

Supporting Information

# Microfluidic Toolbox using Padlock Probes and Rolling Circle Amplification for Direct Detection and Genotyping of viral RNA

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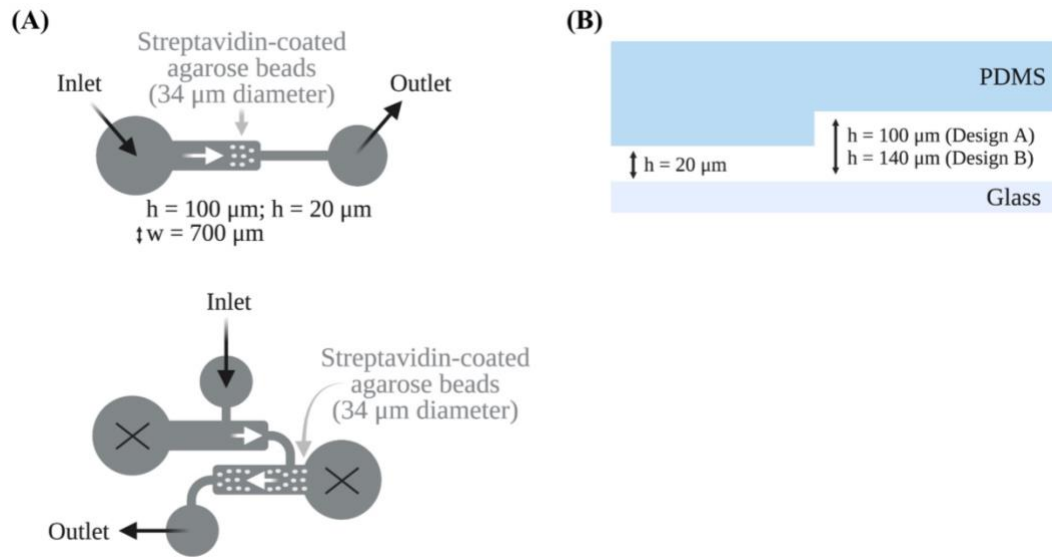
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**Table S1:** List of biotinylated capture oligonucleotides (Biot-Anchor), padlock probes (PLP), L-shaped oligonucleotides (L-Probe), detection oligonucleotides (DO), PLP-primer and restriction oligonucleotide (RO) sequences used for each assay. Color codes highlight complementary regions, with green highlighting sequences complementary to the target, blue for complementary probes, and red for detection probes. Riboses are identified in the “chimeric” PLPs with a purple color.

Sequence ID (assay)	Sequence (5' - 3')
Biot-Anchor 1 (HybRCA)	Biotin - AAAAAAAAAA TAGTGAACCGCCACACATGACCATT CACTCAATACTTGAGCACACTCAT
Biot-Anchor 2 (HybRCA)	Biotin - AAAAAAAAAA TGTTTACGCAAATATGCGTAAAACT CATTCAAAAAGTCTGTGTCAACA
Biot-Anchor 3 (HybRCA)	Biotin - AAAAAAAAAA GCTTCAGACATAAAAAACATTGTTTTGAT AATAAAGAAGTACTTAAAGTT
L-Probe 1 (HybRCA)	ACATGTTGTGCCAACCCACATAGAATTTGCT TTTTAGTGT TACGATGTTGACCCCTGTA TTAGGGATGCGGGTAGTA
L-Probe 2 (HybRCA)	CATTTGAGTTATAGTAGGGATGACATTACGT TTTTAGTGT TACGATGTTGACCCCTGTA TTAGGGATGCGGGTAGTA
L-Probe 3 (HybRCA)	CAGATAGAGACACCAGCTACGGTGGGAGCTC TTTTAGTGT TACGATGTTGACCCCTGTA TTAGGGATGCGGGTAGTA
L-Probe 4 (HybRCA)	GCCATAATTCTAAGCATGTTAGGCATGGCTC TTTTAGTGT TACGATGTTGACCCCTGTA TTAGGGATGCGGGTAGTA
L-Probe 5 (HybRCA)	GCTACAACACGTTGTATGTTTGGGAGCAAGA TTTTAGTGT TACGATGTTGACCCCTGTA TTAGGGATGCGGGTAGTA
L-Probe 6 (HybRCA)	CAATTACTACAGTAGCTCCTCTAGTGGCGGC TTTTAGTGT TACGATGTTGACCCCTGTA TTAGGGATGCGGGTAGTA
L-Probe 7 (HybRCA)	CCCAACCCATAAGGTGAGGGTTTTCTACATC TTTTAGTGT TACGATGTTGACCCCTGTA TTAGGGATGCGGGTAGTA
L-Probe 8 (HybRCA)	GTGGCATCTCCTGATGAGGTTCCACCTGGTT TTTTAGTGT TACGATGTTGACCCCTGTA TTAGGGATGCGGGTAGTA
L-Probe 9 (HybRCA)	GACTCATAAAGTCTGTGTTGTAATTGCG TTTTAGTGT TACGATGTTGACCCCTGTA TTAGGGATGCGGGTAGTA
L-Probe 10 (HybRCA)	GAAACACACAACAGCATCGTCAGAGAGTATC TTTTAGTGT TACGATGTTGACCCCTGTA TTAGGGATGCGGGTAGTA
L-Probe 11 (HybRCA)	GAGGTCCTTTAGTAAGGTCAGTCTCAGTCCA TTTTAGTGT TACGATGTTGACCCCTGTA TTAGGGATGCGGGTAGTA
L-Probe 12 (HybRCA)	GCCGGCCCCTAGGATTCTTGATGGATCTGGGTTTTAGTGT TACGATGTTGACCCCTGTA TTAGGGATGCGGGTAGTA
PLP (HybRCA)	TACAGGGGTCAACATCGTA TGCGTCTATTTAGTGGAGCCTT AGTAGCCGTGACTATCGACT ACTACCCGCATCCCTAA

Sequence ID (assay)	Sequence (5' - 3')
Biot-Anchor 1 (C2CA)	<b>Biotin - TTTTT AACATTAGTAGCGTTATTAACAA TAAGTAGGGACTGGGTC</b>
Biot-Anchor 2 (C2CA)	<b>Biotin - TTTTT CCAGTTTGGCCTGGAGCGATTG TCTGACTTCATCACCTC</b>
Biot-Anchor 3 (C2CA)	<b>Biotin - TTTTT GAAACTTTTTGTTAGACTCAGTA AGAACACCTGTGCCTGT</b>
Biot-Anchor 4 (C2CA)	<b>Biotin - TTTTT AGCAGCAAGATTAGCAGAAGCTC TGATTTCTGCAGCTCTA</b>
PLP 1-Wu (C2CA)	<b>AGTGGGTTGGAAACC GTGTATGCAGCTCCTCAGTAATAGTGTCTTAC TGGCTCTATTTAGTGGAGCC GTAACCAACACCATrU</b>
PLP 2-Wu (C2CA)	<b>CCTGATAAAGAACAG GTGTATGCAGCTCCTCAGTAATAGTGTCTTAC TGGCTCTATTTAGTGGAGCC CTGTGCAGTTAACArU</b>
PLP 1-Alfa (C2CA)	<b>CAATGTCTCTGCCAA GTGTATGCAGCTCCTCAGTAATAGTGTCTTAC AGTAGCCGTGACTATCGAC CATCAGTAGTGTCArU</b>
PLP 2-Alfa (C2CA)	<b>AAGGATATCATTAA GTGTATGCAGCTCCTCAGTAATAGTGTCTTAC AGTAGCCGTGACTATCGAC TTTGTCAAGACGTGrC</b>
PLP 1-Beta (C2CA)	<b>CACGCACTAAATTAA GTGTATGCAGCTCCTCAGTAATAGTGTCTTAC AGTCGATAGTCACGGCTACT AACCTGAGGGAGArC</b>
PLP 2-Beta (C2CA)	<b>AACACCATTACAAGG GTGTATGCAGCTCCTCAGTAATAGTGTCTTAC AGTCGATAGTCACGGCTACT ACAATTAAAACCTTrU</b>
PLP-Primer (C2CA)	<b>TACTGAGGAGCTGCATACAC</b>
RO (C2CA)	<b>GTGTATGCAGCTCCTCAGTA</b>
DO Biot-Common (C2CA)	<b>GTAAGACACTATTACTGAGGATTTT - Biotin</b>
DO Cy3-Wu (C2CA)	<b>GGCTCCACTAAATAGACGCATTTT - Cy3</b>
DO Cy5-Alfa (C2CA)	<b>AGTCGATAGTCACGGCTACTTTT - Cy5</b>
DO AF488-Beta (C2CA)	<b>AGTAGCCGTGACTATCGACTTTT - AF488</b>



**Figure S1:** (A) Top and (B) side view of the microfluidic device designs. White arrows indicate the direction of the flow.