

Basic pH Reverse Phase UPLC Fractionation

IDeA National Resource for Proteomics
Workshop for Core Directors and Staff

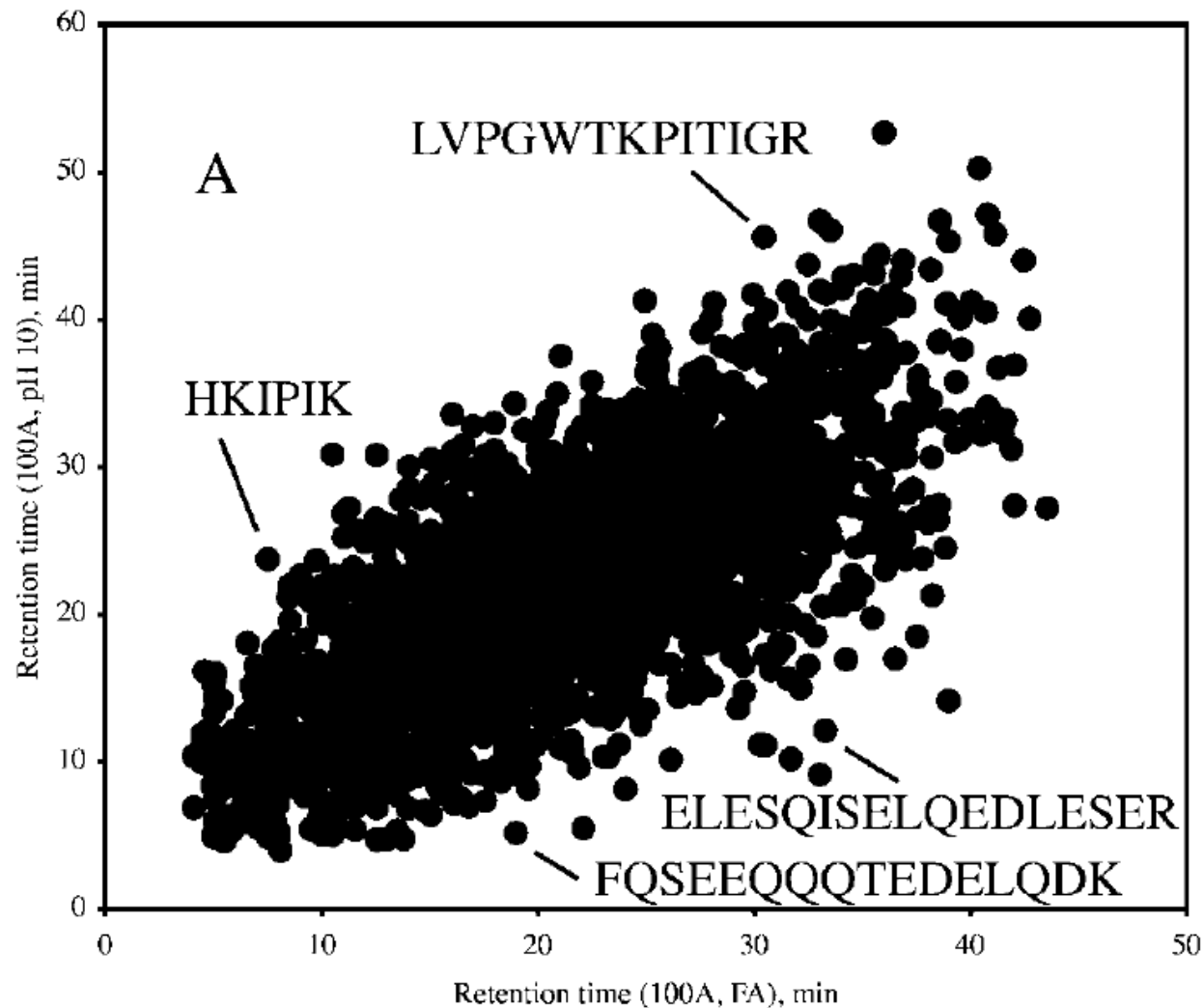
New separation scheme

- Solution digestion and multidimensional peptide separation
 - High pH Reverse Phase UPLC
 - 1mm diameter column (~2-50ug)
 - Fraction collection in 96 well plate
 - Fraction concatenation

Practical Implementation of 2D HPLC Scheme with Accurate Peptide Retention Prediction in Both Dimensions for High-Throughput Bottom-Up Proteomics

Ravi C. Dwivedi,[†] Vic Spicer,[‡] Michael Harder,[‡] Mihaela Antonovici,[†] Werner Ens,[‡] Kenneth G. Standing,[†] John A. Wilkins,[†] and Oleg V. Krokhin^{*†}

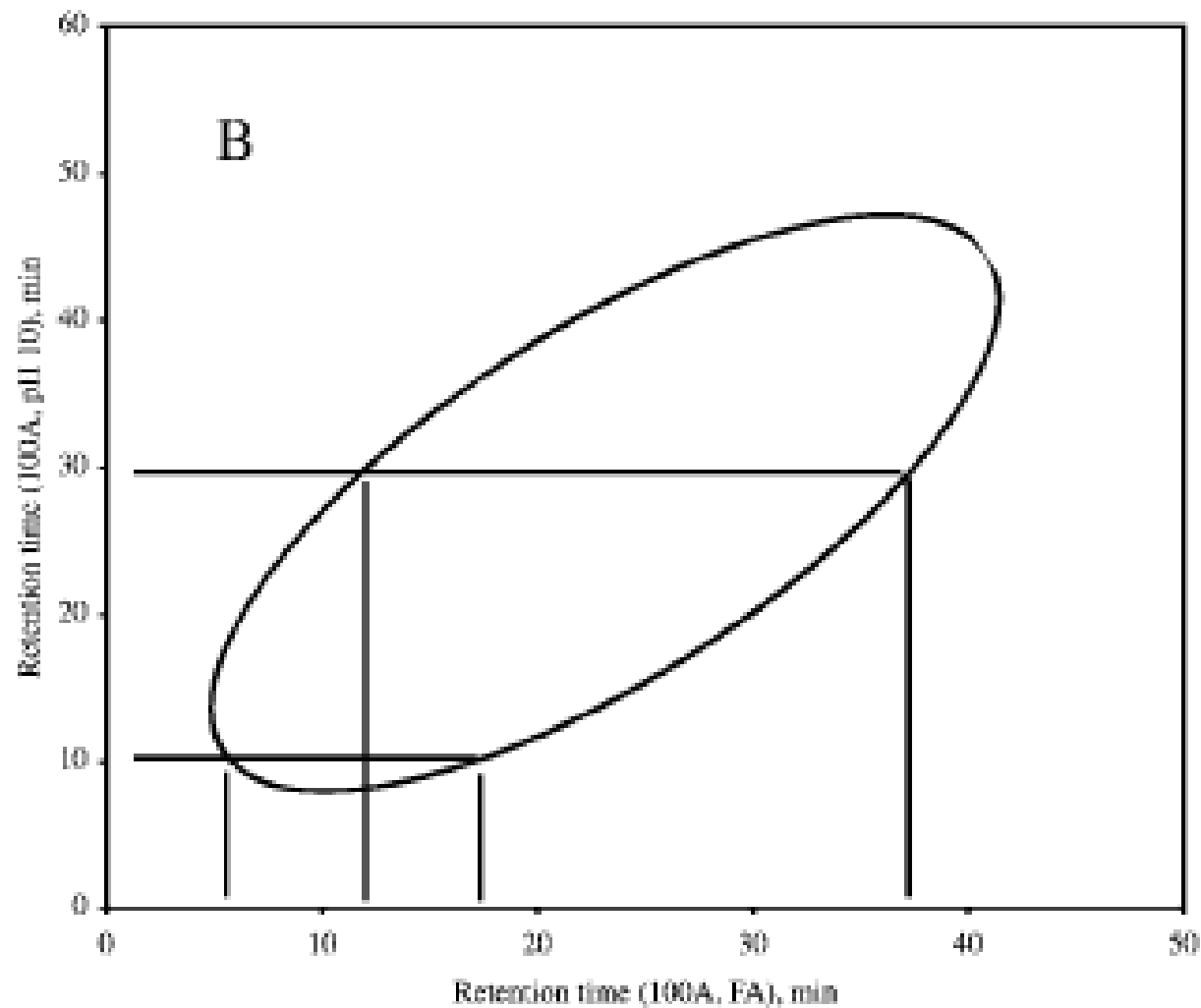
Manitoba Centre for Proteomics and Systems Biology, University of Manitoba, 799 JBRC, 715 McDermot Avenue, Winnipeg, MB, R3E 3P4, Canada, and Department of Physics and Astronomy, University of Manitoba, Winnipeg, MB, R3T 2N2, Canada



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RESEARCH ARTICLE

Reversed-phase chromatography with multiple fraction concatenation strategy for proteome profiling of human MCF10A cells

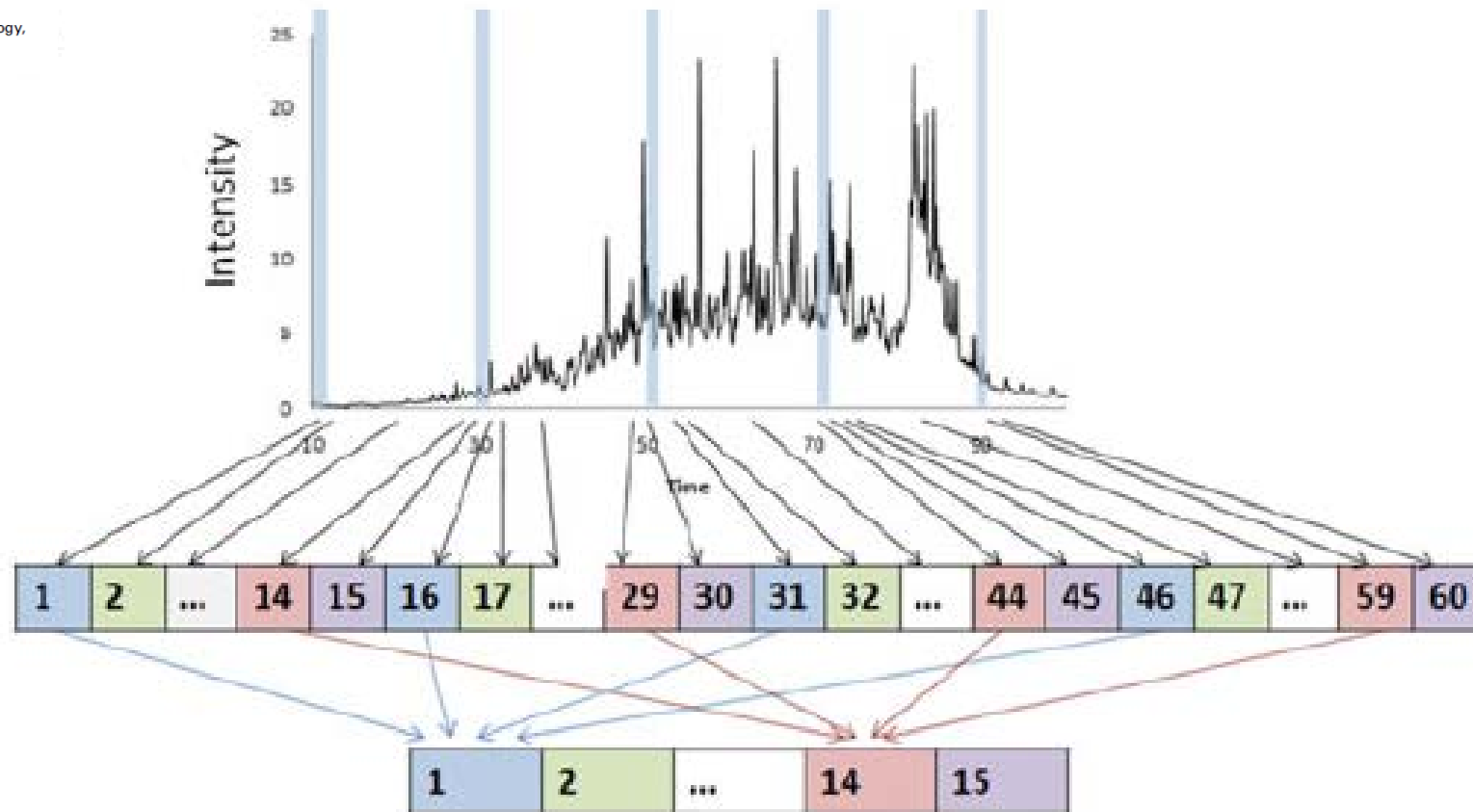
Yuexi Wang^{1*}, Feng Yang^{1*}, Marina A. Gritsenko^{1*}, Yingchun Wang², Therese Claus¹, Tao Liu¹, Yufeng Shen¹, Matthew E. Monroe¹, Daniel Lopez-Ferrer¹, Theresa Reno³, Ronald J. Moore¹, Richard L. Klemke³, David G. Camp II¹ and Richard D. Smith¹

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³ Department of Pathology and UC San Diego, Moores Cancer Center, La Jolla, CA, USA

high pH first-dimension fractionation



concatenation of high pH fractions (super-fractions)

RESEARCH ARTICLE

Reversed-phase chromatography with multiple fraction concatenation strategy for proteome profiling of human MCF10A cells

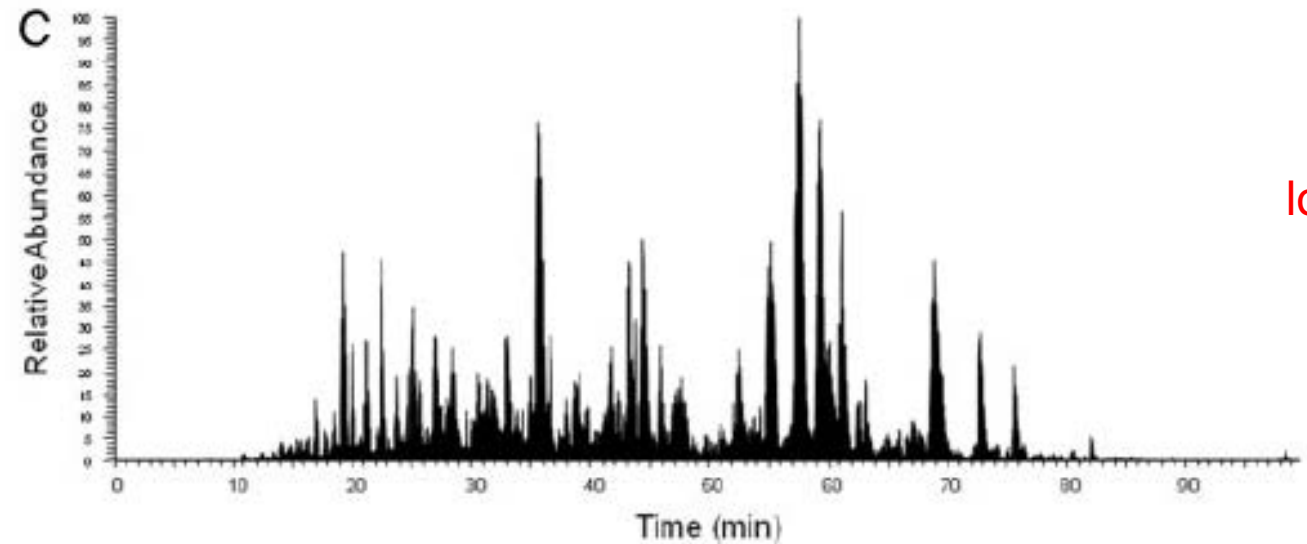
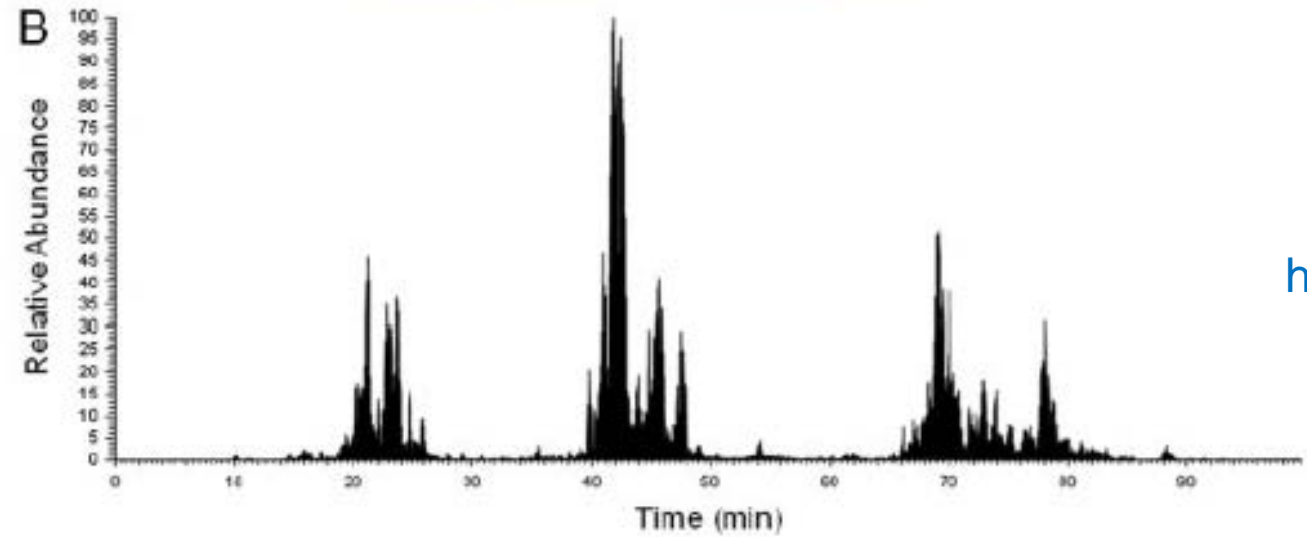
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second-dimension separation of concatenated fraction 10



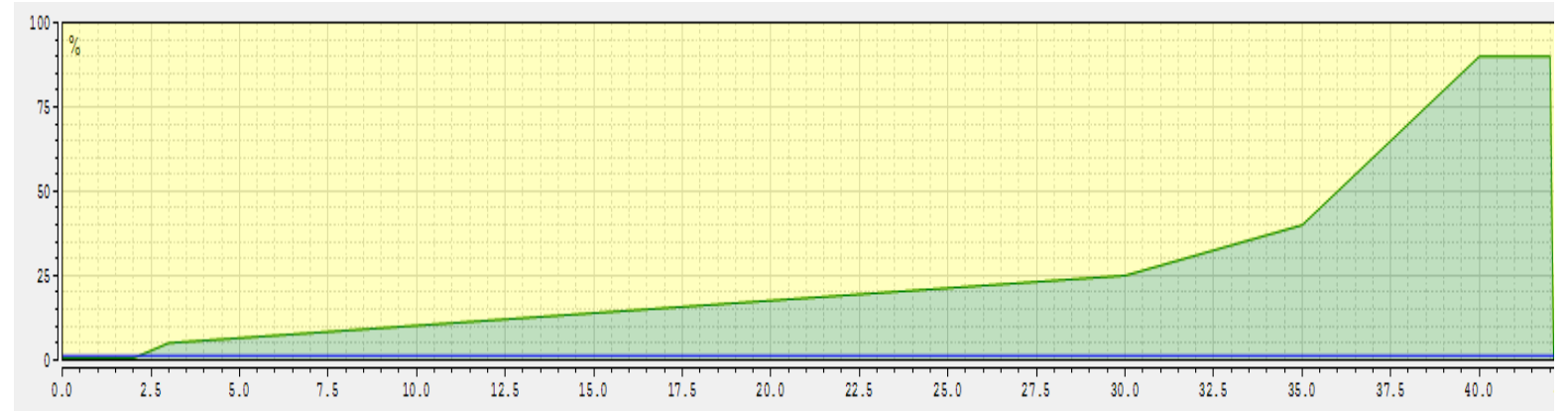


Buffer A H₂O 10mM NH₄OH
Buffer B ACN 10mM NH₄OH pH 10



Waters BEH
C18 Column, 130Å, 1.7 μ m,
1.0 mm X 100 mm

- Yeast Lysate ~660ug
- Stock solution 6.6ug/ul
- Injected 50ul of a 1ug/ul solution (diluted in Basic BufferA)
- Gradient separation
- 0.5-25% 30min
- 25-40% 5 min
- 40-90% 5min

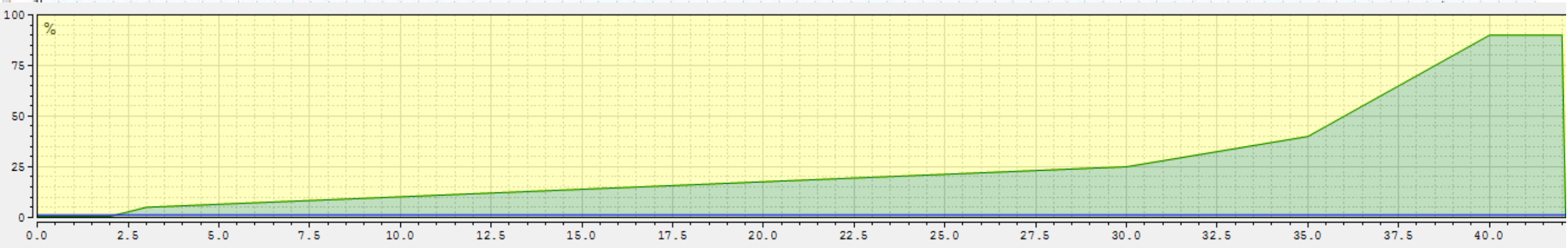
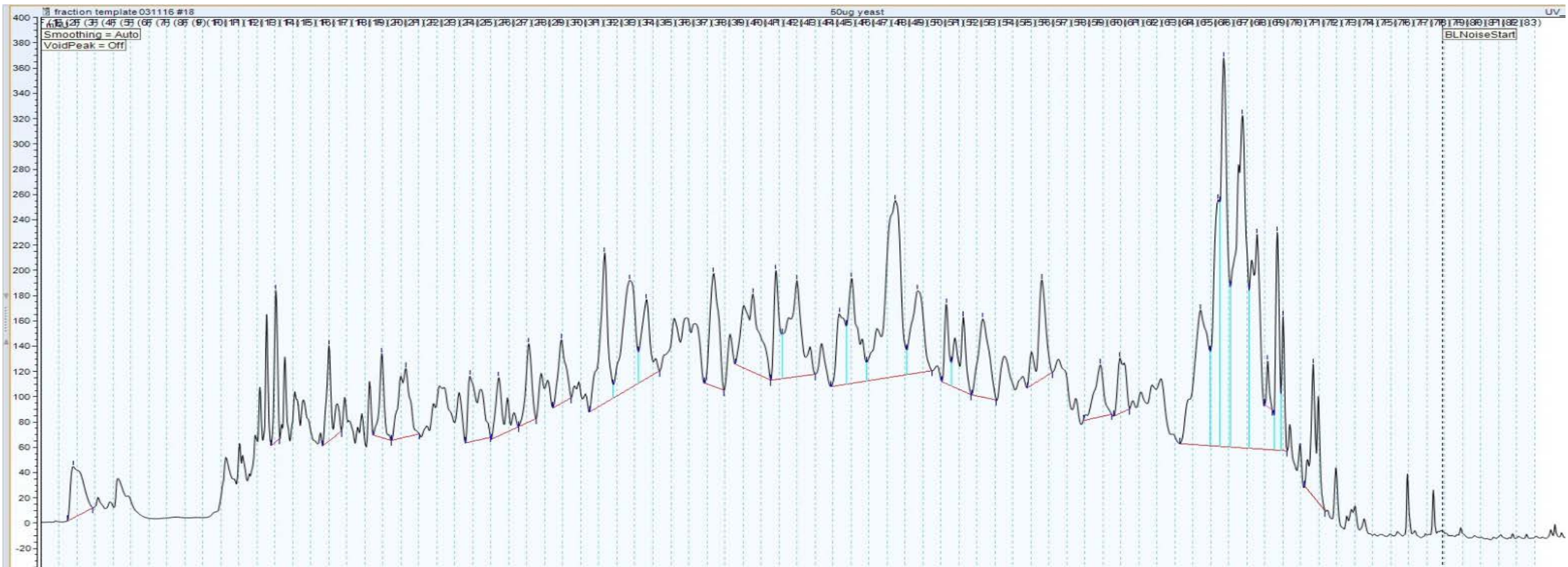


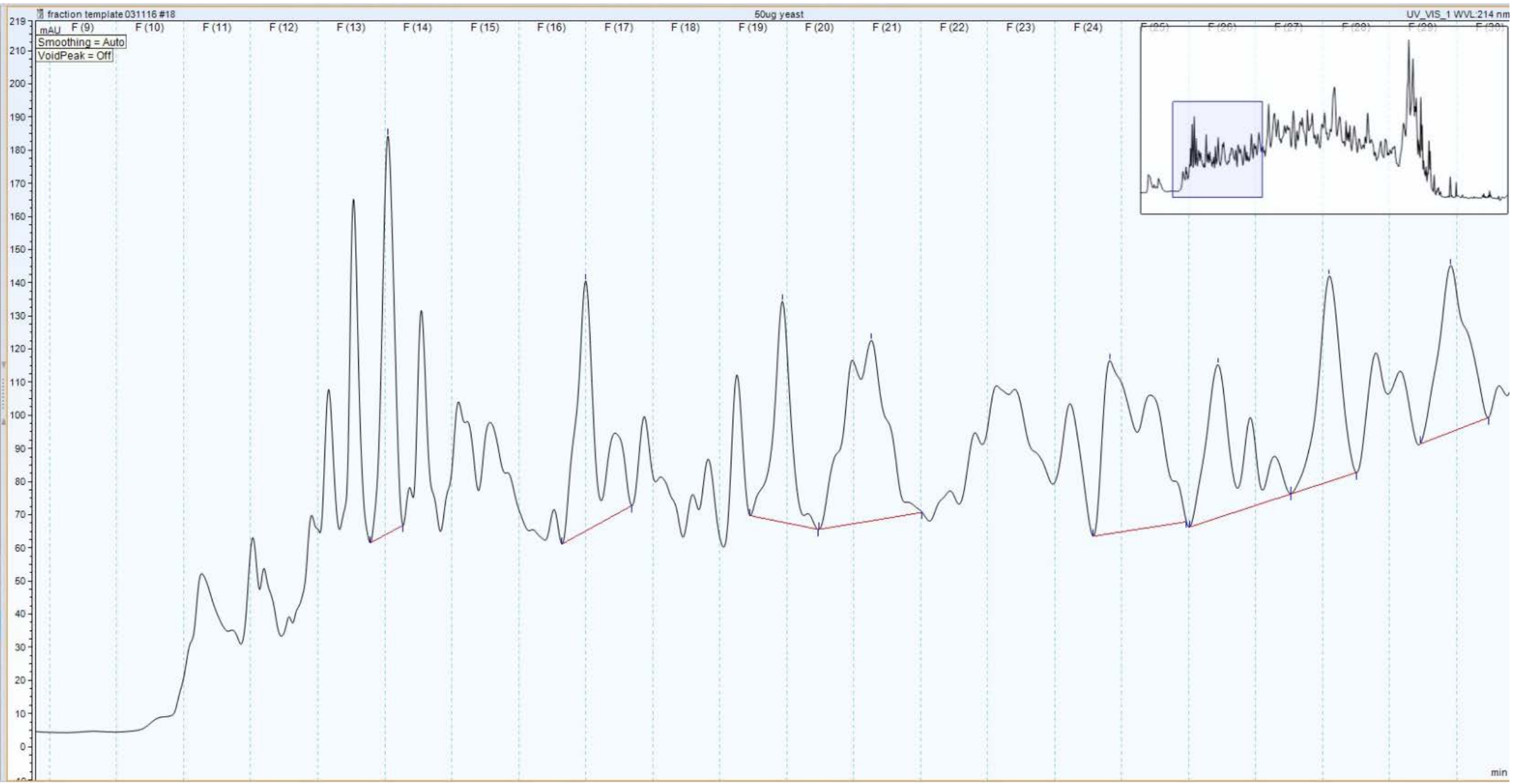
A1									F1	F2	F3	F4
F16	F15	F14	F13	F12	F11	F10	F9	F8	F7	F6	F5	
F17	F18	F19	F20	F21	F22	F23	F24	F25	F26	F27	F28	
F40	F39	F38	F37	F36	F35	F34	F33	F32	F31	F30	F29	
E1									F1	F2	F3	F4
F16	F15	F14	F13	F12	F11	F10	F9	F8	F7	F6	F5	
F17	F18	F19	F20	F21	F22	F23	F24	F25	F26	F27	F28	
F40	F39	F38	F37	F36	F35	F34	F33	F32	F31	F30	F29	

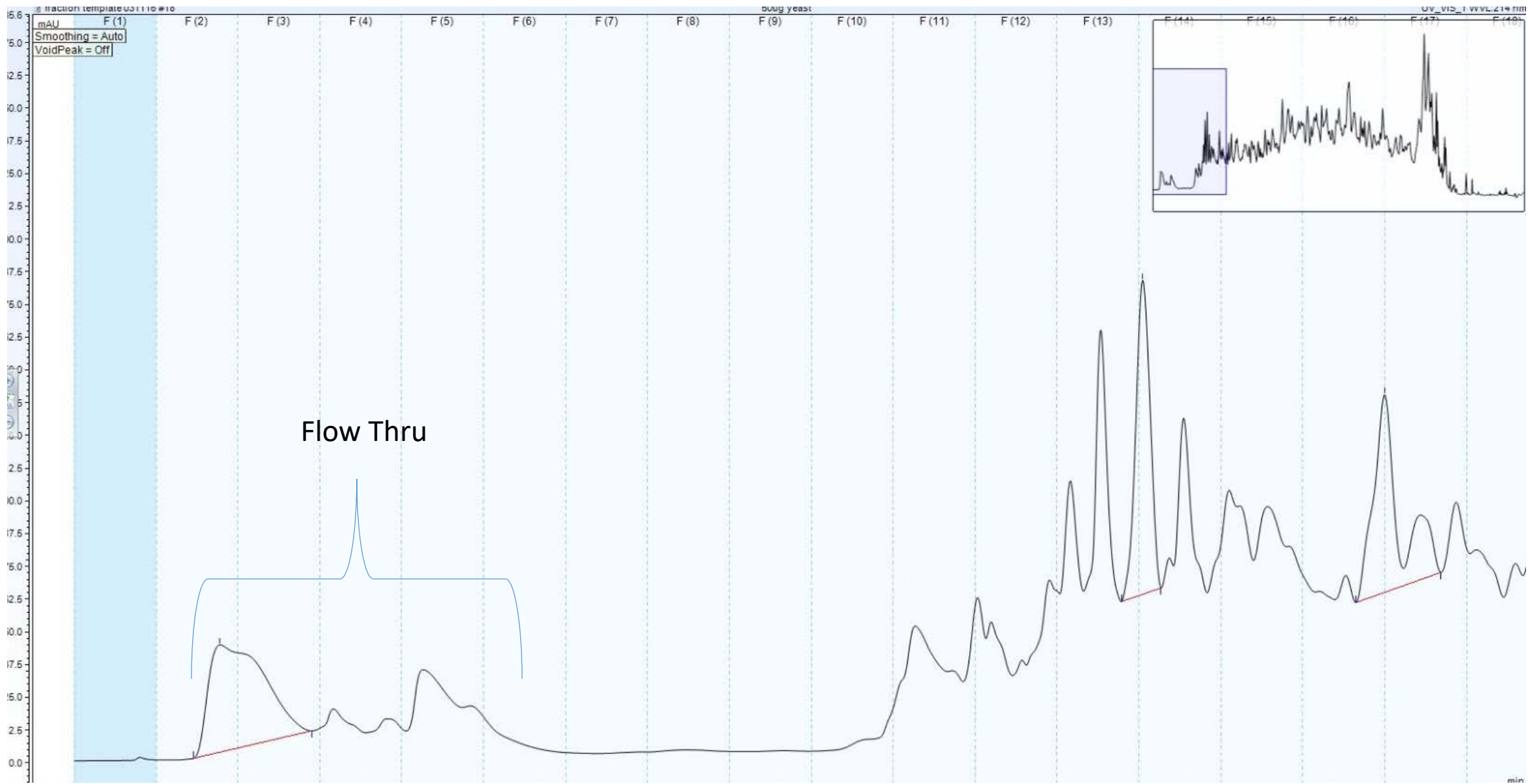
Fraction Collection in PCR plate (200ul wells) every 60s , flow rate 100ul/min



50 ug Yeast Lysate







Super Fractions

A1									F1	F2	F3	F4
F16	F15	F14	F13	F12	F11	F10	F9	F8	F7	F6	F5	
F17	F18	F19	F20	F21	F22	F23	F24	F25	F26	F27	F28	
F40	F39	F38	F37	F36	F35	F34	F33	F32	F31	F30	F29	
E1									F1	F2	F3	F4
F16	F15	F14	F13	F12	F11	F10	F9	F8	F7	F6	F5	
F17	F18	F19	F20	F21	F22	F23	F24	F25	F26	F27	F28	
F40	F39	F38	F37	F36	F35	F34	F33	F32	F31	F30	F29	

SF1 = F5,F17,F29

SF2 = F6,F18,F30

...

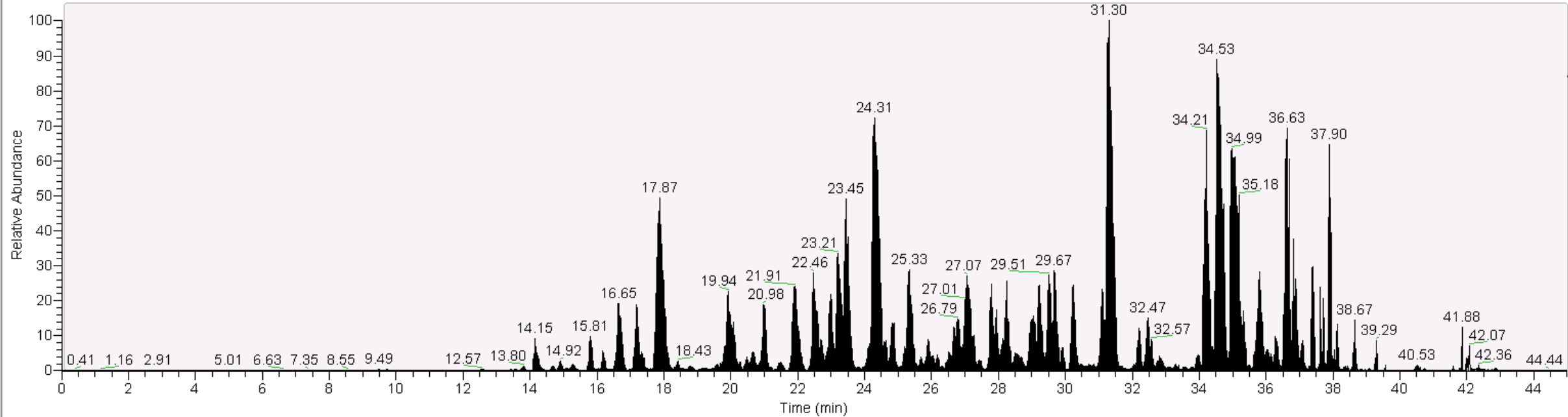
SF12 = F16,F28,F40



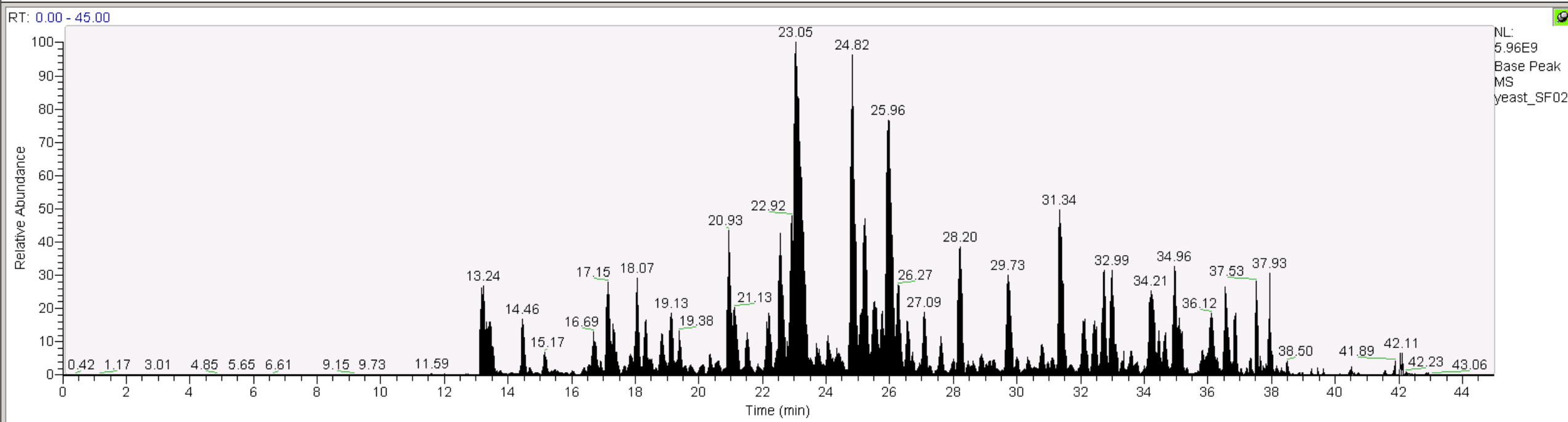
D:\rick\031416\yeast_SF01

03/14/16 17:10:18

RT: 0.00 - 45.00



NL:
7.95E9
Base Peak
MS
yeast_SF01

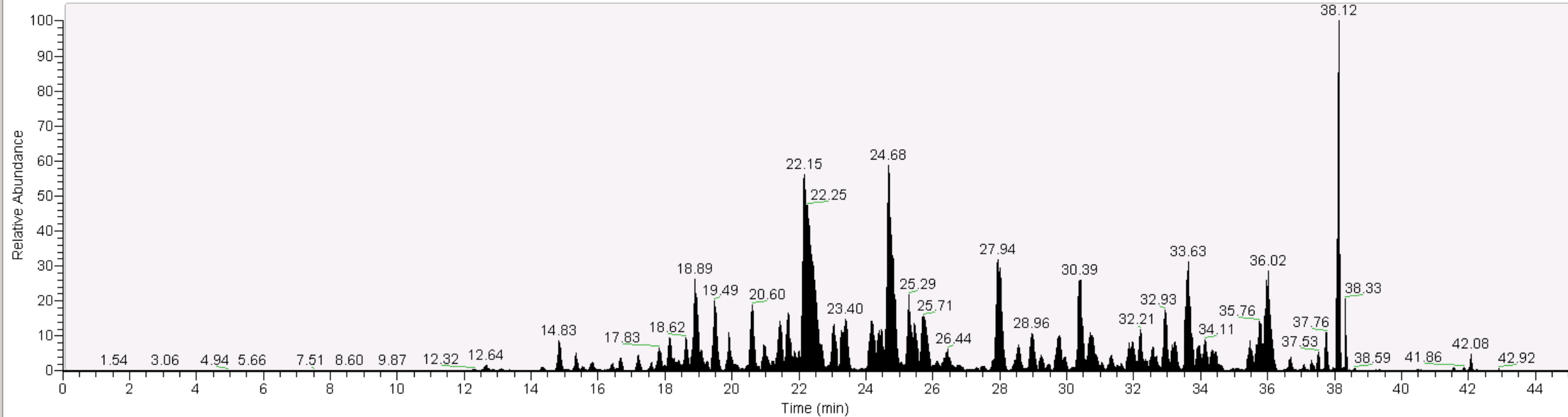


NL:
5.96E9
Base Peak
MS
yeast_SF02

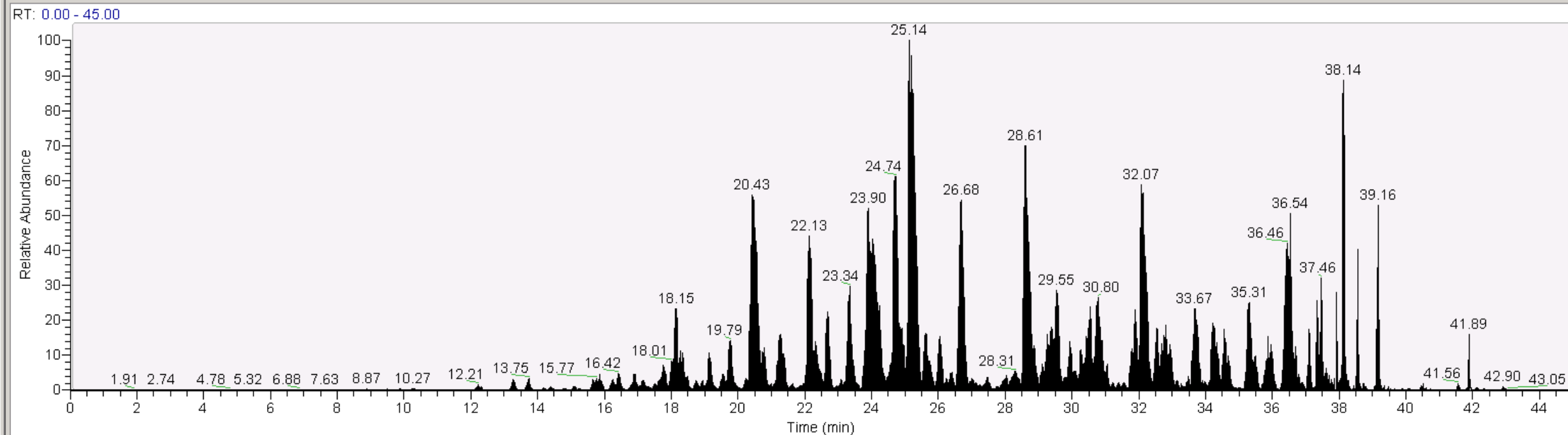
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RT: 0.00 - 45.00

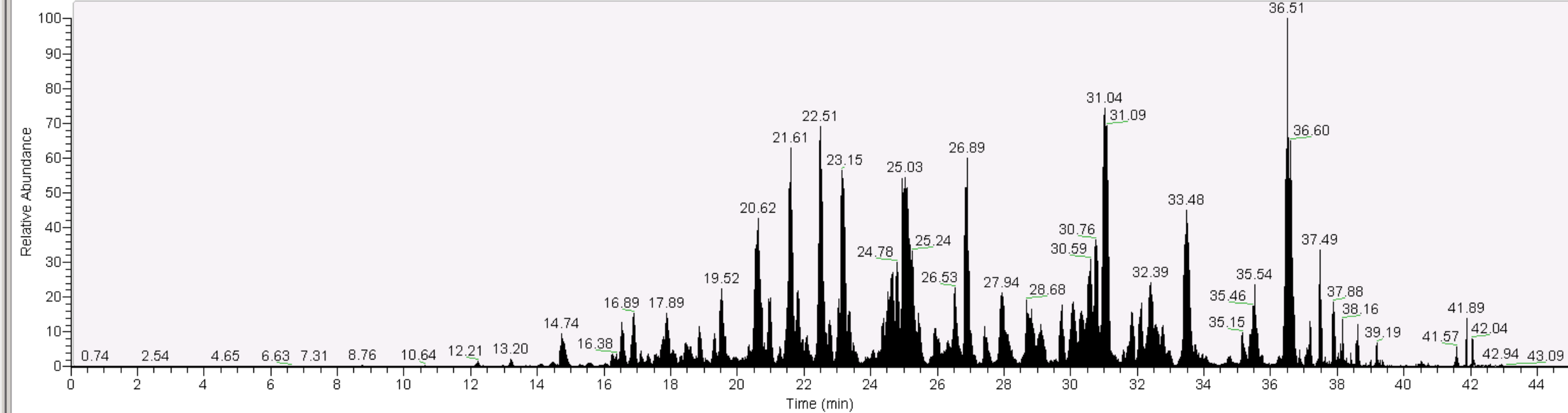


NL:
1.34E10
Base Peak
MS
yeast_SF03



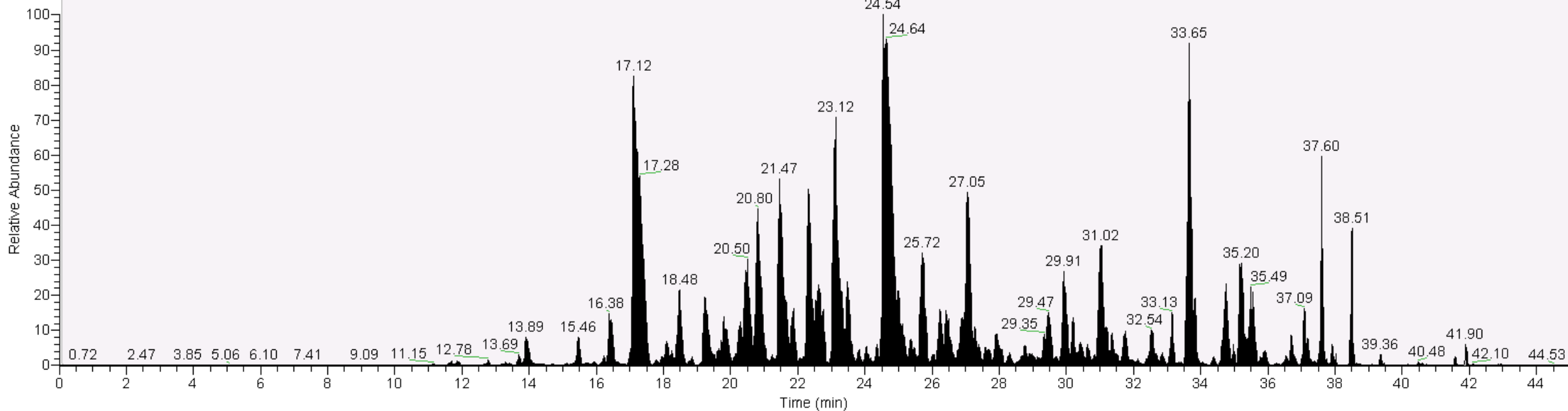
NL:
6.25E9
Base Peak
MS
yeast_SF04

RT: 0.00 - 44.99



NL:
5.62E9
Base Peak
MS
yeast_SF05

RT: 0.00 - 44.99



NL:
6.99E9
Base Peak
MS
yeast_SF12

Protein Depth and Time

mouse thymus – 100 ug digested

- SF6 (7 hours)
 - 6998 proteins (IBAQ value)
 - 5966 (two peptides and IBAQ)
 - Median peptides 6
- SF12 (13 hours)
 - 8881 proteins (IBAQ value)
 - 7849 proteins (two peptides and IBAQ)
 - Median peptides 7
- SF36 (37 hours)
 - 10121 proteins (IBAQ value)
 - 9546 proteins (two peptides and IBAQ)
 - Median peptides 9

