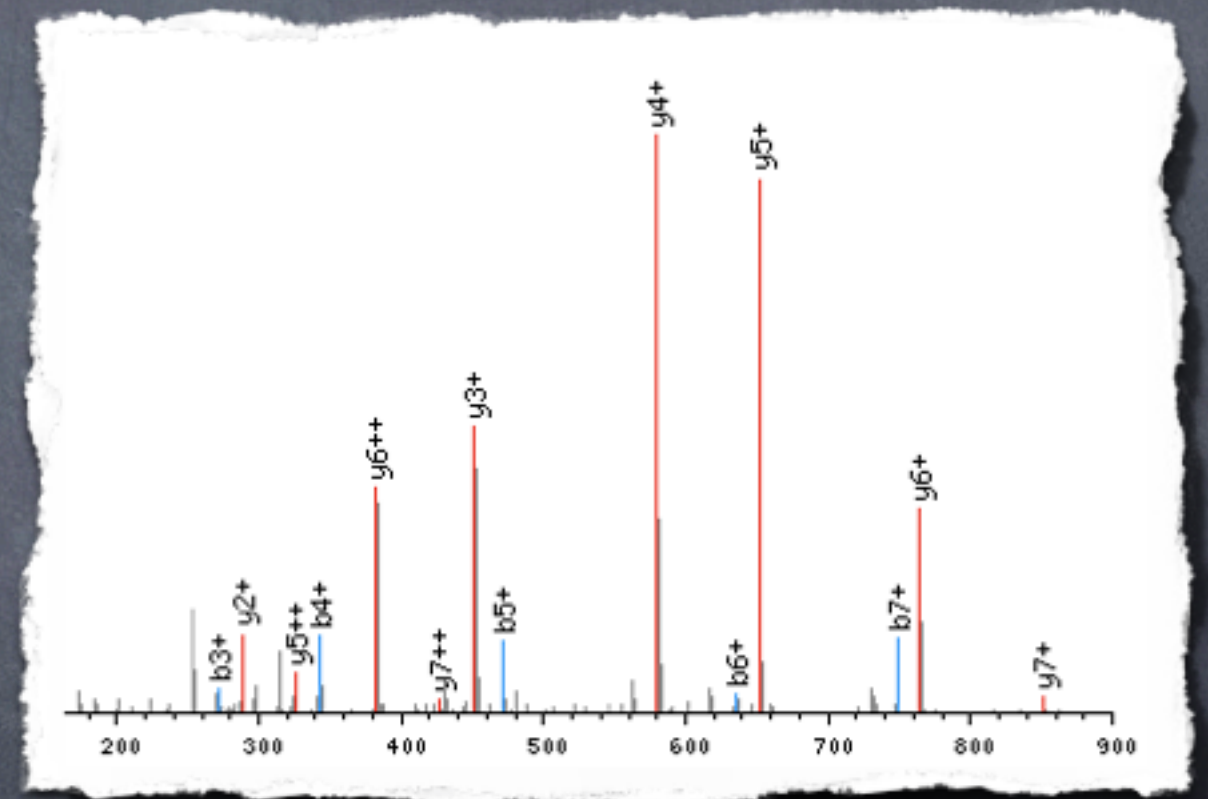




# Methodology

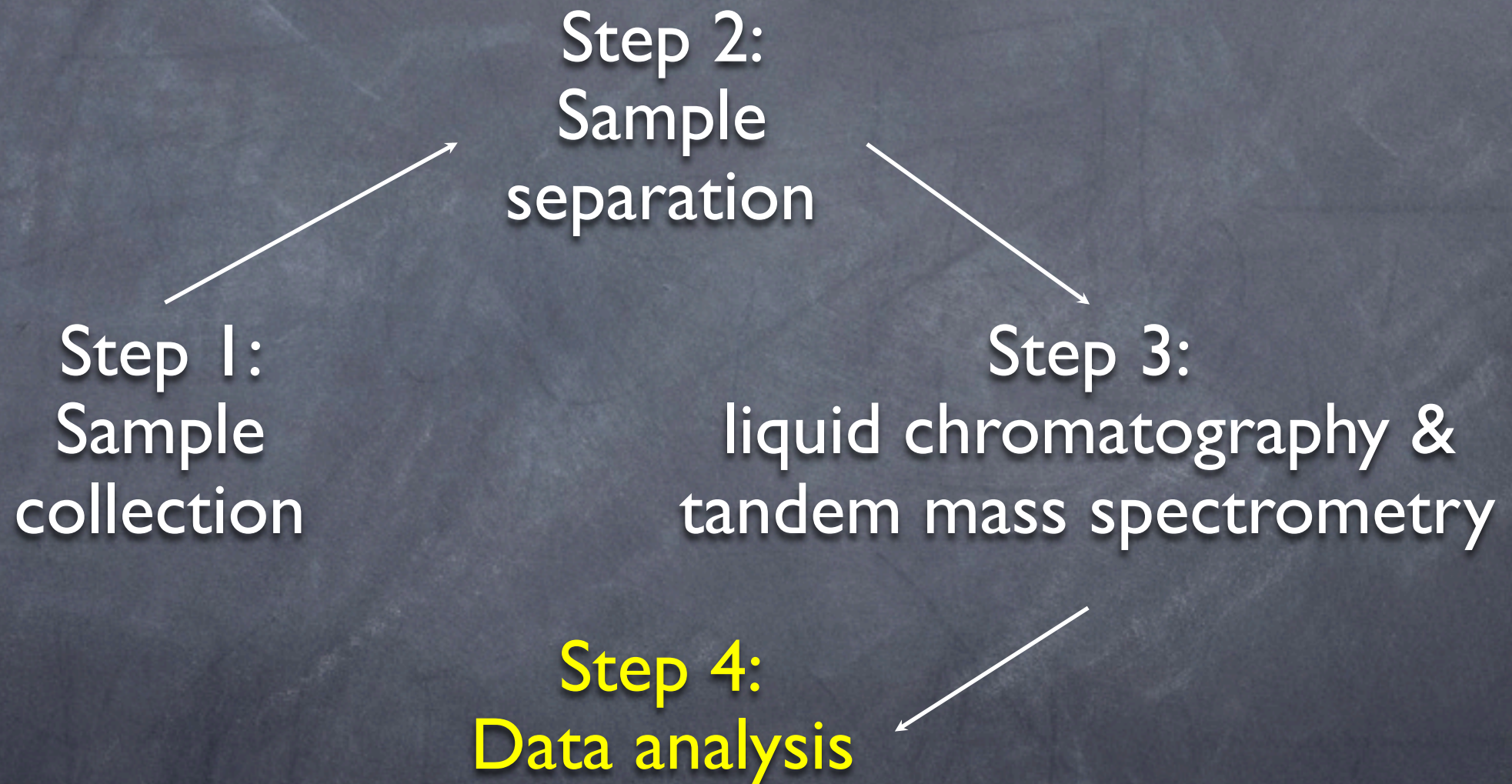
## Step 3

- ⑥ LUMC, Leiden.
- ⑥ Separate peptides with liquid chromatography.
- ⑥ On-line with 'ion trap' mass spectrometer.
- ⑥ Result: dataset with experimental spectra.





# Methodology





# Methodology

## Step 4

- Compare exp. spectra to spectra from a protein database with X!Tandem (*in silico* digest).
- Protein identification using validated tools (PeptideProphet, iProphet and ProteinProphet) to estimate accuracy of peptide/protein identifications.
- Ideal result: list of all proteins in the ESP sample.



# However...

- You need a protein database that contains all proteins likely to be in your sample.
  - *T. solium* protein database is **incomplete**.  
NCBI: ~ 270 proteins.
  - Genome is not (yet) known.



# Solutions?

- How to get around this limitation:

1. Sequence *T. solium* genome.

2. Supplement *T. solium* protein database with proteins from other helminths.

All *Taenia* (821) all *Schistosoma* (29,953), all *Echinococcus* (1,146), all *Trichinella* (16,325), *Sus scrofa* (1,388) and cRAP (112).



# What are the results so far?

Origin	# proteins
<i>T. solium</i>	27
Sequences from other helminths	32
<i>S. scrofa</i>	17

- *T. solium* proteins: mostly proteins used in EITB.
- Gene ontology classification: stress response, metabolism, detoxification, proteolysis, ...



# Discussion

- Unknown proteins in ESPs.

Unmatched spectra = unknown proteins?

- Are helminth ES proteomes roughly the same?

Look to genome for differences?

B. Victor, K. Kanobana, S. Gabriël, K. Polman, N. Deckers, P. Dorny, A. M. Deelder and M. Palmblad. Proteomic analysis of *Taenia solium* metacestode excretion-secretion proteins. Accepted for publication in PROTEOMICS on March 5, 2012.



# What is next?

- Search *T. solium* Expressed Sequence Tag (EST) library.

Transcriptomics: mRNA – cDNA – EST.

- Find unassigned spectra and try *de novo* analysis.

Find peptide from spectrum.

- ✓ Chance of finding 'new' peptides/proteins.



# What is next?

- *T. solium* genome project(s)?

Derive proteins from DNA.

- Analyze *T. hydatigena* ESPs and compare with *T. solium* (discover differential proteins).

2-D PAGE and look for differences.



# Thank you!

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