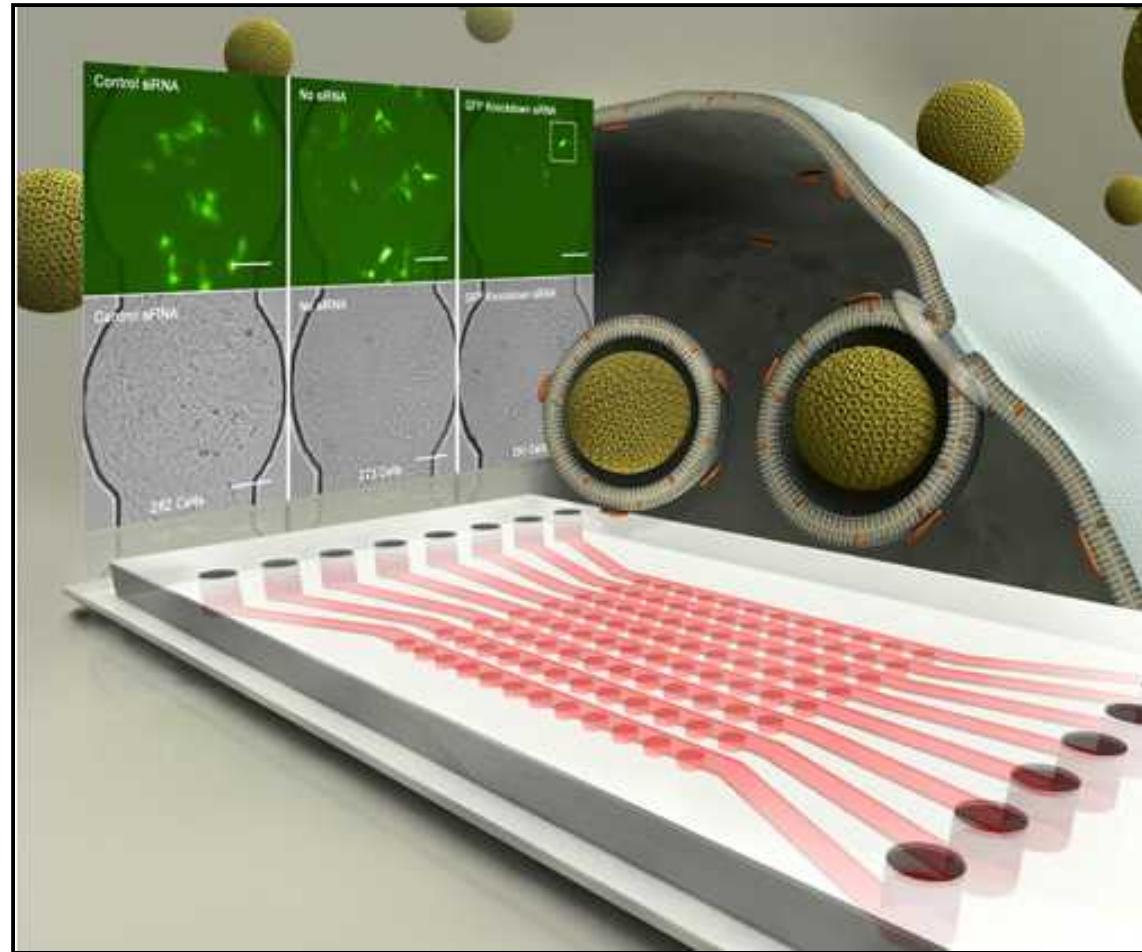


Microfluidic devices for genetic screening of positionally-controlled cell cultures

SAND2013-3133C

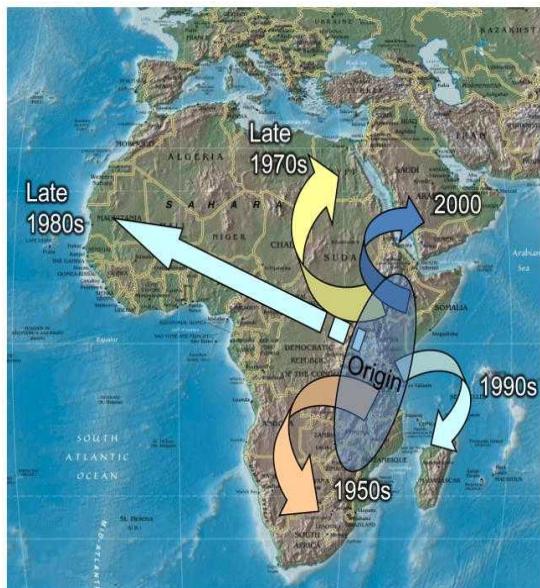
Benjamin R. Schudel
Brooke Harmon
Vinay V. Abhyankar
Benjamin Pruitt
Oscar A. Negrete
Anup K. Singh



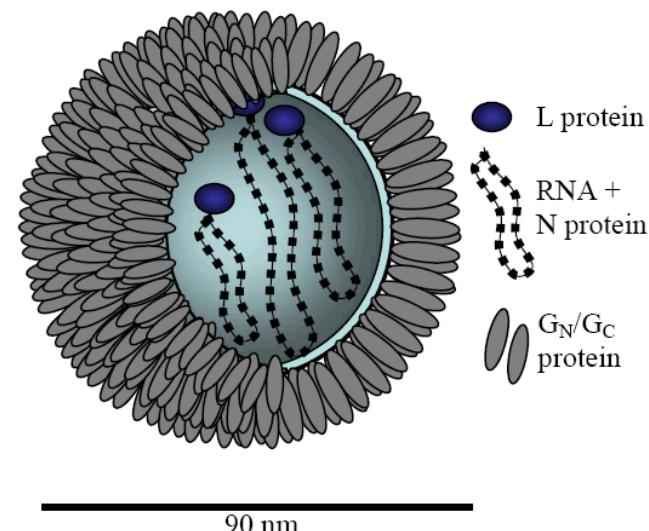
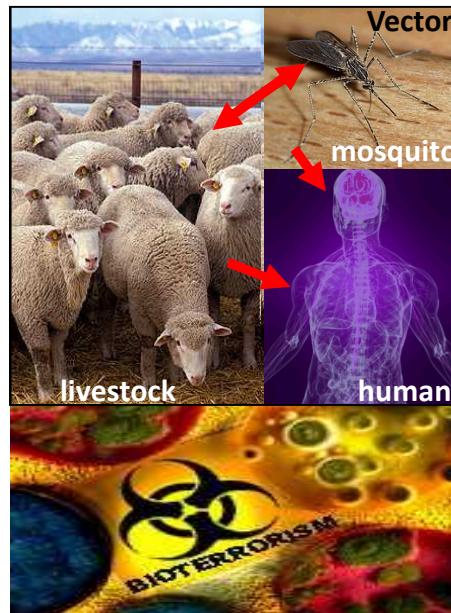
Sandia National Laboratories is a multi-program laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.

Motivation: Rift Valley Fever virus (RVFV)

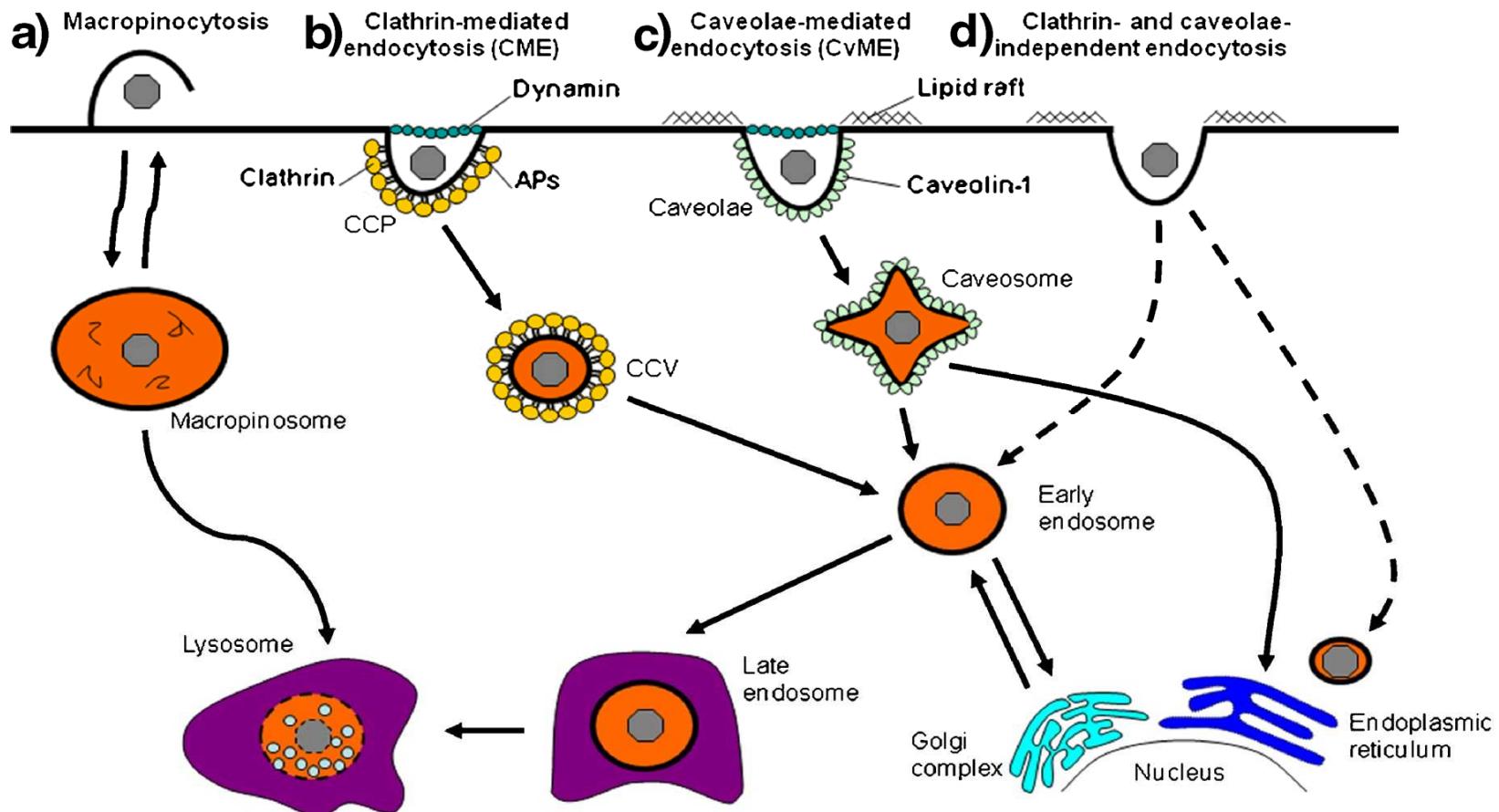
Sub-Saharan Africa, zoonosis, enveloped virus, potentially stable



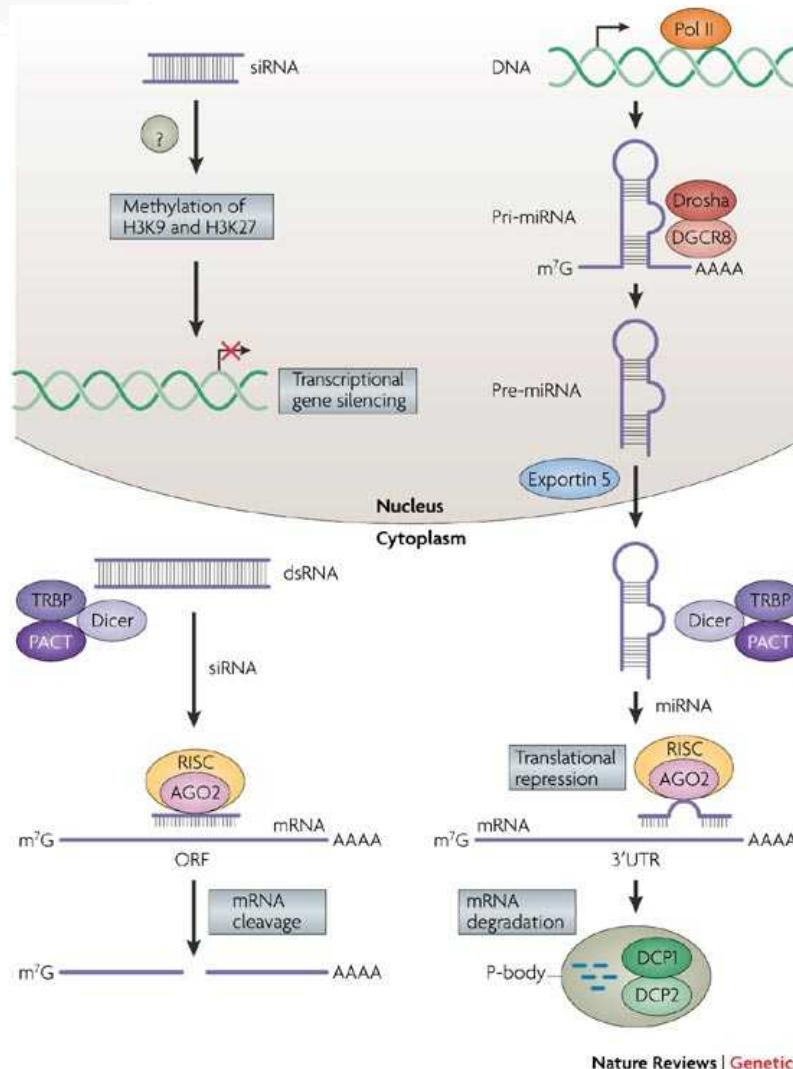
Weaver S, Antiviral Res. 2010



Rift Valley Fever Virus – What we don't know



RNA interference (RNAi) is the technique used to knock down a specific gene using small interfering RNA (siRNA)



siRNA:

- RNA strands are small enough not to trigger an immune response from the cell
- Specifically interfere with genetic expression
- Ideal for examining which genes participate within an infection process

Figure Credit: Kim, Rossi, *Nature Reviews Genetics*, 2007, 8, 173.

Due to the vast parameter space, smaller and faster screening techniques are desired for cheaper discoveries

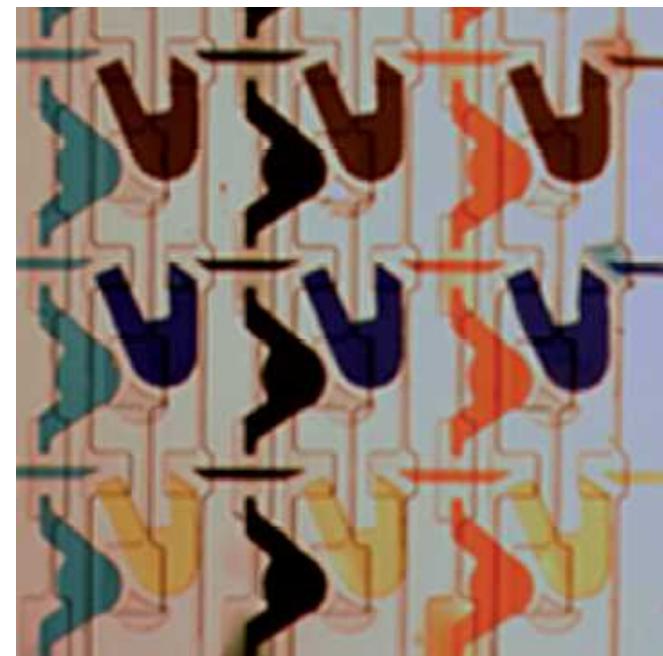
| | | | | | | | | | | | | | | |
|----------|-----------|----------|--------|---------|-----------|----------|----------|-----------|----------|----------|----------|----------|---------|---------|
| ABLIM1 | ATF3 | CAMTA1 | CRYBA2 | DPH2 | FBLN2 | HAO | IGLL1 | KRTAP5-2 | MCM7 | NUDT1 | PUM2 | SLC16A8 | TTC33 | |
| ABLIM3 | ATF4 | CAMTA2 | CRYM | DSC1 | FBX047 | HABP4 | IGSF1 | LAIR1 | MDN1 | NUDT2 | QPRT | SLC35A3 | TTLL12 | |
| ABR | ATF6 | CAPRIN1 | CS | DSC2 | FCAR | HADHB | IGSF3 | LAIR2 | MEIS2 | NUP160 | R3HDM1 | SLC3501 | TTLL5 | |
| ACAN | ATG2A | CAPS | CSH1 | DSC3 | FCGR2B | HARS2 | INCA | LARP5 | MEOX1 | NUP205 | RAB3GAP1 | SLC39A14 | UBR4 | |
| ACIN1 | ATMIN | CARHSP1 | CSH1 | DSCAM | FCHO1 | HAS3 | INPP4A | LDHC | MEST | NUP210 | RAB6IP1 | SLC4A1AP | UBXD2 | |
| ACOT9 | ATP13A2 | CAST | CSHL1 | DST | FGF12 | HBM | IQCE | LDOC1 | MFAP2 | OR10C1 | RABGAP1 | SMC5 | UBXD8 | |
| ACSBG1 | ATP1B1 | CBFA2T3 | CSTF2T | DTNB | FGF13 | HCG3 | IQSEC2 | LEMD3 | MGAT2 | OR10J3 | RAD54L2 | SMG5 | UNC84A | |
| ACSL3 | ATP1B4 | CBX5 | CTAGE5 | DULLARD | FGF14 | HDLBP | IRF5 | LGALS8 | MGC70857 | OR13G1 | RBM34 | SMG6 | UNC84B | |
| ACYP1 | ATP5C1 | CBX6 | CTBP1 | DUSP4 | FIX1 | HERP2 | IRF7 | LGALS9 | MGRN1 | OR2B3P | RBPJ | SMPX | WAPAL | |
| ADD2 | ATP5E | CCBL2 | CFTR | FCM1 | FLJ15422 | HTC2 | KCTD2 | LILRA4 | MMP19 | OR2W5 | RCAN1 | SNAPIN | WBP2 | |
| ADD3 | ATP5F | CCDC170 | CFTR | FCM1 | FLJ15422 | HTC2 | KIAA0082 | LIMCH1 | MMRN1 | OR5B3 | RFTN1 | SPANXN1 | WDR37 | |
| AGT | ATP5G | CDH7 | CFTR | FCM1 | FLJ15422 | HTC2 | KIAA0090 | LMX1A | MN1 | OR5U1 | RGL1 | SPCC1L | WDR47 | |
| ALAS1 | ATP5G3 | CDC42EP4 | YB601 | EPF1D | FMRP1 | HES1H2BD | KIAA0157 | LOC441150 | MOBP | OR9K2 | RHOBTB2 | SPG20 | WSDC1 | |
| ALG11 | ATP5J | CDH7 | YB601 | EPF1D | FMRP1 | HES1H2BD | KIAA0182 | LOC441476 | MOCS1 | OTP | RIMBP2 | SRRM2 | WMC1 | |
| AMPH | ATP6V0A2 | CENTR2 | YCTB1 | EPN4 | FOXA2 | HMHA1 | KIAA0240 | LOC492311 | MOG | PADI4 | RIM51 | SSBP2 | XPO6 | |
| AMY1A | ATP6V | EPN4 | YCTB1 | EPN4 | FOXA2 | HMHA1 | KIAA0241 | LOC493869 | MON1B | PALLD | RNASEK | STGC3 | ZBTB1 | |
| ANGEL1 | BACH1 | EPN4 | YCTB1 | EPN4 | FOXA3 | HMHA1 | KIAA0265 | LOH11CR2A | MON2 | PANX1 | RP4- | STMN1 | ZBTB43 | |
| ANK1 | BAHD1 | EPN6 | EPN6 | EPN6 | FOXM1 | HNRPAL | KIAA0323 | LPIN1 | MORC2 | PAXIP1 | 691N24.1 | STOM | ZC3H13 | |
| ANKRD12 | BAT2D1 | CHAC2 | EPN6 | EPN6 | FOXM1 | HNRPAB | KIAA0409 | LRCH1 | MORC3 | PCNX | RPGRIP1L | SUGT1P | ZC3H3 | |
| ANKRD6 | BCKDHB | CIC | EPN6 | EPN6 | F11F4 | FSTL4 | HNRPH3 | KIAA0423 | LRRC18 | MRP527 | PDS5A | RPH3A | SULT1C3 | ZC3H7B |
| ANKS1A | BHMT2 | CIS | EPN6 | EPN6 | EIF5 | FUT8 | HNRPK | KIAA0460 | LRRC52 | MS4A13 | PDSS1 | RPIA | SUZ12 | ZCCHC14 |
| ANXA11 | BICD1 | CLCA1 | ELAVL3 | ELAVL3 | GABARAPL1 | H0XA10 | KIAA0467 | LRRC6 | MSMB | PDXDC1 | RPL13A | SWAP70 | ZFPM2 | |
| ANXA13 | BIN1 | CLCA1 | ELAVL3 | ELF5 | GABPB2 | H0XA3 | KIAA0556 | LRRC8B | MSRB2 | PEF1 | RRP12 | SYNGR4 | ZFYVE26 | |
| ANXA6 | BLID | CLCC1 | ELDA | ELDA | GAK1 | H0XB6 | KIAA0746 | LSM5 | MTIF | PES1 | RRP1B | SYT11 | ZHX2 | |
| ANXA7 | BOP1 | CLEC15A | ELDA | ELDA | EML1 | GAK1 | KIAA0776 | LSP1 | MTCH1 | PHC2 | RRS1 | TARDBP | ZHX3 | |
| AOAH | BRDT | CLTA | ELDA | ELDA | EML2 | GATA3 | KIAA0802 | LY96 | MTCH2 | PHF3 | RTF1 | TBC1D2B | ZIM2 | |
| AOC2 | BRF1 | CLTB | ELDA | ELDA | EN03 | GCAT | KIAA0892 | LYPLA3 | MTCP1 | PHLDA3 | RUFY3 | TBC1D9 | ZNF281 | |
| AP1B1 | BTF3 | CNKSRL | ELDA | ELDA | ENPP4 | GPFT1 | HPCAL1 | KIAA0895 | MAGEA11 | MTHFD2L | PHLDB1 | RUNX1T1 | TCF25 | ZNF318 |
| AP2A1 | C10orf55 | CNN2 | DDX58 | ENTPD2 | GGT1 | HPS1 | KIAA0907 | MAGEA2 | MTIF2 | PHLPP | RUSC1 | TDRD7 | ZNF423 | |
| AP2S1 | C10orf62 | CNTN1 | DENN3 | EPB41L3 | GGTL3 | HSPA8 | KIAA0913 | MAGEA3 | MTMR15 | PHLPP1 | SAMD4A | TGDS | ZNF510 | |
| AP4E1 | C15orf2 | COBL | DFFA | ETHE1 | GJA5 | HYAL1 | KIAA0922 | MAGEB1 | MTXL | PHOSPHO2 | SAPS1 | TLX2 | ZNF705A | |
| APBB1 | Clorf186 | COL11A1 | DHPS | ETV3L | GLE1 | HYAL4 | KIAA1012 | MAL | MYBPC1 | PISD | SASH1 | TMED3 | ZZEFL1 | |
| APOBEC1 | C20orf103 | COL12A1 | DHX9 | EVC | GLT25D2 | HYPK | KIAA1024 | MAP1B | MYH11 | PITPNB | SATB2 | TMEMF2 | | |
| ARC | C22orf31 | COL17A1 | DIP2C | EXOSC2 | GPATCH8 | ICA1 | KIAA1033 | MAP1LC3C | MYL1 | PIWIL3 | SBNO2 | TMEM194 | | |
| ARHGAP6 | C2orf64 | COL2A1 | DIS3 | EXOSC7 | GPD1L | ICAM4 | KIN | MAP4 | MYL4 | PLD3 | SCMH1 | TMEM2 | | |
| ARHGEF12 | C3orf27 | COL4A5 | DLX4 | EXPH5 | GPM6A | ICOSLG | KIR2DL3 | MAP6 | MYT1L | PLEKHA6 | SDF2L1 | TMEM50A | | |
| ARHGEF15 | C3orf63 | COL4A6 | DMD | EYA2 | GPM6B | IDH3B | KLHDC2 | MAPRE3 | NAP1L1 | PLEKHG7 | SEC14L2 | TMEM89 | | |
| ARHGEF18 | C4BPB | COL6A2 | DMXL2 | EYA3 | GRP | IDH3G | KLHL18 | MAST1 | NAT6 | POLA2 | SEC61G | TRAK1 | | |
| ARHGEF9 | C8orf22 | COMM3 | DNAJC9 | FAIM2 | GSPT2 | IFI6 | KLRC1 | MATN1 | NUDCD3 | PPWD1 | SEPHS2 | TRIM2 | | |
| ARL2BP | C9orf103 | CORO1C | DNM2 | FAM116B | GSTM4 | IFIT1 | KLRC1 | MATN1 | NUDCD3 | PRDM1 | SETX | TRIM72 | | |
| ARL6IP1 | C9orf5 | COTL1 | DNMBP | FAM120A | GTF2A1 | IFIT5 | KLRD1 | MBNL1 | NCDN | PRMT1 | SF3B3 | TSC22D3 | | |
| ASCC3L1 | CALB2 | COX15 | DNTT | FANCB | GTF2I | IFR01 | KLRK1 | MC4R | NCDN | PSME4 | SKIV2L2 | SPN15 | | |
| ASTL | CALD1 | CRISP1 | DOCK9 | FASTKD2 | GYPE | IGF2 | KRT13 | MC4R | NUDCD3 | PSME4 | SKIV2L2 | TSPY14 | | |
| ASTN1 | CAMSAP1L1 | CRMP1 | DPAGT1 | FBLN1 | H3F3B | IGFL4 | KRT18 | MC4R | NUDCD3 | PSME4 | SKIV2L2 | TSPY14 | | |

357 Hits
(Dr. Brooke Harmon)

Large scale screening is often performed using robotic handling in screening facilities



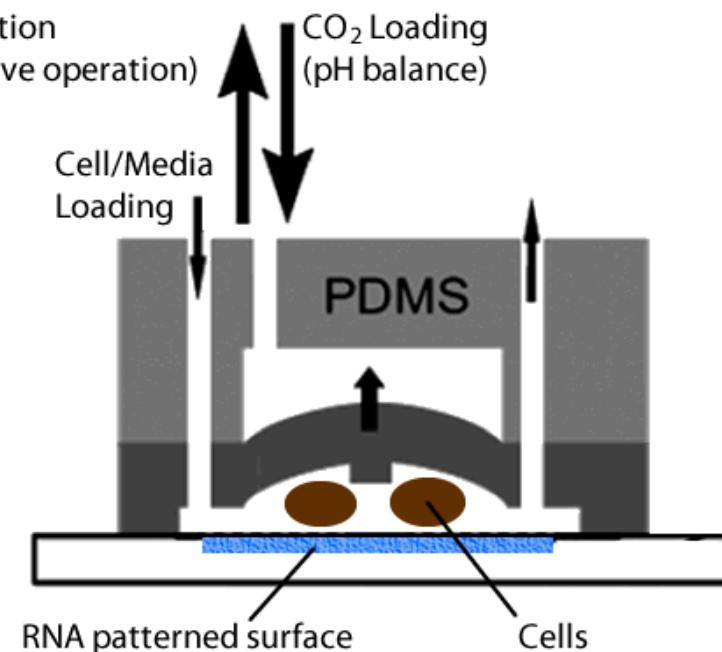
Image Credit: www.velocity11.com, Agilent



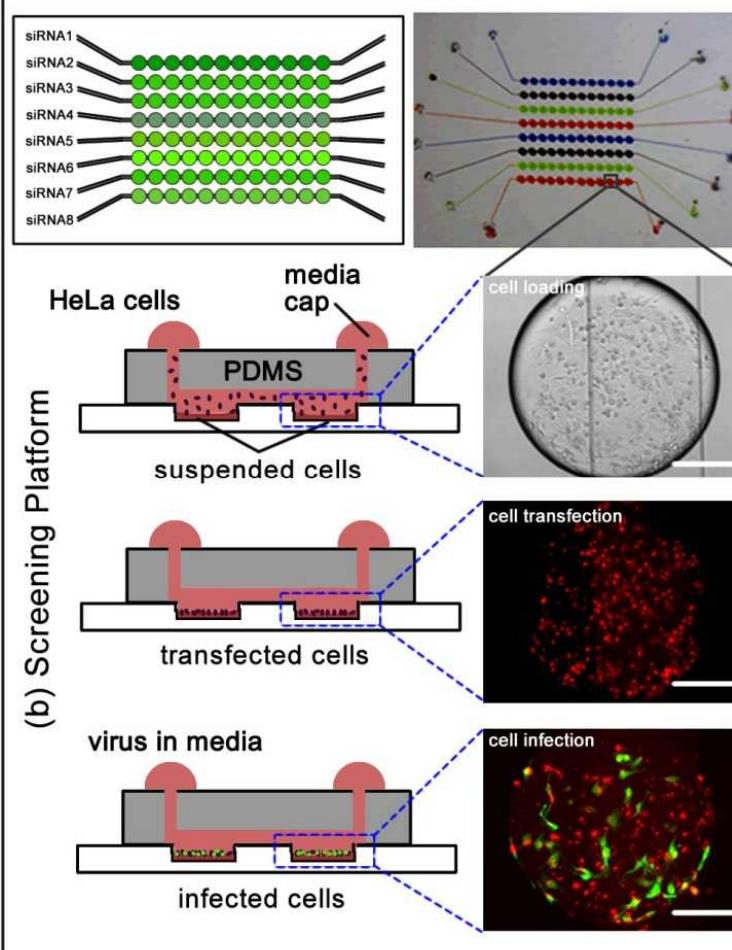
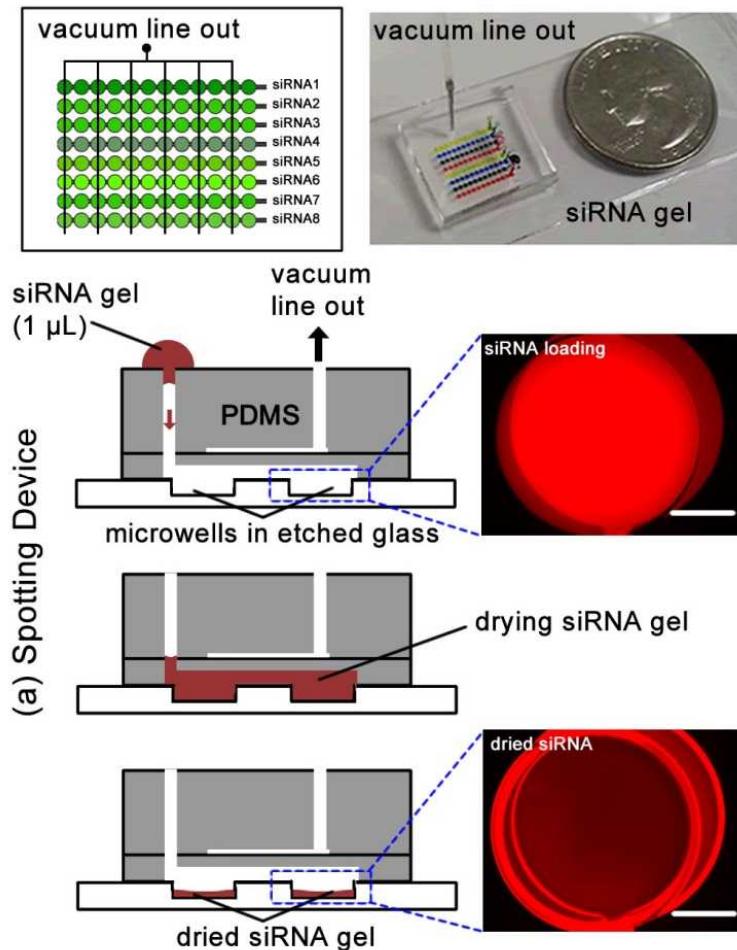
Schudel *et al.*, *Lab Chip* 2009.

The knockdown process and infection evaluation can take up to four days, which requires a stable cell culture platform

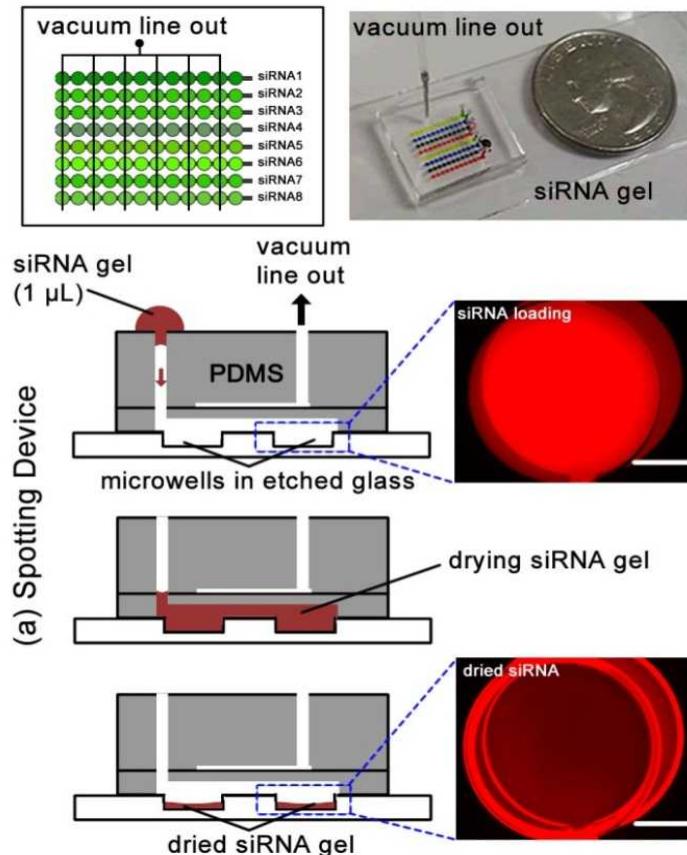
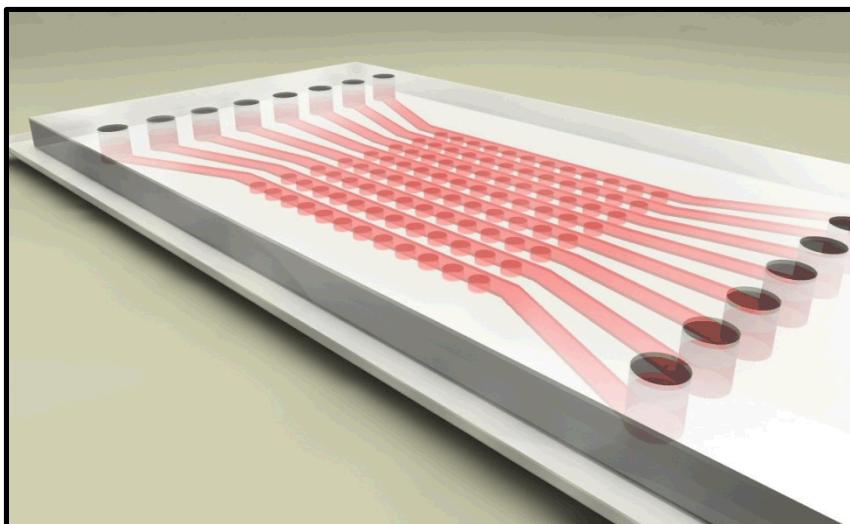
1. Cell seeding/culture
2. siRNA transfection
3. Lower gene expression
4. Infection with GFP-based virus



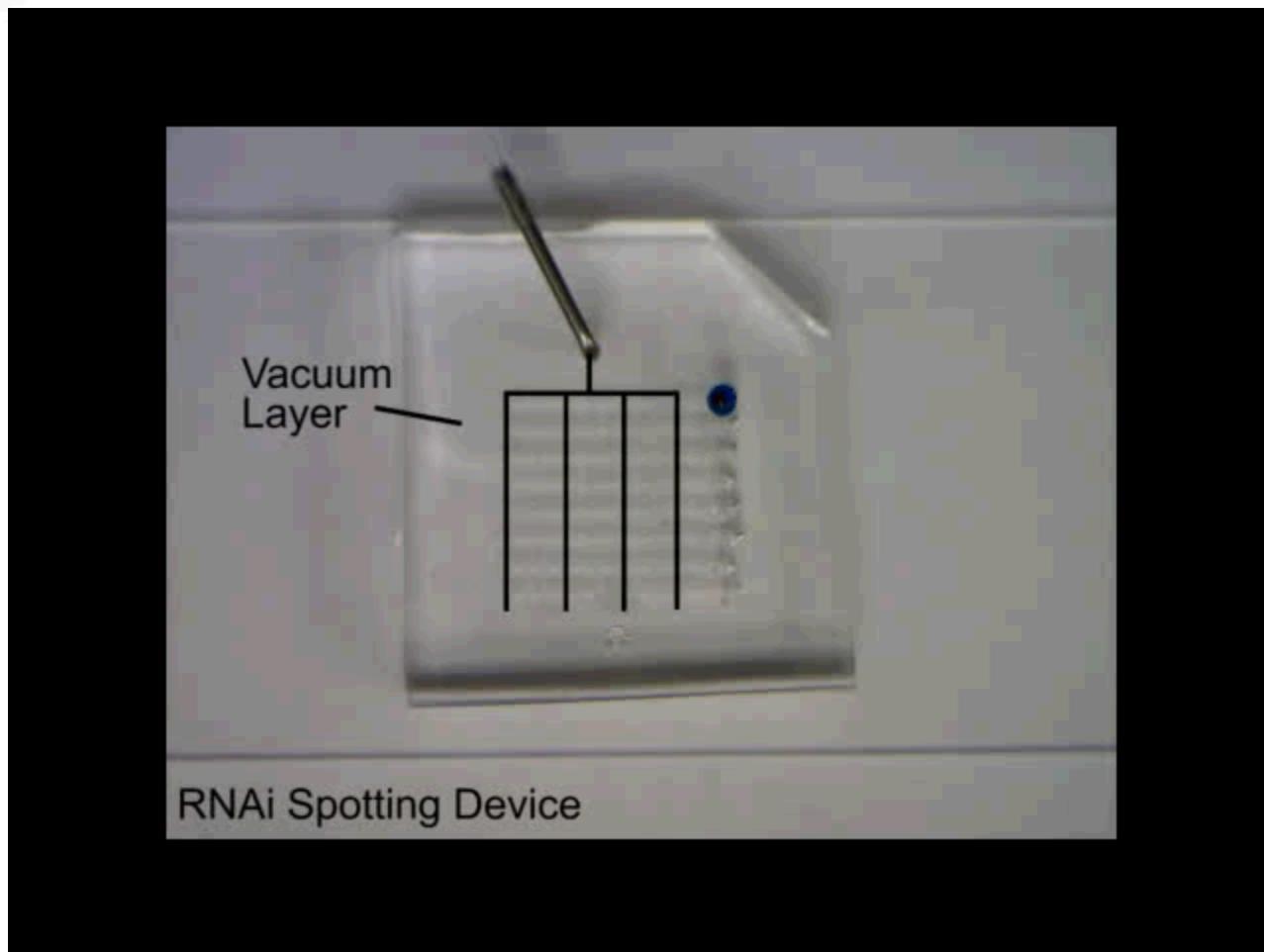
Microfluidic siRNA Screening System: Spotting Device and Screening Platform



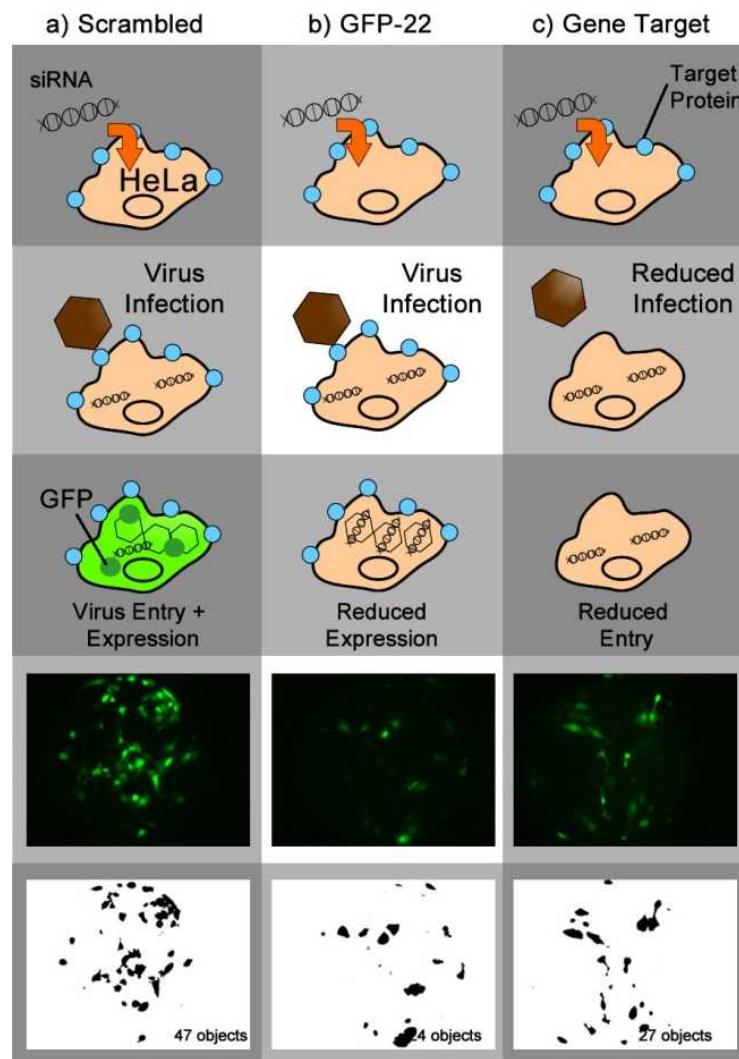
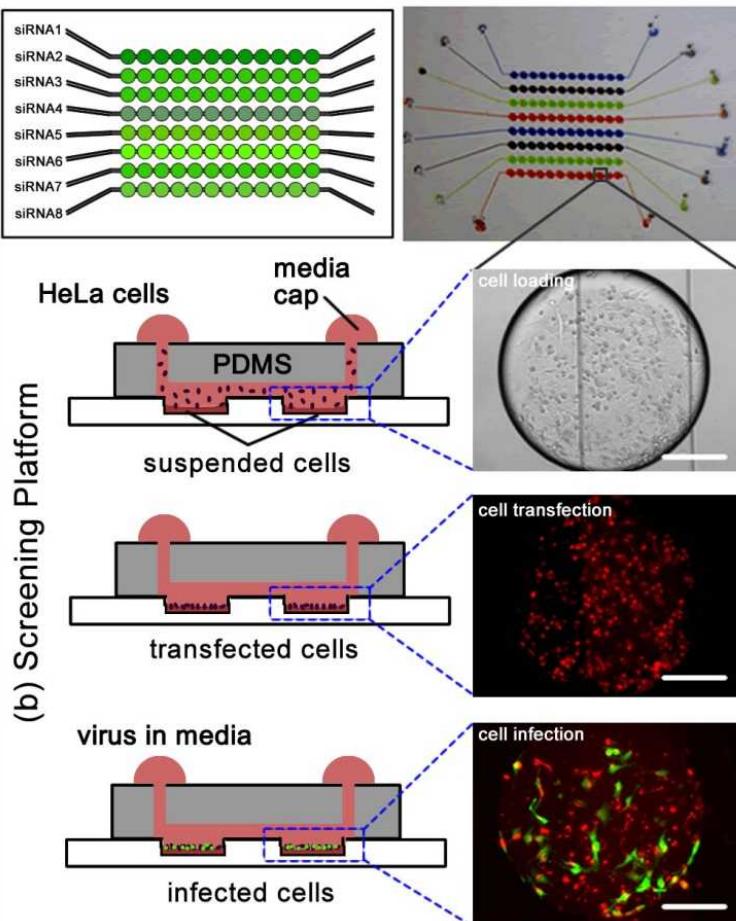
siRNA libraries are patterned using a multilayer microfluidic spotting device



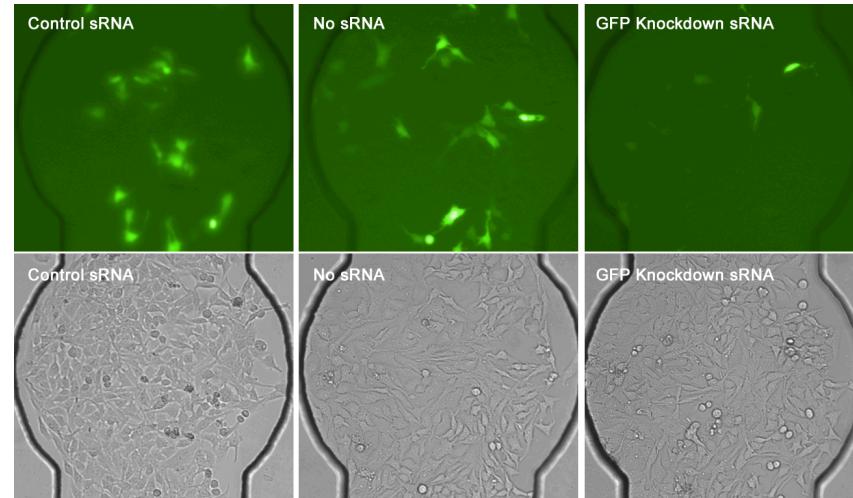
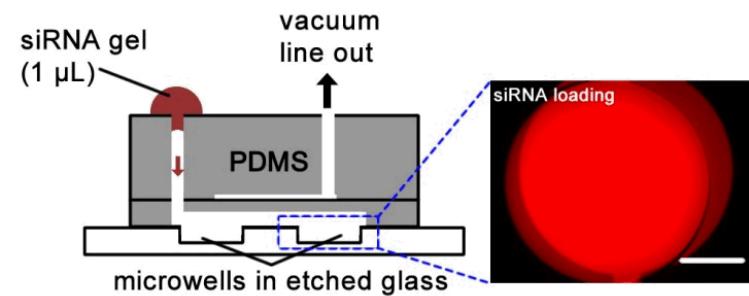
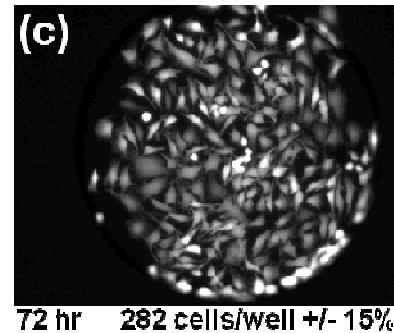
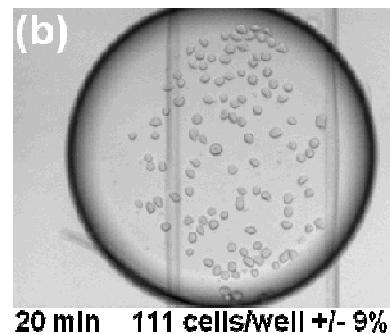
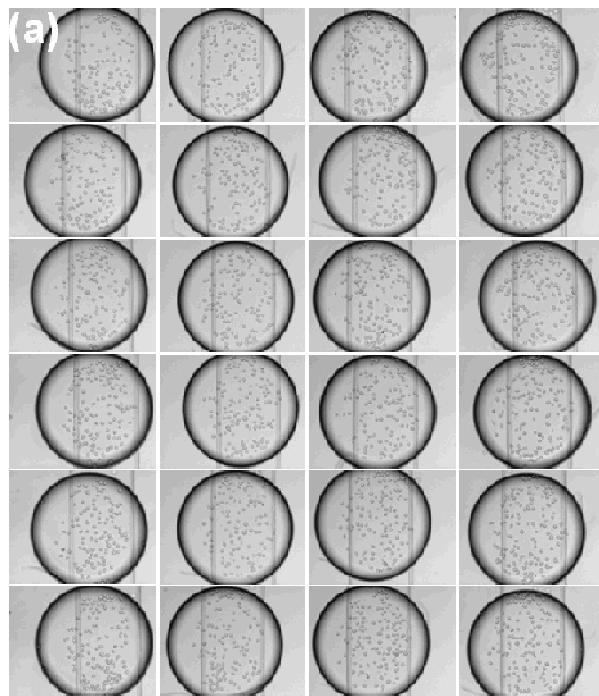
Demonstration of RNAi Spotting Device



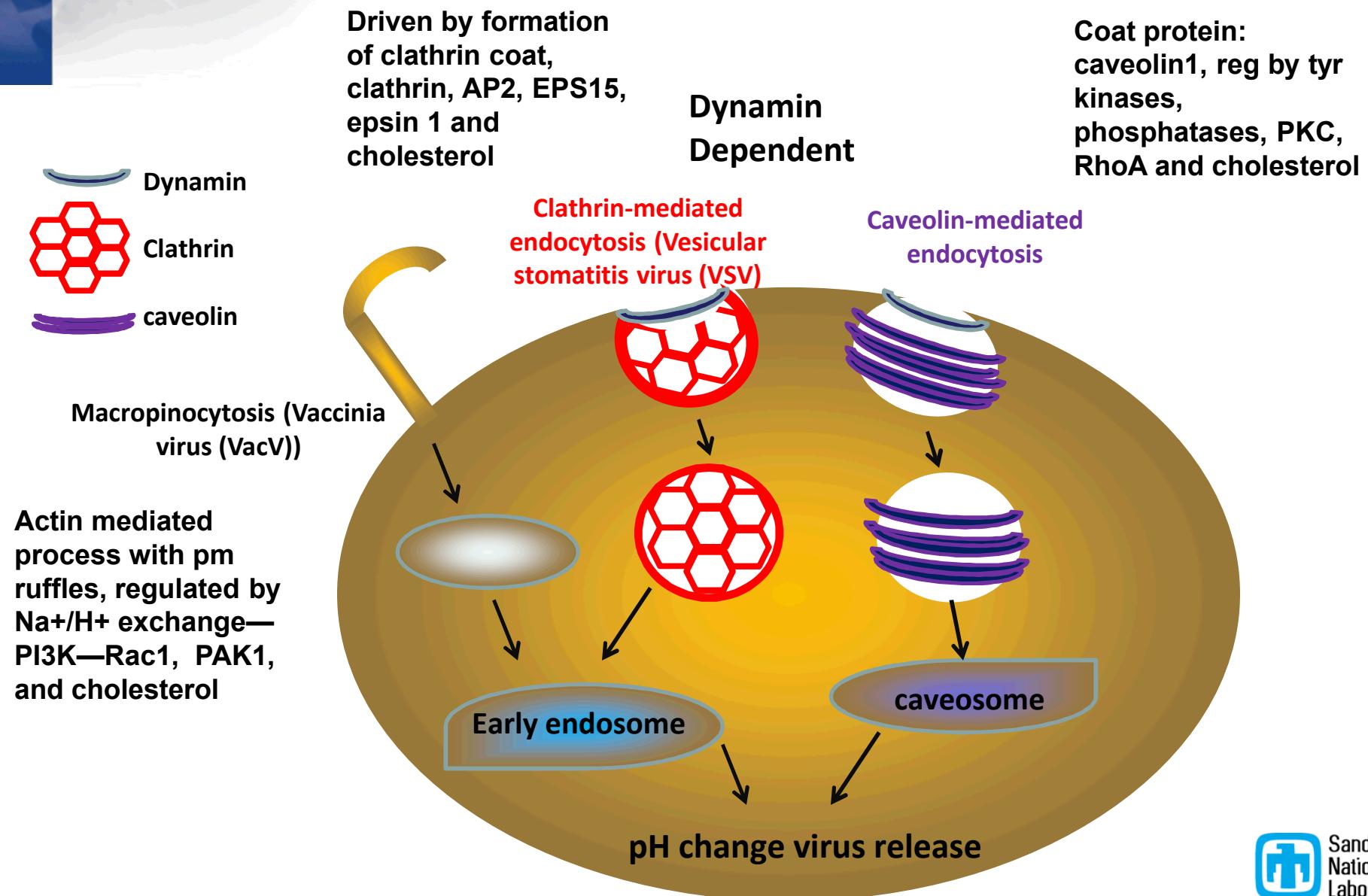
Microfluidic screening using controlled wells



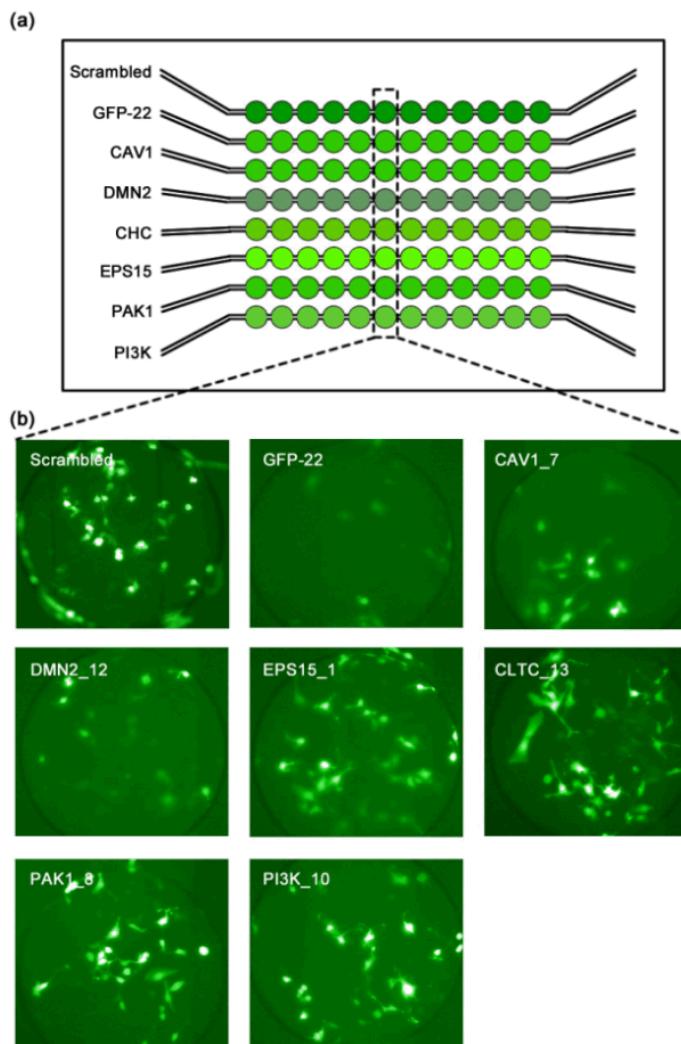
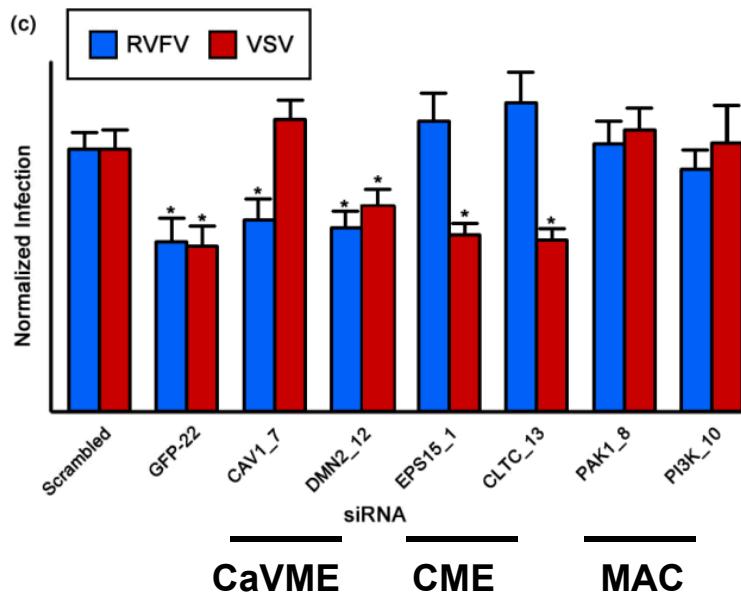
HeLa cells transfected with siRNA demonstrate knockdown properties of GFP associated siRNA analyzed on-chip



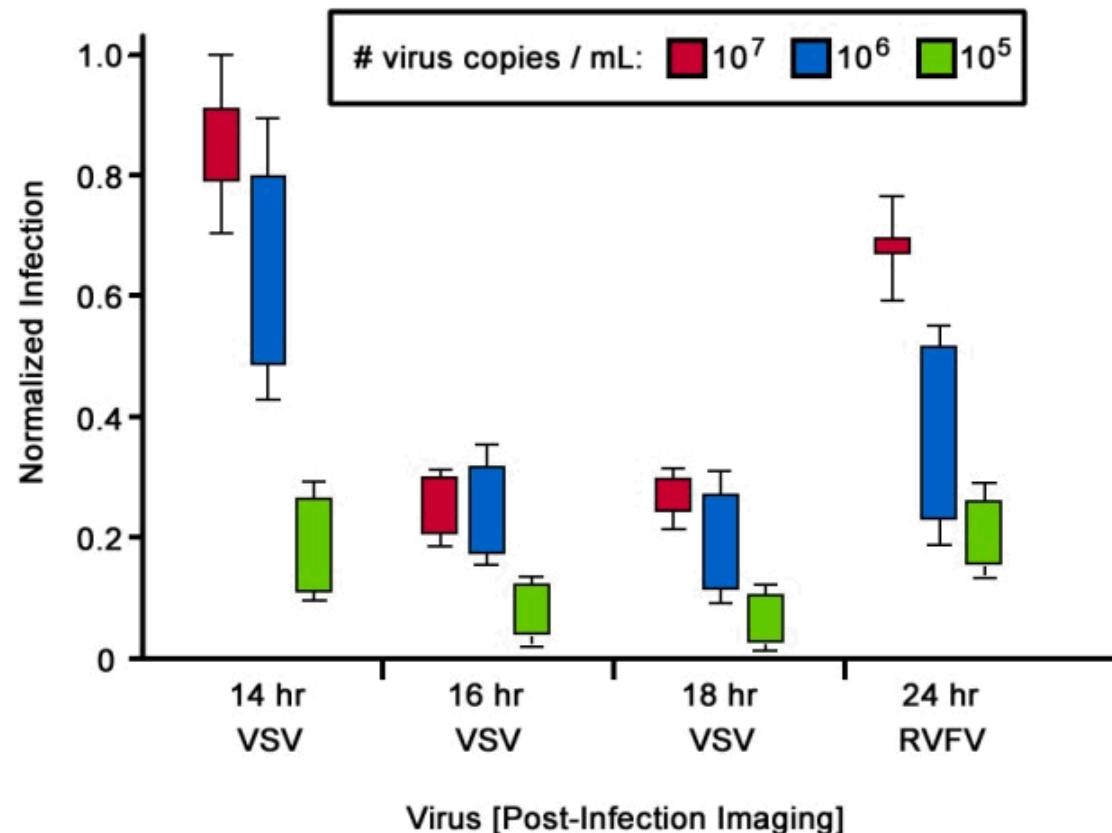
Routes of Virus Entry



Virus Entry Screen: Multiple experiments per siRNA



Sensitivity experiments – bandwidth of GFP response



Current Results / Forward Path

- Assay using RVFV-MP12-GFP can demonstrate knockdown using siRNA in cell culture well plates
- Demonstrated cells on platform being infected by RVFV
- Future work: Demonstrate knockdown of RVFV on chip
- Integrate wells into a microfluidic device, demonstrating cell capture, transfection and knockdown

Acknowledgements



Oscar Negrete
Brooke Harmon
Dianna Maar
Anup Singh
Carrie Kozina
Vinay Abhyankar