Ricin Attribution R&D Results and Conclusions

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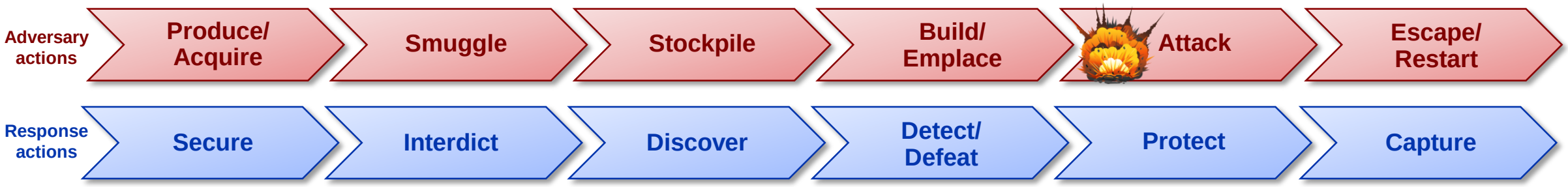
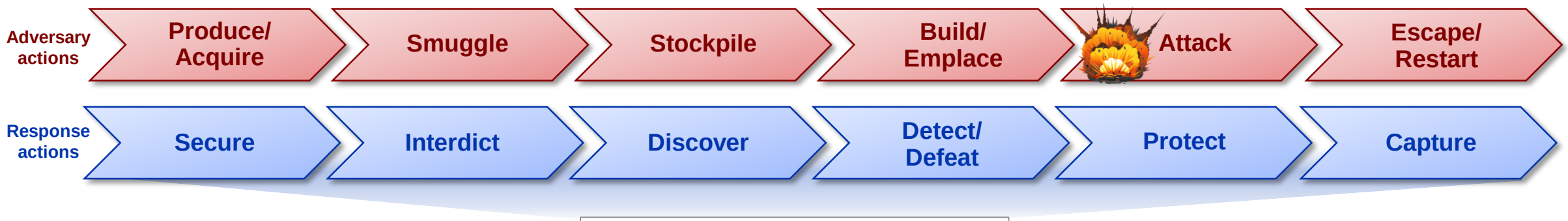
Path to Ricin Use and Disruption Options



Gehl and Plecas, Introduction to Criminal Investigation: Processes, Practices, and Thinking, 2017

Forensic Response Actions



Forensic Response Actions

Chemical/Biological

- Chemical/biochemical analysis
- DNA analysis

Pattern Impressions

- Biometrics (e.g. fingerprints)
- Tool mark examination

Surveillance

- Cell phone data
- Physical/video

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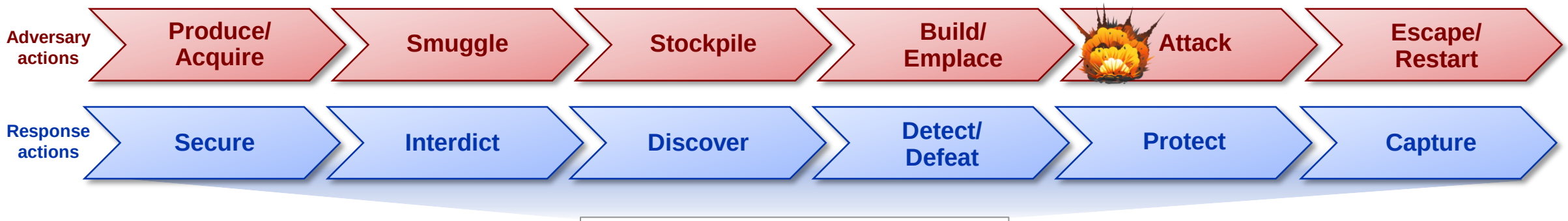

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Chemical and biochemical analysis of threat signatures (e.g. trace elements) can address operational needs of investigators including attribution of ricin

Gehl and Plecas, Introduction to Criminal Investigation: Processes, Practices, and Thinking, 2017

Approaches to International Threat Prevention

Prosecution

Prevent further attacks by apprehension of those responsible

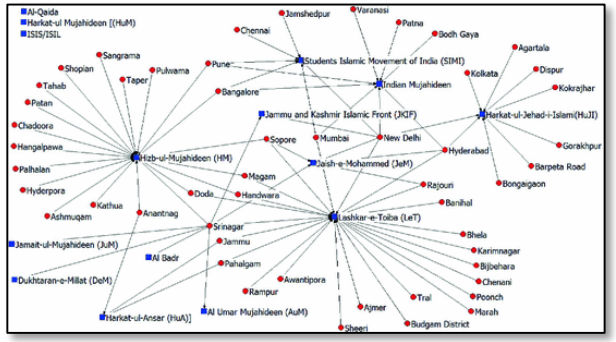
Non/Counter Proliferation

Help governments, industries prevent misuse of knowledge, facilities, precursors

Focus Interdiction Efforts

Increase enforcement in channels connecting producers with supplies and users

Collect Intelligence on Threats



Map production/distribution networks across space/time, assess intent and plans

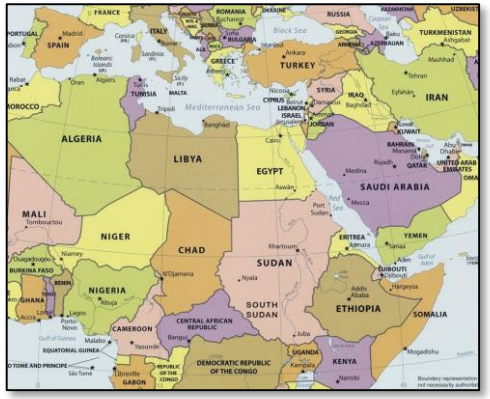
Forensic attribution plays a central role in all of these threat prevention approaches

Example MIT LL Attribution R&D Application Goals

What is it?

ID threat material using chemical, biological, physical signatures

Where has it been?



Estimate location history using DNA metabarcoding, ML

Who assembled it?

Identify suspects in challenging samples using DNA sequencing/bioinformatics

How was it made?

Precursor/supplies/methods intel using chemical, genetic, ML methods

When was it made?

Predict age of produced materials (emerging)

Where is the source?

Origin determination using chemical/physical analysis, ML

ML = Machine Learning

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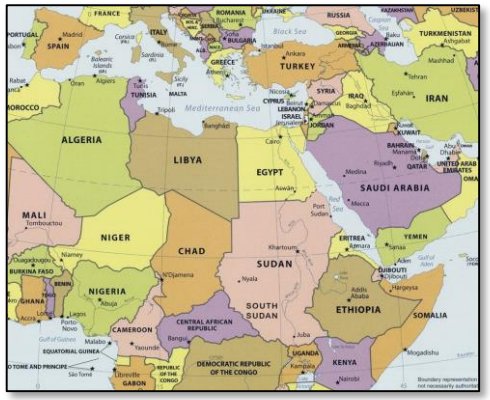

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BLUF and Outline

Bottom Line Up Front: All methods attempted show some promise for discriminating methods and/or cultivars used to extract ricin, additional samples/data/analysis likely needed for acceptance in forensic labs

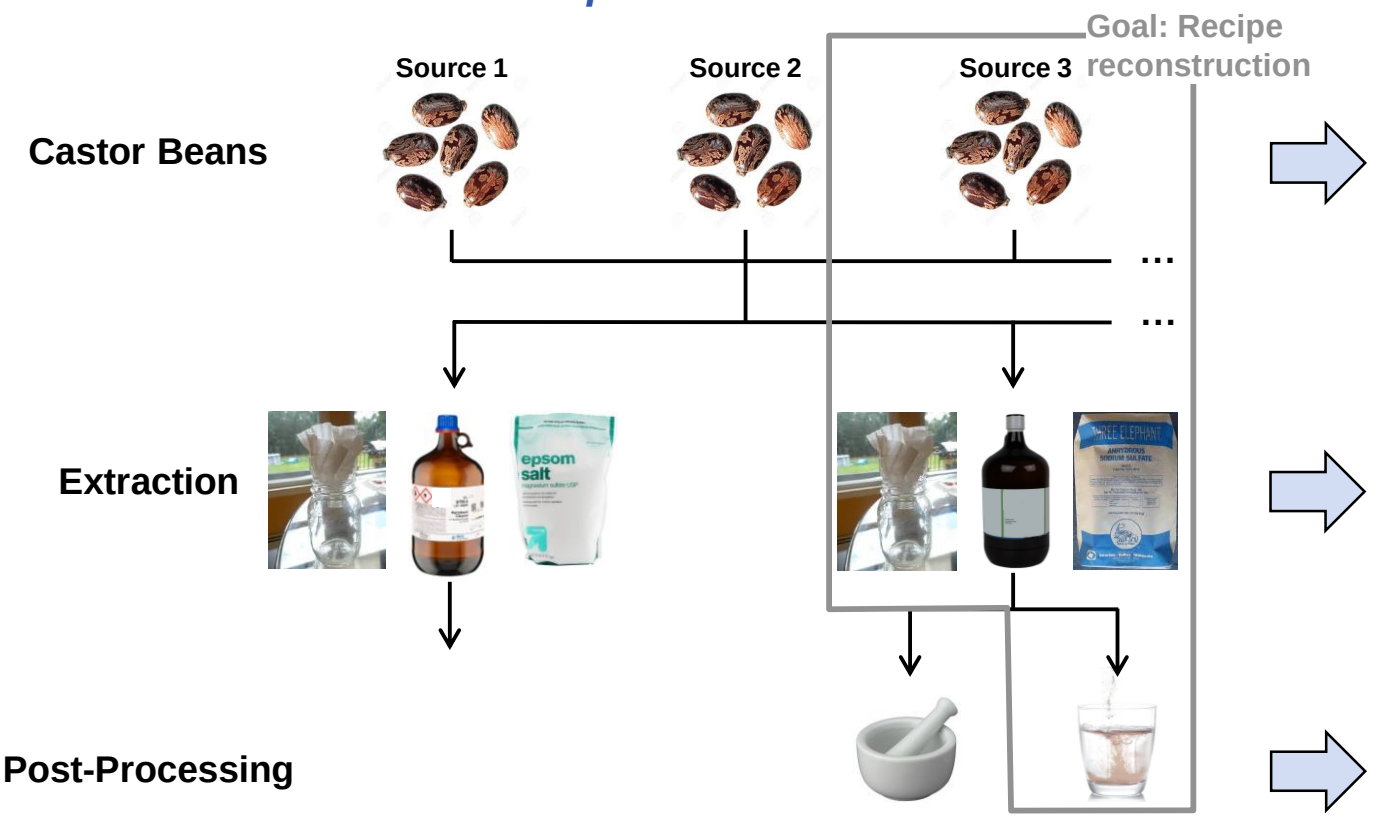
A. What method was used to extract ricin?
1. Ricin and agglutinin protein ratios
2. Trace element analysis
3. Small molecule analysis
4. Co-isolating proteins

B. What cultivar was used in the extraction?
1. Small molecule analysis
2. Peptide variant analysis
3. ~~DNA sequencing~~

MITLL Ricin Attribution Program Introduction

Goal: Enable improved supply chain outreach and post-attack investigations by developing capability to forensically reconstruct ricin preparation recipe and raw material sources

Preparation Methods



Potential Attribution Signatures

Where did it come from?

- Ricin gene DNA sequence
- Ricin protein sequence
- Castor bean DNA sequence
- Post-translational modifications

How was it made?

- Co-isolated proteins
- Inorganic and organic composition
- Isotope ratios

- Inorganic and organic composition
- Particle size

Program will result in reference samples, analytical methods, and a data interpretation framework for transition

Signature Measurement Techniques

LC-MS

Technique Description:
- **Liquid chromatography – mass spectrometry (LC-MS)**
- **Measures polar organic compounds, biomolecules (e.g. proteins/peptides, etc.)**

Signatures:
- **Ricin/Agglutinin quantitation for protein ratios**
- **Co-isolating Proteins**
- **Peptide variants**

GC-MS

Technique Description:
- **Gas chromatography – mass spectrometry (GC-MS)**
- **Measures non-polar organic compounds**

Signatures:
- **Small molecules**

ICP-MS

Technique Description:
- **Inductively coupled plasma – mass spectrometry (ICP-MS)**
- **Measures elemental concentrations**

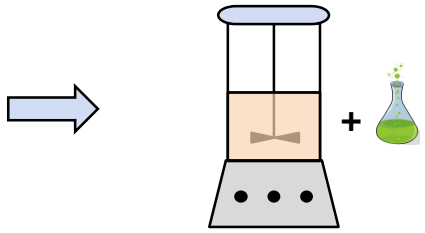
Signatures:
- **Trace elements**

Basics of Ricin Extraction and Purification

Source

- **Castor beans come from *Ricinus communis* plant**

Extract

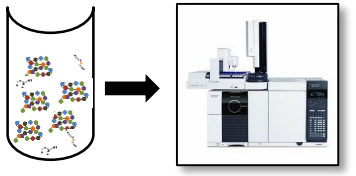


- **Beans are blended and proteins are extracted**

Purify

- **Protein purified from sample matrix**

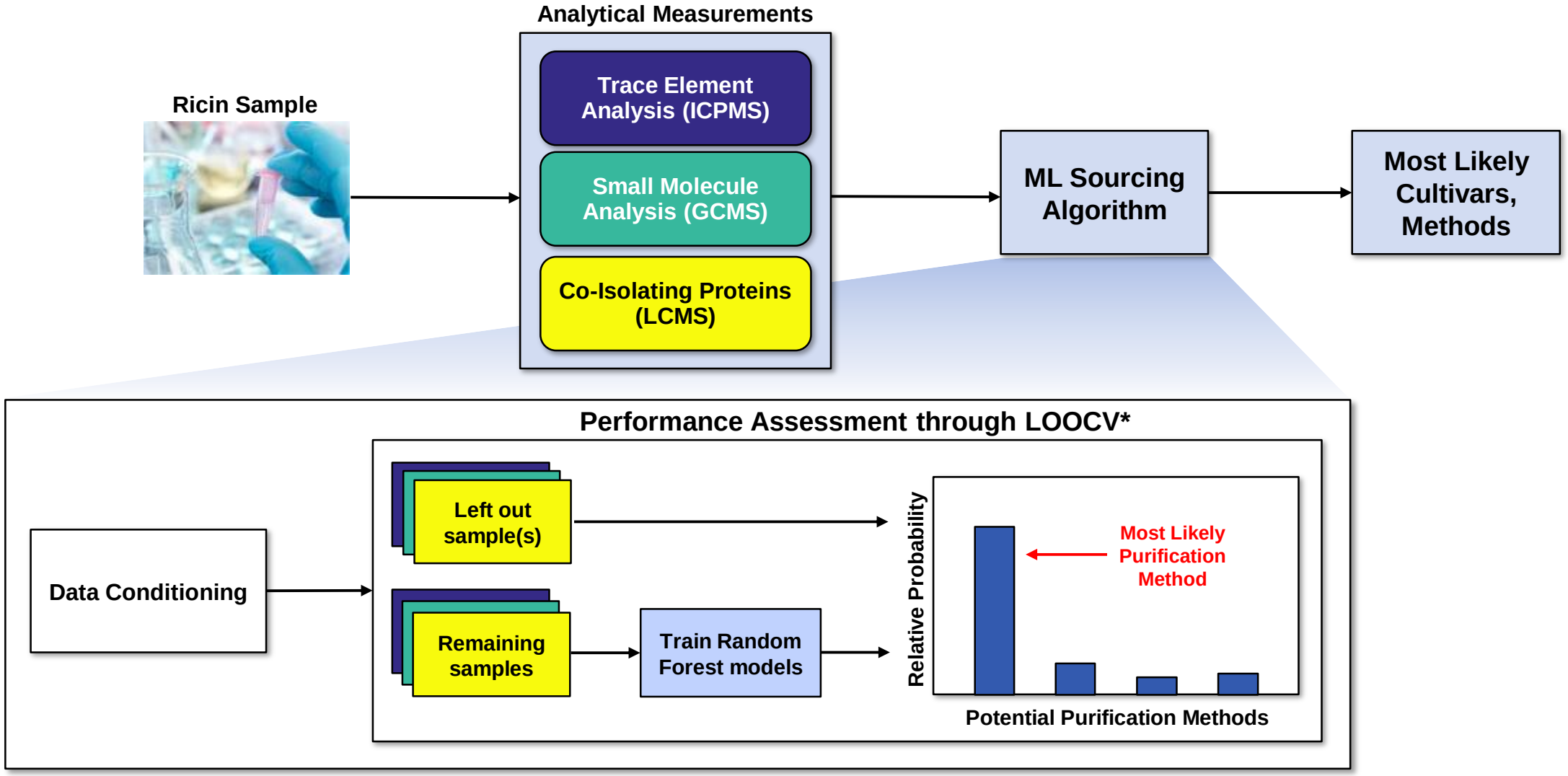
Analyze



- **Signatures remaining after purification are analyzed**

Method	Source	Preparation
Purification Method 1	Cultivar 1	A,B,C
Purification Method 2		F,G
Purification Method 3		I,J,K
Purification Method 4		L,R,S
	Cultivar 2	M
	Cultivar 3	N
	Cultivar 4	O
	Cultivar 5	P
	Cultivar 6	Q
	Cultivar 7	R

Attribution Using Simple Machine Learning Algorithm



ML = Machine Learning
LOOCV = Leave One Out Cross Validation
** "Leave one batch out" for Trace Element Analysis*

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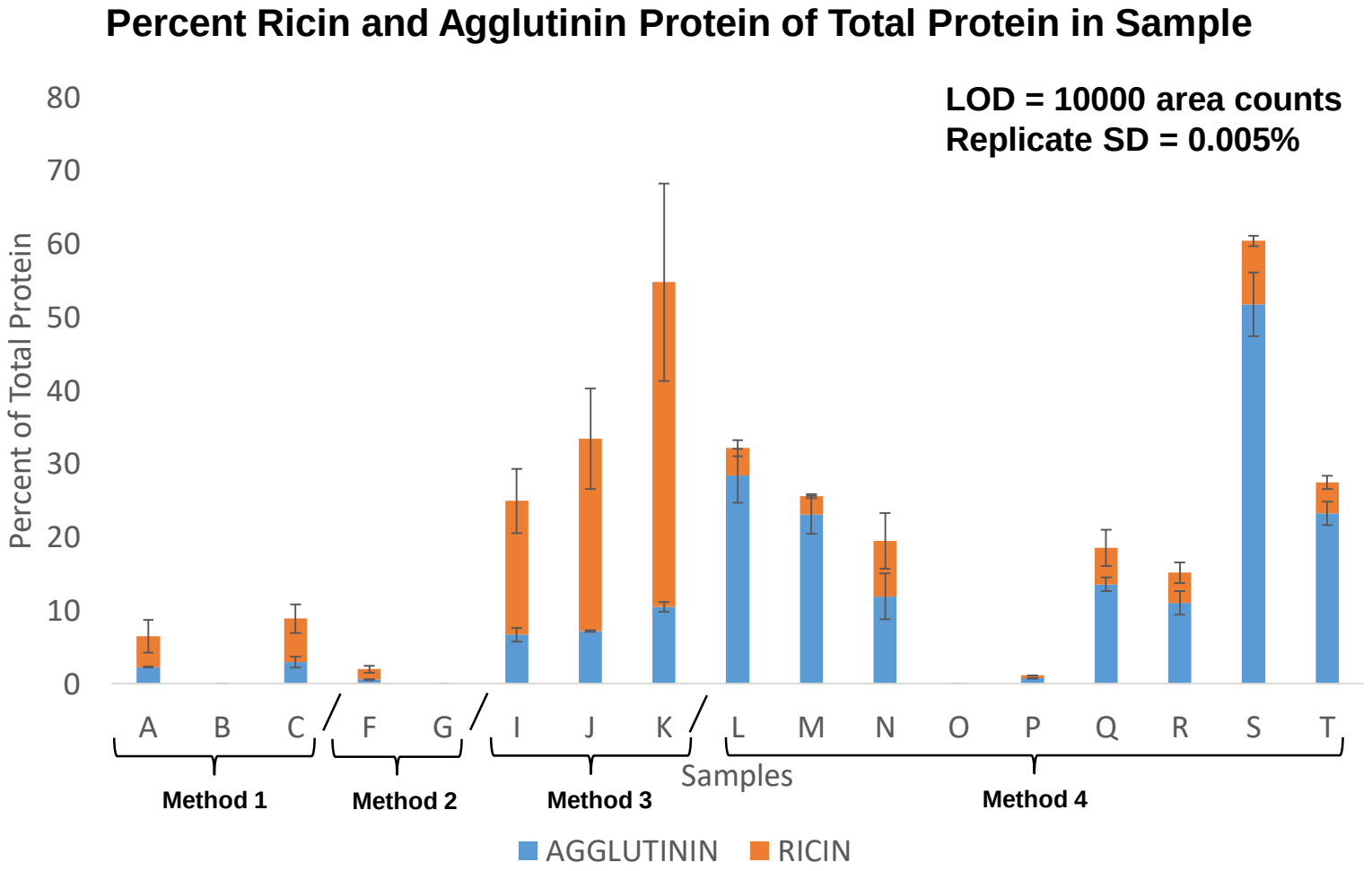
A1) Ricin and Agglutinin Protein Results (Method)

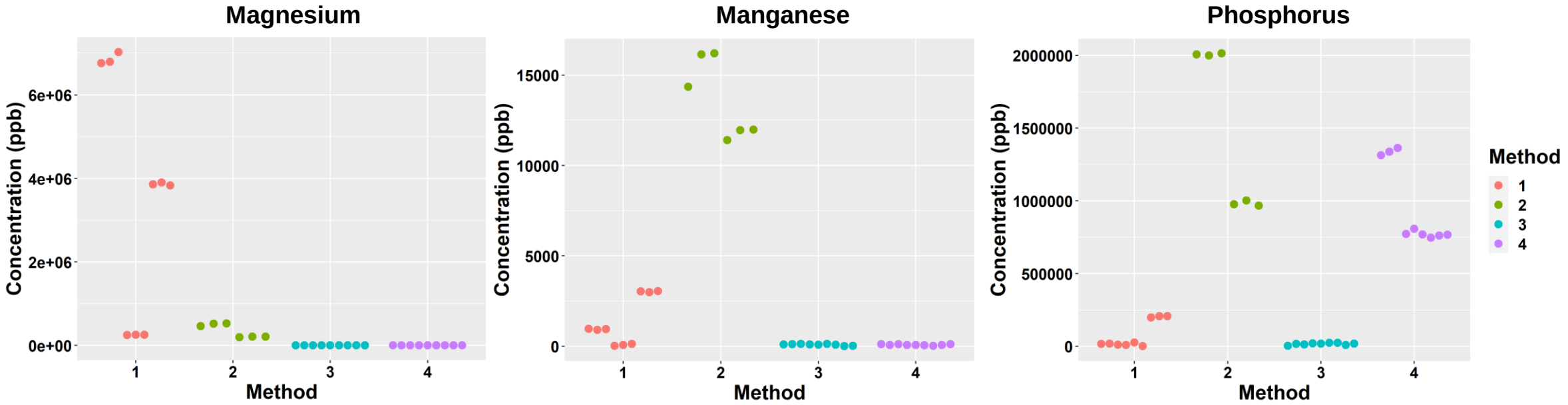


Simple ratio of ricin to agglutinin may be indicative of certain purification methods

A, B, C, F, G, I, J, K, L, S, T = Cultivar 1

M, N, O, P, Q, R = Cultivars 2-7

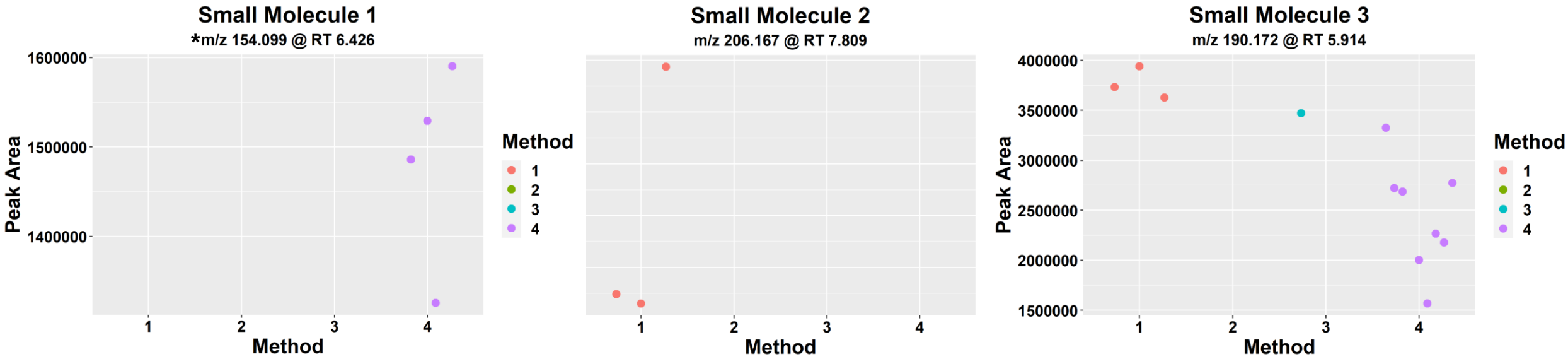
A2) Trace Element Analysis (Method)



- Method 1 used Epsom salts ($MgSO_4$ heptahydrate) which contain magnesium
- Additional elements also provide support for method discrimination (Na, S, K, Ca, Cr, Zn, Ga, Sr, Ba, W, Pb)
- Data on additional cultivar / purification method combinations is needed

Trace element analysis can be used to identify purification method

A3) Small Molecule Analysis (Method)



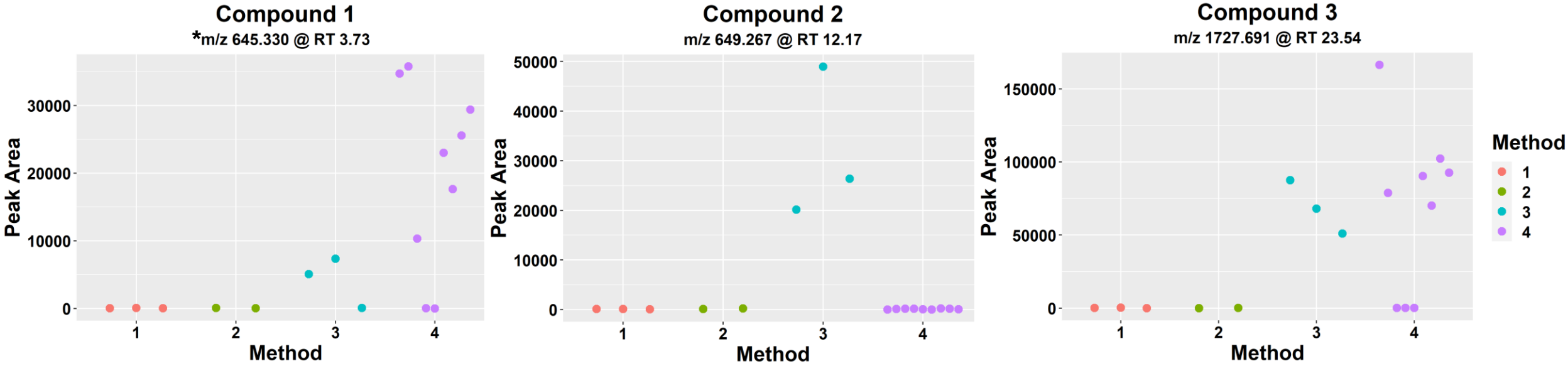
- **Purification methods 1, 2, and 4 can be distinguished based on presence / absence of small molecules**
  - **Ex. Small molecule 2 is unique to method 1**
  - **Additional compounds (beyond those in the figures) are potentially useful**

- **Additional data needed**
  - **Additional cultivar / purification method combinations**
  - **Sample replicates**
  - **Technical replicates**

Small molecule analysis shows potential in identifying purification method

***Figure subtitles read as: mass/charge of 154.099 at retention time of 6.426 minutes**

A4) Co-Isolating Proteins (Method)



- **Purification methods 3 and 4 can be distinguished from other methods**
  - **Additional compounds (beyond those in the figures) are potentially useful**
  - **Purification methods 1 and 2 are not distinguishable from each other**

- **Additional data needed**
  - **Additional cultivar / purification method combinations**
  - **Sample replicates**
  - **Technical replicates**

Co-Isolating proteins show potential in identifying purification method

***Figure subtitles read as: mass/charge of 645.330 at retention time of 3.73 minutes**

A) Results Summary for Purification Method Classification

	Total number of features	Number of features detected in at least one sample	Classification accuracy*
Ricin/Agglutinin Ratio Analysis	Visual results useful, ML not applied		
Trace Element Analysis	56	44	91%
Small Molecule Analysis	27	27	77%
Co-Isolating Proteins	70	69	53%

Purification methods can be distinguished based on some feature types and ML techniques

***Leave-One-Out-Cross-Validation**

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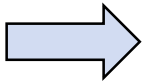
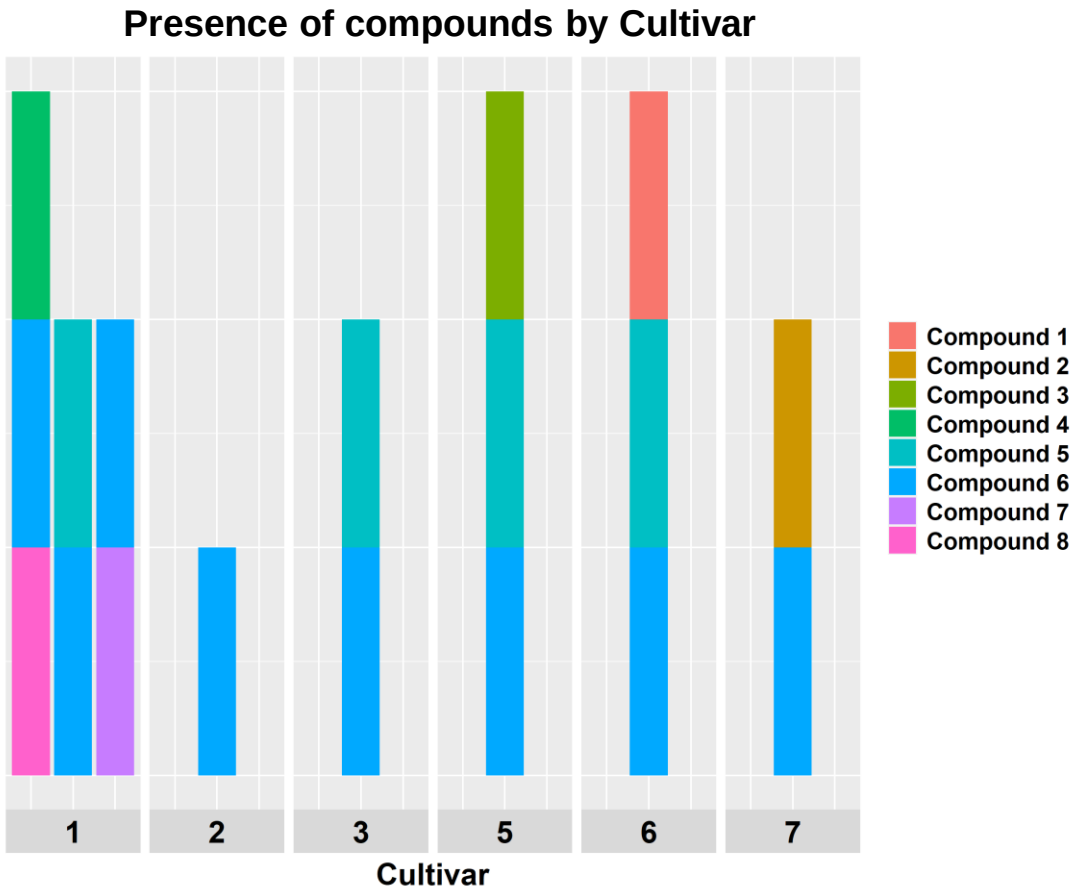


B. What cultivar was used in the extraction?
1. Small molecule analysis
2. Peptide variant analysis
3. ~~DNA sequencing~~

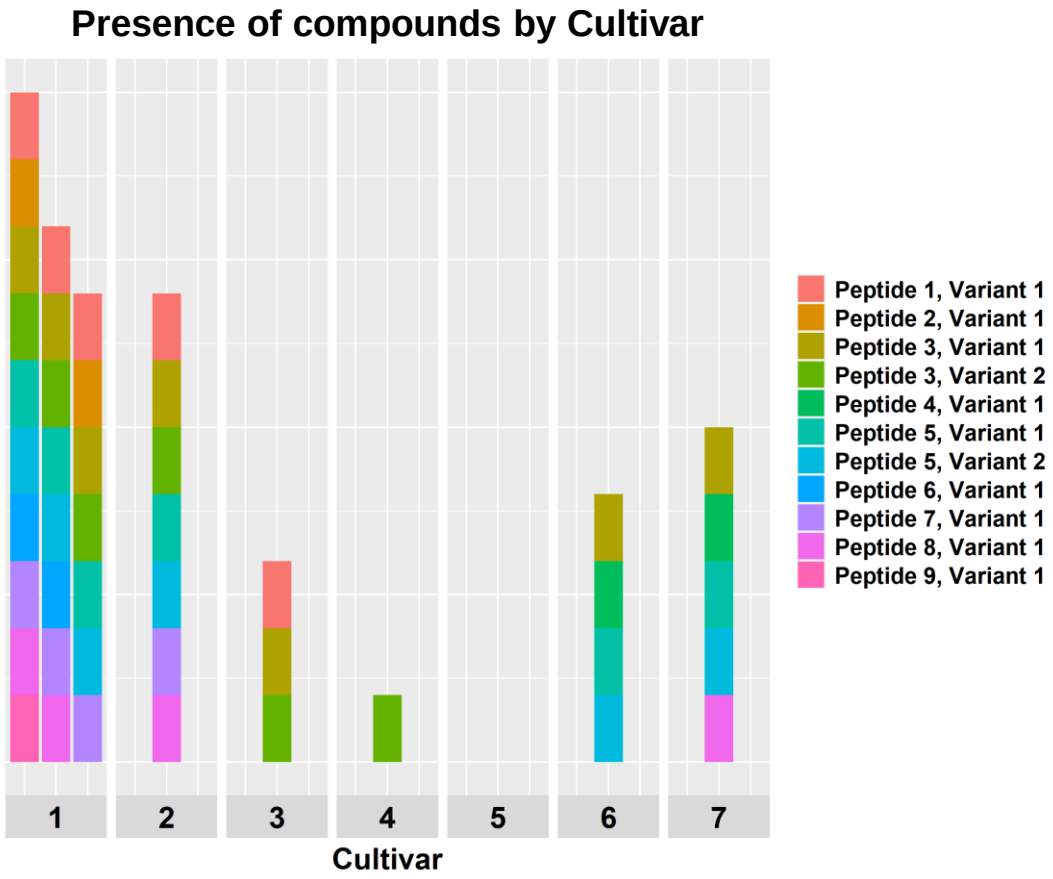
B1) Small Molecule Analysis (Cultivar)

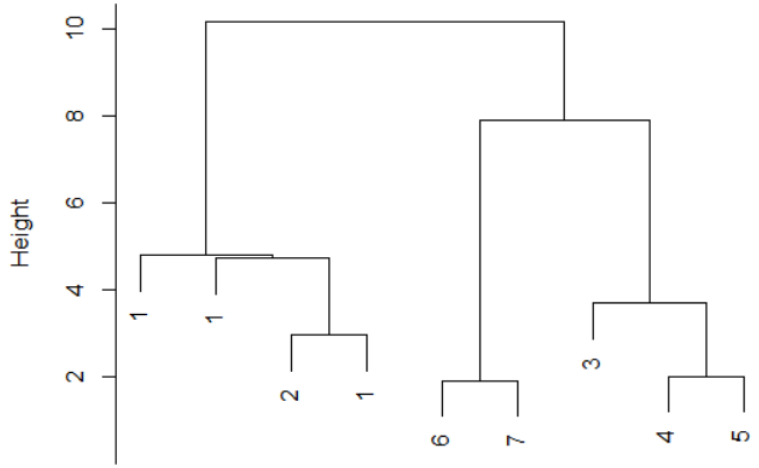


- **Presence / absence patterns show differences across cultivars**
  - **Example Compound 1 was only present in cultivar 6**
  - **Example Compound 2 was only present in cultivar 7**
  - **However, differences are exhibited within cultivar 1**
- **Additional data is required for proof-of-concept**
  - **Additional cultivar / purification method combinations**
  - **Sample replicates**
  - **Technical replicates**

Small molecule analysis may have potential in distinguishing cultivars

B2) Peptide Variant Analysis (Cultivar)

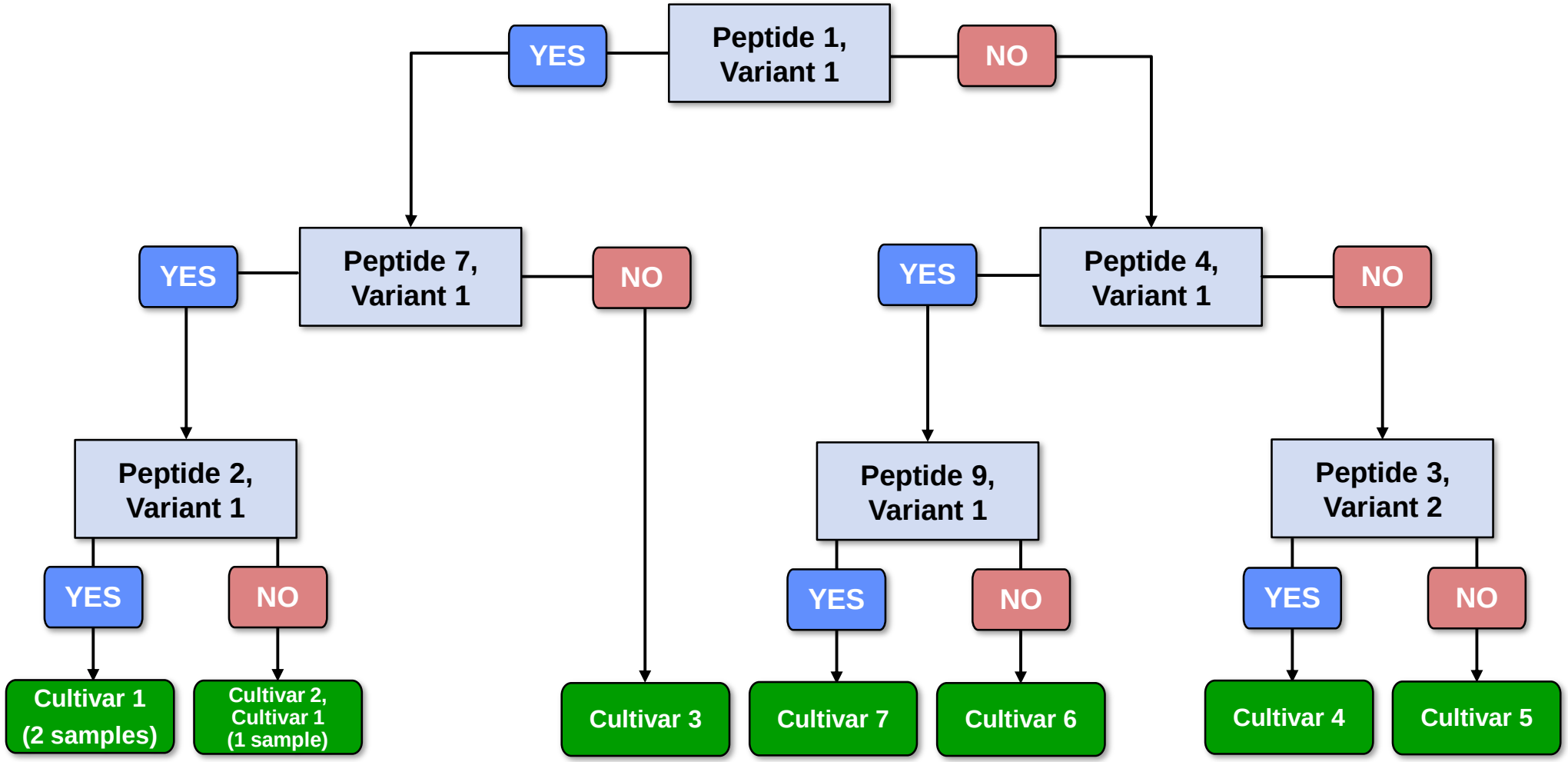




- **Presence / absence patterns show differences across cultivars (left)**
  - **Ex. Peptide 8, Variant 1 was present in cultivars 1, 2, and 7 but not in 3-6**
- **Cultivar 1 samples show similarities (left) and cluster together (above)**
- **Additional data is required for proof-of-concept**
  - **Additional cultivar / purification method combinations**
  - **Sample replicates**
  - **Technical replicates**

Peptide variant analysis of the ricin protein shows potential in distinguishing cultivars

B2) Peptide Variant Analysis (Cultivar)



Example (qualitative) decision tree shows each cultivar may be distinguishable based on single variants

Summary

- **Extracted ricin samples provide valuable sample set for forensics**
  - **Four different purification methods used on a single cultivar, with triplicates/duplicates runs for each method**
  - **One purification method used on 7 different cultivars (singlicate)**

- **Valuable (but needing additional data) attribution proof-of-concept signatures were obtained for all methods attempted**
  - **Protein ratios (not originally planned but has some value), trace element, and co-isolating protein analyses is useful for discerning the purification method used**
  - **Peptide variant analysis may be useful for cultivar discrimination**
  - **Small molecule analysis may be used to answer both questions**

- **Machine learning provides additional confidence in ability to distinguish methods, particularly trace element analysis**

PTM = Post-Translational Modifications

Future Work

Next Steps:

- Assess the impact of common decontamination processes on signatures
- Sequence for trace DNA signatures
- Vet additional important signatures (PTMs for cultivars) prior to use

Capability Implementation Requirements:

- Biochemical analytical laboratory equipment
  - Instrumentation includes ICP-MS, GC-MS, LC-MS (or a subset/equivalent techniques)
  - Laboratory facilities to conduct chemical and biological analyses
    - BL2 or equivalent lab space, biosafety cabinet, chemical hood to handle trace metals, basic lab supplies and instrumentation, etc.
- Expertise
  - University degree in analytical chemistry/biochemistry
  - University degree in statistics or data analysis background

PTM = Post-Translational Modifications