FONDAZIONE EDMUND MACH

The rare case of a cluster mutation in grapevine - a metabolomics study

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Silvia Vezzulli, Claudio Moser,

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Colored Grapes

Vezzulli S et al. J. Exp. Bot. 2012;63:6359-6369, © 2012 The Author(s)

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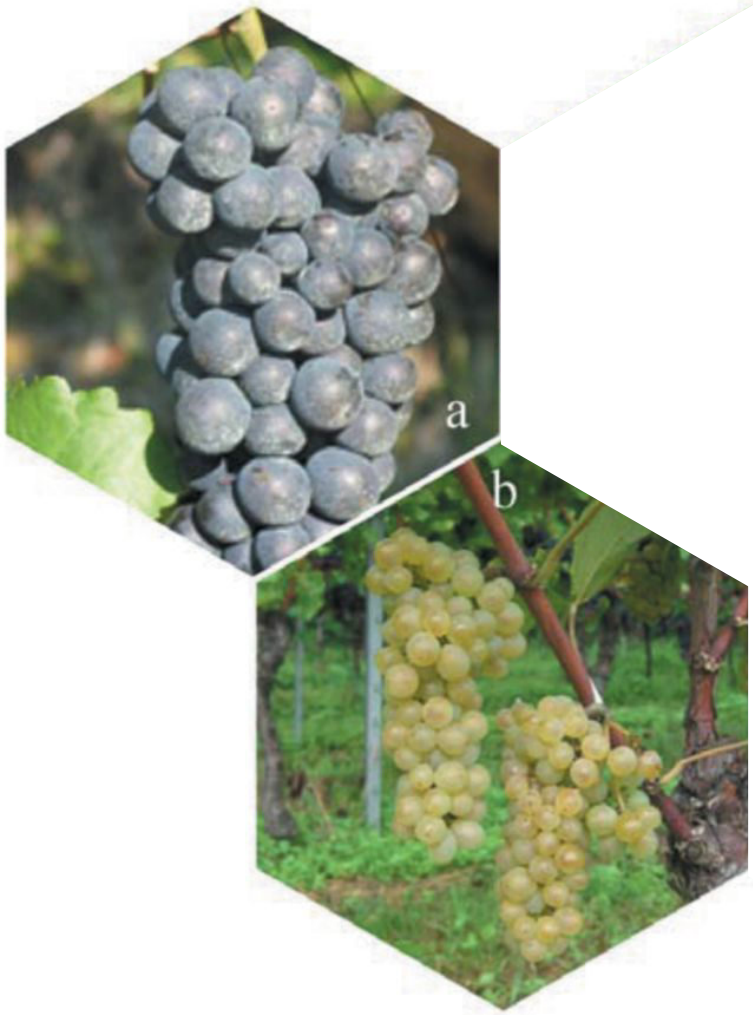
White Grapes



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Both on one Cluster

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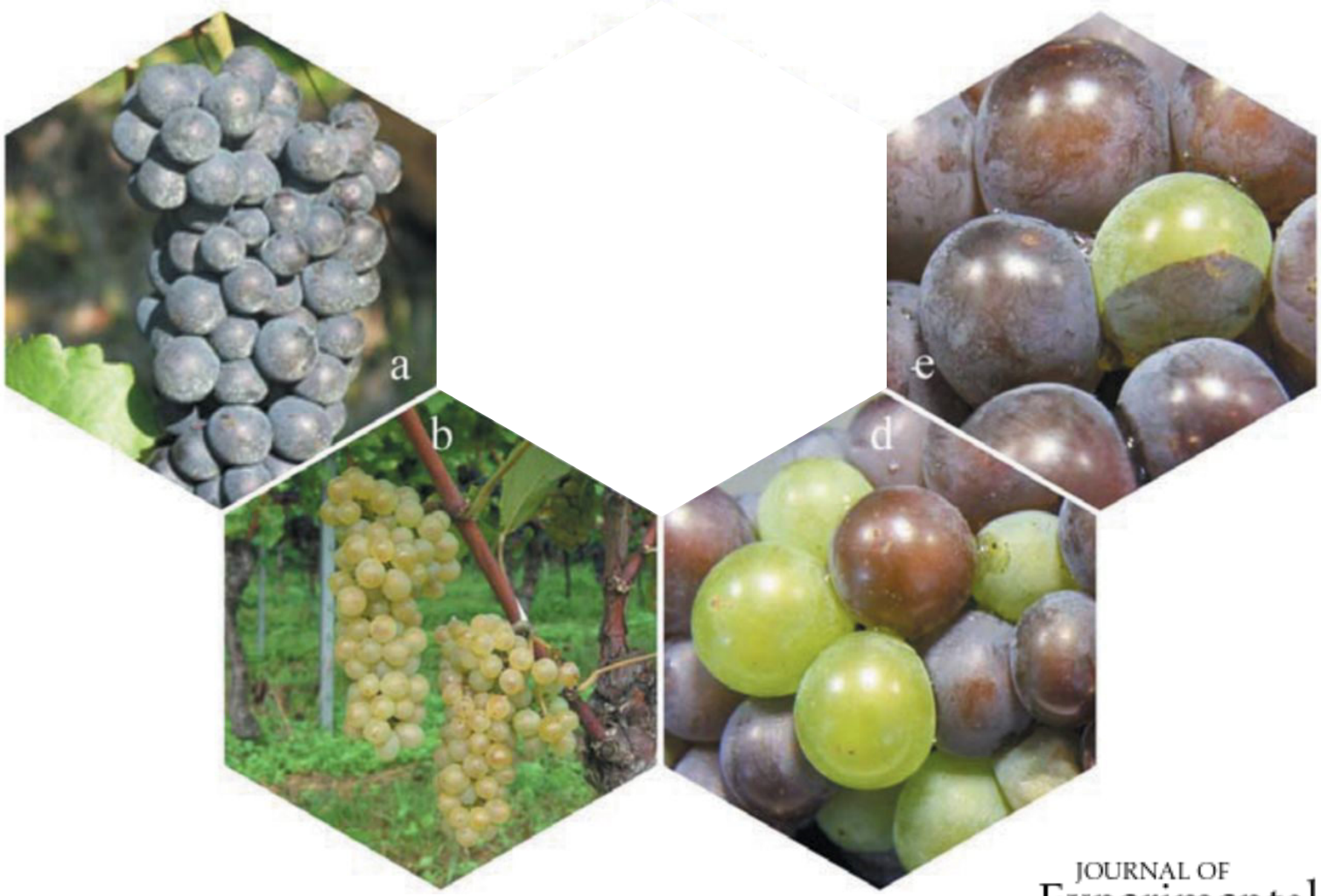
Both on one Berry



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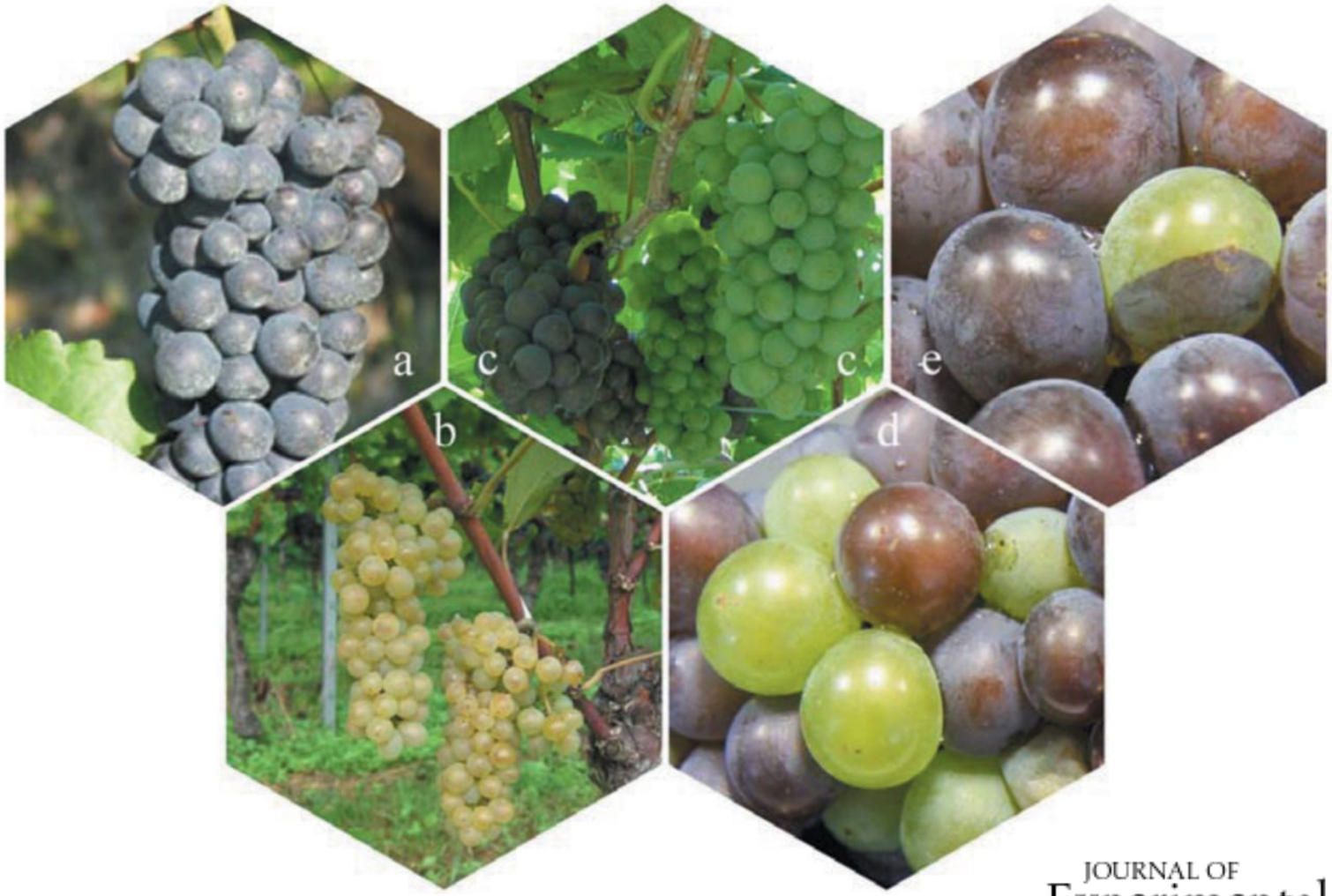
Both on one Shoot



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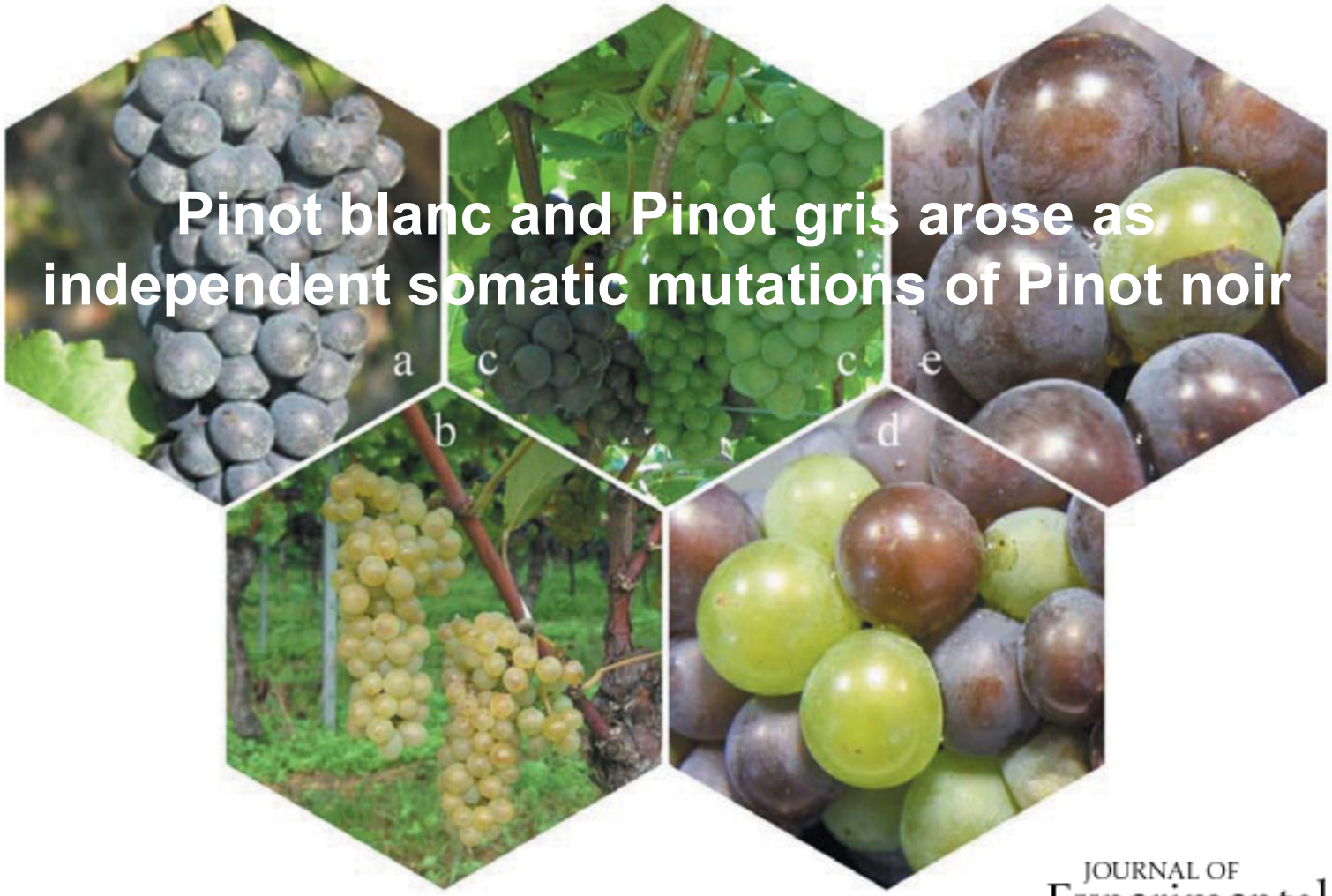
Both on one Shoot



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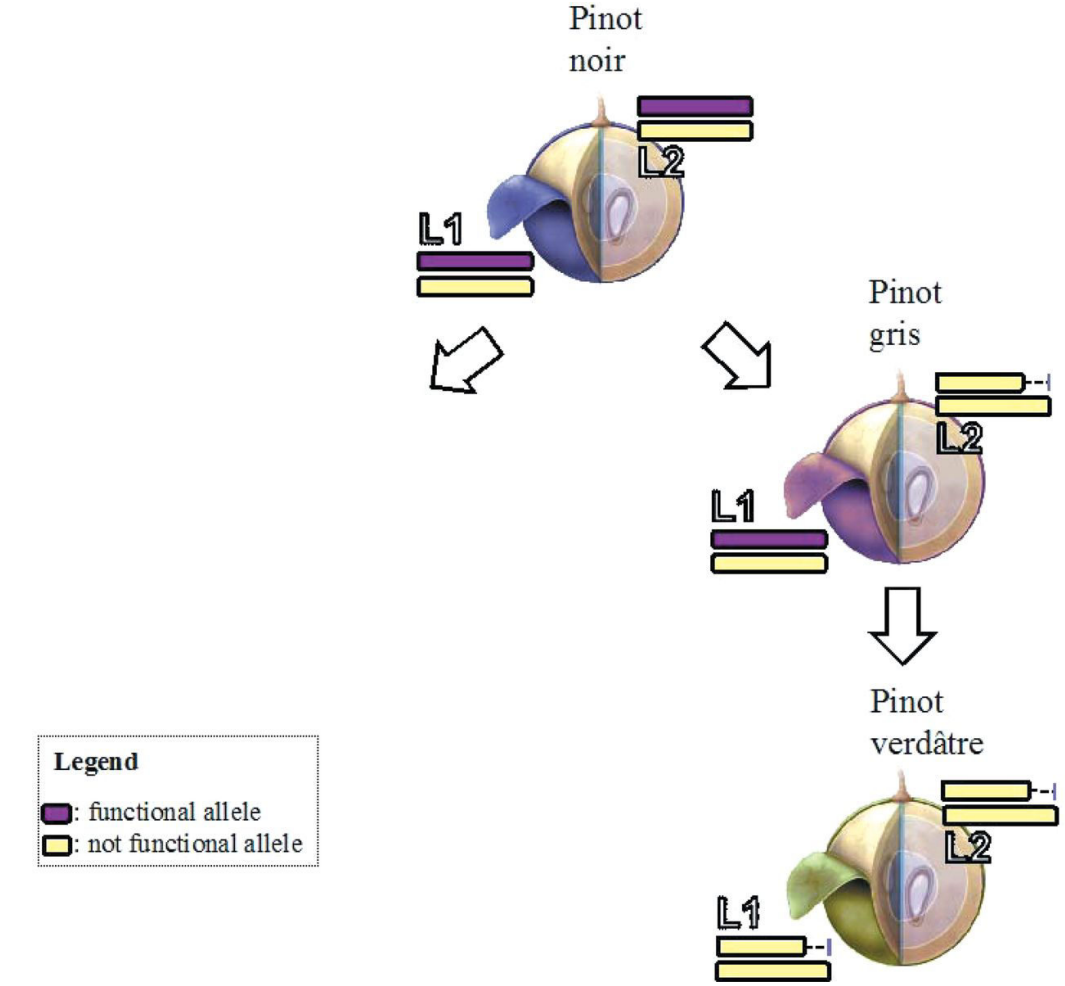
Model of the Formation of Pinot gris, Pinot verdâtre, and Pinot blanc from Pinot noir



somatic mutation

chimera

displacement

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Why is This of Interest for Analytical Chemistry?

- Mutation on chromosome 2
- Carries colour coding genes → Anthocyanes (LCMS)
- Study difference between red and white

- Carries also genes coding for terpene synthase → is it also affected?

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Sample Preparation

- storage at -80°C
- ground under liquid N_2
- 4g powder +
  5mL MilliQ-H_2O +
  $MgSO_4$ +
  0.1% NaN_3-solution +
  Citric acid +
  Ascorbic acid + IS
  in 20mL HS vial

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Solid Phase Microextraction

- PDMS/DVB/CAR 2cm fiber
- 10 min incubation at 60°C
- 40 min extraction at 60°C
- 2 min desorption in GC inlet at 260°C
- Multiple Headspace Extraction

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Instrumentation

- Autosampler Gerstel with SPME, cooled tray at 4°C
- GC-MS LECO Peg4D CF GCxGC–TOFMS
- 1st column RTX5-MS 30m, 0.25mm, 250µm
- 2nd column Stabilwax 1m, 0.15mm, 150µm
- He 1.2 mL/min constant flow
- Oven 40°C for 2 min, 5°C/min 180°C, 25°C/min 260°C for 5 min
- Secondary oven offset +20°C from GC oven
- Modulator offset +10°C from 2nd oven
- Chiller start -85°C
- Modulation period 2.5s for 6 min, 4.5s
- Transfer line 280°C
- MS source 230°C
- Acquisition rate 200 scans/s
- Detector offset +200V from tune value

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Instrumentation

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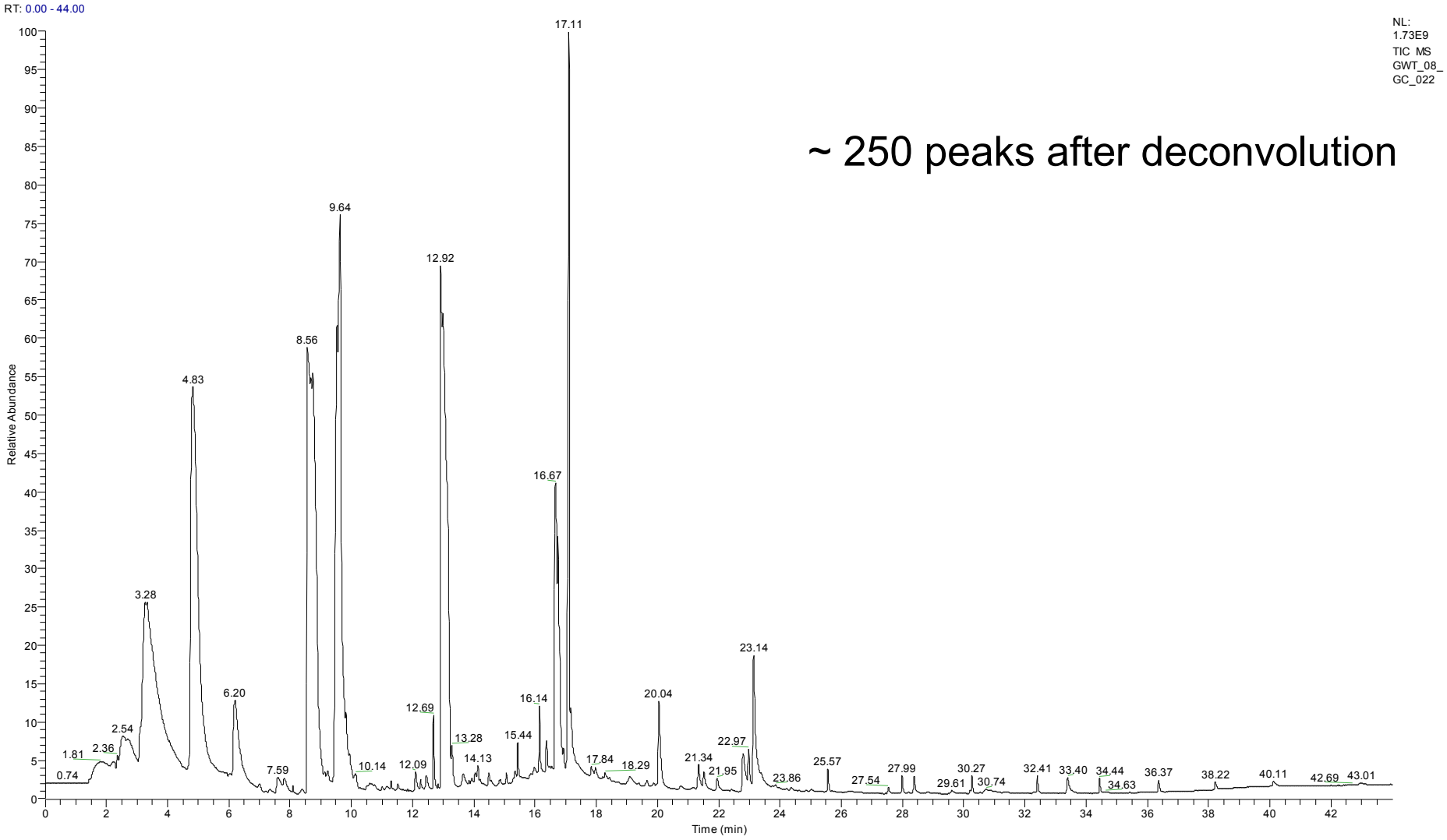
1D GC-qMS Chromatogram of Gewürztraminer



~ 250 peaks after deconvolution

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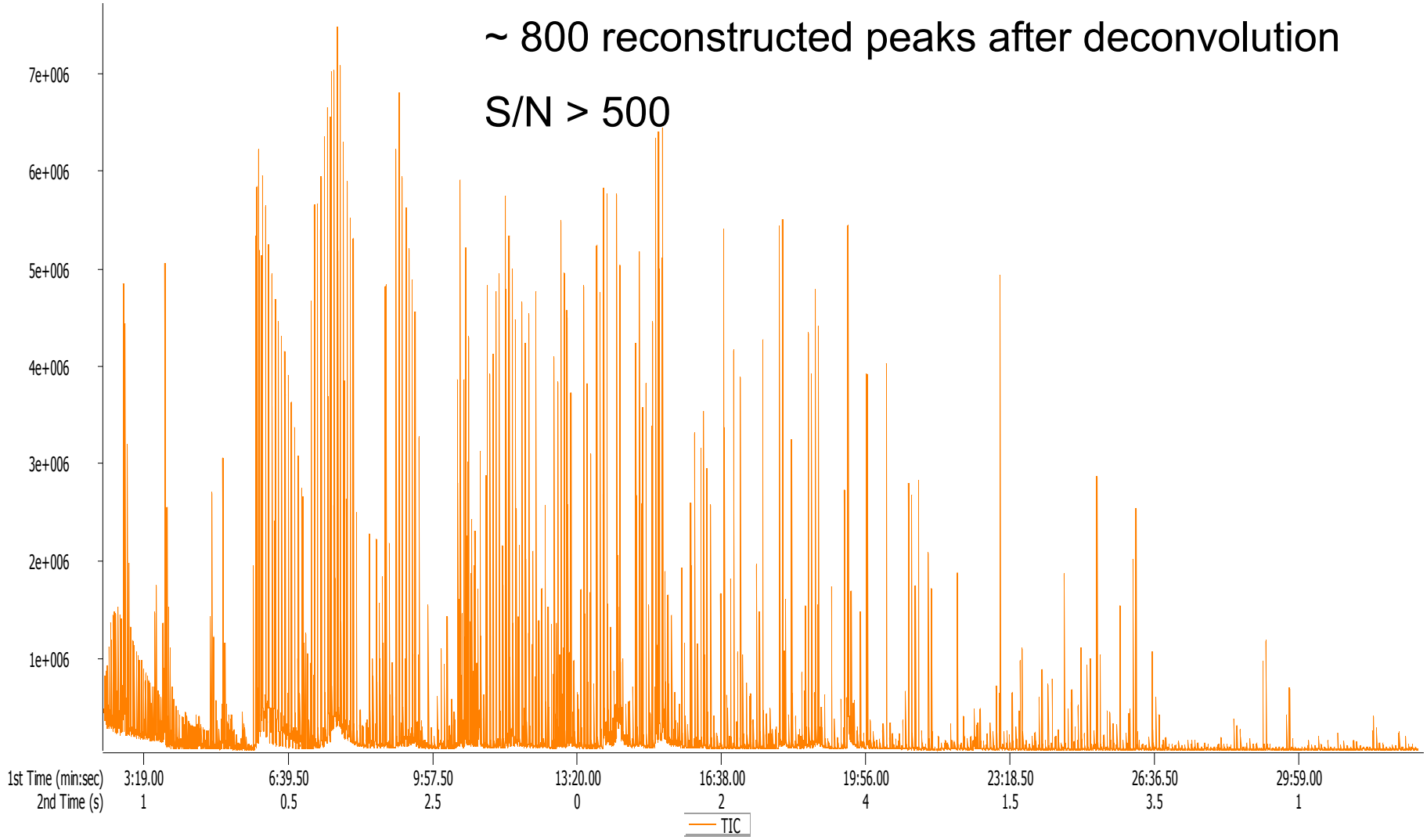
2D GCxGC TOFMS

~ 800 reconstructed peaks after deconvolution

S/N > 500



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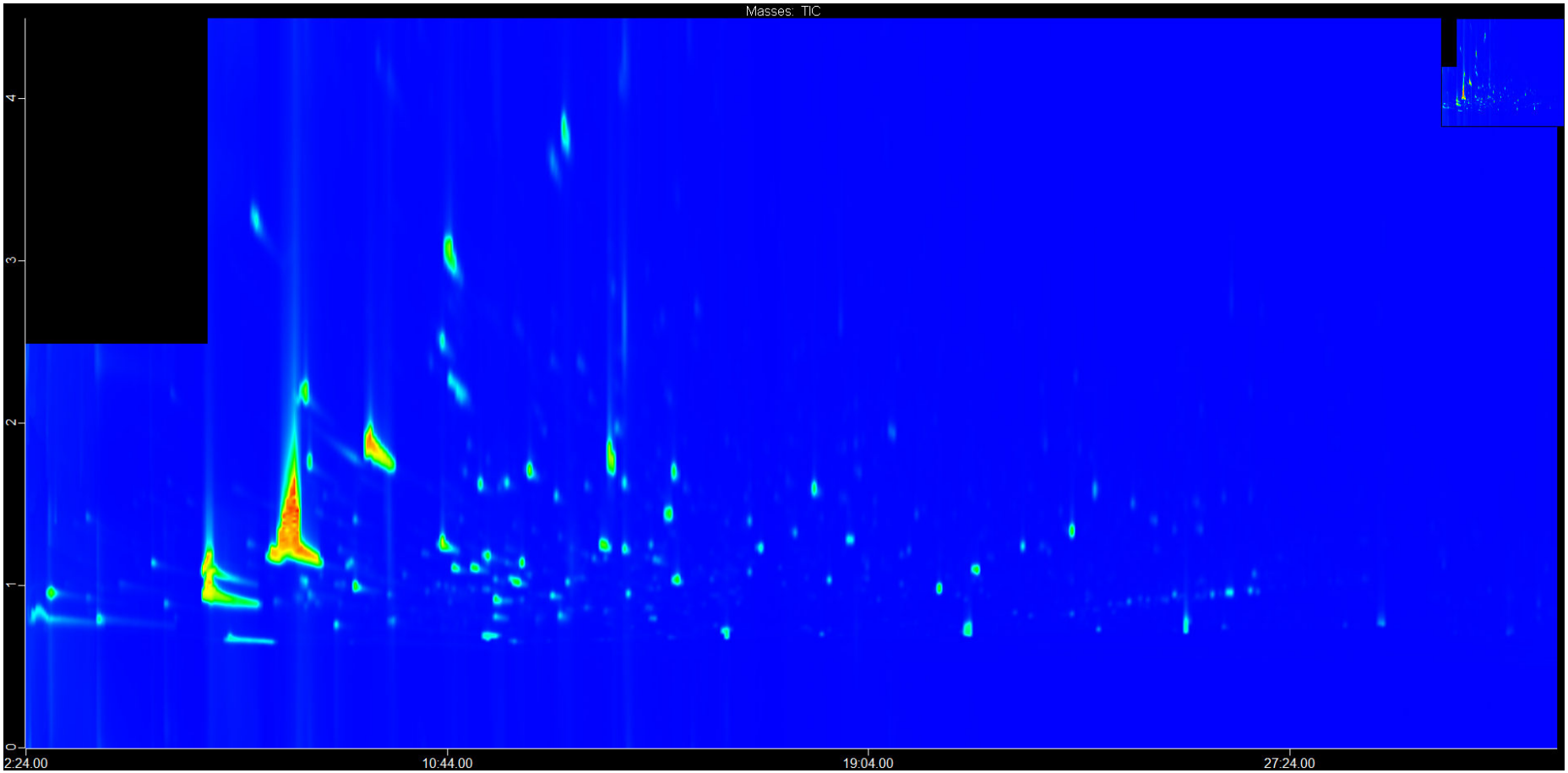
2D GCxGC TOFMS



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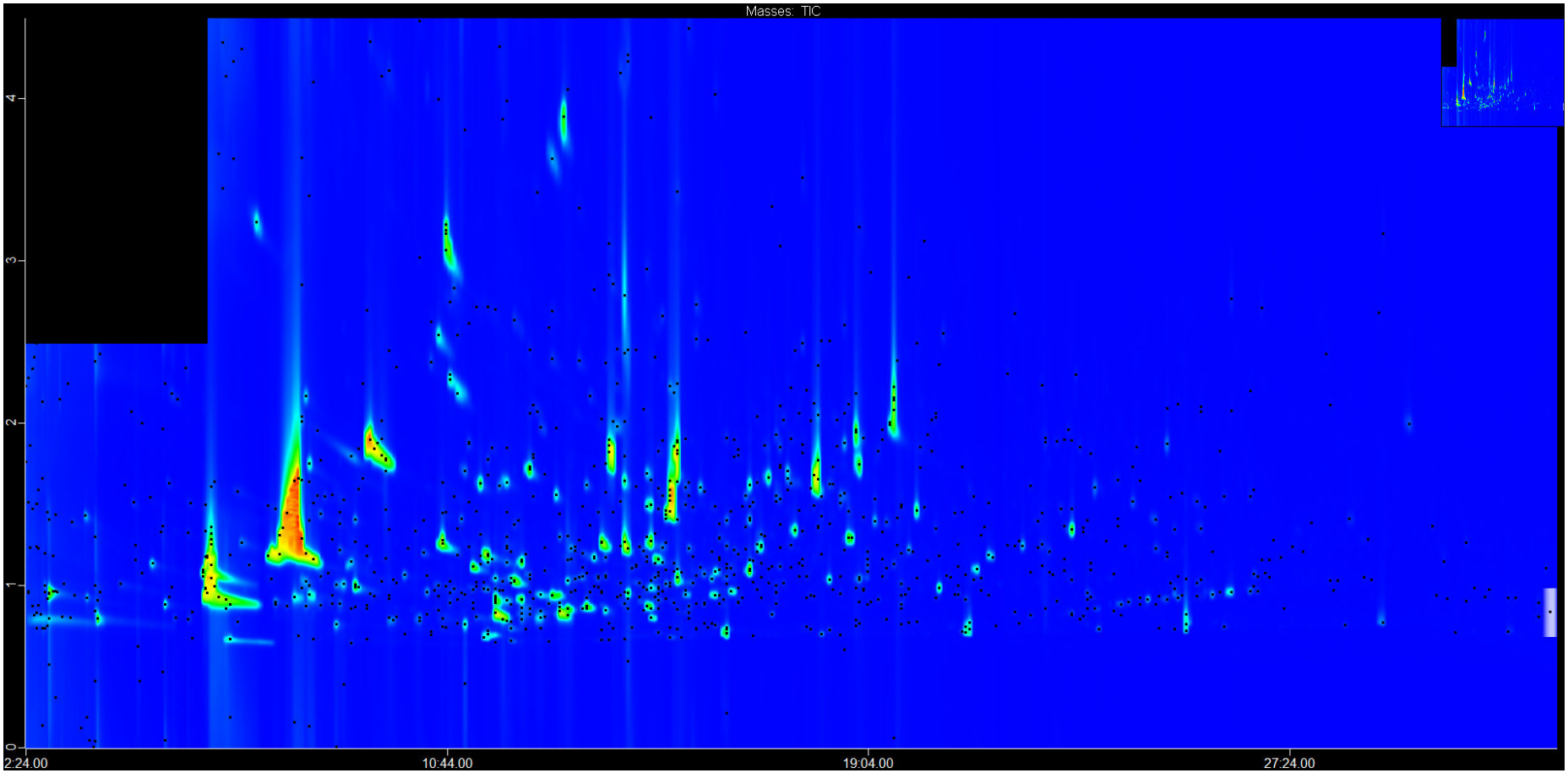
2D GCxGC TOFMS



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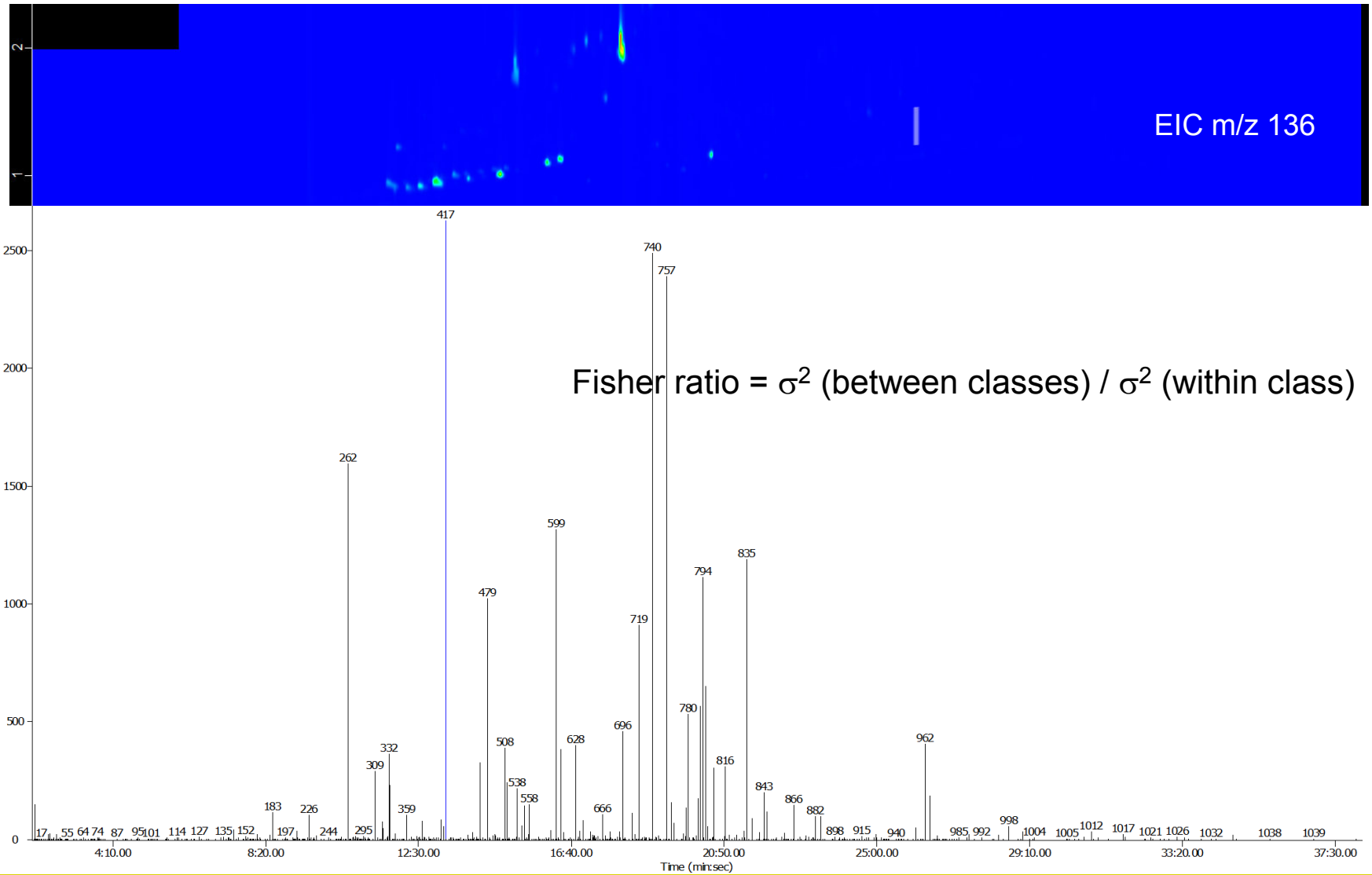
StatCompare



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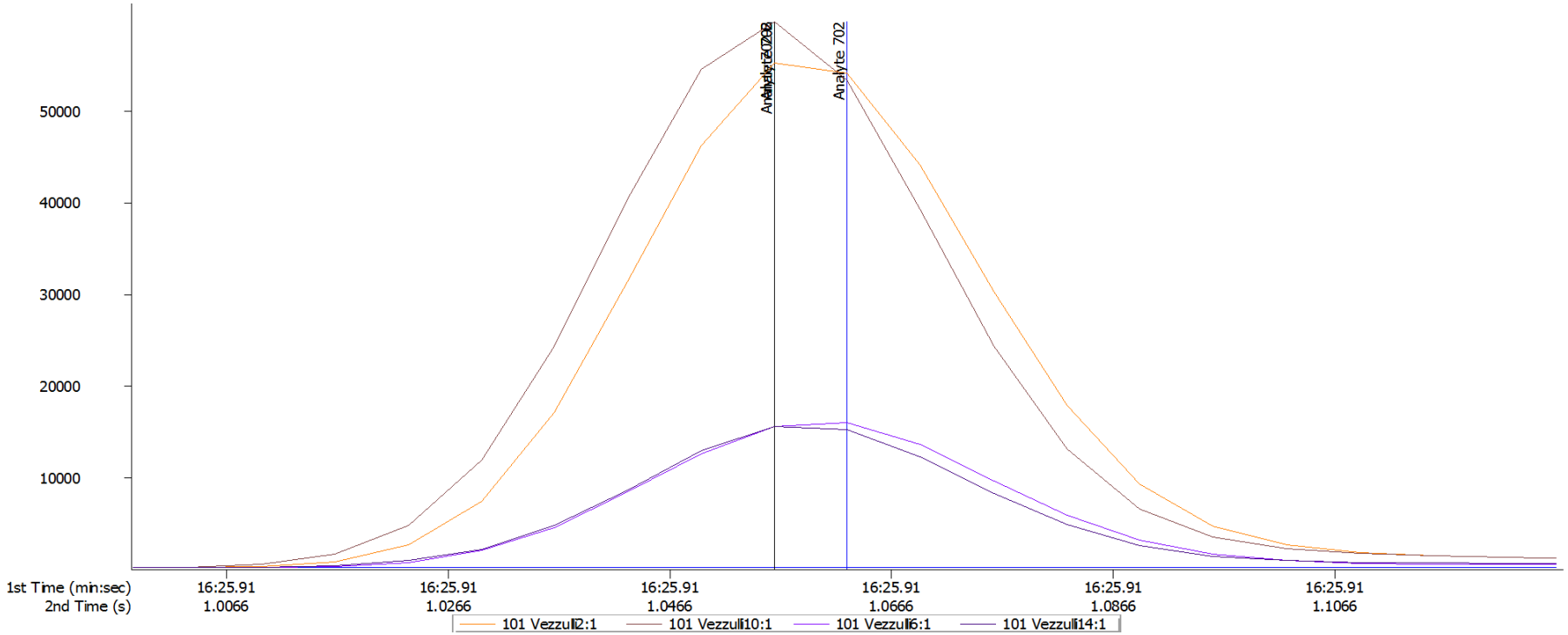
WT Higher



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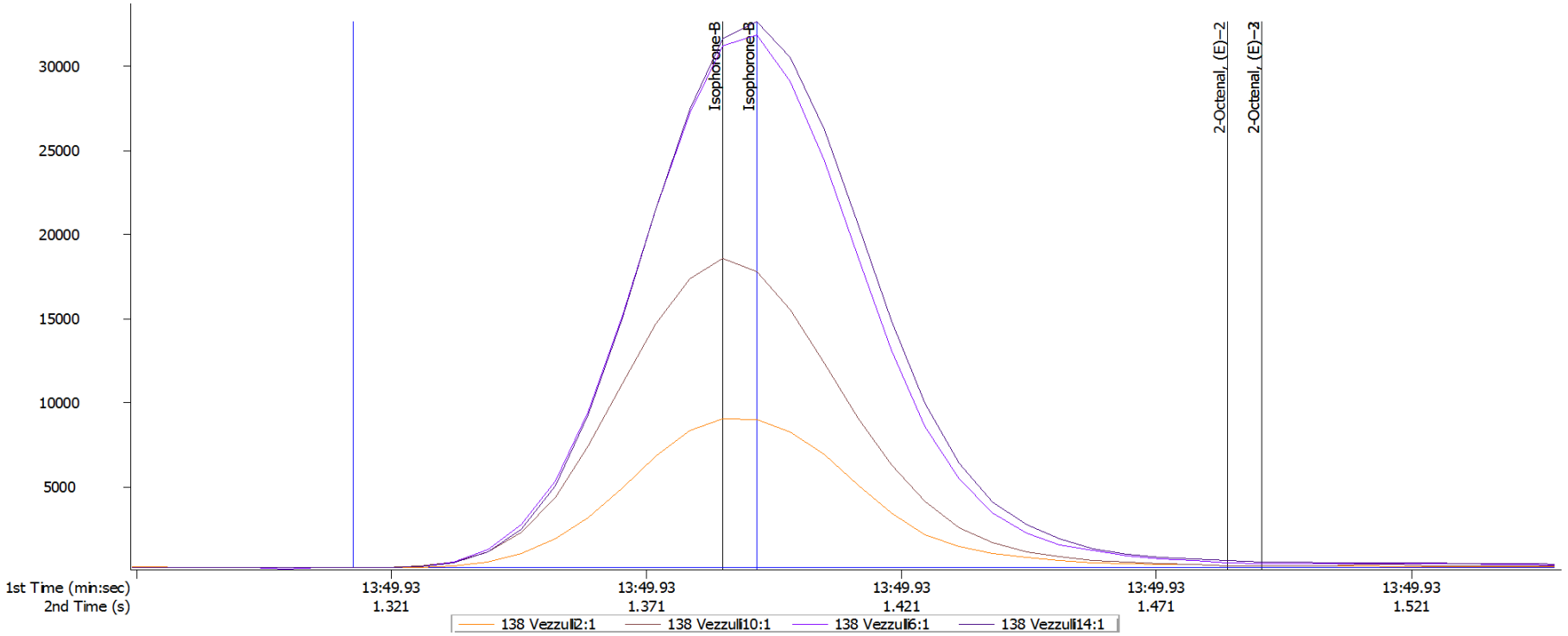
MUT Higher



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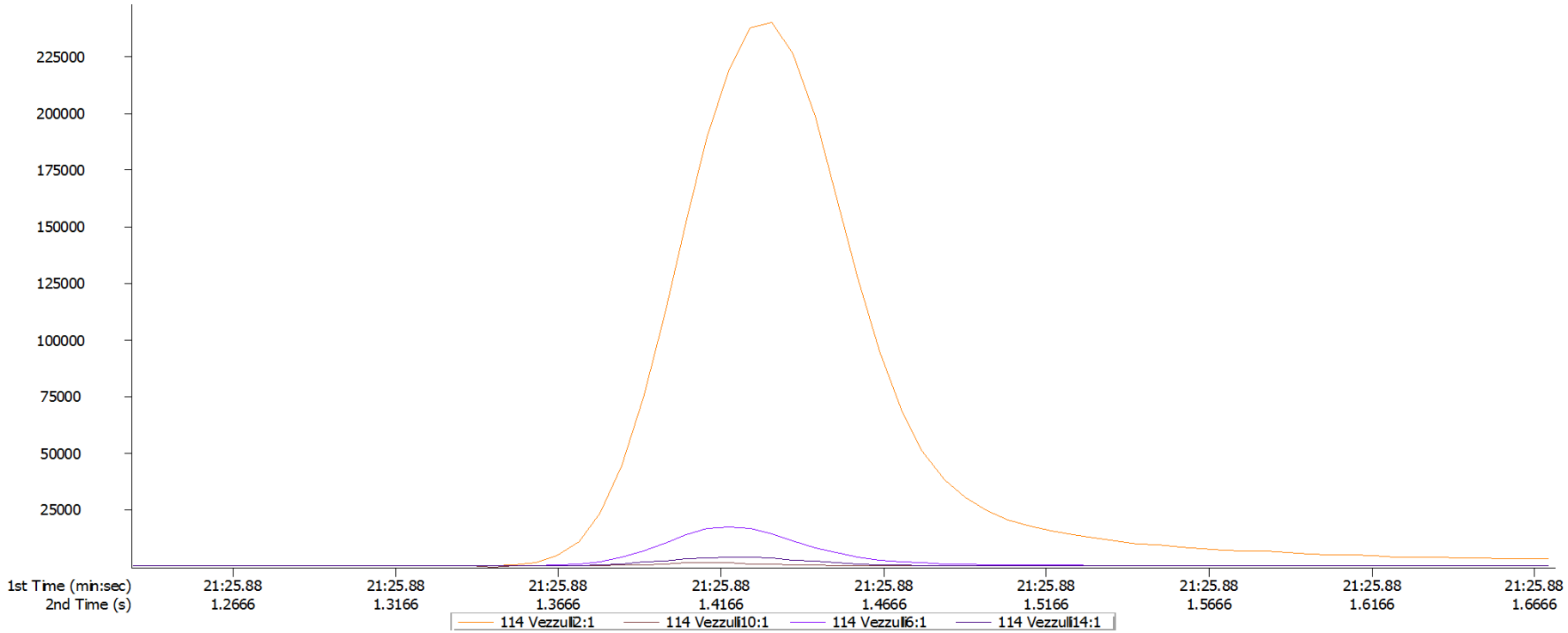
Difference Between Clones



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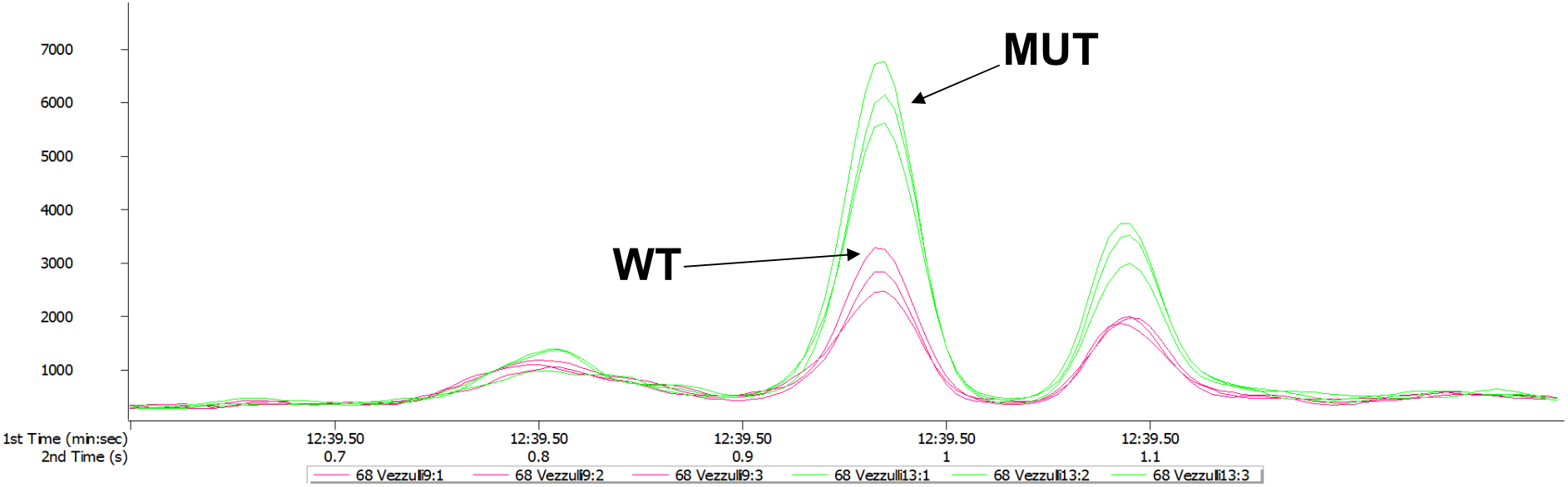
Repeatability



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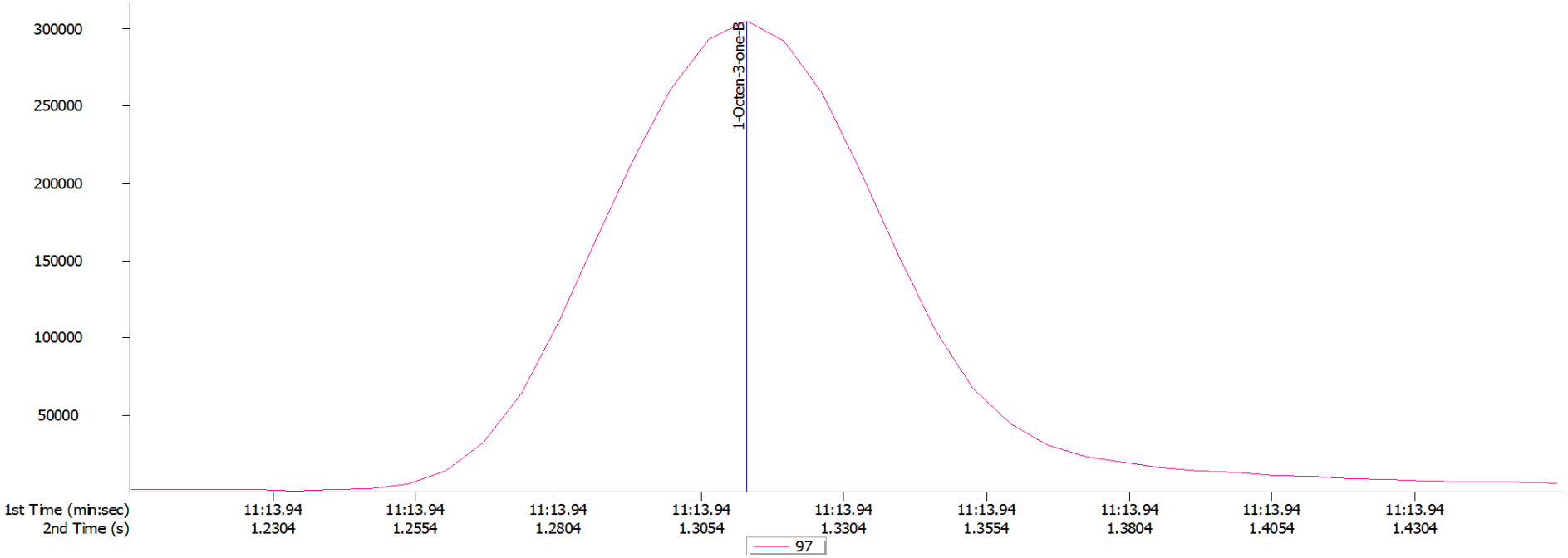
Narrow Peaks



peak width of 150ms, scan rate of 200 scans/second results in 30 points over peak → sufficient
for identification also lower scan rate possible → higher sensitivity, purer deconvolution

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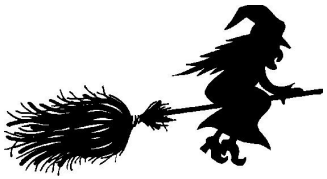
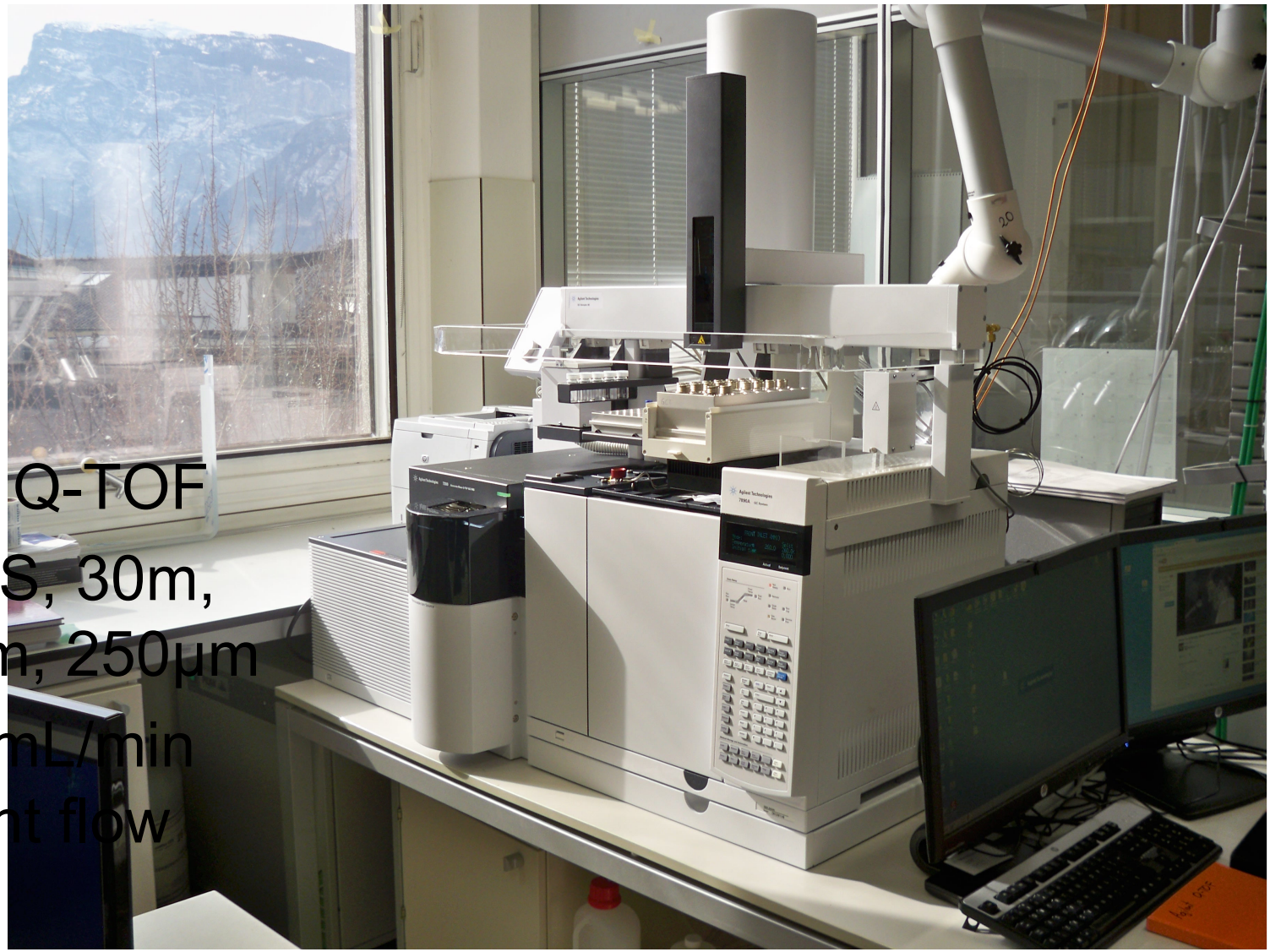
GC-HRMS

- Accurate mass should lead to better deconvolution
- Very good S/N
- CI provides molecular ion → calculation of sum formula
- build own spectra library

GC-HRMS





- Agilent Q-TOF
- HP5-MS, 30m, 0.25mm, 250µm
- He 1.2mL/min constant flow

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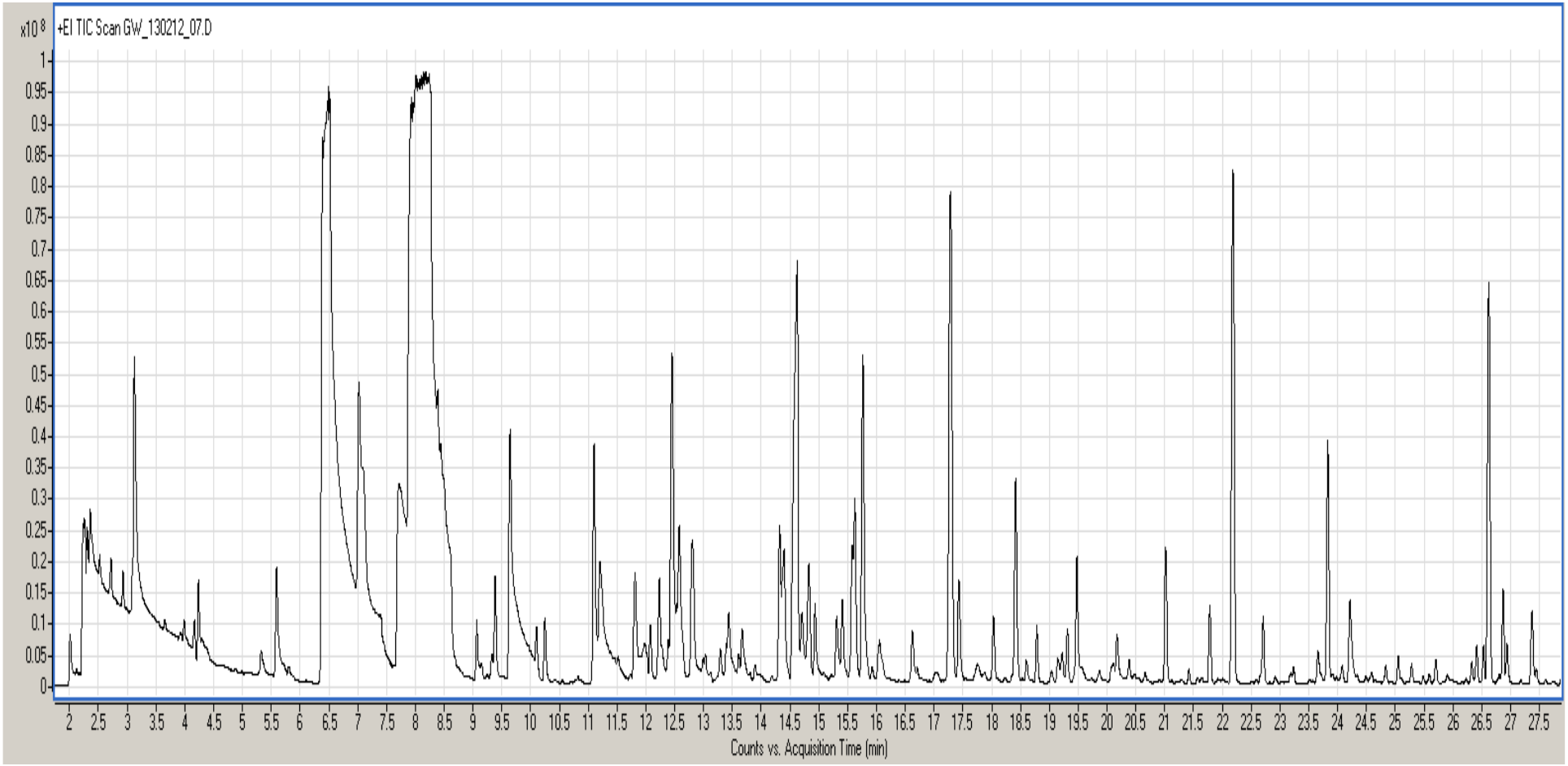
GC-HRMS



~ 350 peaks after deconvolution

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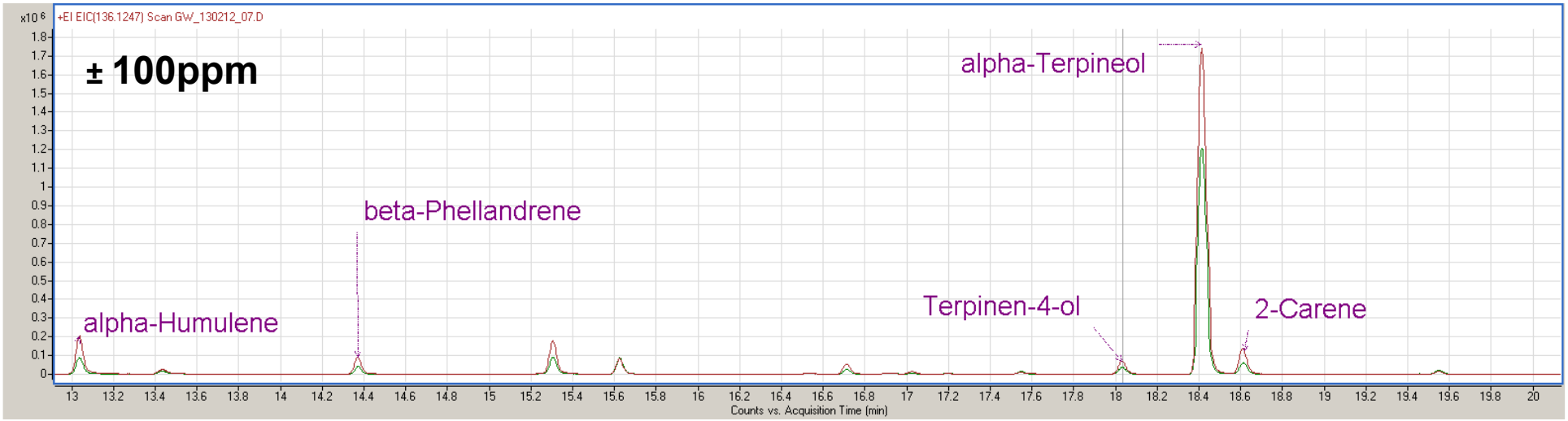
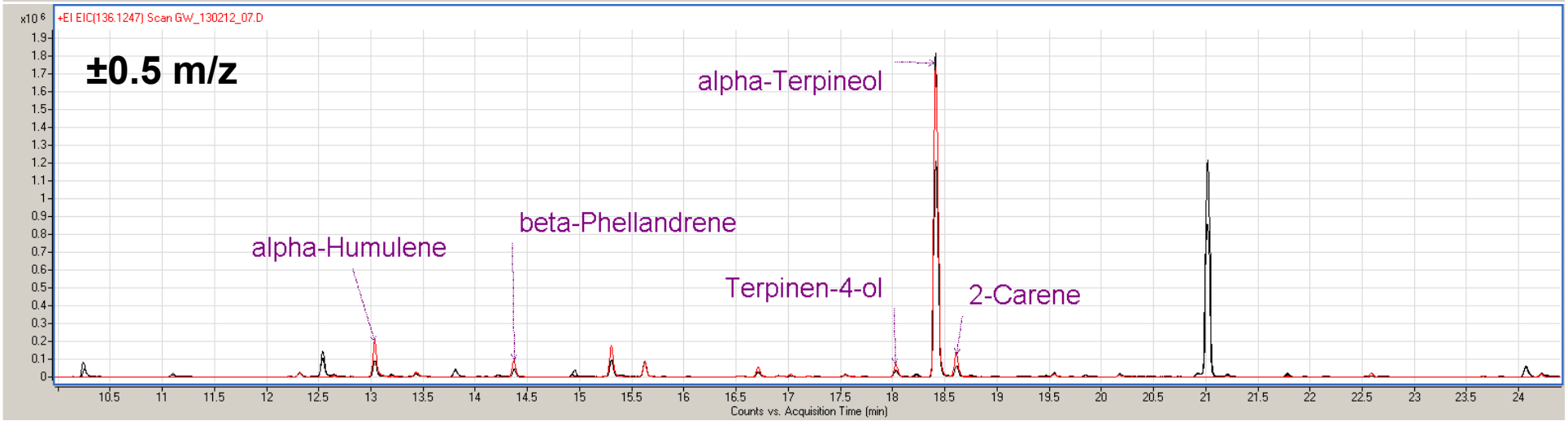
Monoterpenes





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Summary

- HS-SPME of grape samples

- 1D GC-qMS ~ 250 peaks
- 1D GC-HR-TOF-MS ~ 350 peaks
- 2D GCxGC-TOF-MS ~ 1000 peaks

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Summary

- Tentative identification of > 100 metabolites

- High Fisher ratio between WT and MUT for > 50 compounds
- Thereof tentatively identified 15, e.g. Hexanal, E-2-Hexenol, 2-Heptanol, several mono- and sesquiterpenes →
- Yes, a terpene synthase could be affected

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Problems

- Need of TOF (accurate) mass spectra library
- Use HRMS with CI
- Settings for deconvolution

- 2 clones with different behaviour → need of biological replicates

- Combine different –omics techniques

- What is a true metabolite?

Thanks to

- Silvia Vezzulli
- The organizers
- The audience