Supporting Information

Rapid detection of HPV16/18 based on CRISPR-Cas13a/Cas12a dual-channel system

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Figure S1. The best RAA primer pairs were screened by DNA gel electrophoresis. (A) Screen the RAA primer pairs for HPV16 L1 gene. (B) Screen the RAA primer pairs for HPV18 L1

gene.

| Primers/crRNAs | Sequences | Targets | |
|---------------------|---|---------------|--|
| HPV16L1-F3 | 5'-GTTGTAAGCACGGATGAATATGTTGCACGC-3' | | |
| HPV16L1-R5 | 5'-CTGGTTTGGGCCTGTGTAGGTGTTGAGGTA-3' | HP V 10 L1-12 | |
| HPV16I 1-F6 | 5'-AATCCAGATACACAGCGGCTGGTTTGGGCCTGT-3' | HPV16 L1-T4.1 | |
| | | HPV16 L1-T4.2 | |
| HPV10L1-K8 | 5-004001010041441404044101414101-5 | HPV16 L1-T4.3 | |
| HPV16I 1-F7 | 5'-CGGCTGGTTTGGGCCTGTGTAGGTGTTGAG-3' | HPV16 L1-T4.1 | |
| | | HPV16 L1-T4.2 | |
| IIF V 10L1-K9 | J-AAACCACCIAIAUUUUAACACIUUUUCAAA-J | HPV16 L1-T4.3 | |
| | 5'- | | |
| HPV16L1-T2-crRNA | GAUUUAGACUACCCCAAAAACGAAGGGGACUAAA | | |
| | ACCAGGAAAACCAAACUUAUUGGGGUCAGG-3' | | |
| | 5'- | | |
| HPV16L1-T4.3-crRNA | GAUUUAGACUACCCCAAAAACGAAGGGGACUAAA | | |
| | ACAUGGCCACUAAUGCCCACACCUAAUGGC -3' | | |
| | | HPV18L1-T1.1 | |
| HPV18L1-F1 | 5'- TGCCCCTGCTATTGGGGGAACACTGGGCTAA -3' | HPV18L1-T1.2 | |
| HPV18L1-R1 | 5'- TTTACAAATAGACTGACAAATATCCAATGG -3' | HPV18L1-T1.3 | |
| | | HPV18L1-T1.4 | |
| HPV18L1-F2 | 5'- GTTACATAAGGCACAGGGTCATAACAATGG-3' | HPV18L1-T2 | |
| HPV18L1-R3 | 5'- TGCTTAAATTTGGTAGCATCATATTGCCCA-3' | | |
| HPV18L1-F4 | 5'- CTGCAGATGTTATGTCCTATATTCATAGTA -3' | 1101/101 1 72 | |
| HPV18L1-R4 | 5'- AGGACATAACATCTGCAGTTAAAGTAATAG-3' | HPV18L1-13 | |
| | 5'- | | |
| HPV18 L1-T1.4-crRNA | UAAUUUCUACUAAGUGUAGAUAGCAGUAUAGCAG | | |
| | ACAUGUU-3' | | |

Table S1. The primers and crRNAs used in this study

Table S2. Excitation and emission wavelengths of LED lamps required for different light emitting groups

| channel | luminescent groups | Excitation | Excitation |
|---------|--------------------|-----------------|-----------------|
| | | wavelength (nm) | wavelength (nm) |
| 1 | FAM | 455~485 | ~520 |
| 2 | CY3 | 540~550 | ~580 |
| 3 | TET | 510~530 | ~540 |
| 4 | JOE | 510~530 | ~550 |
| 5 | ROX | 570~590 | ~610 |

| Component | Volume to add (µL) |
|--|--------------------|
| Cas13a $(1\mu M)$ | 1μL |
| crRNA (100ng/µL) | 1μL |
| Cas12a $(1\mu M)$ | 2μL |
| crRNA (30ng/µL) | 1μL |
| T7 RNA polymerase mix | 1μL |
| rNTP Mix | 1μL |
| RNAase inhibitors | 1μL |
| $Mgcl_2$ (1M) | 0.25µL |
| Cas13a- Fluorescent reporter $(2\mu M)$ | 1.25µL |
| Cas12a- Fluorescent reporter $(10\mu M)$ | 1μL |
| multiplex RAA amplification product | 5µL |
| RNase-Free Water | to 25µL |
| Total | 25μL |

 Table S3. Fluorescence detection system for CRISPR-Cas13a/Cas12a dual-channel detection system



Figure S2. Fluorescence imaging of five DNA probes under UV cut gel recovery instrument