

A pooled genome-wide screening strategy to identify and rank influenza host restriction factors in cell-based vaccine production platforms

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Supplemental S8. Primers and thermocycling conditions

dPCR assays

For measurement of total influenza viral particles in CFS

Influenza A segment 7, forward	GACCRATCCTGTCACCTCTGAC
Influenza A segment 7, reverse	AGGGCATTYTGGACAAKCGTCTA

1) 95°C, 10min 2) 95°C, 30s 3) 61°C, 30s 4) 72°C, 30s 5) Back to step 2, 39 cycles 6) 72°C, 10min 7) 4°C, hold

For measurement of integrated lentiviral vector copy number

WPRE, forward	GTCCTTTCCATGGCTGCTC
WPRE, reverse	CCGAAGGGACGTAGCAGA
Albumin, forward	TTTGCAGATGTCAGTGAAAGAGA
Albumin, reverse	TGGGGAGGCTATAGAAAATAAGG

1) 95°C, 10min 2) 95°C, 30s 3) 60°C, 30s 4) Back to step 2, 39 cycles 5) 72°C, 10min 6) 4°C, hold

For measurement of influenza segment 4 mRNA (GFP and HA)

GFP, forward	CTGCTGCCCGACAACCAC
GFP, reverse*	TCACGAACTCCAGCAGGAC
HA, forward	ATCGACTATGAGGAGCTGAGGG
HA, reverse*	GCCGTTACTCCGTTTGTGTTGT
Actin, forward	GTCATACTCCTGCTTGCTGAT
Actin, Reverse	AAAGACCTGTACGCCAACAC

*Reverse transcription was carried out using these primers as gene-specific primers to selectively amplify positive-sense RNA

1) 95°C, 10min 2) 95°C, 30s 3) 60°C, 30s 4) Back to step 2, 39 cycles 5) 72°C, 10min 6) 4°C, hold

Knockout pools

TBK1

sgRNA sequence	TTCCGCGGCCACGGTAATGA
TIDE assay primer, forward	GGCCGTTTTCCAAAATACCGA
TIDE assay primer, reverse	GATGCAGGTCGAGGACCG

1) 95°C, 60s 2) 95°C, 15s 3) 63°C, 15s 4) Back to step 2, 28 cycles 5) 72°C, 5min 6) 4°C, hold

DDX6

sgRNA sequence	AGGTCTAGCCGTTCAAGTAA
TIDE assay primer, forward	TGTTGCAGGGATGAGGTGTC
TIDE assay primer, reverse	CCTGTCTCACTGGAATGCTGT

1) 98°C, 3min 2) 98°C, 30s 3) 63°C, 30s 4) 72°C, 60s 5) Back to step 2, 34 cycles 6) 72°C, 5min 7) 4°C, hold

SMG9

sgRNA sequence	GCTGAAATGAAGGAACGAGG
TIDE assay primer, forward	TCAAACATGCACTACCCCC
TIDE assay primer, reverse	CCAGTCAGTGCTAACGACAGT

1) 98°C, 3min 2) 98°C, 30s 3) 63°C, 30s 4) 72°C, 60s 5) Back to step 2, 34 cycles 6) 72°C, 5min 7) 4°C, hold

CARM1

sgRNA sequence	TCGCGTCGCCGATGGTGAGG
TIDE assay primer, forward	TTGTGTGGGGCGGGGTA
TIDE assay primer, reverse	GCTCCCTTGCTCACTCTGG

1) 98°C, 3min 2) 98°C, 30s 3) 63°C, 30s 4) 72°C, 60s 5) Back to step 2, 34 cycles 6) 72°C, 5min 7) 4°C, hold *Use high GC buffer

Non-targeting control (NTC)

sgRNA sequence	TTCCGCGGCCACGGTAATGA
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Illumina amplicon library prep/barcoding

P5_0nt_stagger*	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGAC GCTCTTCCGATCTTTGTGGAAAGGACGAAACACCG
P5_1nt_stagger*	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGAC GCTCTTCCGATCTCTTGTGGAAAGGACGAAACACCG
P5_2nt_stagger*	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGAC GCTCTTCCGATCTGCTTGTGGAAAGGACGAAACACCG
P5_3nt_stagger*	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGAC GCTCTTCCGATCTAGCTTGTGGAAAGGACGAAACACCG
P5_4nt_stagger*	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGAC GCTCTTCCGATCTCAACTTGTGGAAAGGACGAAACACCG
P5_6nt_stagger*	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGAC GCTCTTCCGATCTTGCACCTTGTGGAAAGGACGAAACACCG
P5_7nt_stagger*	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGAC GCTCTTCCGATCTACGCAACTTGTGGAAAGGACGAAACACCG
P5_8nt_stagger*	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGAC GCTCTTCCGATCTGAAGACCCTTGTGGAAAGGACGAAACACCG
P7_Barcode1	CAAGCAGAAGACGGCATAACGAGATCGAGTAGTGACTGGAGTTCA GACGTGTGCTCTTCCGATCTCCAATTCCCCTCCTTTCAAGACCT
P7_Barcode2	CAAGCAGAAGACGGCATAACGAGATTCTCCGGTGACTGGAGTTCA GACGTGTGCTCTTCCGATCTCCAATTCCCCTCCTTTCAAGACCT
P7_Barcode3	CAAGCAGAAGACGGCATAACGAGATAATGAGGTGACTGGAGTTCA GACGTGTGCTCTTCCGATCTCCAATTCCCCTCCTTTCAAGACCT
P7_Barcode4	CAAGCAGAAGACGGCATAACGAGATGGAATCGTGACTGGAGTTCA GACGTGTGCTCTTCCGATCTCCAATTCCCCTCCTTTCAAGACCT
P7_Barcode5	CAAGCAGAAGACGGCATAACGAGATTCTGAGTGACTGGAGTTCA GACGTGTGCTCTTCCGATCTCCAATTCCCCTCCTTTCAAGACCT
P7_Barcode6	CAAGCAGAAGACGGCATAACGAGATACGAATGTGACTGGAGTTCA GACGTGTGCTCTTCCGATCTCCAATTCCCCTCCTTTCAAGACCT
<p><u>Illumina adaptor</u> - <u>Stagger nucleotides</u> - <u>Index sequence</u> - Vector binding sequence</p>	

*The eight P5 primers were pooled for use in barcoding PCR

- 1) 95°C, 60s
- 2) 95°C, 30s
- 3) 53°C, 30s
- 4) 72°C, 30s
- 5) Back to step 2, 28 cycles
- 6) 72°C, 10min
- 7) 4°C, hold