Genetic Basis for the 3-ADON and 15-ADON Trichothecene Chemotypes in *Fusarium*

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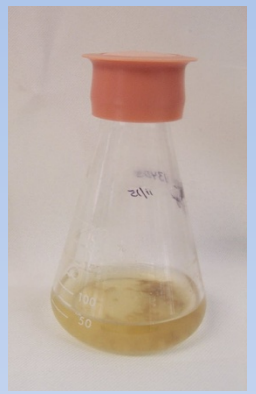
Bacterial Foodborne Pathogen and Mycology Research Unit

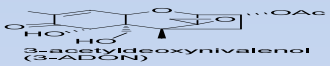
Major mycotoxins found in *Fusarium*-infected grains

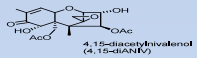
DON

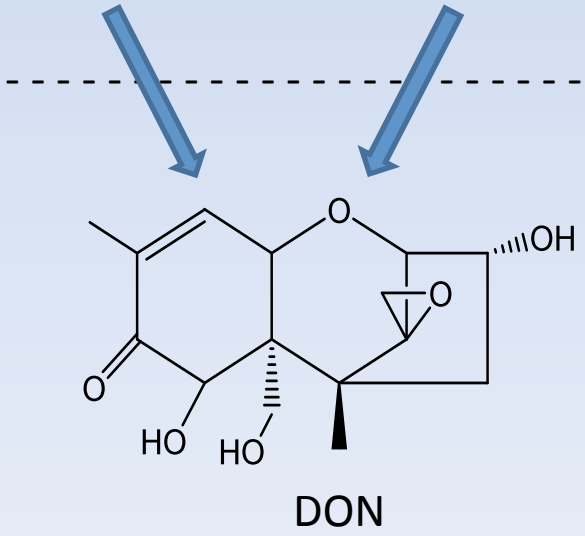
NIV

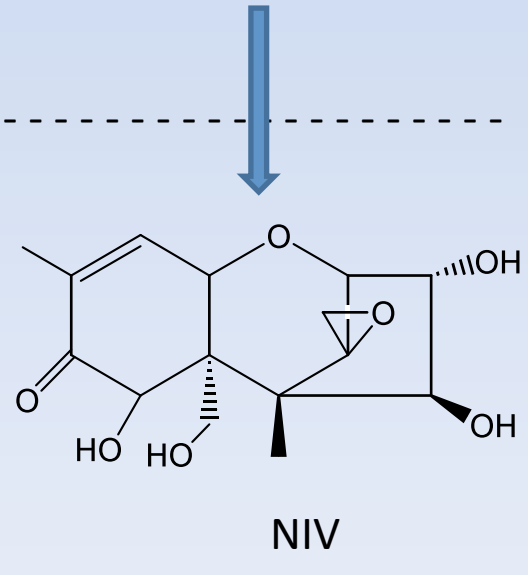
Mycotoxins produced by *Fusarium* in laboratory culture



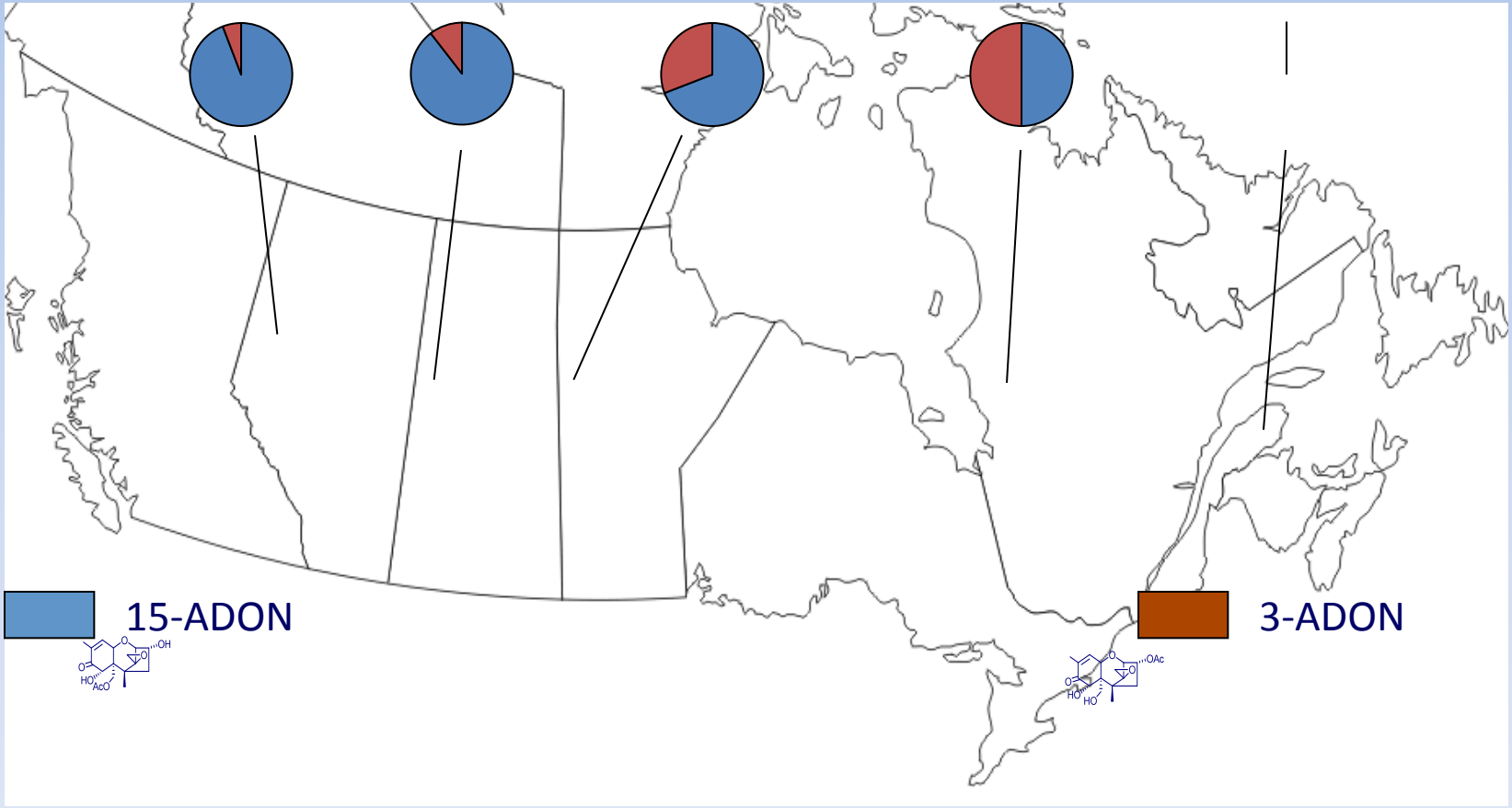




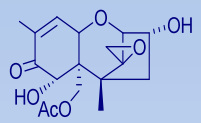


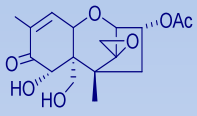


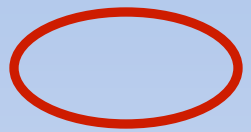
Emerging populations of F. graminearum 3ADON chemotypes

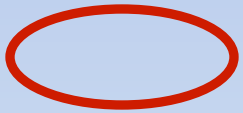


Ward et al., Fungal Genetics and Biology 2008, 45: 473–484



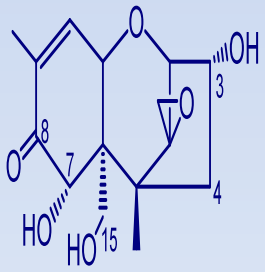


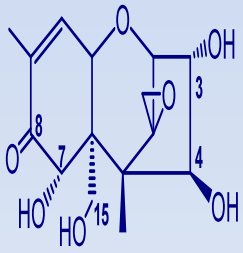




15-ADON

3-ADON

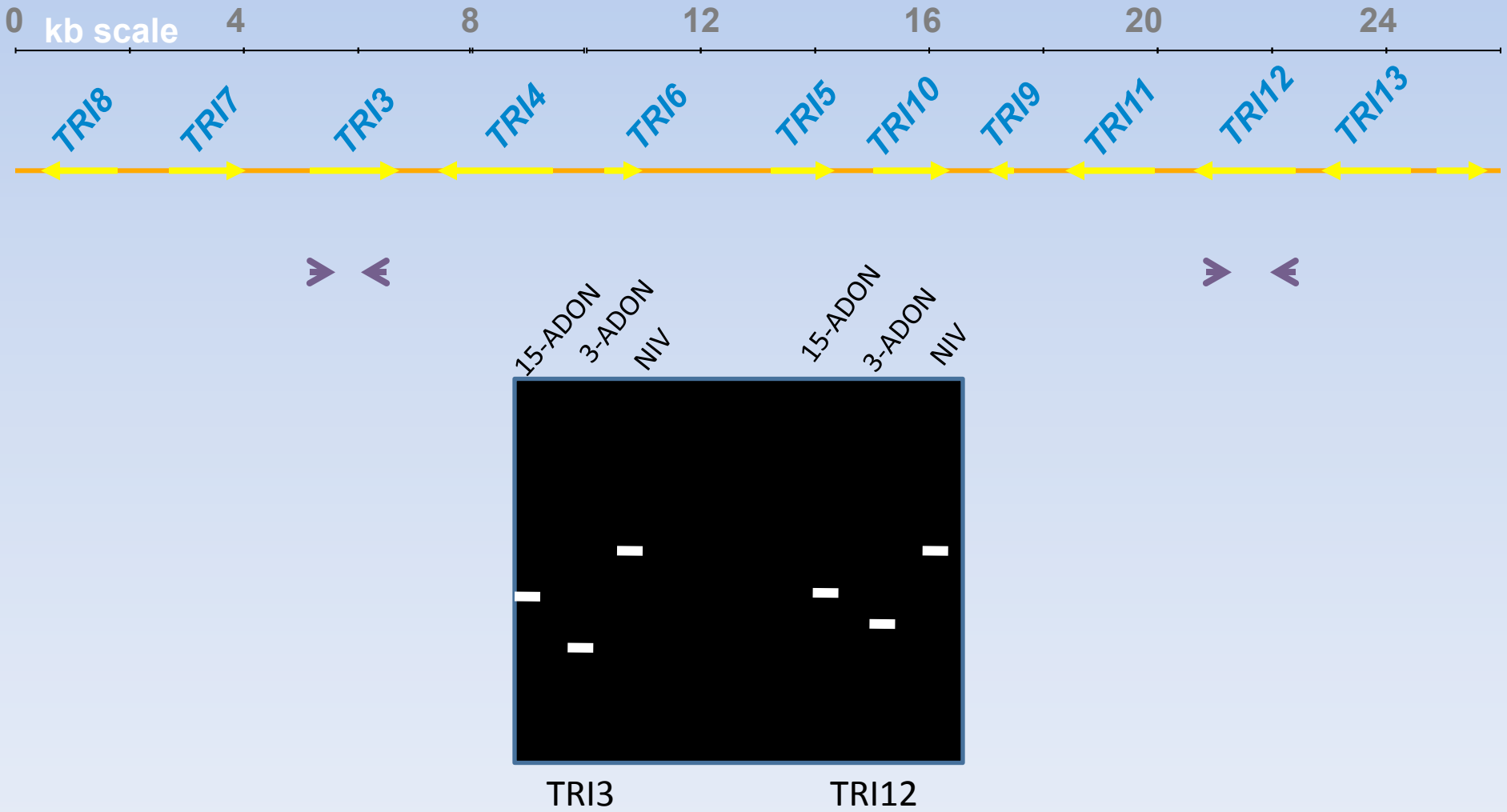




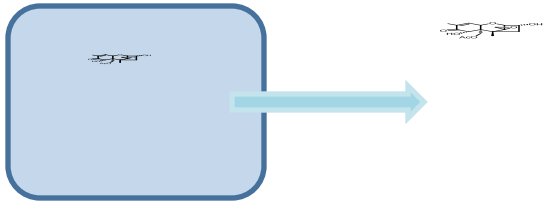
DON

NIV

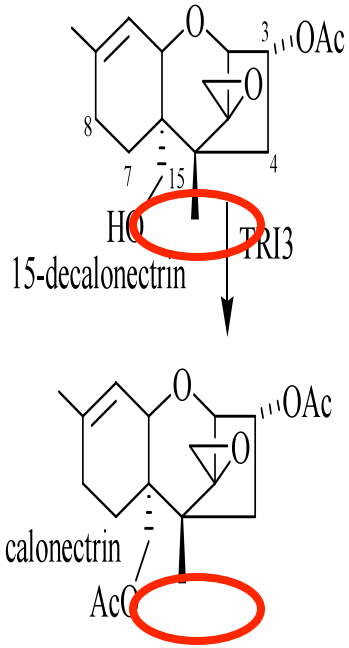
Chemotyping: using PCR analysis to correlate with chemistry



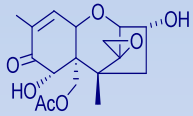
TRI12 = trichothecene efflux pump



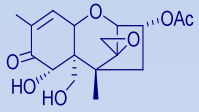
TRI3 = C15 acetyltransferase



Is *TRI3* a determining factor?

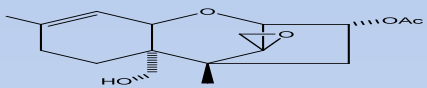


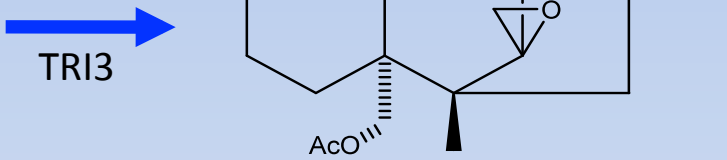
15-ADON



3-ADON

Yeast Feeding Experiments to assess TRI3 activity

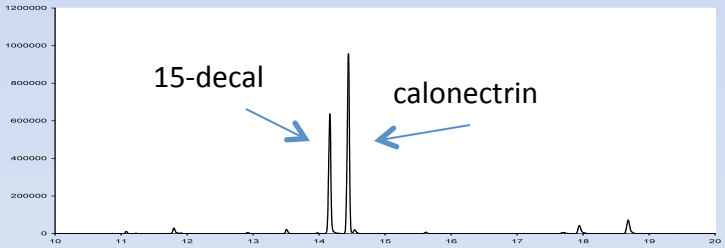




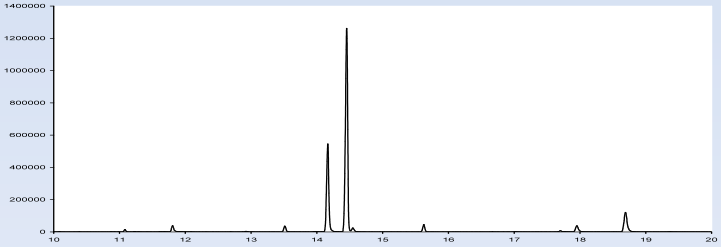
15-decal

Calonectrin

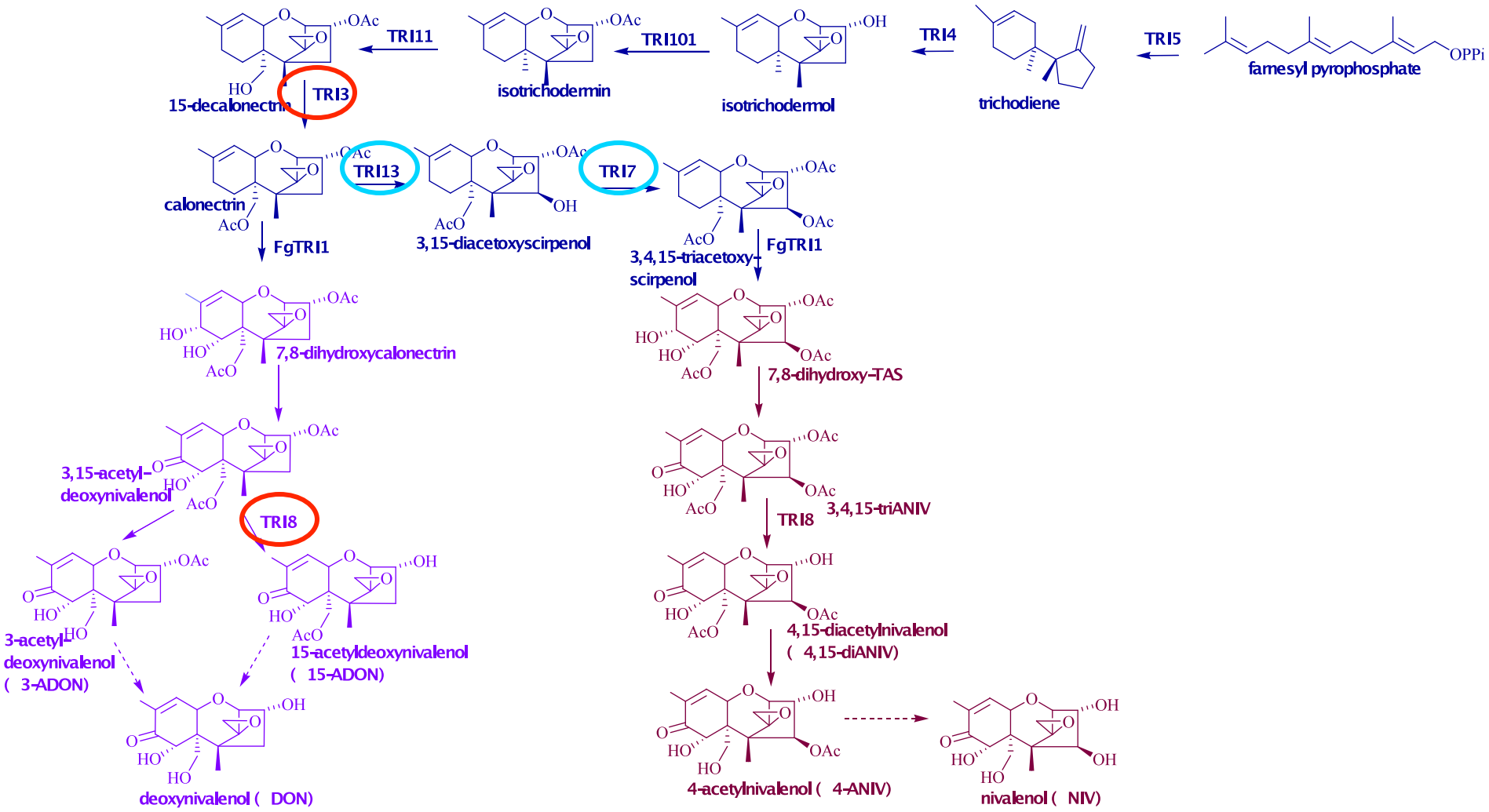
15-ADON TRI3 + 15-decal



3-ADON TRI3 + 15-decal



Trichothecene pathways



Nucleophilic elbow

```
3-ADON  1     ATGGTTCTCG ATCGTTTGTT GTTTCTTCTG AGCTTGTGGC TGGGCTTTGT TGGCGCCACT
15-ADON 1     ATGGCTCTCG ATCGTTTGTT GTTTCTTCTG GGCTTCTGGT TGGGCTTGGT TGGCGCCGCT
       61     CAAGCTGCGT TATC...AGA ACCTATTCCG CCTAGCAAGG ACCCGTGGTA TACTGCACCT
       61     CAAGCTGCGT TGTCGTCAGA ACCGCTTCCG CCCAGCAAAG ACCCGTGGTA TACTGCACCT
      118     CCTGGATTTG AGAATGCCGA GCCTGGAACT GTCTTCAGAG TGCGGCCTGC GCCTGGAAAC
      121     CCTGGATTTG AGAATACCGA GCCTGGAACA GTCTTGAGAG TGCGACCTGC GCCTGGAAAC
      178     TTAACCAGCG TCATCGGCAA TTGCTCTGCT TCTTACAACA TTCTCTATCG TACGACCGAC
      181     TTGACCAGCG TCACCAGCAA TTGCTCTGCT TCTTACAACA TCCTCTACCG TACGACCGAT
      238     AGCCATTTCA AGCCTACTTG GGCAGTCACC ACTCTTTTGG TCCCCAAACT GGGTCCTGAA
      241     AGTCATTTCA AGCCCGCTTG GGCCGTCACC ACTCTTTTGA TCCCCGAGCT GGGTCCTGAA
      298     AGTCTTGCGC AGCAGAAGTA CCAACAAAGC GCGCTCTTGT CATTCCAAGT GCCTTATGAC
      301     AGTCTTGCGC ACCAAAAGTA CCAACAAAGC GCGCTAATGT CAATCCAAGT TGCCTATGAC
      358     TCCCCCGATG TCGATGCCAG CCCTAGTAAC GCTATGTACG ACGCGAGCGA TTTTTTTTCG
      361     TCTCCCGACG TCGATGCCAG CCCTAGTAAC ACTATGTACA CCGCGAGCAA TTTTTCTTCC
      418     AATTACTATG GAGCTGCTCT AGGTGAAGGT ATCTTCGTTT CTGTTCCGGA TTATGAGGGA
      421     ATCTACTATG AAGCTGCTCT AGGTCAAGGG CTCTTCGTTT CTGTTCCAGA TTACGAGGGA
      478     CCCTTGGCTG CTTTTACCGC TGGTCTCATC TCTGGATATG CTACTTTGGA CTCAATTCGC
      481     CCCTTGGCTG CTTTTAGCGC TGGTGTCATC TCTGGGTACG CTACTTTGGA CTCAATTCGC
      538     GCTATTCTGT CGCTCGGTCT TGGCTTCAAC ACGATCGACA CGCCCAGTGT CGCCCTTTGG
      541     GCTGTTCTAT CGCTTGGTCT GGGCCTCAAC ATGACCAACA CGCCCAGTGT TGCCCTTTGG
      598     GGCTATTCTG GCGGTGCGTT TGCCACGGAA TGGGCATCGG AACTTGCAGT ACAGTATGCA
      601     GGCTACTCTG GCGGTGCGTT TGCCACCGAA TGGGCATCAG AACTTGCGGT ACAGTATGCA
      658     CCCGAGCTTG TAGCAGGACC CGTTATTGGT GCTGTGATGG GAGCCCCGTT GCCCAATATC
      661     CCAGAGCTGA TAACAGGACC CGTTATCGGT GCTGCGTTGG GAGCCCCGTT GGCCAATATC
      718     ACTTCATGCA TGCGCGATGT CAATGGAGGA CCTAAGTCCG GTCTGGTGGT AAACATTCTG
      721     ACTTCACTCC TGTACGATGT CAATGGCAAA CCTGGAGCCG GTCTGGTGCC AAATATGTTG
      778     CTGGGTCTTA CGGGTCAATA TCCCGATGTC CGCAAGCACC TTGTATCCAA ACTCAACGAT
      781     TTGGGTCTTA CAAGCCAATA TCCTGACGTC CGCAAGTACC TTATATCCAA ACTCAACGAT
      838     GATGGCCAGT ACAACAAGGC TGGCTTTCTC GCTGCTGAGG GTTTTACTAT CAGCGAAGCG
      841     GATGGCCAGT ACAACAAGAC TGGCTTTCTC GCTGCTGAGG GTTTTACCAT CAACGAAGCT
      898     CTTAGCACCT TTTCTGGCAA C...ATCAAC AAGTACTTCC AGAAGGGGAC TGATATTCTC
      901     GGCGTCGCCT TTTATGGAAT TGATATCAAC AAGTACTTCC AGAAGGGGAC TGACATTCTC
      955     AGCGACCCGA AGATTACAGC TCTTATCAAT CGGGAGGGCG TTTTGGGATA TCACGGCACC
      961     AGCGACCCGA AGATTGTAGC TCTTCTTAAC CAAGAGGGCC TTCTGGGATA TAACGGTACC
     1015     CCCAGATGGC CCATGTTTAT TTACCAAGCT ATCTCTGACG AGGTCACGCC GATTGCTGCT
     1021     CCCAGATGGC CTTTGTTTAT TTACCAAGCT ATCCACGACG AGGTCACACC TATAGCTGAT
     1075     ACCGATGCCG TAGTCGAGAG ATATTGTTCA GTGGGGGCTG ACGTTCACTT TGAAAGGAAC
     1081     ACCGATGCTG TAGTCAACAG ATATTGTGCC GTGGGGGCCG ACATTCACTT CGAAAGGAAT
     1135     ACTCTCGGCT CGCACGACGA GGAAGCCGGC AATAGCTATG ACGCGGCTTT CCAGTGGCTT
     1141     ACTATCGGCG GGCACTACCA GGAAGCCGAC AATAGCTATG AGGCGGCTTT TCAGTGGCTT
     1195     TTGGACATCT TCTCCGGCCA GCGTGACACA AAGGGTTGTG TTATCAAGGA CGTGACGAGA
     1201     TTGGACATCT ACTCCGGCCA GCGTGACACA AAGGGTTGTG TCATCAAGGA AGTGACGAGA
     1255     GACGTGGACG TGACGGGAGA CGTGACGGGA GACGTGACGA GAGACGTAAC ACGAGAGTTG
     1261     AACATTACCG GAAGTGTCCT ACAAACACGT GAGAATGTTC AAAAGAGTGG AGTTGACTTC
     1315     TGA
     1321     TGGAGATCTG CCTGGTAA
```

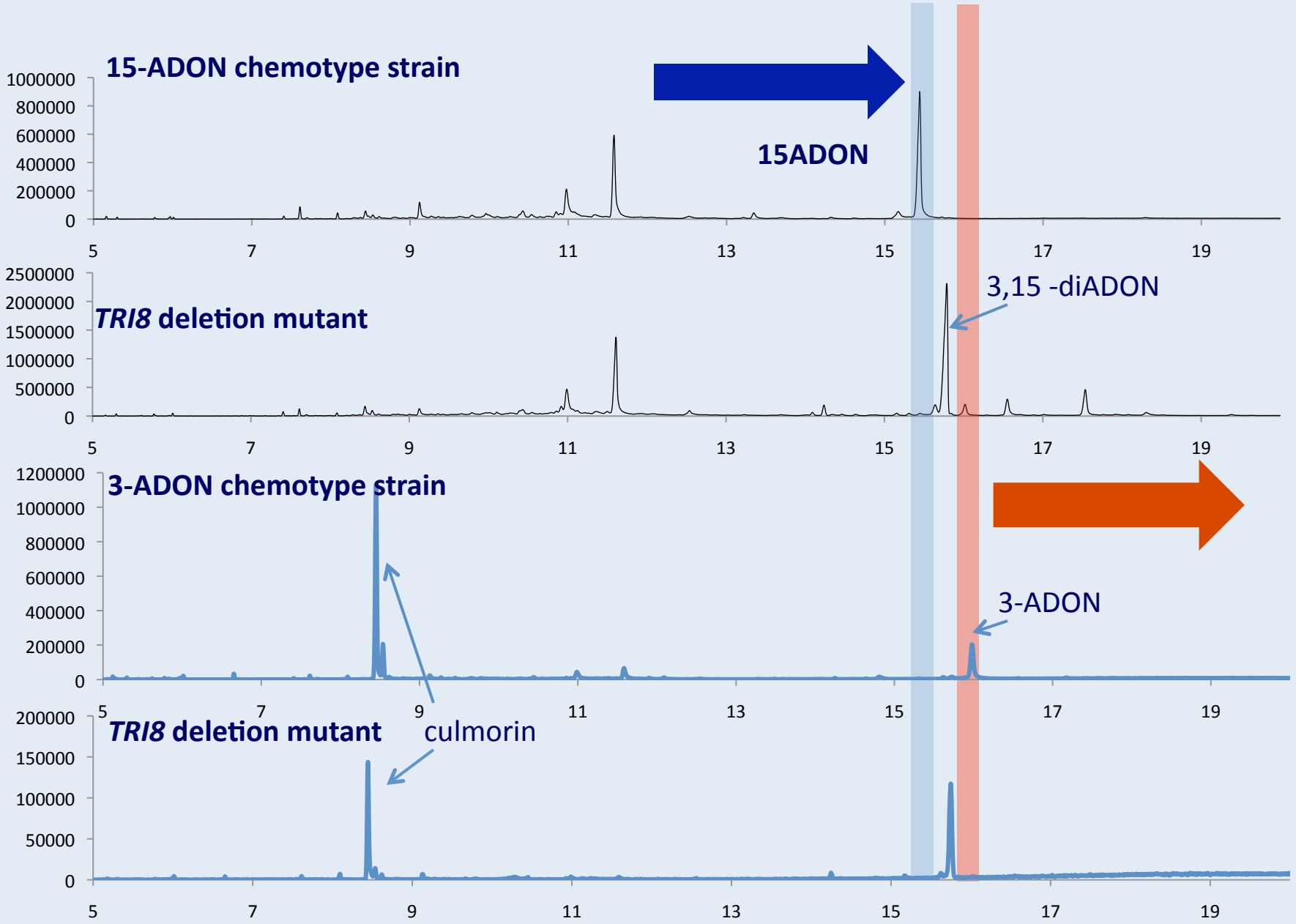
TRI8 sequence comparisons

chemotype	# nucleotides	% aa ID	TRI6 bs
3-ADON	1299	78	none
15-ADON	1338	85	-525 nt
NIV	1335		-525 nt

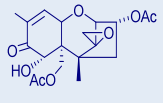
```
3-ADON  1255   GACGTGGACG TGACGGGAGA CGTGACGGGA GACGTGACGA GAGACGTAAC ACGAGAGTTG
15-ADON 1261   AACATTACCG GAAGTGTCCT ACAAACACGT GAGAATGTTC AAAAGAGTGG AGTTGACTTC

        1315   TGA
        1321   TGGAGATCTG CCTGGTAA
```

TRI8 Wild Type and deletion mutants



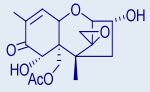
Deletion studies confirmed that 3,15-diADON was precursor for TRI8 from both 3-ADON and 15-ADON producers



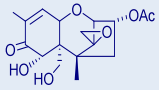
3,15-diADON

X

X

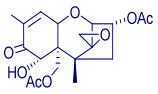


15-ADON

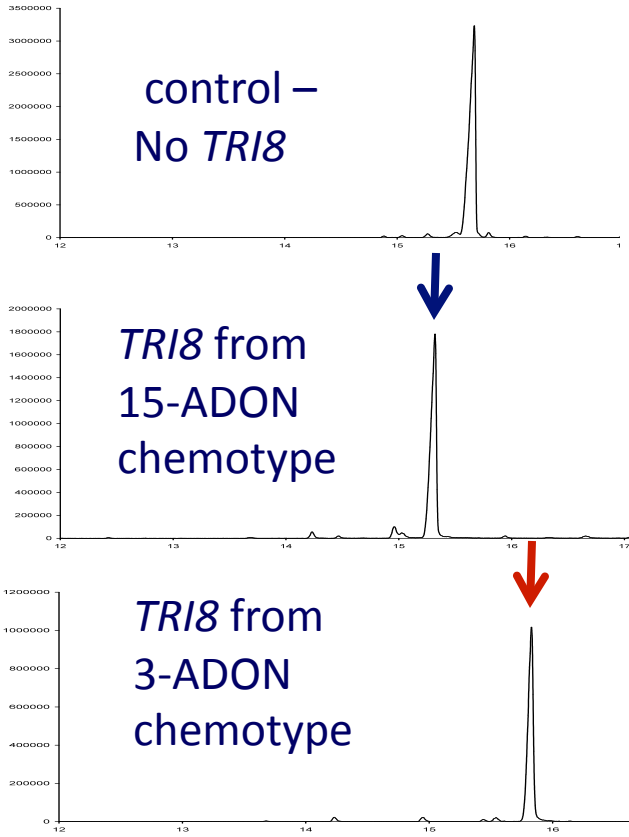


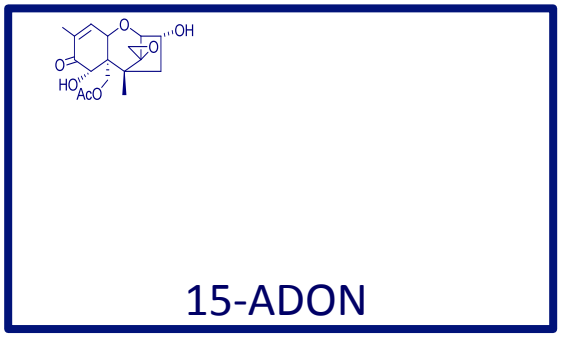
3-ADON

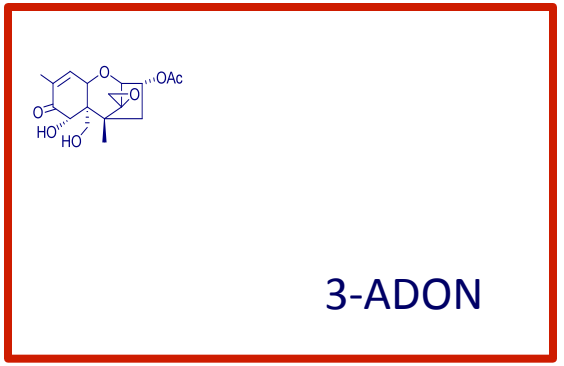
Expression of *TRI8* in yeast – feeding 3,15-diADON



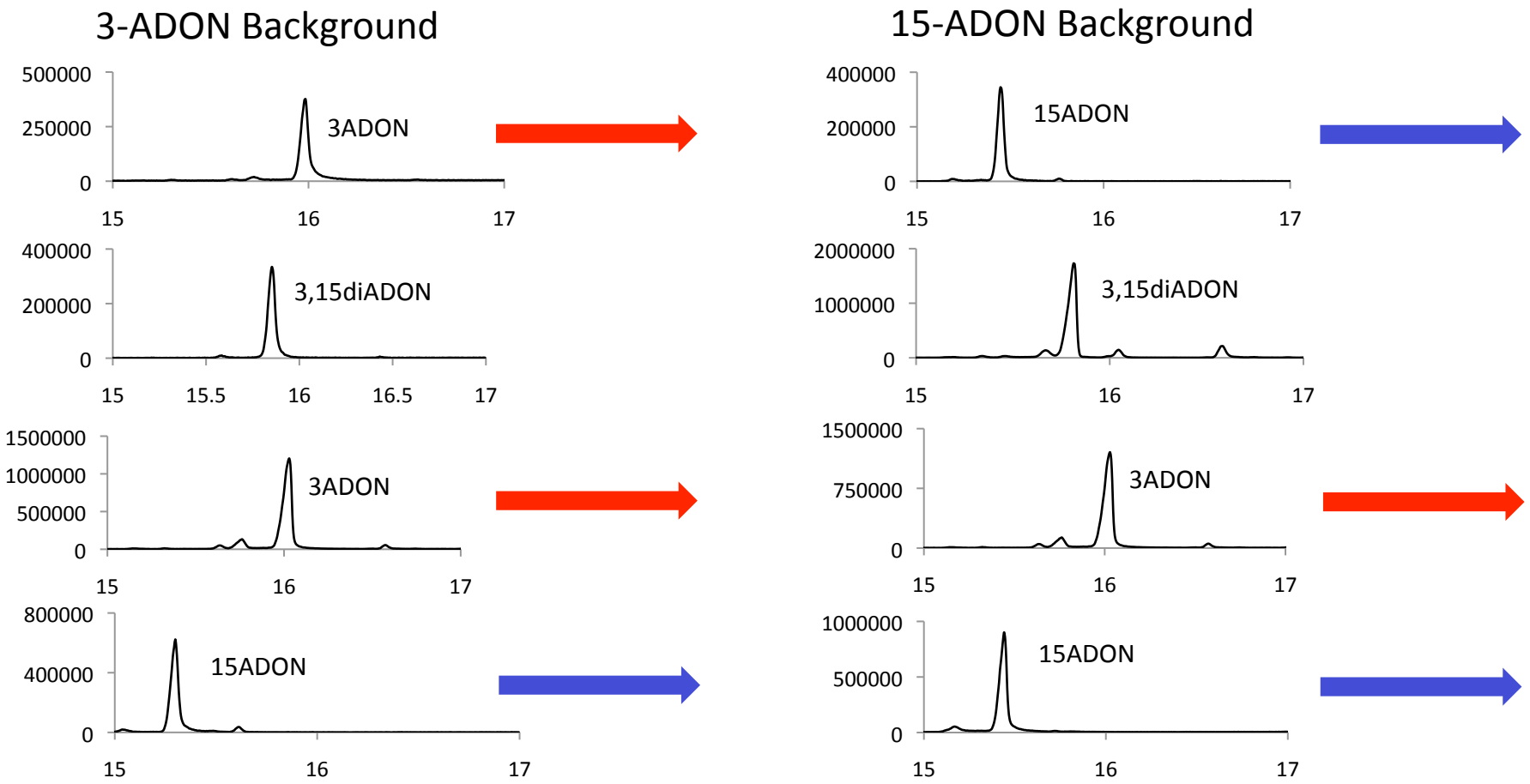
3,15-diADON





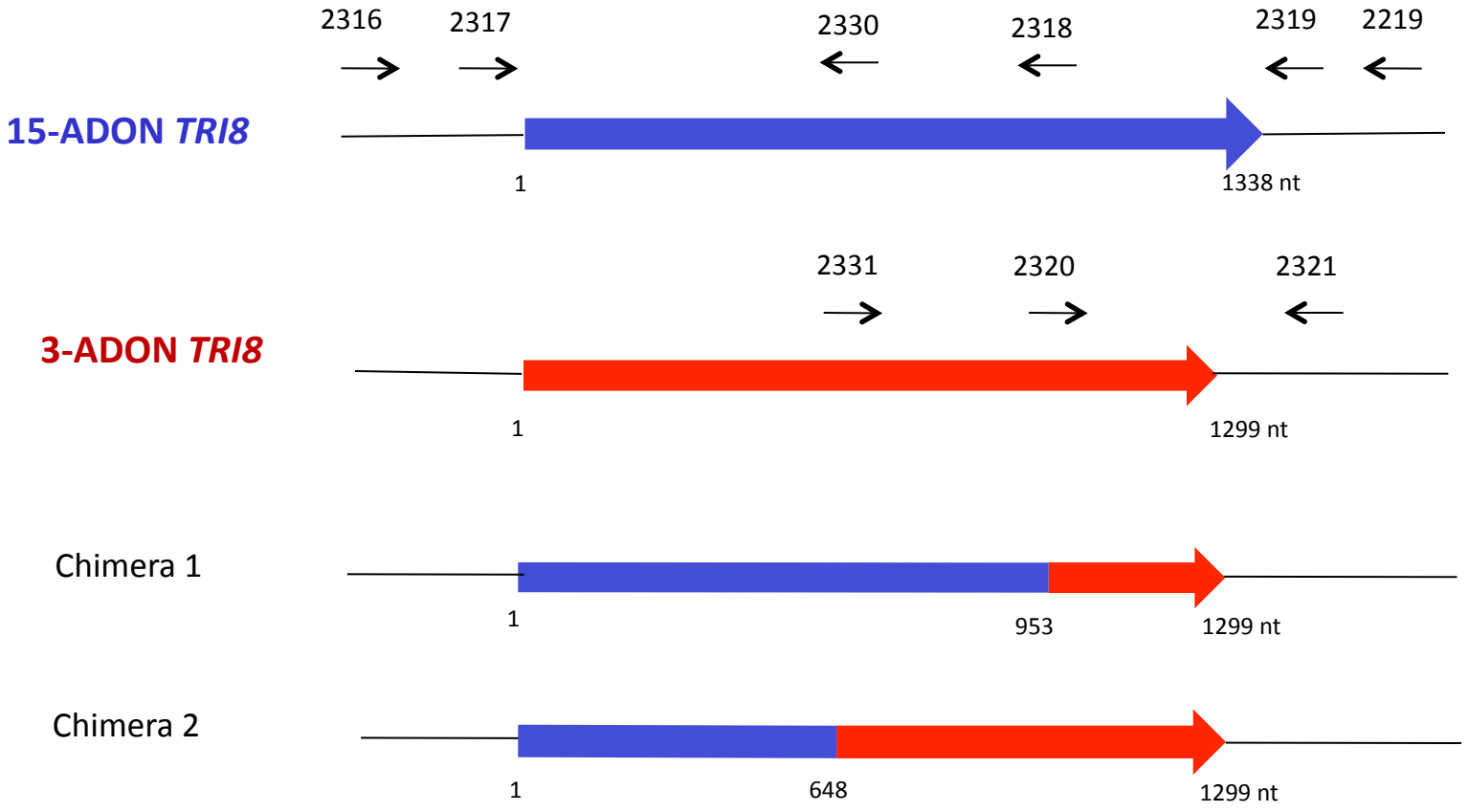


Adding back intact *TRI8* to deletion mutants



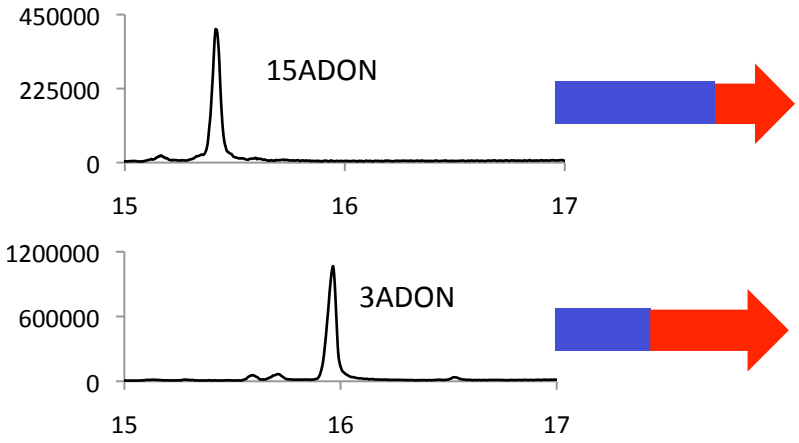
Conclusion: *TRI8* has different function

Determination of *TRI8* region responsible for different esterase activity

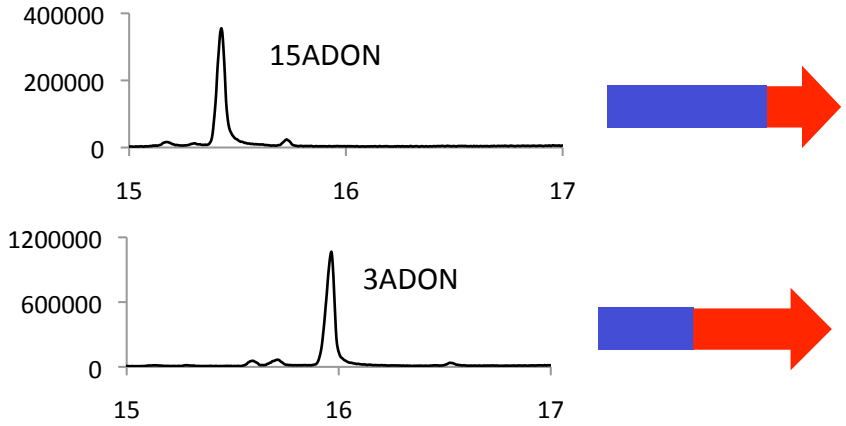


Phenotype of chimeras

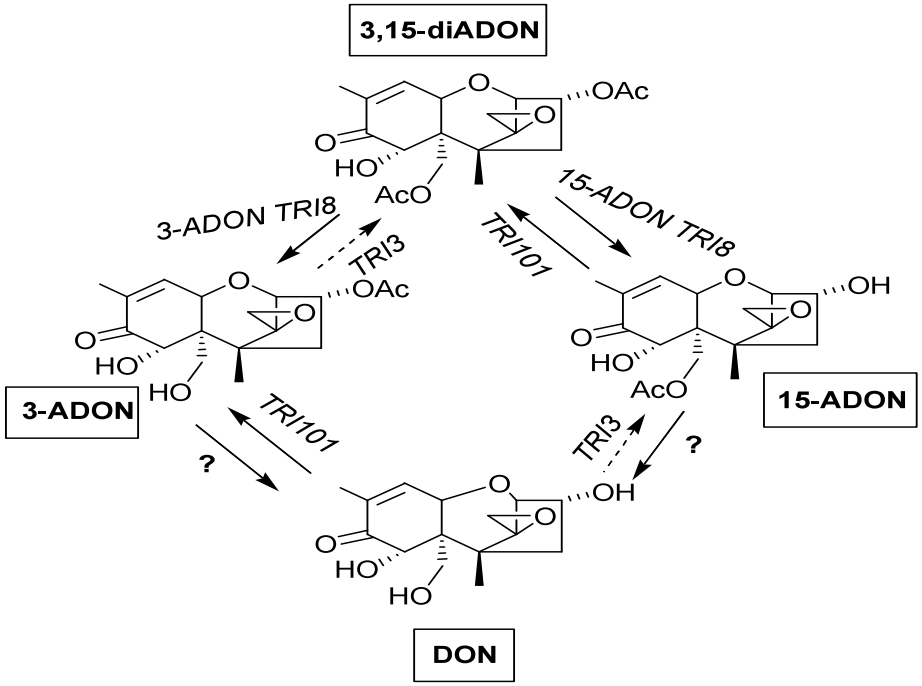
3-ADON background



15-ADON background



Proposed pathway to explain small amounts (*in planta*) of 3-ADON in a 15-ADON producer



Conclusions

- *TRI8* from a 3-ADON producer encodes C-15 esterase
- *TRI8* from a 15-ADON producer encodes C-3 esterase
- Middle region of *TRI8* defines whether a strain produces 3-ADON or 15-ADON
- Small sequence differences can impact functionality

Acknowledgments

Jennifer Teresi

Stephanie Folmar

Kim MacDonald

Visit Poster # 26 at the PBG Poster Session this afternoon.