International Workshop on Environmental Proteomics
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Targeted peptide quantification of *Dehalococcoides* proteins in a mixed culture during dehaloresipiration

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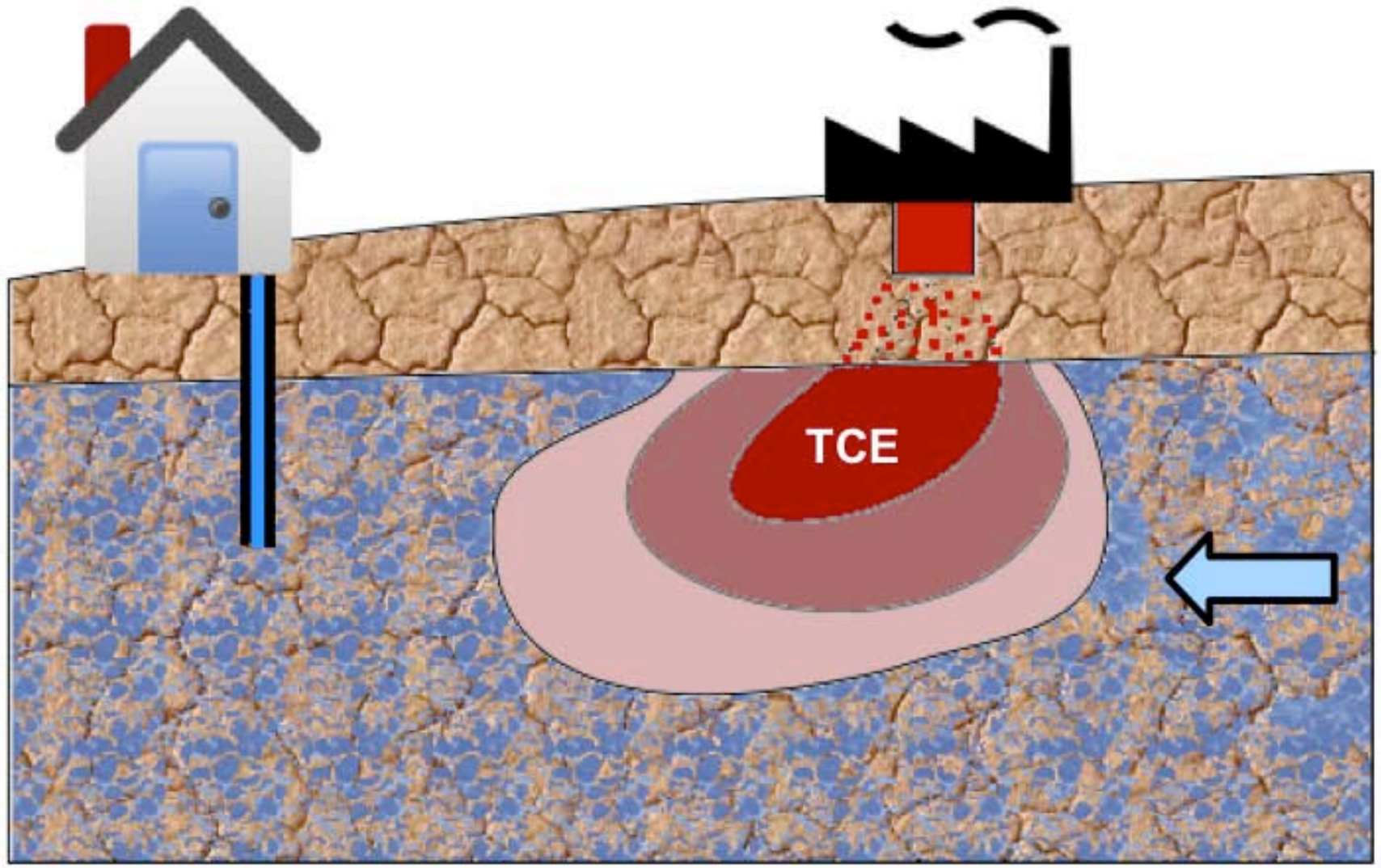
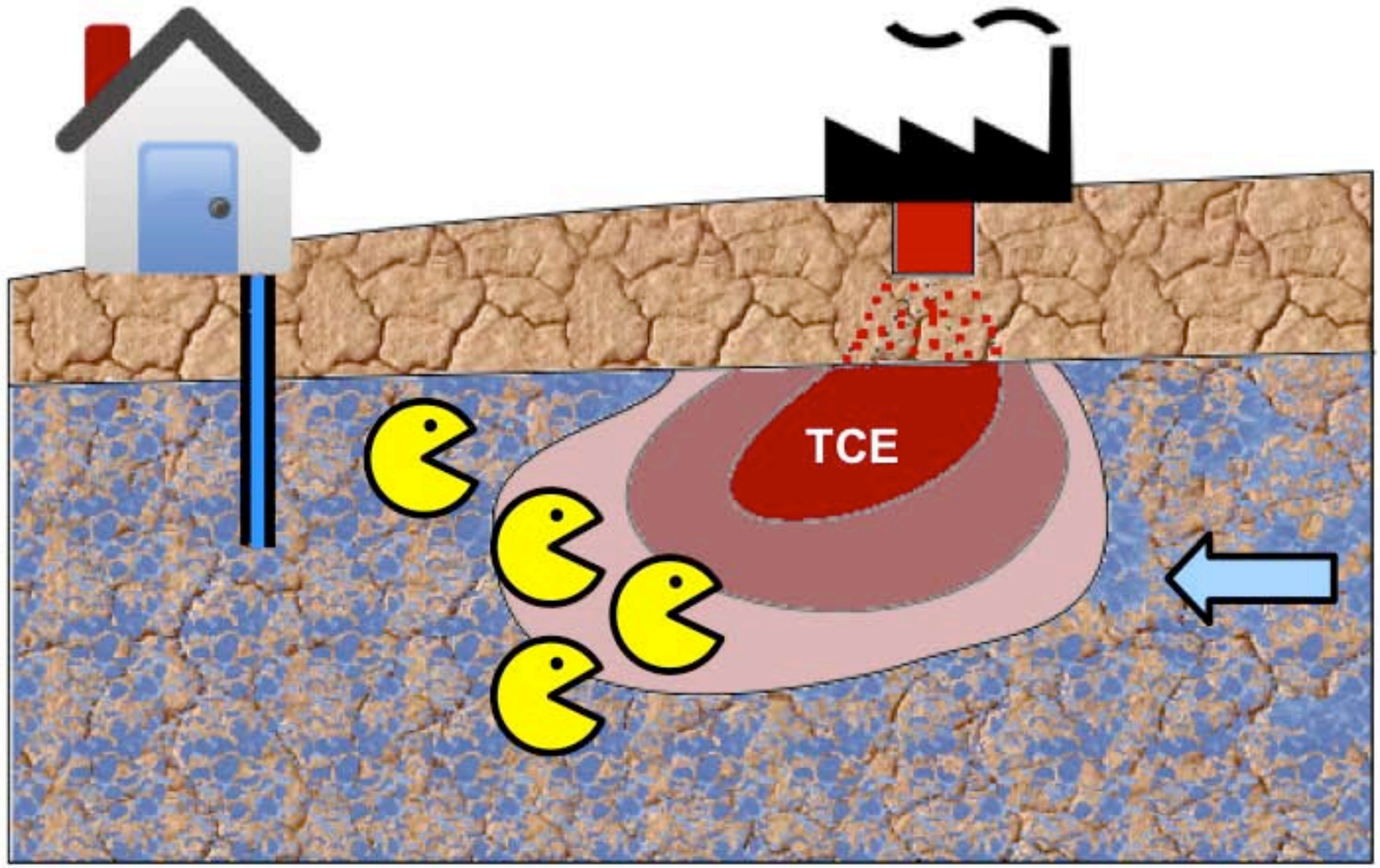
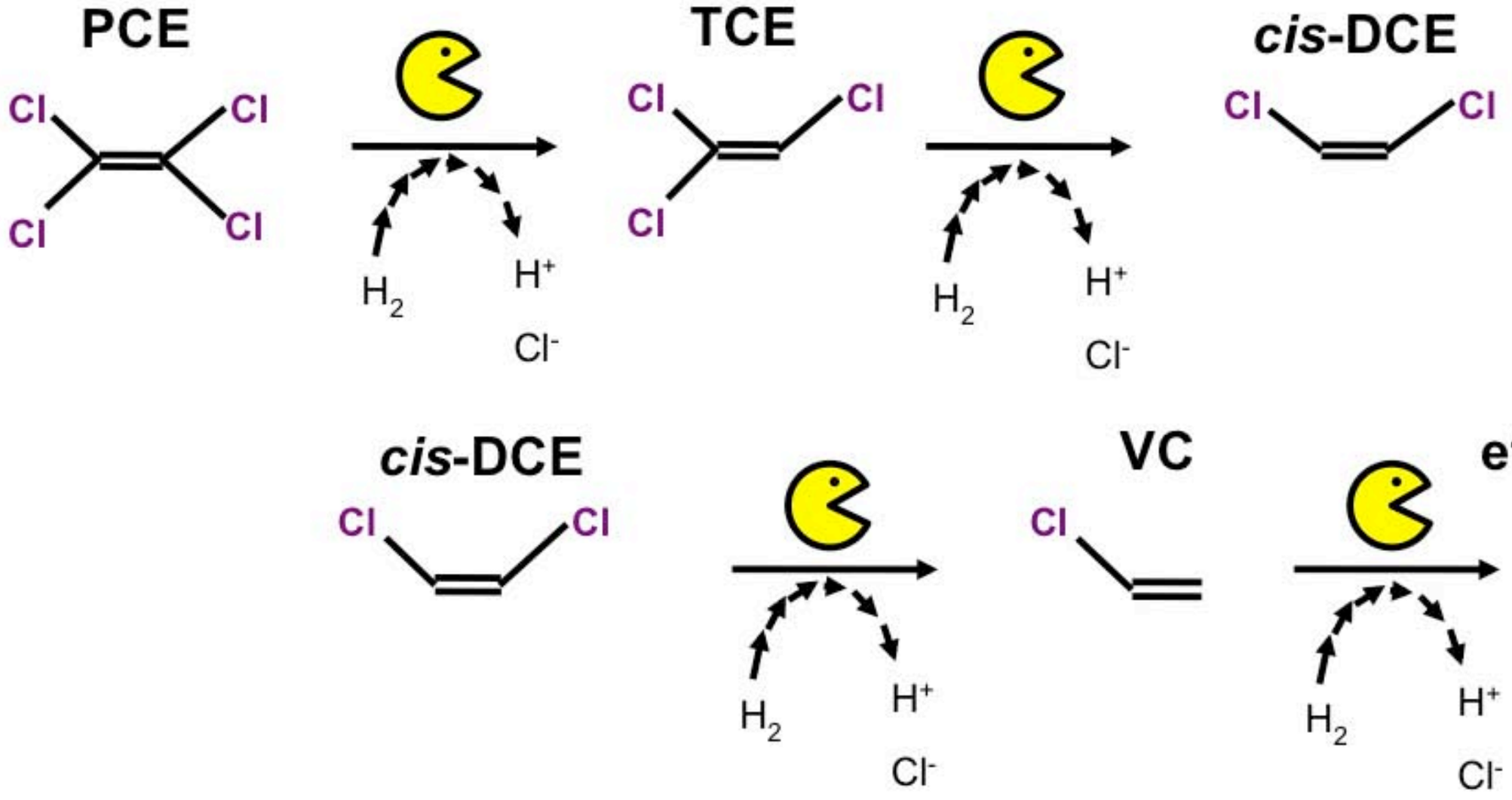
Bioremediation



Bioremediation



Dehalorespiration



Dehalococcoides spp. are the only organisms known to do all these steps metabolically.

Big-Picture Questions

- How can we assess dehalorespiration rates in the field?
- How can we tell if *Dehalococcoides* are healthy and active?

Mixed Cultures With Active *Dehalococcoides* spp.

D2 (Richardson lab)

- Complex mixed culture
- Maintained on PCE and Butyrate

KB1 (E Edwards lab, U Toronto)

- Commercial mixed culture for field site bioaugmentation
- Enriched on TCE and Methanol

Proteins known to correlate with dehalorespiration activity

- Reductive dehalogenases (eg TceA, PceA)
- Other oxidoreductases: (eg FdhA, HupA)

Lots of shotgun data (more later)

Sequence of TceA (from genome)

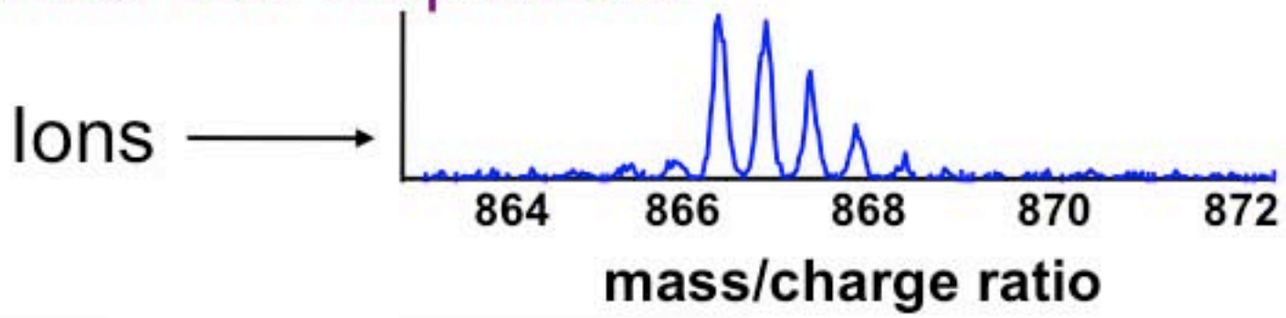
MSEKYHSTVTRRDFMKRLGLAGAGAGALGAAVLAENNLPHEFKDVDDL
LSAGKALEGDHANKVNNHPWWVTTRDHEDPTCNIDWSLIKRYSGWNN
QGAYFLPEDYLSPTYTGRRHTIVDSKLEIELQGKKYRDSAFIKSGIDWM
KENIDPDYDPGELGYGDRREDALIYAATNGSHNCWENPLYGRYEGSRP
YLSMRTMNGINGLHEFGHADIKTTNYPKWEGTPEENLLIMRTAARYFGA
SSVGAIKITDNVKKIFYAKVQPFCLGPWYTITNMAEYIEYPVPVDNYAIPIV
FEDIPADQGHYSYKRFGGDDKIAVPNALDNIFTYTIMLPEKRFKYAHSIP
MDPCSCIAYPLFTEVEARIQQFIAGLGYNSMGGGVEAWGPGSAFGNLS
GLGEQSRVSSIIEPRYGSNTKGSLRMLTDLPLAPTKPIDAGIREFCKTCG
ICAEHCPTQAISHEGPRYDSPHWDCVSGYEGWHLDYHKCINCTICEAVC
PFFTMSNNSWVHNLVKSTVATTPVFNGFFKNMEGAFGYGPRYSPSRDE
WWASENPIRGASVDIF

>95% confidence	>50% confidence
>0% confidence	no evidence

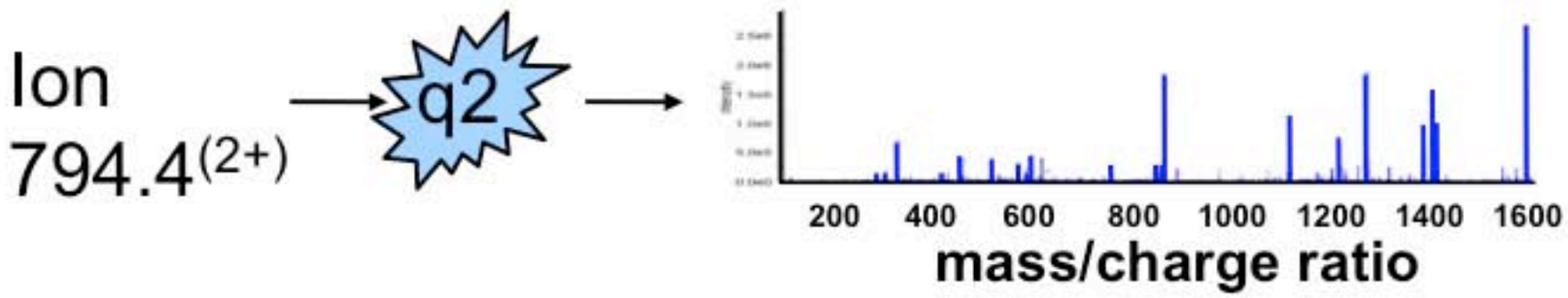
How can we get absolute quantification of a specific protein in a complex sample?

ABI 4000 QTrap Can Operate Like a Normal Triple Quadrupole

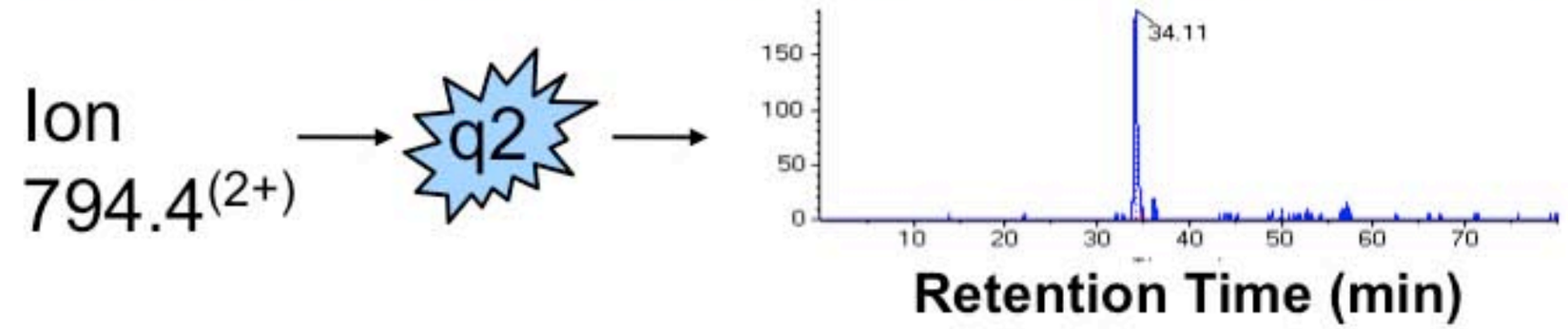
Linear Ion Trap Mode



Select a precursor, Fragment, Scan fragments



Select a precursor, Fragment, Select specific *m/z* on **Q3**

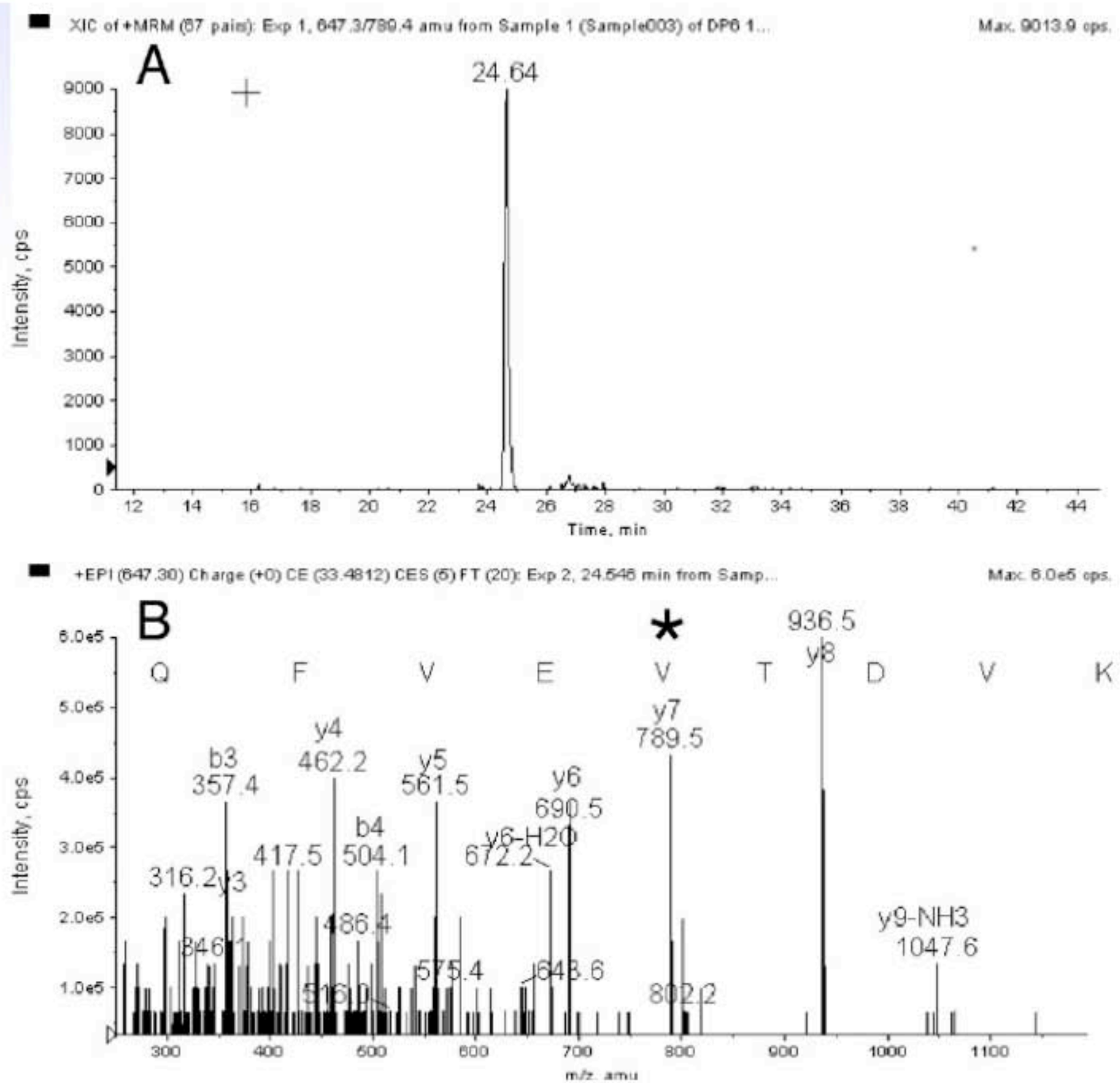


Yields a chromatographic peak

Precedent in Medicine

Peptide of fibronectin, biomarker for preeclampsia (in urine) and emphysema (in plasma)

Anderson and Hunter, **2006**, *Mol. Cell. Proteomics* 573



Name for assay: Multiple reaction monitoring (MRM)

MRM Targets We Chose

- 5 reductive dehalogenases, Hup, other oxidoreductases, RNA polymerase, ribosome subunit L7, S-Layer, GroEL
- Monitored 2 peptides per protein, 2 fragments per peptide

Are these useful bioindicators?
Trypsin "Digestion" of NCBI-nr

Better Target Peptides:

WEGTPEENLLIMR: Would come from 19 TceA genes on NCBI-nr, all *Dehalococcoides* spp.

DEWWASENPIR: Would come from 19 TceA genes on NCBI-nr, all *Dehalococcoides* spp.

Less-Specific Peptide:

FATSDLNDLYR: Comes from 3 *Dehalococcoides* genes and **1007** additional bacterial Rpo genes!

Picking Transitions for Multiple Reaction Monitoring (MRM)

TceA peptide WEGT-PEENLMIR elutes around 57 min on nLC

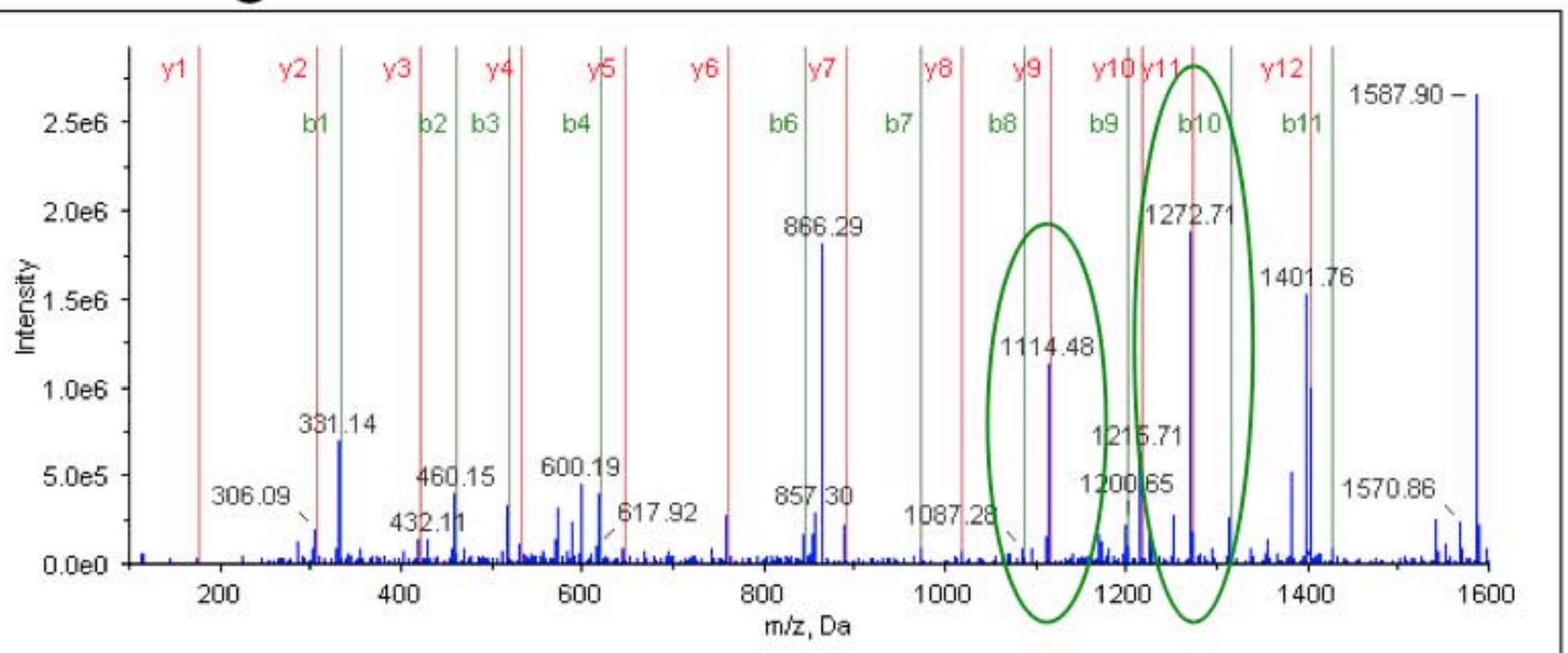
Ionization (ESI)

Select on Q1: Precursor ion 794.4$^{(2+)}$

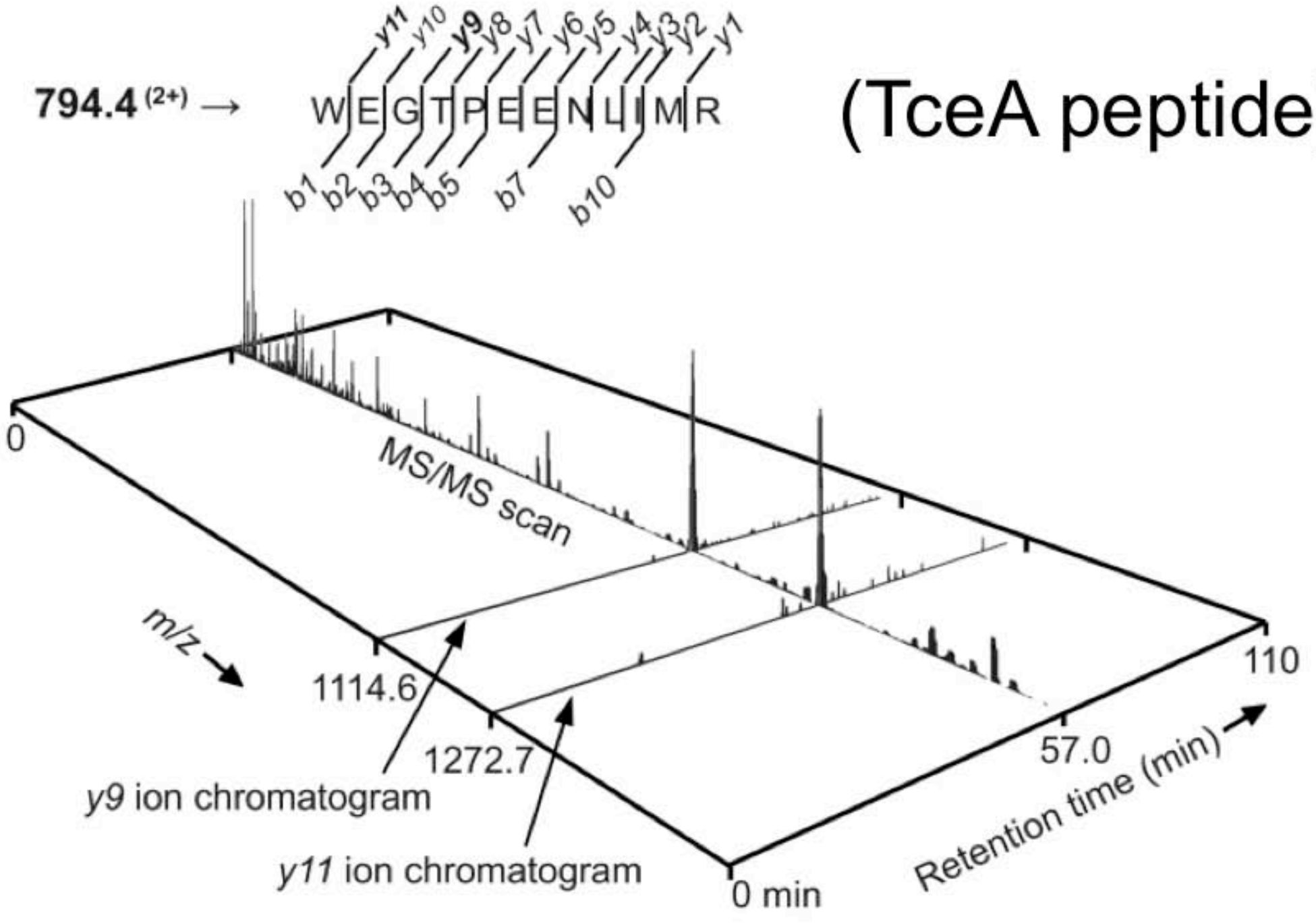
Fragmentation in collision cell (q2)

Pick representative fragments for Q3

Residue	b	y
W	**331.1887**	1731.8856
E	**460.2312**	**1401.7042**
G	**517.2527**	**1272.6616**
T	**618.3004**	**1215.6402**
P	715.3532	**1114.5925**
E	**844.3957**	**1017.5397**
E	**973.4383**	**888.4971**
N	**1087.4813**	**759.4546**
L	**1200.5653**	**645.4116**
L	**1313.6494**	**532.3276**
I	**1426.7335**	**419.2435**
M	1557.7739	**306.1594**
R	1713.8751	**175.1190**



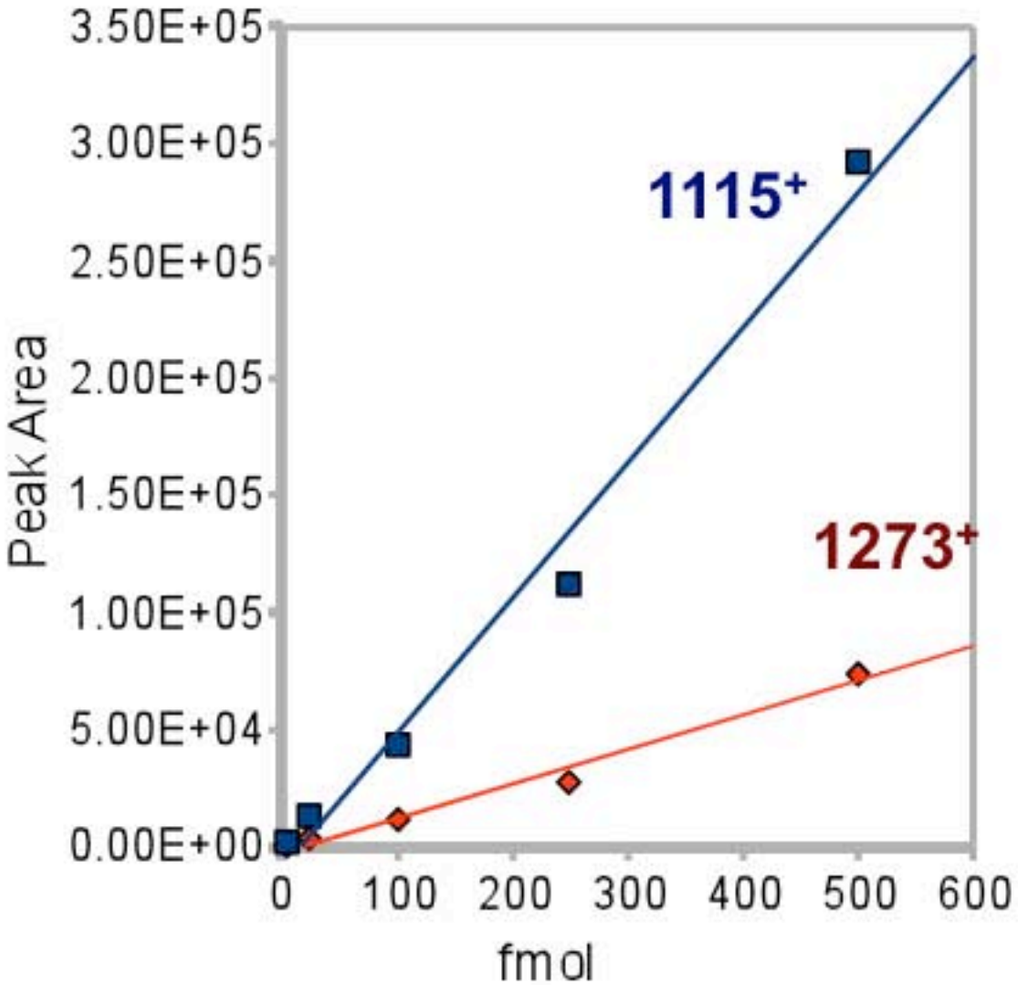
Peptide Quantification *via* Multiple Reaction Monitoring



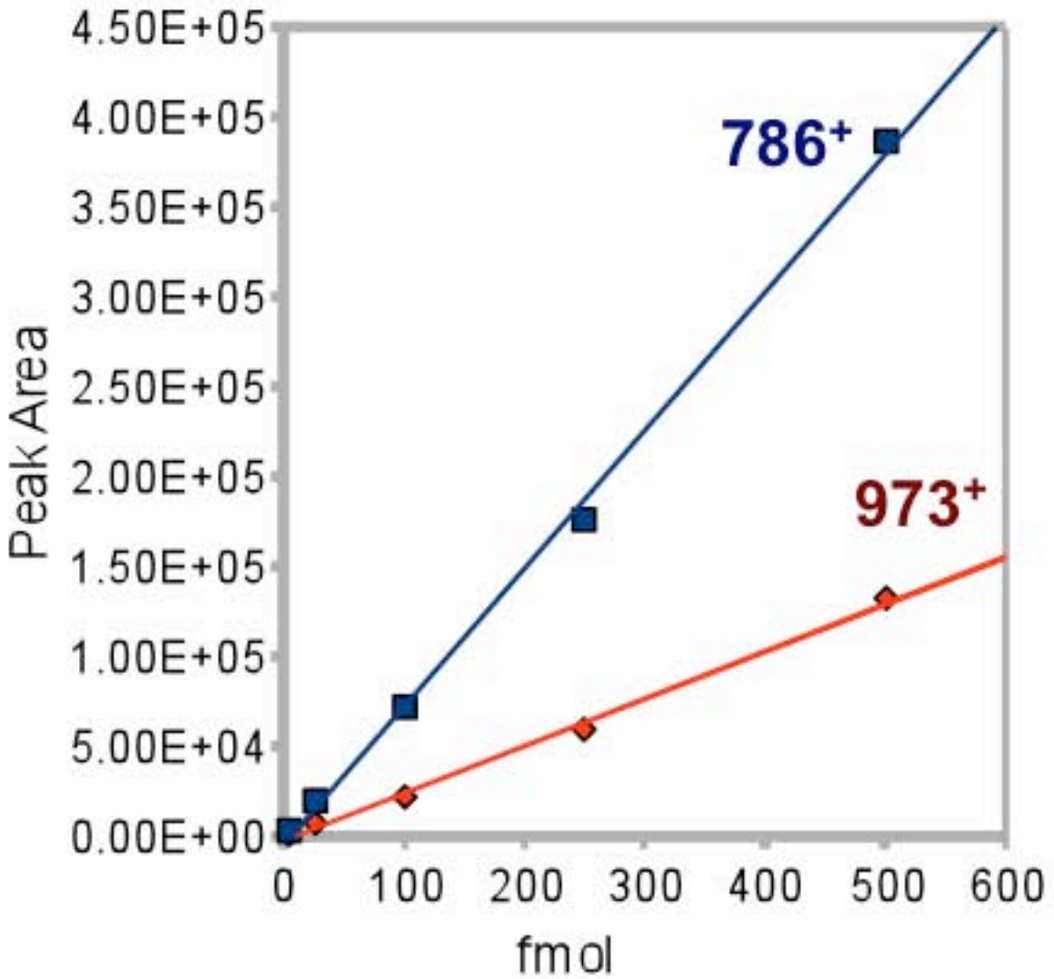
(TceA peptide)

Linear Response in a Background Sediment Digest

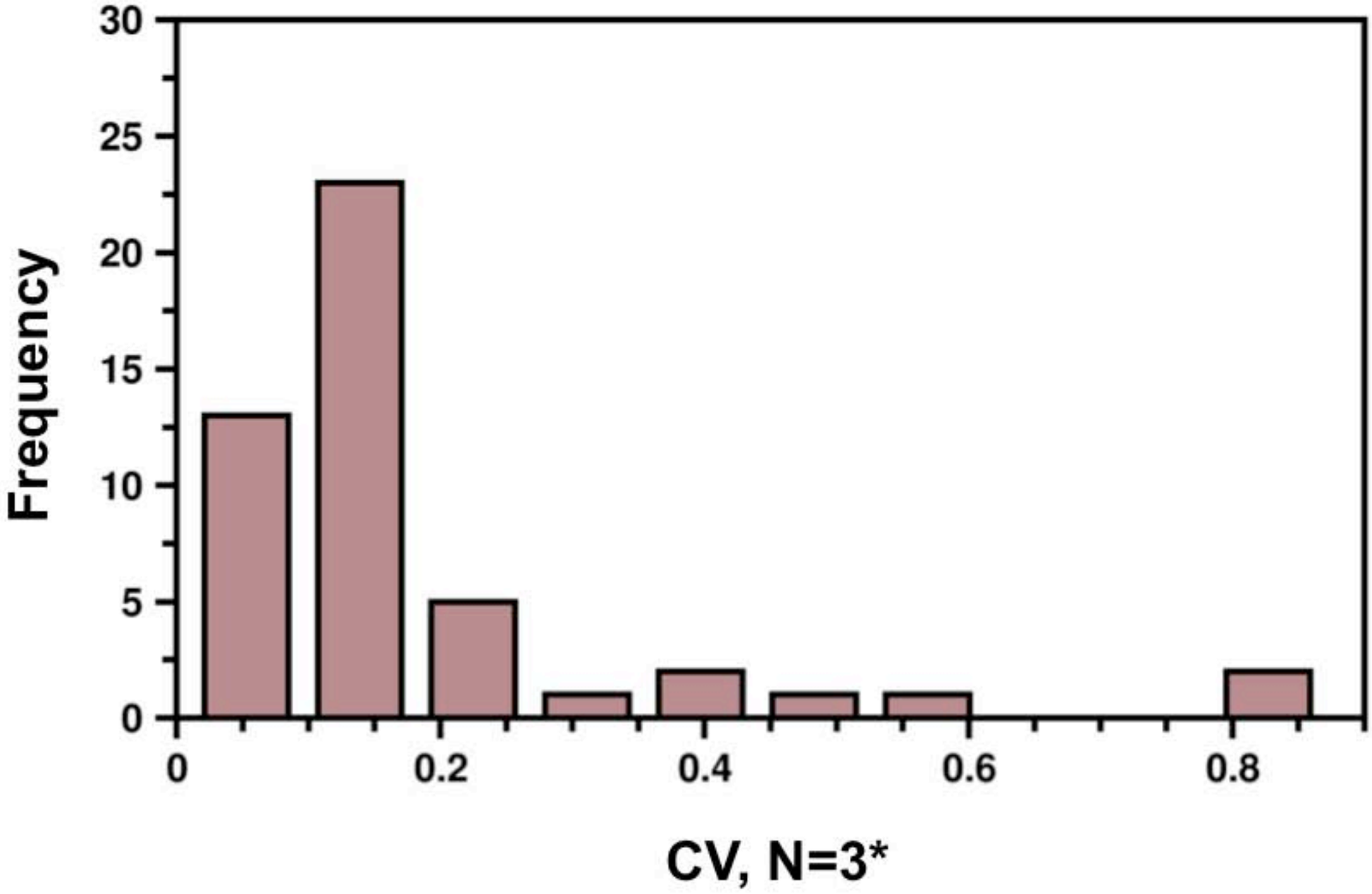
TceA: WEGTPEENLLIMR 794$^{(2+)}$ →



TceA: DEWWASENPIR 702$^{(2+)}$ →

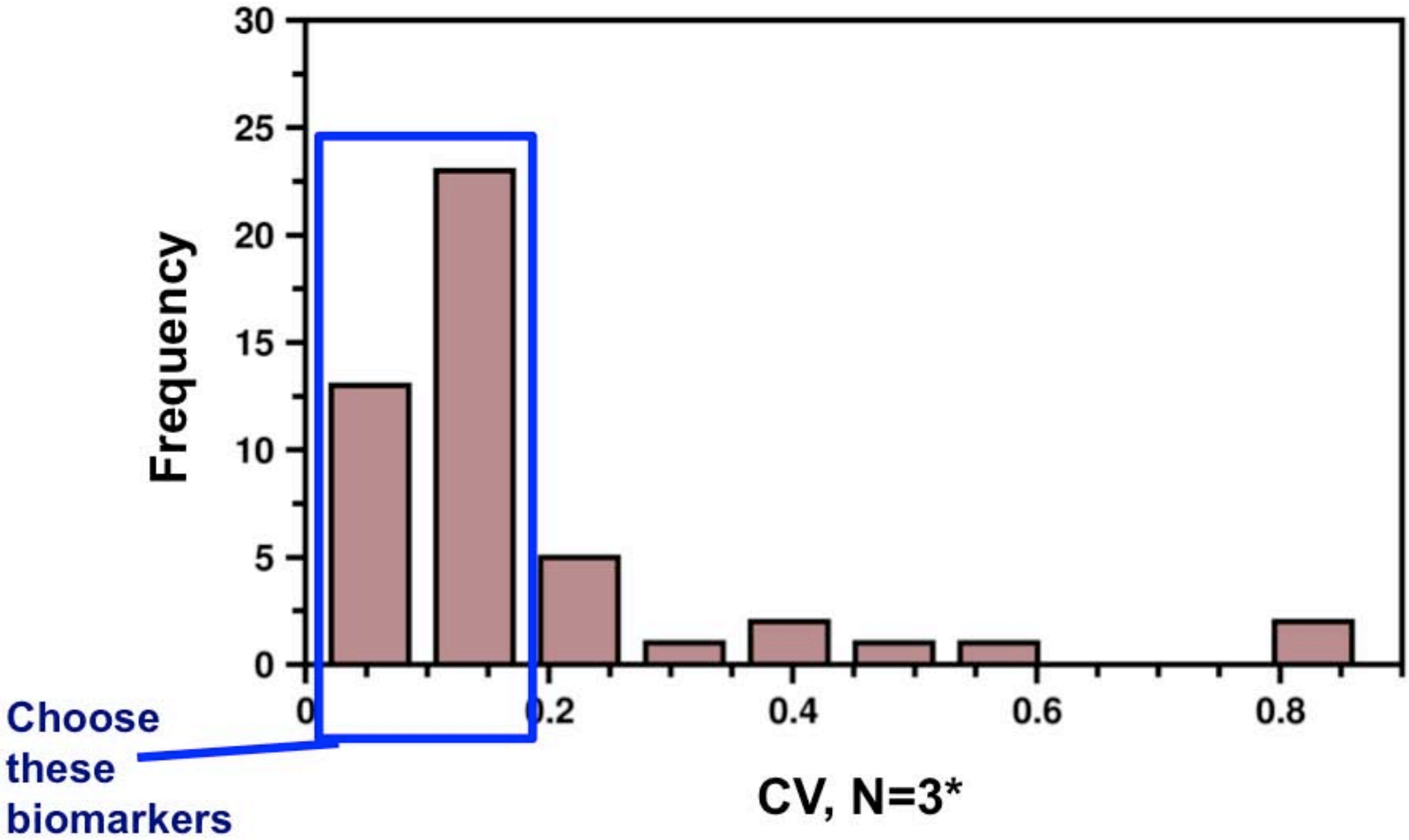


Many Peptides Had Low Variability Over a 4 d Period



*Independent analysis of 100 fmol standard on Day 1, Day 3 & Day 4

Many peptides had low day-to-day variability



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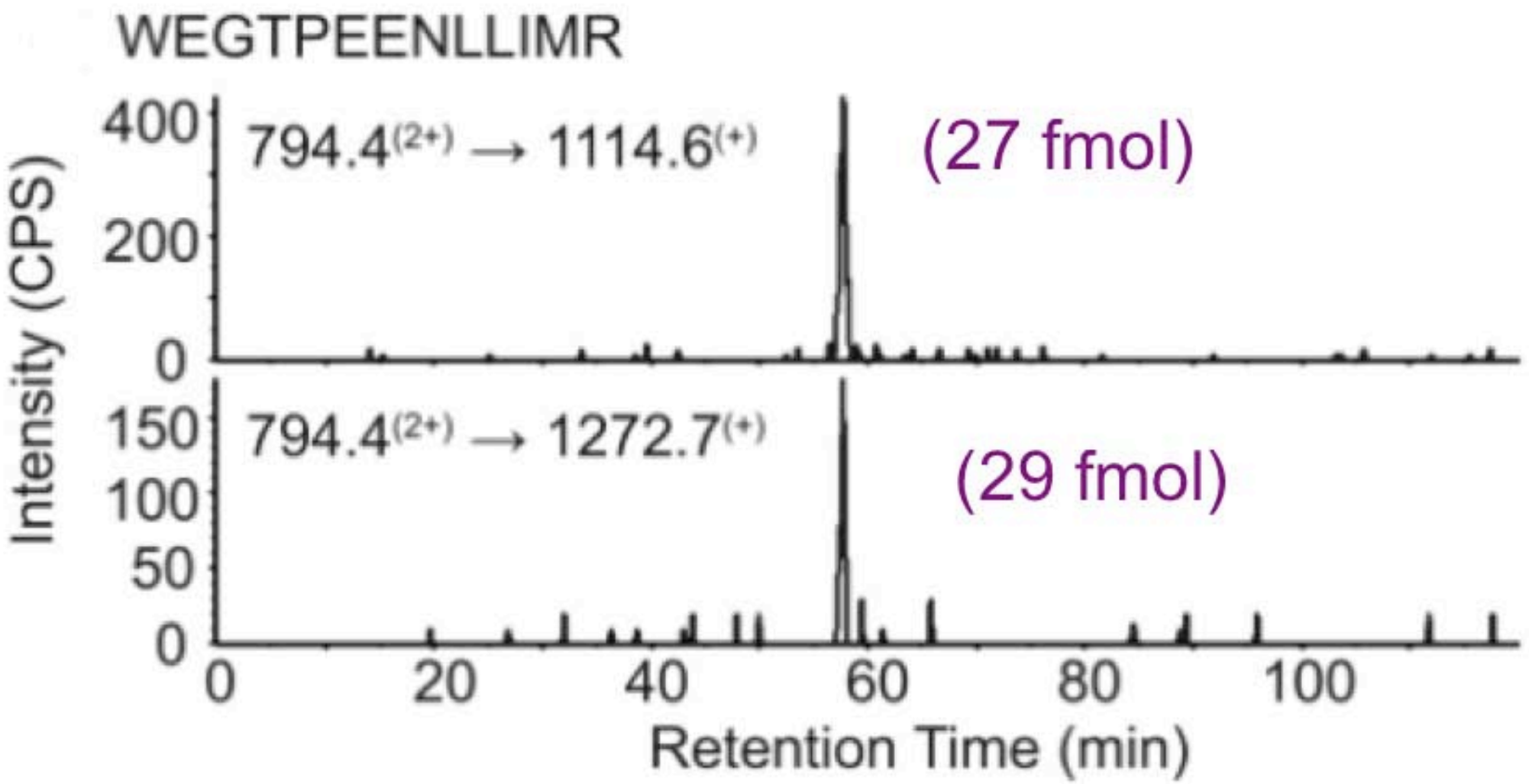
Most of the following data available in publication:

Werner, Ptak, Rahm, Zheng and Richardson
2009 *Environmental Microbiology* 11:2687-2697

TceA Enzyme ID and Quantification

Gene Previously Thought to be Missing from Commercial Culture KB1™



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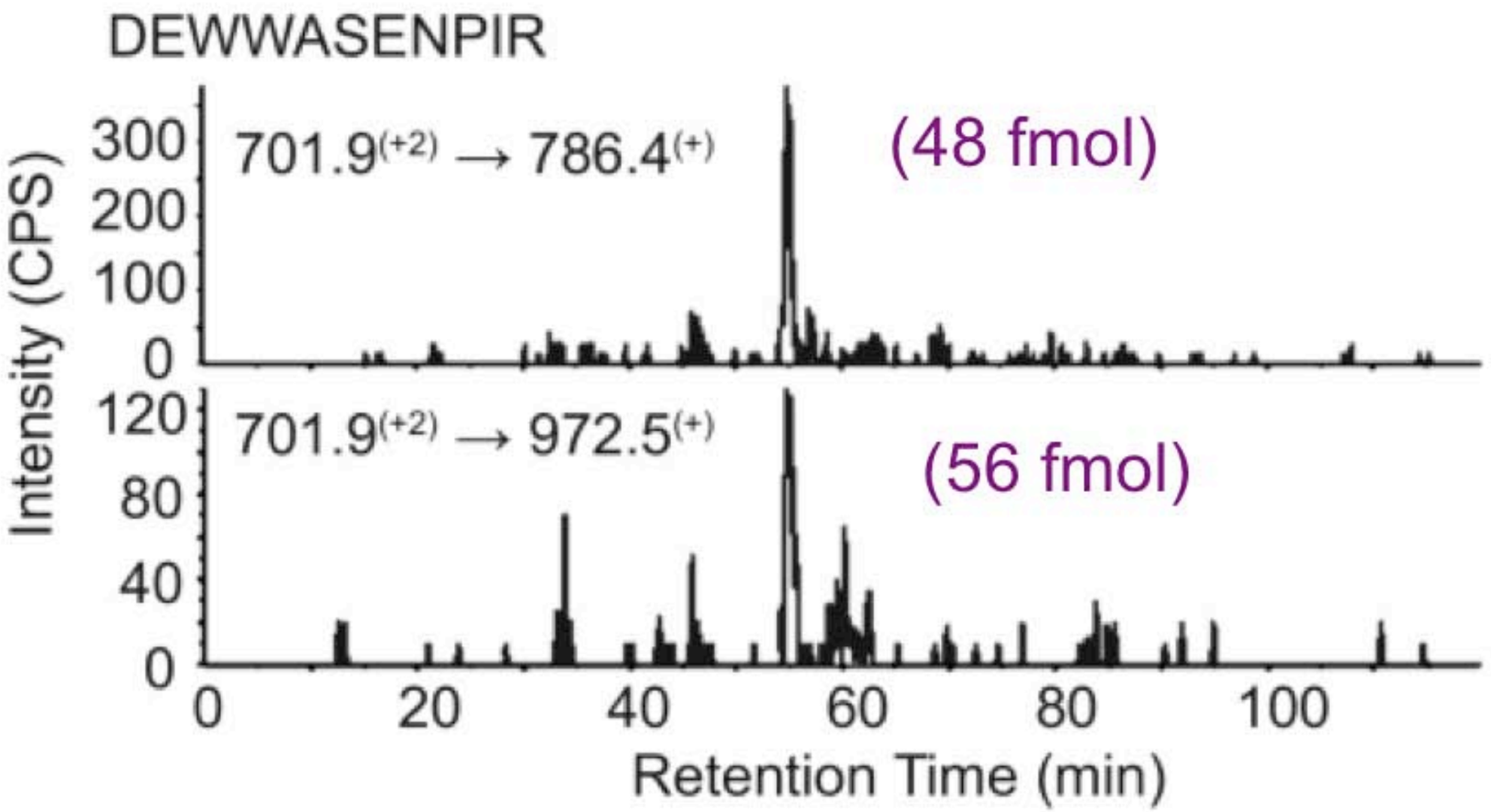
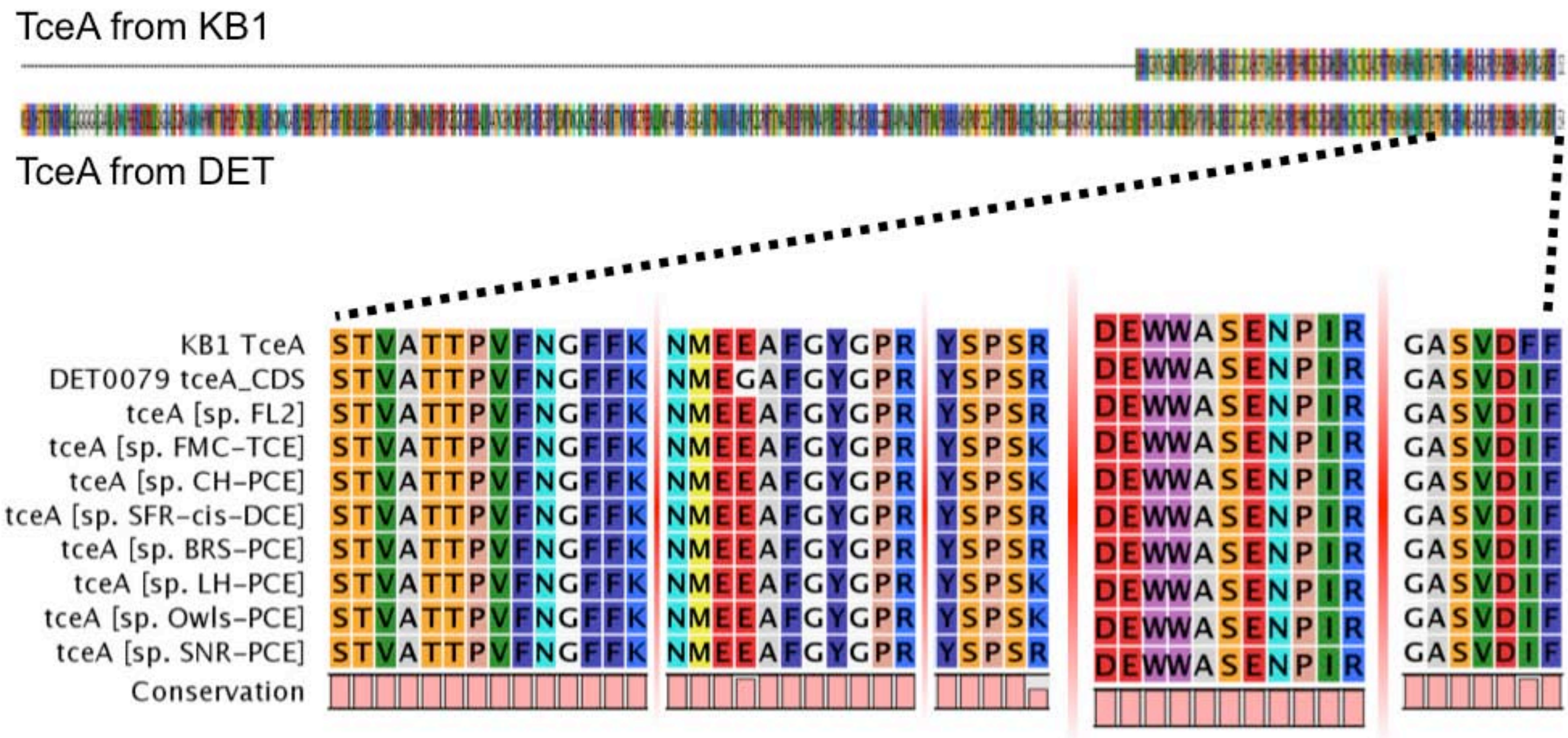
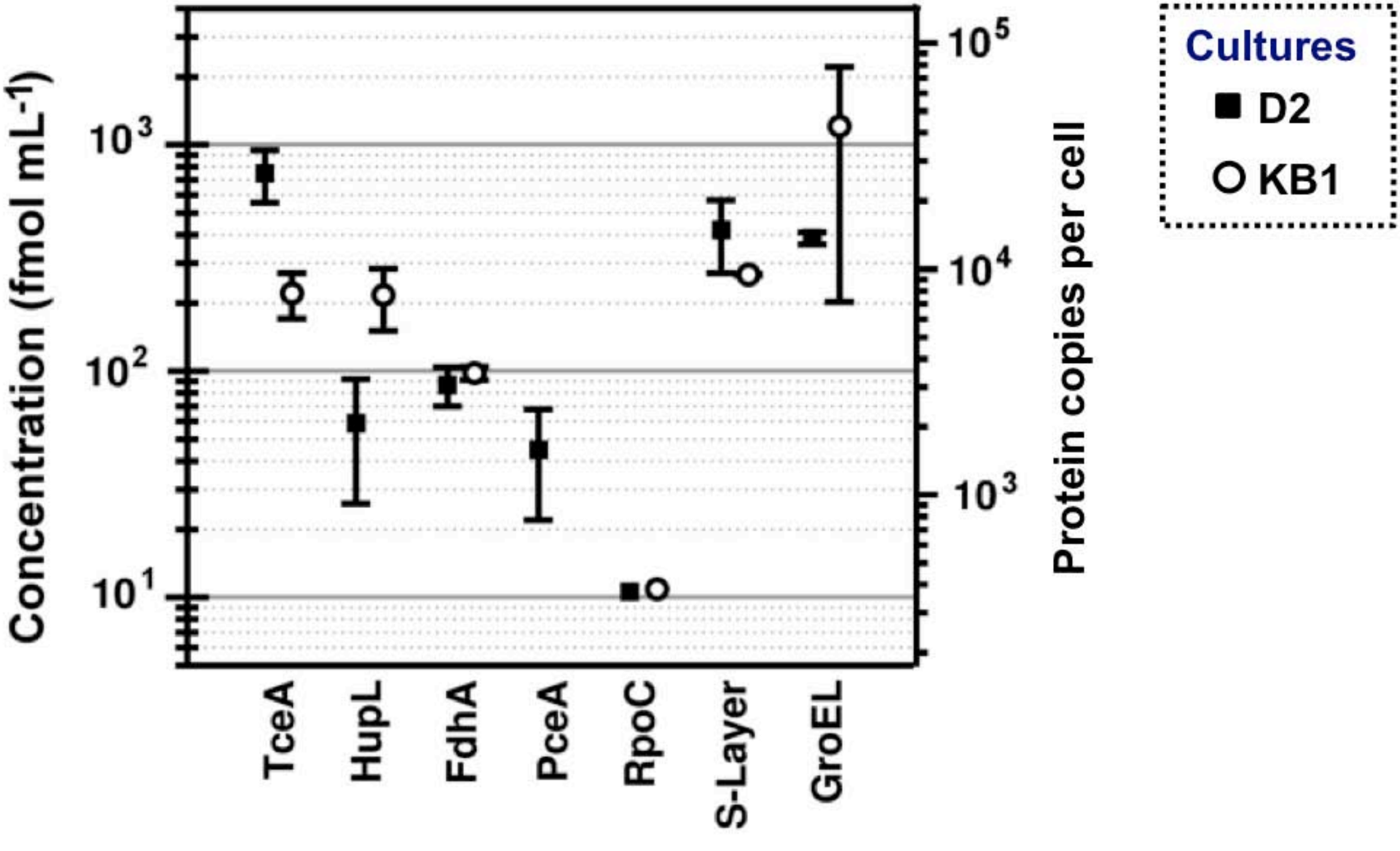


Preliminary KB1 Metagenomic Sequencing Library



Preliminary KB1 gDNA fragment library from Elizabeth Edwards and Alison Waller at U. Toront

Concentrations in Mixed Cultures



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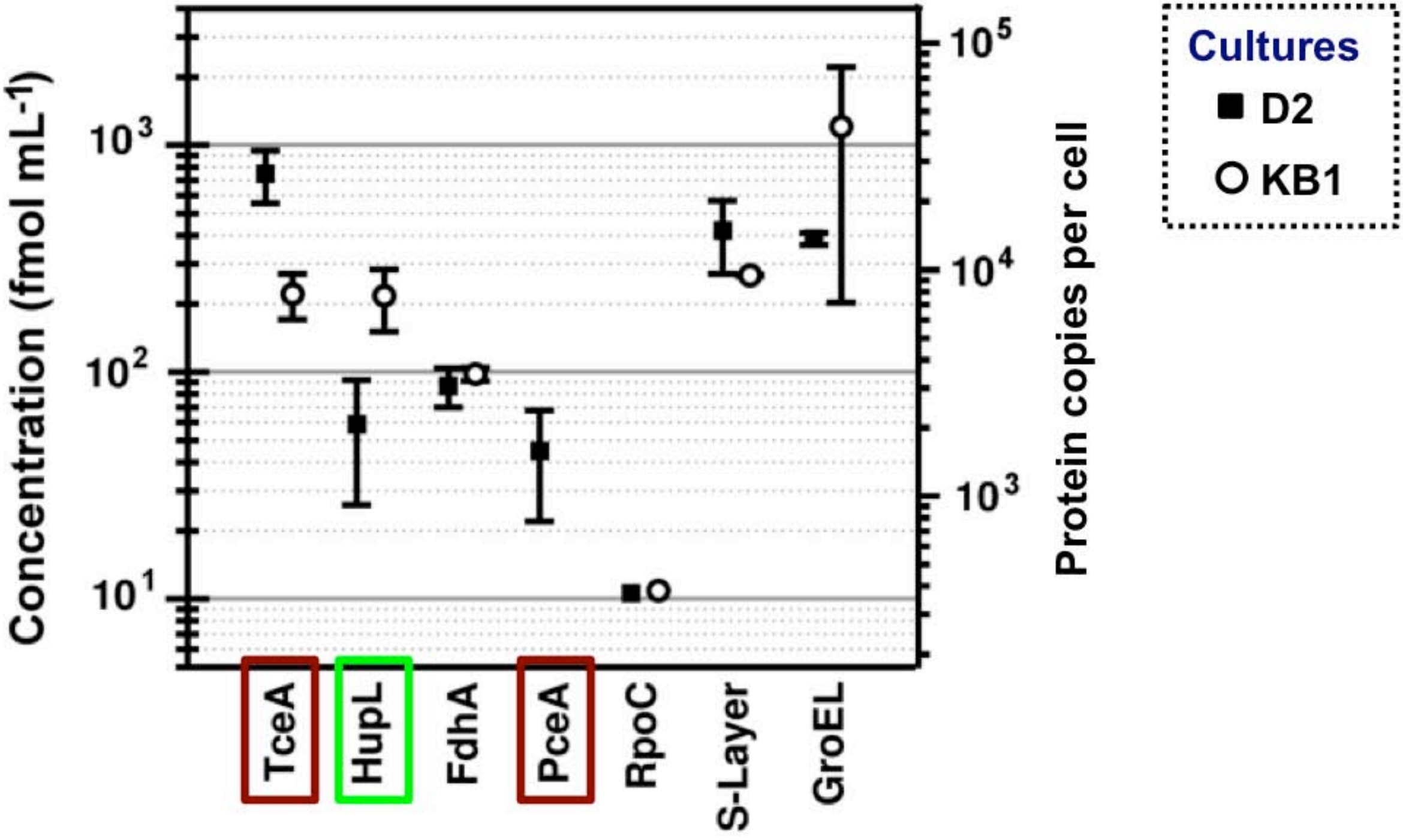
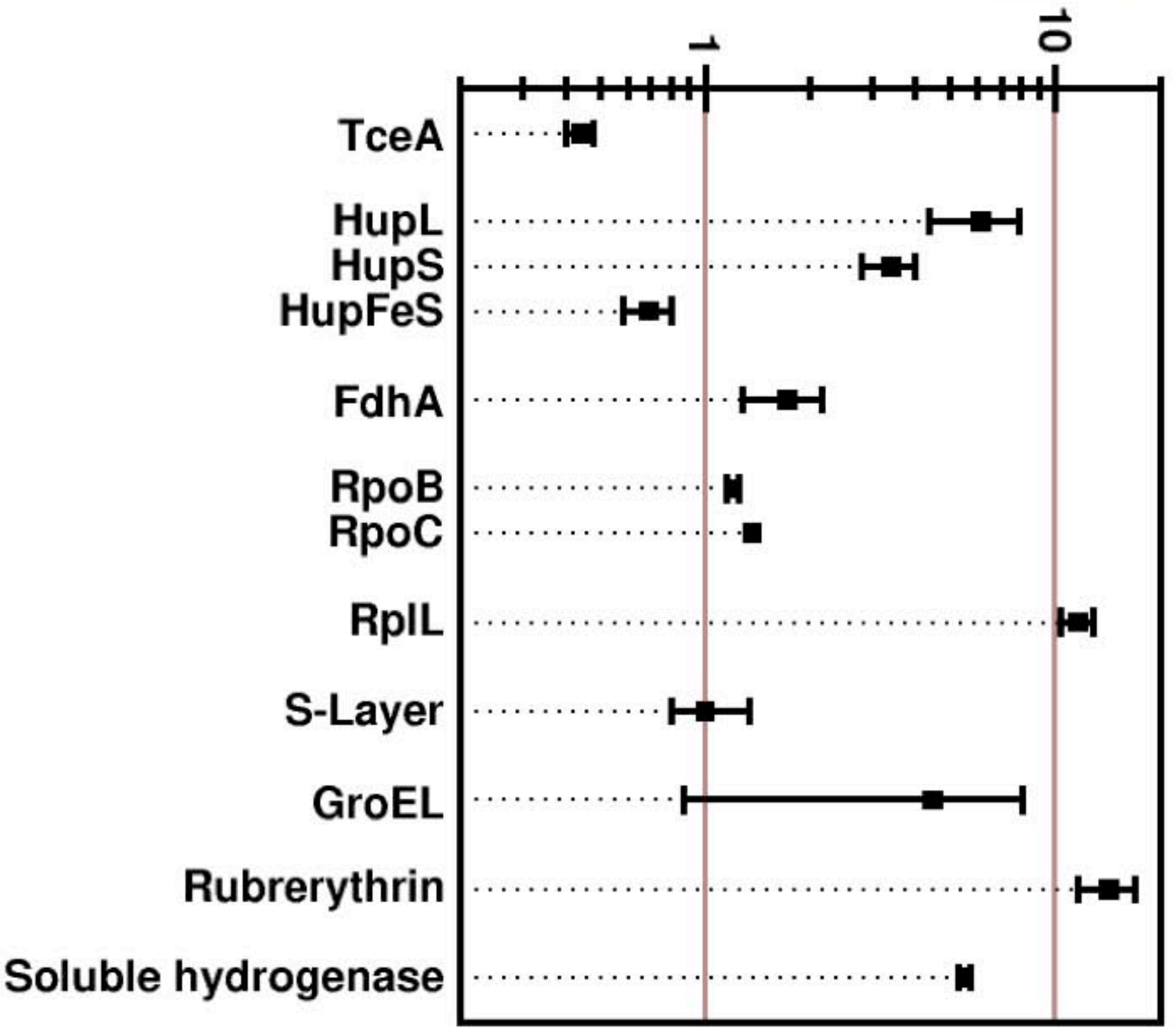


S-layer normalized protein abundance ratio KB1/D2



Challenges

- Quantification of RDases in Field Samples
    - 1-10 L of groundwater for active sites
    - Protein extraction protocol?
    - Need to define matrix effect on protein extraction and trypsin digestion
    - Trypsin digestion directly in sample/ acetonitrile? Peptides are easier to extract and to define extraction efficiency.

Thank you

- Brian Rahm, Annie Rowe, Gretchen Heavner, Bob Morris, *Richardson Lab*

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