### Title: Serum-Based DNA Methylation Biomarkers in the Screening and Early Detection of NSCLC

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| --- | --- | --- | --- |
| **0ID** | **Symbol** | **sequence** | **Article** |
| 1 | SHOX2eu | TCGTGCGACCCCGGTCGGGCAGGCGGGACGGAGATTA | [[1-7](#_ENREF_1)] |
| 2 | SHOX2cn | GCATCCACACGAACACACACACACACGCACACACACACGGACGAAAACAGCA | [[1-7](#_ENREF_1)] |
| 3 | GSTP1 | GTCCGCGGGACCCTCCAGAAGAGCGGCCGGCGCCGTGACTCAGCACTGGGGCGGAGCGGGGCGGGAC | [[8-10](#_ENREF_8)] |
| 4 | MGMT | ACCCCGCCCCACGCCGCCATCCCCGTGCCCCTCGGCCCCG | [[11](#_ENREF_11), [12](#_ENREF_12)] |
| 5 | SEPT9\_138 | GCTGCCCACCAGCCATCATGTCGGACCCCGCGGTCAACGCGCAG | [[13](#_ENREF_13)] |
| 6 | SEPT9\_35 | TTTGTTCTTCCCAGCCTTGCACCACTGGCTCGGGGGCTCTCAGGTGGCGCGGCCGCGAGGCGGACCCTGA | [[13](#_ENREF_13)] |
| 8 |  |  |  |
| 9 |  |  |  |
| 10 |  |  |  |
| 11 |  |  |  |
| 12 |  |  |  |
| 13 |  |  |  |

CG:The methylation status of the CpG site with red marked was the probe which genesky company will detect.

Genesky company will provide with positive and negative control

Positive control

|  |  |  |  |
| --- | --- | --- | --- |
|  | Forward Primer | Reverse Primer | Extension primer |
| LINE-1 | TTTGAGTTAGGTGTGGGATATAGTTT | AAAAAATAACGAACGCACCTAA | TTTTTTTTTTTTTTTTTTTTTTTAATCRAATCACTCCCACCC |

Negative control

Reference site was a C site that was not in CpG site, therefore low methylation signal should be detected and non-significant association should be detected between cancer and normal. Methylation status of LINE-1 was taken as biological control since we are clear that it is hypo-methylation in cancer tissues.

### Supplementary

up-stream and down-stream sequence

SHOX2cn agtgaggcggtgagtcgaggtacgtttgtgaaggaggtggtggatgactgcccaccttgccccaggcggccgctcagacttgacctttgcgcttctccgggatattcttcctccgggtgggttgagaagccggaccaggattccacgagggggaaggattctctattcttttttgcgacaaatctggtaacaggatttgctgtgctgttttcgtccgtgtgtgtgtgcgtgtgtgtgtgtgttcgtgtggatgcacgtgtggccccgctggggtgccccctccagtgtccccggagctgaaagatcgcaaagaggatgcgaaagggatggaggacgaaggccagaccaaaatcaagcagaggcgaagtcggaccaatt

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