

Comments from Reviewer 2

The MEG3 primer issue is two-fold: i). where do the MEG3 forward and reverse primers map with respect to the MEG3 lncRNA and ii). Alternatively spliced variant MEG 3a, 3b, 3c and 3d. In an oligo blast search using the forward primer for MEG3, no matches are found. Downloading the paper that the authors cite (Zhou et al., 2007) that describes the cloning and structure of the MEG3 splice variants (and the primers used by the authors for MEG3, no match is found in a search of the entire sequence (NR_002766 in GENBANK). It's possible that the primer sequence is accurate and it is somewhere in that sequence (I wouldn't rely on Addgene for the positioning), but what would be helpful is some description from the authors as to where the MEG3 primers used in the study actually map (in terms of splice variants, or which exon, some clue as to where one can find the primer), so that a reader could reproduce the study if desired.

The same question applies to the DNMT1 variants, where do the primers map with regard to the variant sequences so the reader can determine if what DNMT1 variants are being targeted in the experiments.

Response:

We would like to thank you for your valuable comments and recommendation, which will add to our paper. As previously indicated the forward primer of the MEG3 was designed based on the sequence of the Addgene vector pCI-MEG3 # 44727. The forward primer is located downstream of the chimeric intron and upstream of the MEG3 gene. We used the forward primer to target the 5' end of the transcript and the reverse primer to target the MEG3 sequence itself. The main purpose of that is to detect specifically the expression of the MEG3 from the transfected pCI-MEG3 and not the endogenous MEG3 lncRNA to confirm the successful transfection of pCI-MEG3. The manuscript was modified to include this information in pages 233-235

Kindly find below the position of forward primer on the pCI-MEG3 (<https://www.addgene.org/browse/sequence/61283/>). It is worth noting the MEG3 cDNA was cloned into pCI mammalian expression vector https://pl.promega.com/-/media/files/resources/protocols/technical-bulletins/0/pci-mammalian-expression-vector-protocol.pdf?sc_lang=en

> pCI-MEG3 sequence 5613 bps

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TCAATATTGGCCATTAGCCATATTATTCATTGGTTATATAGCATAAATCAATATTGGCTATTGGCCATTG
CATACGTTGTATCTATATCATAATATGTACATTTATATTGGCTCATGTCCAATATGACCGCCATGTTGGC
ATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTT
CCGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCATTGACGTCA
ATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTCAATGGGTGGAGTATTTAC
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GGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTCCGCCCTTATTGACGTCAATGA
CGGTAAATGGCCCGCCTGGCATTATGCCAGTACATGACCTTACGGGACTTTCTACTTGGCAGTACATC
TACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACACCAATGGGCGTGGATAGCGGT
TTGACTCACGGGGATTTC AAGTCTCCACCCATTGACGTCAATGGGAGTTTGTTTGGCACCAAATCA
ACGGGACTTTCCAAAATGTCGTAATAACCCCGCCCCGTTGACGCAAATGGGCGGTAGGCGTGACGGTGG
GAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCACTAGAAGCTTTATTGCGGTAGTTTATC
ACAGTTAAATTGCTAACGCAGTCAGTGCTTCTGACACAACAGTCTCGAACTTAAGCTGCAGAAGTTGGTC

Chimeric intron

GTGAGGCACTGGGCAGGTAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGGCTTG

TCGAGACAGAGAAGACTCTTGC GTTTCTGATAGGCACCTATTGGTCTTACTGACATCCACTTTGCCTTC

MEG3 forward primers

T7 promoter

TCTCCACAGGTGTCCACTCCCAGTTCAATTACAGCTCTTAAGGCTAGAGTACTTAATACGACTCACTATA

EcoRI

MEG3

GGCTAGCCTCGAGAATTCGCAGAGAGGGAGCGCGCCTTGGCTCGCTGGCCTTGGCGGCGGCTCCTCAGGA

GAGCTGGGGCGCCACGAGAGGATCCCTCACCCGGGTCTCTCTCAGGGATGACATCATCCGTCCACCTC

CTTGTCTTCAAGGACCACCTCCTCTCCATGCTGAGCTGCTGCCAAGGGGCCTGCTGCCCATCTACACCTC

ACGAGGGGCACTAGGAGCACGGTTTCTGGATCCCAACATACAAAGCAGCCACTCACTGACCCCCAGG

ACCAGGATGGCAAAGGATGAAGAGGACCGGAAGTACCAGCCAGCTGTCCCTCTTACCTAAAGACTTAAA

CCAATGCCCTAGTGAGGGGGCATTGGGCATTAAAGCCCTGACCTTTGCTATGCTCATACTTTGACTCTATG

AGTACTTTCCTATAAGTCTTTGCTTGTGTTACCTGCTAGCAAAGTGGAGTGTTTCCCTCCCAAGGGGG

TGTCAGTCTTTGTCGACTGACTCTGTCATCACCTTATGATGTCCTGAATGGAAGGATCCCTTTGGGAAA

TTCTCAGGAGGGGGACCTGGGCCAAGGGCTTGCCAGCATCTGCTGGCAACTCCAAGGCCCTGGGTGGG

CTTCTGGAATGAGCATGCTACTGAATCACCAAAGGCACGCCCGACCTCTCTGAAGATCTTCCTATCCTTT

TCTGGGGGAATGGGGTCGATGAGAGCAACCTCCTAGGGTTGTTGTGAGAATTAAATGAGATAAAAGAGGC

CTCAGGCAGGATCTGGCATAGAGGAGGTGATCAGCAAATGTTTGTGAAAAGGTTTGACAGGTCACTCCC

TTCCACCCCTCTTGCTTGTCTTACTTGTCTTATTTATTCTCCAACAGCACTCCAGGCAGCCCTTGTCCTA

CGGGCTCTCCTTGTCATCAGCCAAGCTTCTTGAAAGGCCTGTCTACACTTGCTGTCTTCCTCCTCACCTC

CAATTTCTCTTCAACCCACTGCTTCCTGACTCGCTCTACTCCGTGGAAGCACGCTCACAAAGGCACGTG

GGCCGTGGCCCGGCTGGGTGCGCTGAAGAACTGCGGATGGAAGCTGCGGAAGAGGCCCTGATGGGGCCCA

MEG3
reverse
primers

CCATCCCGGACCCAAGTCTTCTTCCTGGCGGGCCTCTCGTCTCCTTCCTGGTTTGGGCGGAAGCCATCAC
CTGGATGCCTACGTGGGAAGGGACCTCGAATGTGGGACCCAGCCCCTCTCCAGCTCGAAATCCCTCCAC
AGCCACGGGGACACCCTGCACCTATTCCCACGGGACAGGCTGGACCCAGAGACTCTGGACCCGGGGCCTC
CCCTTGAGTAGAGACCCGCCCTCTGACTGATGGACGCCGCTGACCTGGGGTCAGACCCGTGGGCTGGACC
CCTGCCCACCCCGCAGGAACCCTGAGGCCTAGGGGAGCTGTTGAGCCTTCAGTGTCTGCATGTGGGAAGT
GGGCTCCTTACCTACCTCACAGGGCTGTTGTGAGGGGCGCTGTGATGCGGTTCCAAAGCACAGGGCTTG
GCGCACCCCACTGTGCTCTCAATAAATGTGTTTCTGTCTTAACAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

NotI

For DNMT1: The primers are specific for different variants of Homo sapiens DNMT1 on NCBI:

- 1- **Homo sapiens DNA methyltransferase 1 (DNMT1), transcript variant 2, mRNA**
NCBI Reference Sequence: NM_001379.4.
- 2- **Homo sapiens DNA methyltransferase 1 (DNMT1), transcript variant 4, mRNA**
NCBI Reference Sequence: NM_001318731.2
- 3- **Homo sapiens DNA methyltransferase 1 (DNMT1), transcript variant 3, mRNA**
NCBI Reference Sequence: NM_001318730.2

blast.ncbi.nlm.nih.gov/Blast.cgi#1677703423

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Homo sapiens DNA methyltransferase 1 (DNMT1), transcript variant 2, mRNA

Sequence ID: [NM_001379.4](#) Length: 5226 Number of Matches: 1

Range 1: 1862 to 1879 [GenBank](#) [Graphics](#) ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
36.2 bits(18)	21	18/18(100%)	0/18(0%)	Plus/Plus

Query 1 CCATCAGGCATTCTACCA 18
Sbjct 1862 CCATCAGGCATTCTACCA 1879

Related Information
[Gene](#) - associated gene details
[PubChem BioAssay](#) - bioactivity screening
[Genome Data Viewer](#) - aligned genomic context

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Homo sapiens DNA methyltransferase 1 (DNMT1), transcript variant 4, mRNA

Sequence ID: [NM_001318731.2](#) Length: 5234 Number of Matches: 1

Range 1: 1870 to 1887 [GenBank](#) [Graphics](#) ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
36.2 bits(18)	21	18/18(100%)	0/18(0%)	Plus/Plus

Query 1 CCATCAGGCATTCTACCA 18
Sbjct 1870 CCATCAGGCATTCTACCA 1887

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[PubChem BioAssay](#) - bioactivity screening
[Genome Data Viewer](#) - aligned genomic context

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Sbjct 1870 CCATCAGGCATTCTACCA 1887

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Homo sapiens DNA methyltransferase 1 (DNMT1), transcript variant 3, mRNA

Sequence ID: [NM_001318730.2](#) Length: 5235 Number of Matches: 1

Range 1: 1862 to 1879 [GenBank](#) [Graphics](#) ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
36.2 bits(18)	21	18/18(100%)	0/18(0%)	Plus/Plus

Query 1 CCATCAGGCATTCTACCA 18
Sbjct 1862 CCATCAGGCATTCTACCA 1879

Related Information
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[PubChem BioAssay](#) - bioactivity screening
[Genome Data Viewer](#) - aligned genomic context

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PREDICTED: Macaca mulatta DNA methyltransferase 1 (DNMT1), transcript variant X9, mRNA

Sequence ID: [XM_028839278.1](#) Length: 4575 Number of Matches: 1

Range 1: 1202 to 1219 [GenBank](#) [Graphics](#) ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
36.2 bits(18)	21	18/18(100%)	0/18(0%)	Plus/Plus

Related Information
[Gene](#) - associated gene details
[Genome Data Viewer](#) - aligned

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