Figure S1. Experimental flowchart of RE-dMSP. DNA was extracted from 100-150 µl of OSNA lysate and eluted in 50 µl of water. 6.6 µl of DNA solution was incubated with three methylation-specific restriction enzymes to digest unmethylated RASSF1A DNA at 37˚C for 16 h and subjected droplet digital PCR in triplicate. Simultaneously, 2.0 µl of DNA solution was subjected to dPCR without digestion to measure the total amount of DNA. RASSF1A, Ras association domain-containing protein 1; RE-dMSP, restriction enzyme-based digital methylation-specific polymerase chain reaction.
Figure S2. CK19 expression in 19 primary tumors with OSNA-negative and RE-dMSP-positive SNs. Magnification, x200. CK19, cytokeratin 19; OSNA, one-step nucleic acid amplification; RE-dMSP, restriction enzyme-based digital methylation-specific polymerase chain reaction; SN, sentinel lymph node; IHC, immunohistochemical staining; PC, positive control; NC, negative control.
Figure S3. Correlation between PMR of primary tumor and tumor diameter in 16 samples with OSNA-positive and RE-dMSP-negative. PMR, percent of methylated reference; OSNA, one-step nucleic acid amplification; RE-dMSP, restriction enzyme-based digital methylation-specific polymerase chain reaction.