

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Selzer RR, Rosenblatt DS, Laxova R, Hogan K. Adverse Effect of Nitrous Oxide in a Child with 5,10-Methylenetetrahydrofolate Reductase Deficiency. *N Engl J Med* 2003;349:45-50.

Supplementary Appendix 1. Oligonucleotide Primers Used for Amplification and Sequencing of MTHFR Exons from Genomic DNA.*

Exon	Primer Name	Primer Use	Primer Sequence	Product Size	Magnesium Level	Annealing Temperature
				bp	mmol/liter	°C
1	MTHFR 1F#2 MTHFR 1R	PCR, sequencing PCR, sequencing	5'GCC ACT CAG GTG TCT TGA TGT GTC GG3' 5'TGA CAG TTT GCT CCC CAG GCA C3'†	384	3.0	64.0
2	MTHFR 2F MTHFR 2R MTHFR 2F#2 MTHFR 2R#3	PCR PCR Sequencing Sequencing	5'GGA AGG CAG TGA CGG ATG GTA T3'‡ 5'ACC AAG TTC AGG CTA CCA AGT GG3'‡ 5'TAT TTC TCC TGG AAC CTC TCT TCA3' 5'GCC TCC GGG AAA GCC AGA ACC3'	373	1.5	60.0
3	MTHFR 3F MTHFR 3R	PCR, sequencing PCR, sequencing	5'GGG TGA GAC CCA GTG ACT ATG ACC3' 5'CCC TAG CTC CAT CCC CGC CAC CAG G3'	193	1.5	67.5
4	MTHFR 4F MTHFR 4R	PCR, sequencing PCR, sequencing	5'GGT GGA GGC CAG CCT CTC CTG3' 5'GCG GTG AGA GTG GGG TGG AGG G3'	285	1.5	67.5
5	MTHFR 5F#2 MTHFR 5R#2	PCR, sequencing PCR, sequencing	5'GCT GGC CAG CAG CCG CCA CAG CC3' 5'GGA TCT CTG GGC CAC TGC CCT C3'	315	1.5	67.5
6	MTHFR 6F MTHFR 6R	PCR, sequencing PCR, sequencing	5'TGC TTC CGG CTC CCT CTA GCC3'† 5'CCT CCC GCT CCC AAG AAC AAA G3'†	250	1.5	60.0
7	MTHFR 7F MTHFR 7R MTHFR 7R#2	PCR, sequencing PCR Sequencing	5'GCC CTC TGT CAG GAG TGT GCC C3' 5'GGG CAG GGG ATG AAC CAG GGT CCC C3' 5'GGT CCC CAC TTC CAG CAT CAC3'	271	1.5	67.5
8	MTHFR 8F#2 MTHFR 8R#2	PCR, sequencing PCR, sequencing	5'CAG GGT GCC AAA CCT GAT GGT CGC C3' 5'CCA CGG GTG CCG GTC AAG AGA GG3'	283	1.5	67.5
9	MTHFR 9F#2 MTHFR 9R#2	PCR, sequencing PCR, sequencing	5'GTT GGT GAC AGG CAC CTG TCT CT3' 5'TGT TCA ACG AAG GGC CTG GTA C3'	182	1.5	67.5
10	MTHFR 10F MTHFR 10R	PCR, sequencing PCR, sequencing	5'GGC CCA GGT CTT ACC CCC ACC CC3' 5'GGT GGG CGG GGC AAG CTT GCC CCC3'	189	1.5	67.5
11	MTHFR 11F MTHFR 11R	PCR, sequencing PCR, sequencing	5'GCA TGT GTG CGT GTG TGC GGG GG3' 5'CCT CTG CAG GAG CAA GTG CTC CCC3'	516	1.5	67.5

* MTHFR encodes 5,10-methylenetetrahydrofolate reductase. PCR denotes polymerase chain reaction.

† The sequence was reported by Sibani et al.⁷

‡ The sequence was reported by Kluijtmans et al.⁸

Supplementary Appendix 2. Primers Used to Sequence the Complementary DNA Transcript.

Fragment	Primer Name	Primer Sequence
Sense	MTHFR 1F#2	5'GCC ACT CAG GTG TCT TGA TGT GTC GG3'
	MTHFR 518F	5'GCT GCC GTC AGC GCC TGG AGG AG3'
	MTHFR 972F	5'GGA CGT GAT TGA GCC AAT CAA AGA C3'
	MTHFR 1206F	5'GGA AGA TGT ACG TCC CAT CTT CTG G3'
	MTHFR 1683F	5'GCG GAA GCA CTT CTG CAA GTG CTG3'
Antisense	MTHFR 515R	5'GTC ATG TGC AGG ATG GTC TCC AG3'
	MTHFR 1022R	5'CCA TAG TTG CGG ATG GCA GCA TCG3'
	MTHFR 1535R	5'TCC TTC AGC AGG CTG GTC TCA GCC G3'
	MTHFR 1806R	5'GAC AGC ATT CGG CTG CAG TTC AGG3'
	MTHFR endR	5'CAC TCC AGT CTA GCT GCC ATT GTC3'

Supplementary Appendix 3. Primers Used to Amplify Complementary DNA as Seven Overlapping Fragments.

Fragment	Primer Name	Primer Sequence	Product Size	Magnesium Level	Annealing Temperature
			<i>bp</i>	<i>mmol/liter</i>	<i>°C</i>
1	X13F X14R	5'CGG ACA GCC ATA GCT GAG GAG C3' 5'GGC TGG TCT CAG CCG CCA GG3'	1584	1.5	66.0
2	MTHFR 1F#2 MTHFR endR	5'GCC ACT CAG GTG TCT TGA TGT GTC GG3' 5'CAC TCC AGT CTA GCT GCC ATT GTC3'	2206	1.5	64.0
3	X17F X2R	5'GCG AGA GAA ACG GAG GCT CC3' 5'CAT CTG CAC CTG CCA GTC ACT GCC3'	977	1.5	67.5
4	X3F X4R	5'CCT GGC TGT GGA GGC CTG ATG CTG3' 5'GGA TCC TTG CGA CTG CGA GTG GCT C3'	1275	1.5	68.5
5	X5F X6R	5'GGC CAC AAA TCA AAG CAA GG3' 5'CTC TTT GGG TGG CAG GCA GCC G3'	1256	1.5	68.5
6	X7F X8R	5'CCA GCT ACT CTG TCC AGG CCA CTG3' 5'GGC TCA AGC GAT CTA CCT GCC TTG3'	1274	1.5	68.5
7	X11F X12R	5'CTC CAT CAG CTT ATG GGA TCC TTG TC3' 5'GGC TGA AGC AGA GGA GTG ATC TCA GC3'	1174	1.5	67.5