| | Description | Experiments used | Dilution for infection | Map |
|---|---|---|---|---|
| vXC1 | Sindbis virus expressing mCherry and a N20 barcode in the 3'UTR | Rolony quantifications | 1:10 | https://benchling.com/s/iwgYgAZi |
| IDP468-F2 | Sindbis virus expressing mCherry | Calibration of sequencing channels | 1:100 | https://benchling.com/s/JX8PQY0R |
| JK100L2 | Sindbis virus expressing GFP and a N30 barcode in the 3'UTR | 15-base sequencing and comparison between SOLiD and Illumina | 1:100 | https://benchling.com/s/EKtQttOe |

Supp Table S1. List of viruses used.

| | | |
|---|---|---|
| XC1149 | /5phos/aatcagccataccacatttgtagaggtgTCCTCTATGATTACTGACTGCGTCTATTTAGTGGAGCCATTGCTATCTTCTTgggccgcgactctagatcat | IDP468-F2 padlock, nogap |
| XC1151 | /5phos/ccacatttgtagaggtgcataatgttgcTCCTCTATGATTACTGACTGCGTCTATTTAGTGGAGCCATTGCTATCTTCTTgggccgcgactctagatcat | IDP468-F2 padlock, 11nt gap |
| XC1157 | /5phos/gtgcataatgttgcgcagtgttgTCCTCTATGATTACTGACTGCGTCTATTTAGTGGAGCCATTGCTATCTTCTTaatcagccataccacatttgtagag | pXC1 padlock |
| XC1164 | /5phos/gtactgcggccgctacctaaTCCTCTATGATTACTGACTGCGTCTATTTAGTGGAGCCATTGCTATCTTCTTacacgacgctcttccgatct | padlock for JK100L2 barcode |
| XC1215 | /5AmMC12/T+AC+AG+CT+AG+CG+GT+GGTCGTACCTCACGACG | JK100L2 LNA RT primer |
| XC1216 | /5AmMC12/G+GT+TA+AT+AT+AG+TG+GTTATGTGGCAACACTGC | IDP468-F2 and pXC1 LNA RT primer with single crosslinker |
| XC1615 | /5phos/gtgcataatgttgcgcagtgttgTcctctatgattactgactgcgtctaTTTAGTGGAGCCATTGCTATCTTCTTaatcagccataccacatttgtagag | Padlock probes for subsampling rolonies from vXC1, use XC92 to visualize |
| XC1616 | /5phos/gtgcataatgttgcgcagtgttgTgactgtcctctggacaataccgtccTTTAGTGGAGCCATTGCTATCTTCTTaatcagccataccacatttgtagag | Padlock probes for subsampling rolonies from vXC1, use XC1380 to visualize |
| XC92 | /5TYE563/CCTCTATGATTACTGACTGCGTCTa | fluorescent probe for visualizing all rolonies except those made with XC1616 |
| XC1380 | /5TYE563/atatagactgtcctctggacaataccgtcc | fluorescent probe for XC1616 rolonies |
| XC1595 | /5phos/aatcagccataccacatttgtagaggtgTCCTCTATGATTACTGACTGCGTCTATTTAGTGGAGCCATTGCTATCTTCTTgggccgcgactctagatc*a*t | padlocks for in vitro tests, no gap |
| XC1596 | /5phos/ccacatttgtagaggtgcataatgttgcTCCTCTATGATTACTGACTGCGTCTATTTAGTGGAGCCATTGCTATCTTCTTgggccgcgactctagatc*a*t | padlocks for in vitro tests, 11nt gap |
| XC1529 | cacctctacaaatgtggtatggctgattatgatctagagtcgcggccc/3phos/ | mock cDNA for testing gap filling in vitro |
| XC1308 | /5phos/aatcagccataccacatttgtagaggtgTCCTCTATGATTACTGACTGCGTCTATTTAGTGGAGCCATTGCatcgTATCTTCTTgggccgcgactctagatcat | barcode padlocks for calibration, to be used on IDP468-F2 |
| XC1309 | /5phos/aatcagccataccacatttgtagaggtgTCCTCTATGATTACTGACTGCGTCTATTTAGTGGAGCCATTGCtcgaTATCTTCTTgggccgcgactctagatcat | barcode padlocks for calibration, to be used on IDP468-F2 |
| XC1310 | /5phos/aatcagccataccacatttgtagaggtgTCCTCTATGATTACTGACTGCGTCTATTTAGTGGAGCCATTGCcgatTATCTTCTTgggccgcgactctagatcat | barcode padlocks for calibration, to be used on IDP468-F2 |
| XC1311 | /5phos/aatcagccataccacatttgtagaggtgTCCTCTATGATTACTGACTGCGTCTATTTAGTGGAGCCATTGCgatcTATCTTCTTgggccgcgactctagatcat | barcode padlocks for calibration, to be used on IDP468-F2 |
| XC1312 | ACTGCGTCTATTTAGTGGAGCCATTGC | Illumina seq primer for barcode padlock calibration. |

| | | |
|---|---|---|
| XC1016 | /5phos/TCTCGGGAACGCTGAAGAacactgcgcaacattatgca | 3'UTR RT primer for IDP468-F2, for FISSEQ |
| XC1017 | cagccataccacatttgtagagg | 3'UTR RCA primer for IDP468-F2, for FISSEQ |
| XC1493 | /5phos/gtactgcggccgctacctaaTCCTCT | SOLiD sequencing primers for padlocks XC1164 |
| XC1494 | /5phos/tactgcggccgctacctaaTCCTCT | SOLiD sequencing primers for padlocks XC1164 |
| XC1495 | /5phos/actgcggccgctacctaaTCCTCT | SOLiD sequencing primers for padlocks XC1164 |
| XC1496 | /5phos/ctgcggccgctacctaaTCCTCT | SOLiD sequencing primers for padlocks XC1164 |
| XC1497 | /5phos/tgcggccgctacctaaTCCTCT | SOLiD sequencing primers for padlocks XC1164 |

Supp Table S2. List of oligonucleotides used.