

**Appendix:** Maternal Obesity and Gestational Diabetes are Associated with Placental Leptin DNA Methylation

<p><b>Supplementary Table 1:</b> Primers, bisulfite PCR conditions and pyrosequencing assays of the <i>LEP</i> promoter</p>
<p><b>Bisulfite PCR</b></p> <p><i>Forward primer</i>  <i>LEP-For</i> 5' - GAGTTTTTGGAGGGATATTAAGGAT-3'</p> <p><i>Reverse Primer</i>  <i>LEP-Rev</i> 5'-Biotin-CAAAATTATATAAAACCCTATAACCTACCA-3'</p> <p><i>Amplicon length:</i> 383 bp</p> <p><i>Amplification conditions:</i>  Initiation: 15 min. at 94°C  50 cycles: Denaturation: 1 min at 94°C            Annealing: 1 min at 56°C            Extension 1 min at 72°C  Final extension: 10 min at 72°C.</p>
<p><b>Pyrosequencing</b> (7:127881127-127881350)</p> <p><i>Forward sequencing primer 1:</i>  <i>LEP-S1</i> 5'-GGGAGGTATTTAAGGG-3'  Sequence to Analyze 1: (11 CpGs)  TGYGYGYGTGGTTTTTGGHGYGTYGAGGTTTTTTTTTYGAGGTTTYGYGAGGTGTA  TATTGYBGGTTTAGGGTTAGTAGTYGTTYGGTAYGTYGTTATTTTGAGGGG</p> <p><i>Forward sequencing primer 2:</i>  <i>LEP-S2</i> 5' -GGGAGGGGAGGGAGTTGG-3'  Sequence to Analyze 2: (12 CpGs)  YGTTAGAAATGYGTYGGGGTTTTGYGGGGTAGTTGYGTAAGTTGTGATYGGGTYG  TTATAAGWGGGGYGGGTAGGTATGGAGTTTTYGTAGGAATYGTAGYGTARYGGT  TGTAAGGTAAGGTTT</p>