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DETECTION OF TARGET DNA WITH A NOVEL CAS9/SGRNAS-ASSOCIATED REVERSE PCR (CARP) TECHNIQUE

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This study develops a new method for detecting target DNA based on Cas9 nuclease, which was named as CARP, representing Cas9/sgRNAs-Associated Reverse PCR. This technique detects target DNA in three steps: cleaving the detected DNA sample with Cas9 in complex with a pair of sgRNAs specific to target DNA; ligating the cleaved DNA with DNA ligase and; amplifying target DNA with PCR. In the ligation step, the Cas9-cut target DNA was ligated into intramolecular circular or intermolecular concatenated linear DNA. In the PCR step, the ligated DNA was amplified with a pair of reverse primers. The technique was verified by detecting *HPV16* and *HPV18 L1* genes in nine different human papillomavirus (HPV) subtypes. The technique also detected the *L1* and *E6-E7* genes of two high-risk HPVs, *HPV16* and HPV18, in the genomic DNA of two HPV-positive cervical carcinoma cells (HeLa and SiHa), in which no *L1* and *E6-E7* genes were detected in the HPV-negative cervical carcinoma cell, C-33a. By performing these proof-of-concept experiments, this study provides a new CRISPR-based DNA detection and typing method. Especially, the CARP method verified by this study is ready for the clinical HPV detection.

Biography

Beibei Zhang has completed his Master's degree at Henan Agricultural University. He is a Doctor of the State Key Laboratory of Bioelectronics, Southeast University, a double first-rate university with world first-rate disciplines. He has published several papers in reputed journals with impact factor more than 15.

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