

Supplemental Table S1. FMO3 Splicing assay primers

Primer name	sequence
Exon 2 Forward	CCATCATTGGAGCTGGTGTGAG
Exon 2-4 Boundary Reverse	ATTTACTGGATACAAATGTTGAAA
Exon 3-4 Boundary Reverse	TGTTTATTTACTGGATACAAATGTC
Exon 7-8 Boundary Forward	GGCAAAGTACAAGTTCCTTT
Exon 6-8 Boundary Forward	GGCAAAGTACAAGTTCCTTT
Exon 9 Reverse	TTGCCCAATGAAGGAGGAGAG

Supplemental Table S2. FMO3 haplotypes in subjects with measured D₂-nicotine-N-oxide

FMO3 haplotype	rs1800822	rs2266782 (E158K)	rs1736557 (V257M)	rs909530	rs2266780 (E308G)	n alleles	%
1	C	G	G	C	A	145	42.6
2	C	A	G	C	A	78	22.9
3	C	A	G	T	G	61	17.9
4	C	G	A	C	A	35	10.3
5	T	G	G	T	A	19	5.6
6	T	G	G	C	A	1	0.3
7	C	G	G	T	A	1	0.3

Polymorphic sites analyzed are given at the top of each column by rs number and amino acid changes when relevant. Haplotypes are ordered by frequency.

Supplemental Table S3. Kinetic parameters for recombinant FMO3 mediated *trans*-nicotine N-oxidation

Enzyme	k_{cat} (min ⁻¹)	K_m (mM)	k_{cat}/K_m (min ⁻¹ mM ⁻¹)
Reference	70.8 ± 1.1	1.11 ± 0.05	63.6 ± 2.8
158K	99.6 ± 2.2	1.26 ± 0.07	51.1 ± 3.6
308G	51.8 ± 1.2	1.01 ± 0.07	69.0 ± 4.7
158K/308G	79.1 ± 1.9	1.15 ± 0.07	78.9 ± 4.7
(Reference) ^a	124.7 ± 5.6	1.38 ± 0.15	90.1 ± 10.8

^a Corning Supersomes