Microfluidic devices for genetic screening of positionally-controlled cell cultures

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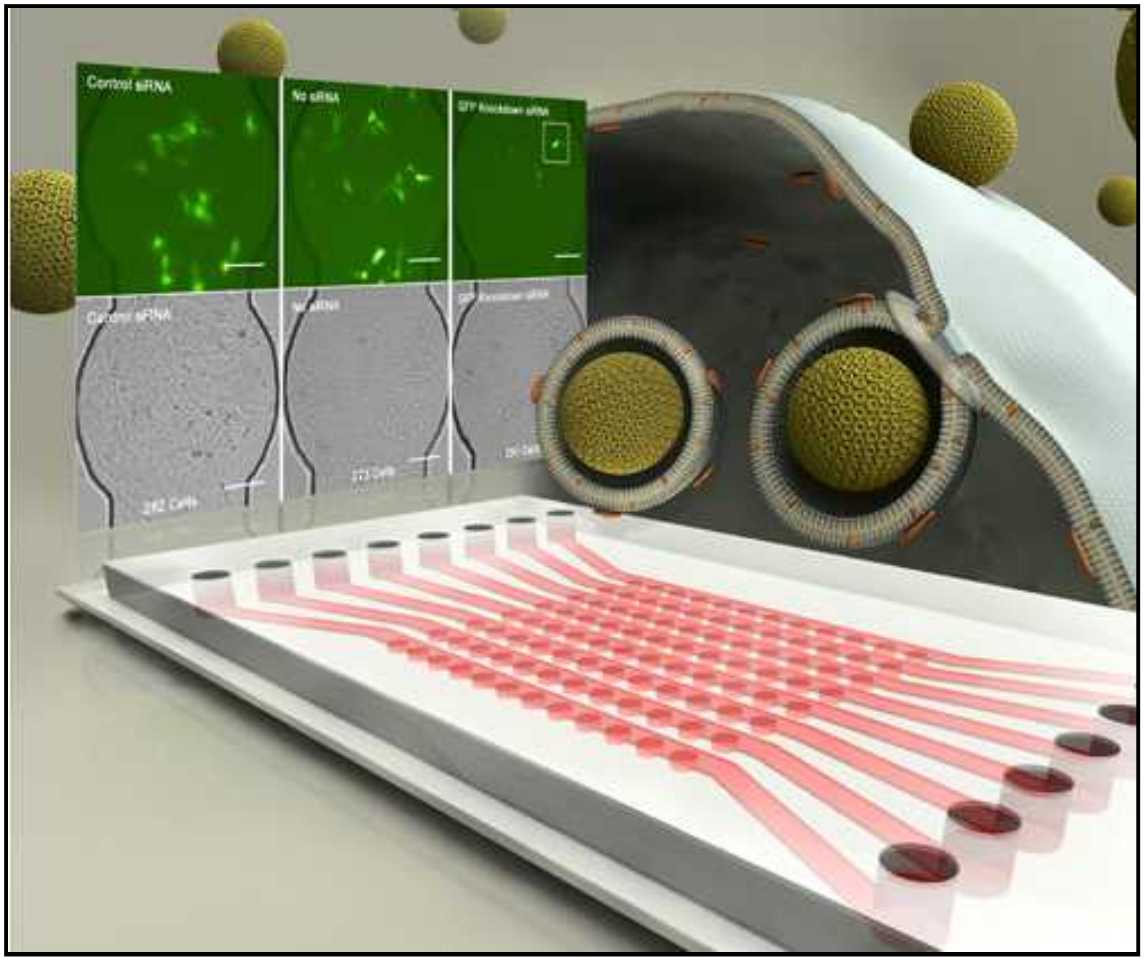

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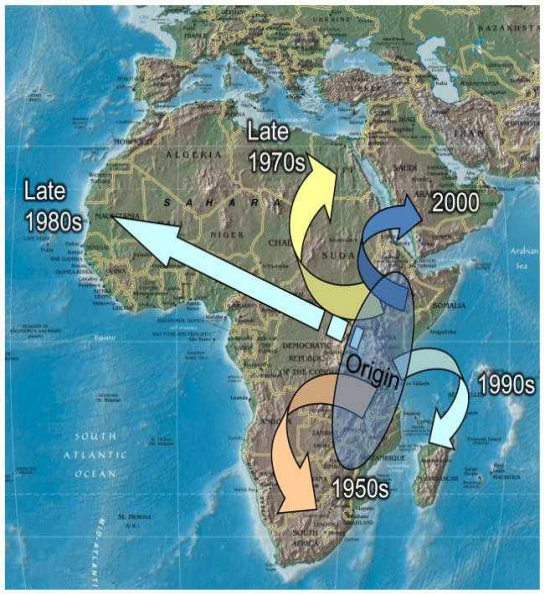
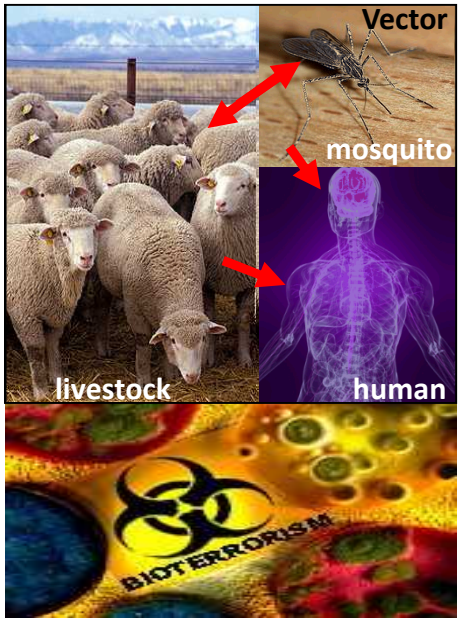
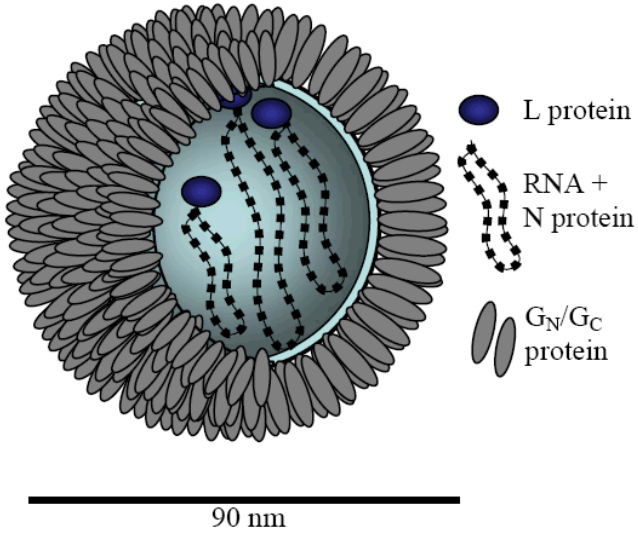
Motivation: Rift Valley Fever virus (RVFV)

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



Weaver S, Antiviral Res. 2010





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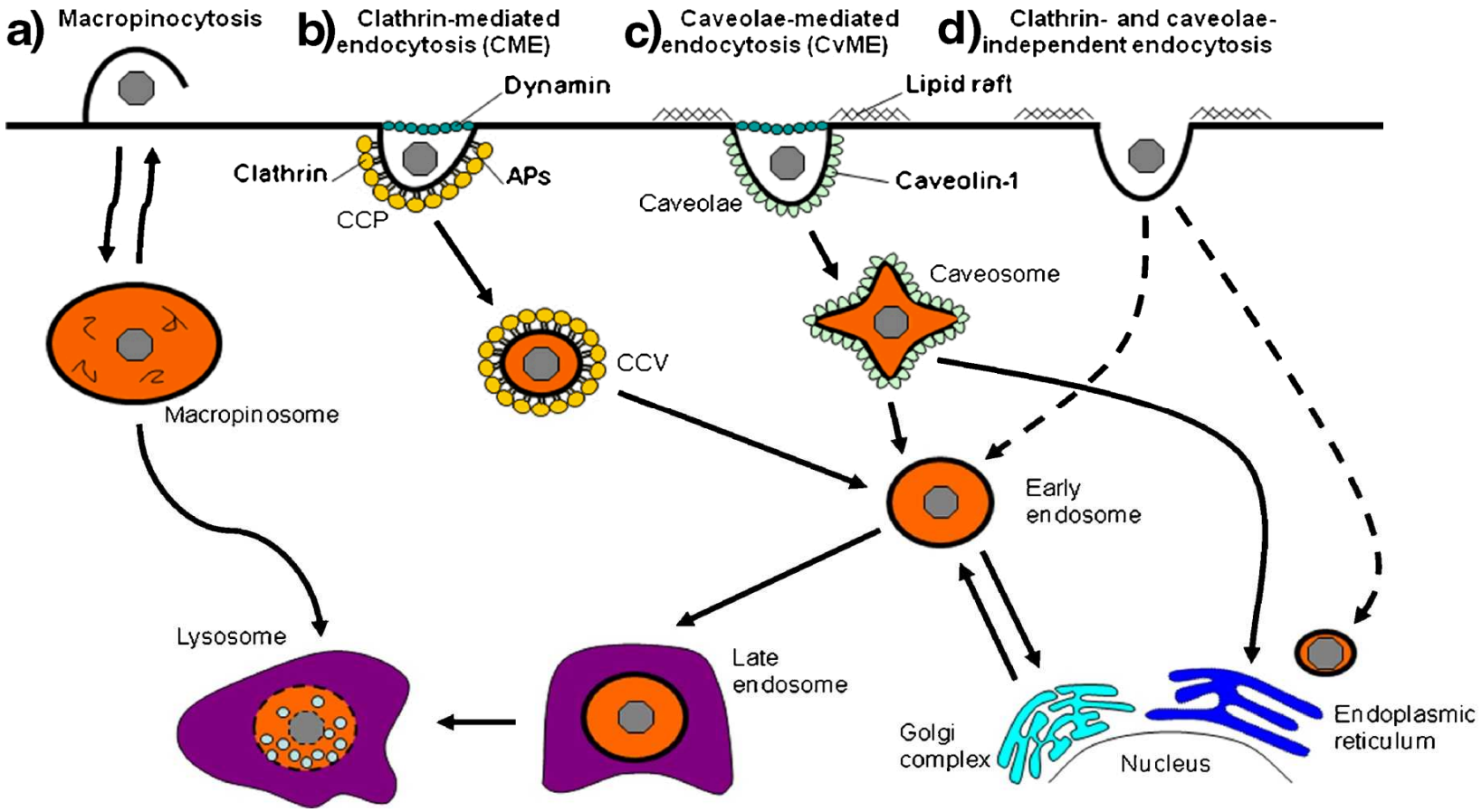
Rift Valley Fever Virus – What we don't know



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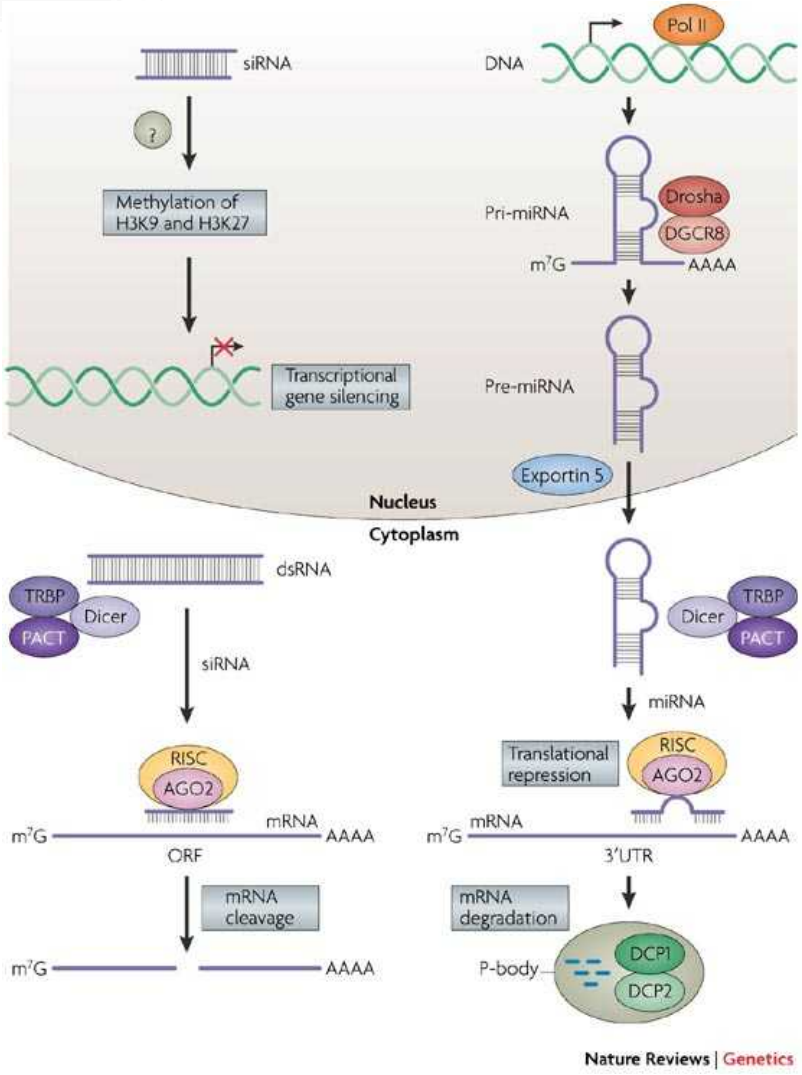
RNA interference (RNAi) is the technique used to knock down a specific gene using small interfering RNA (siRNA)



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.

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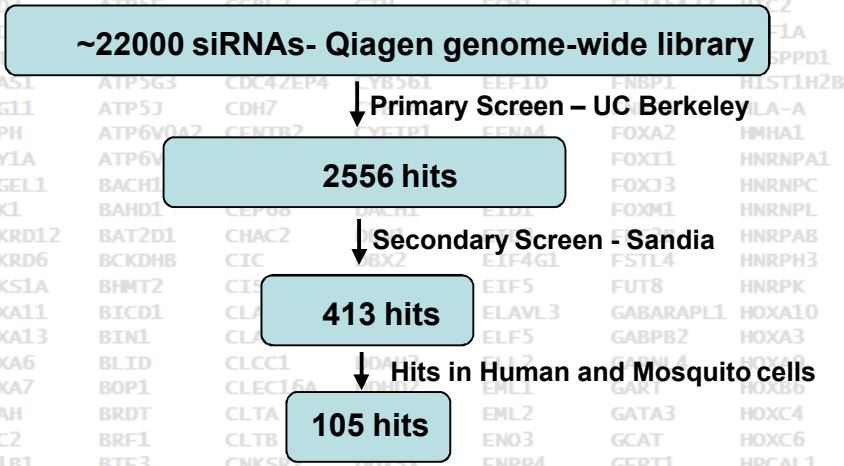
Due to the vast parameter space, smaller and faster screening techniques are desired for cheaper discoveries

Host Factors that inhibit RVFV virus entry and replication



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**.

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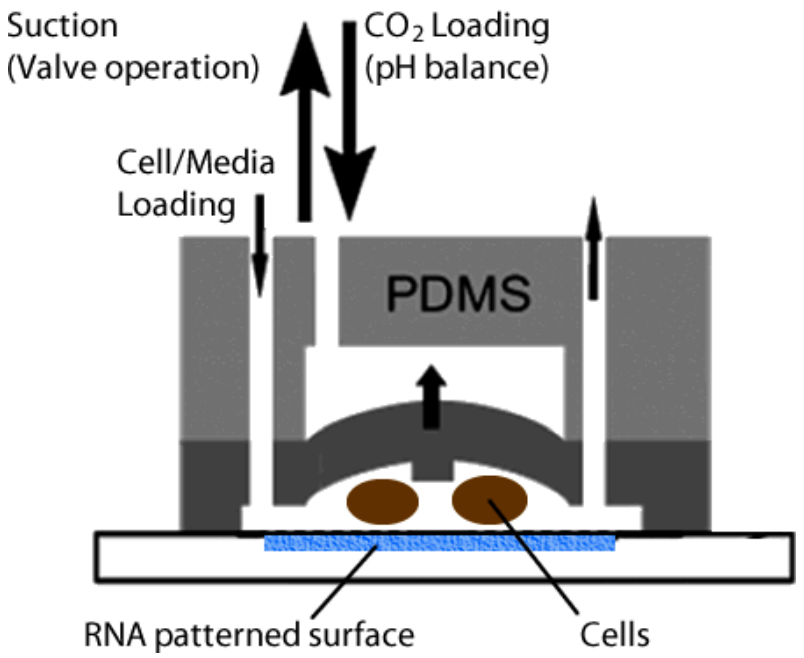
The knockdown process and infection evaluation can take up to four days, which requires a stable cell culture platform

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



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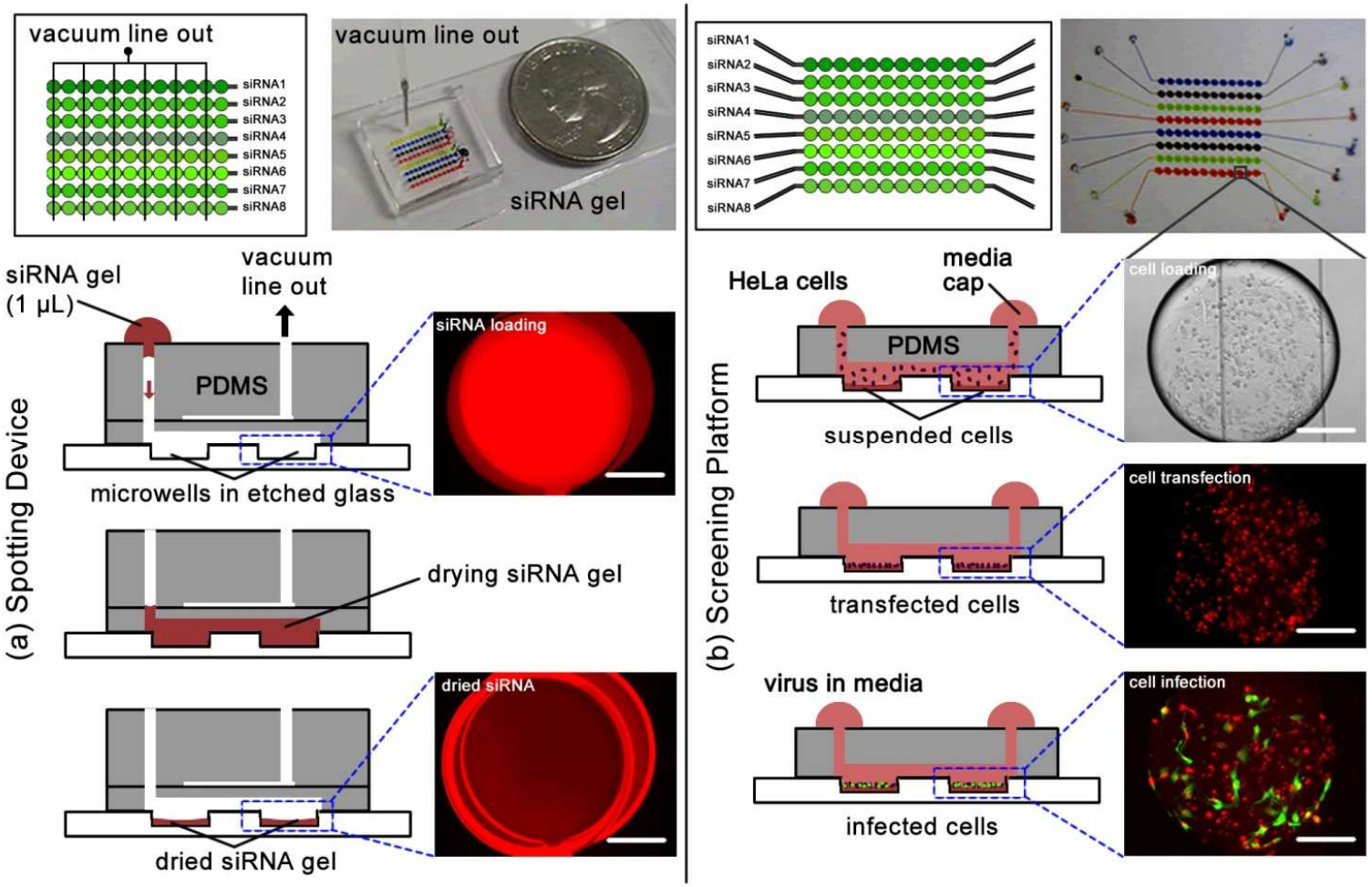
Microfluidic siRNA Screening System: Spotting Device and Screening Platform



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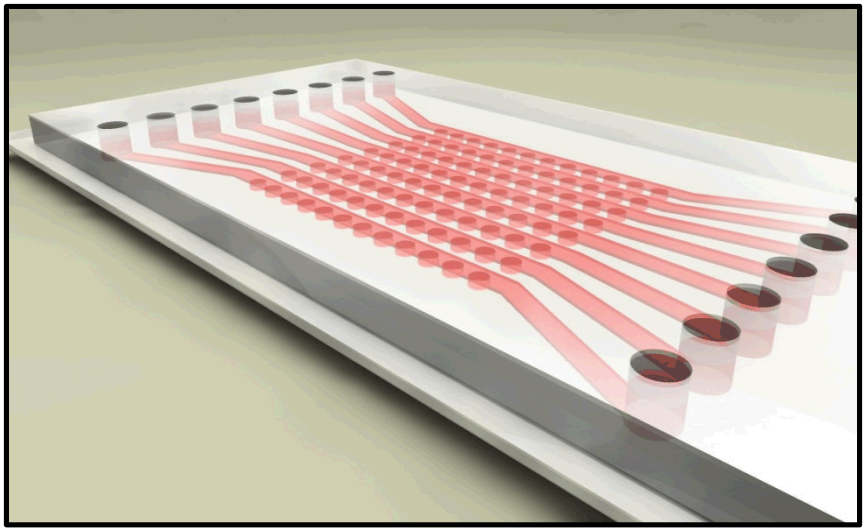
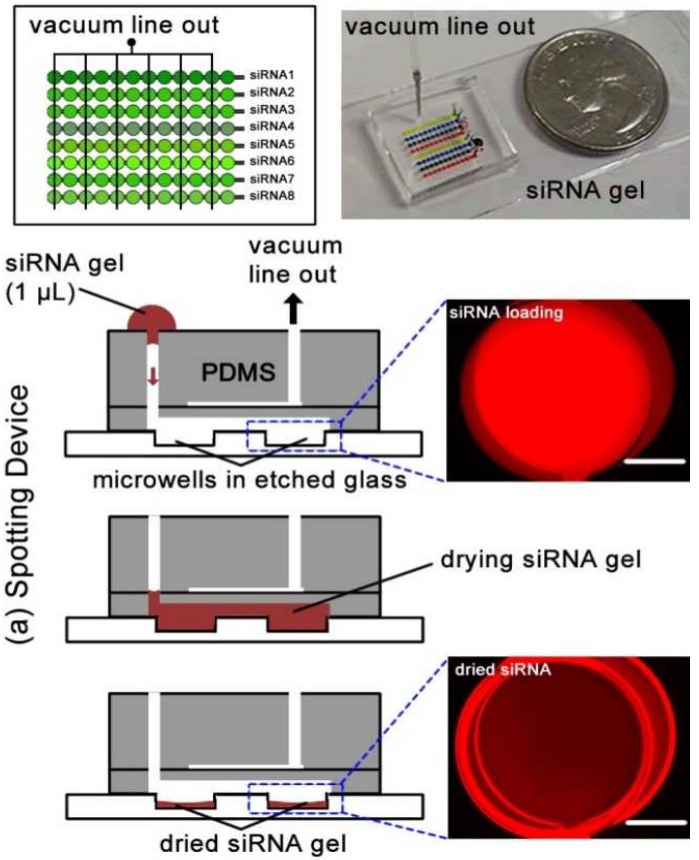
siRNA libraries are patterned using a multilayer microfluidic spotting device





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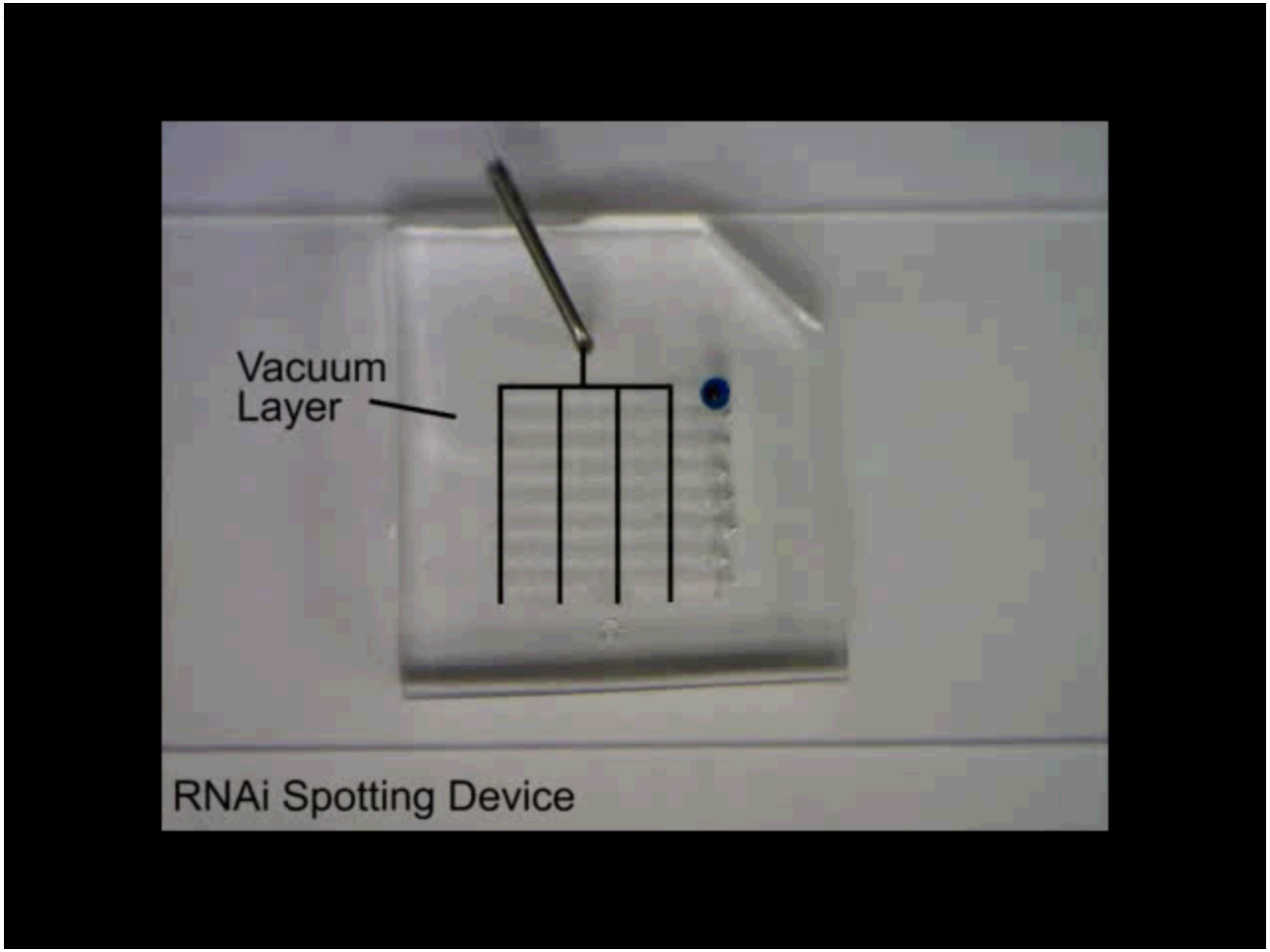
Demonstration of RNAi Spotting Device



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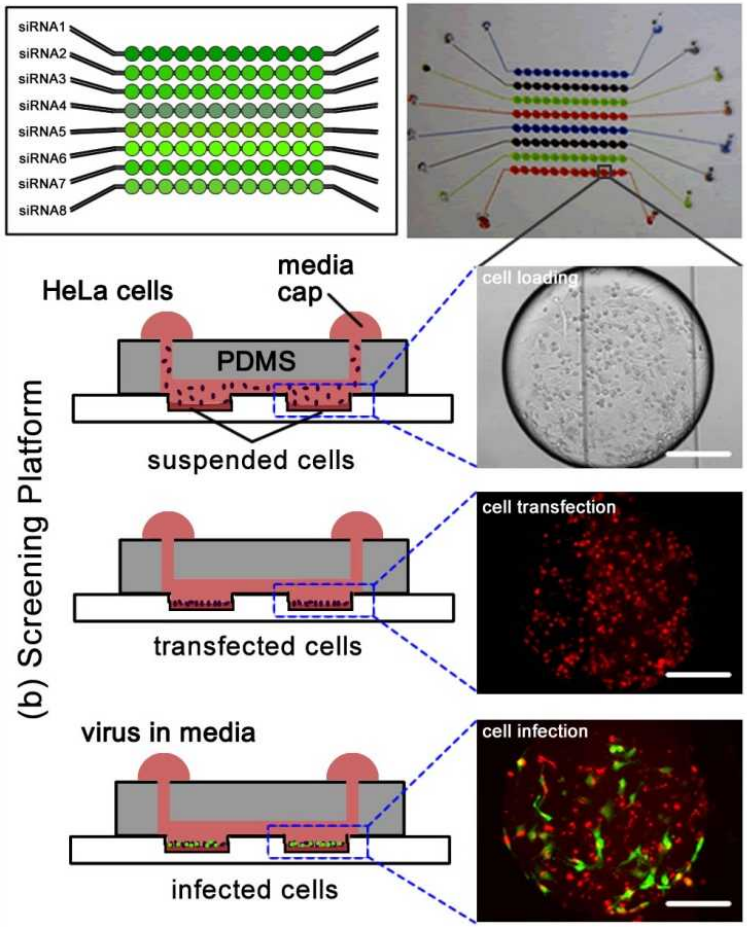
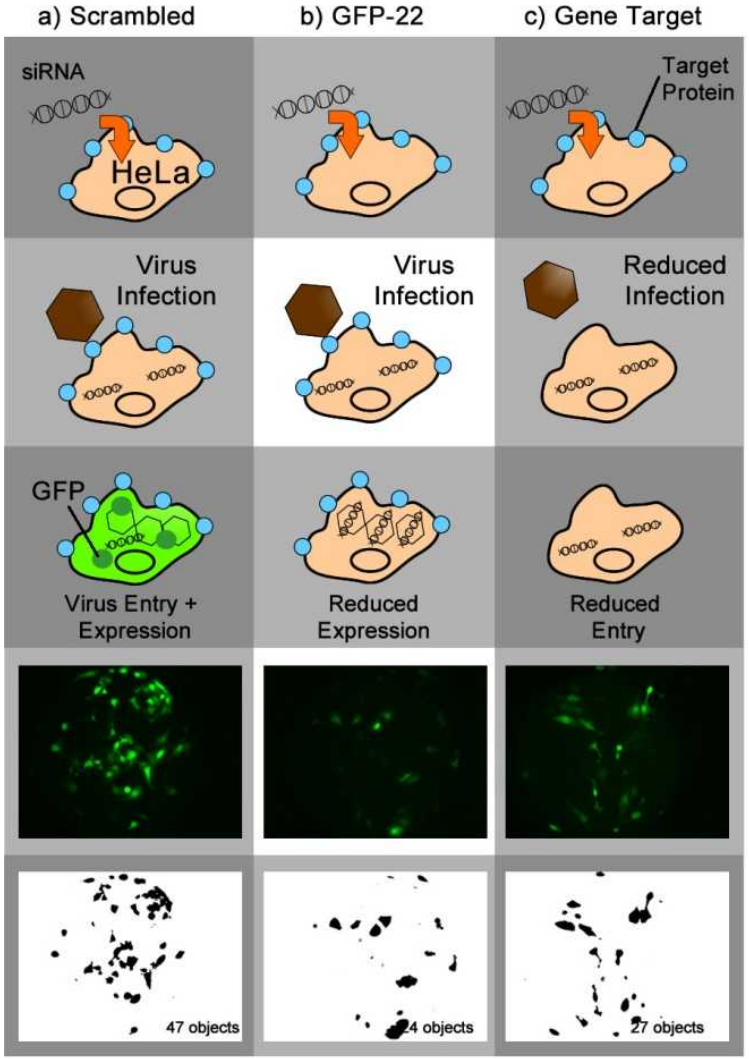
Microfluidic screening using controlled wells





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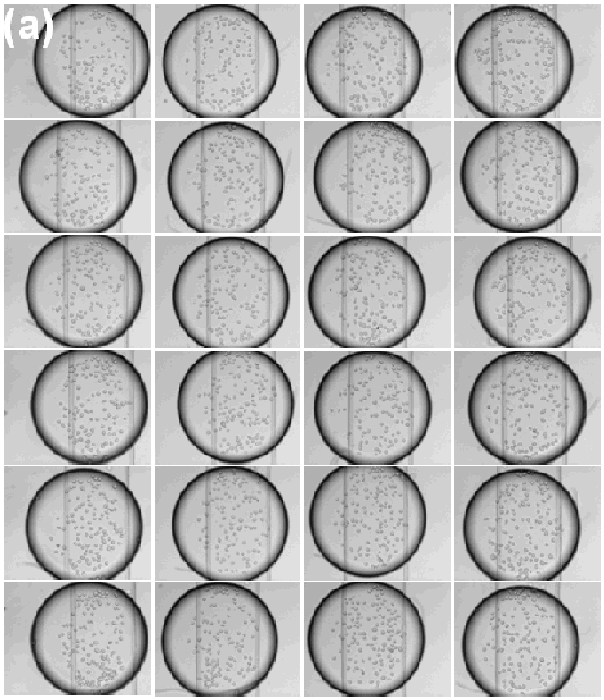
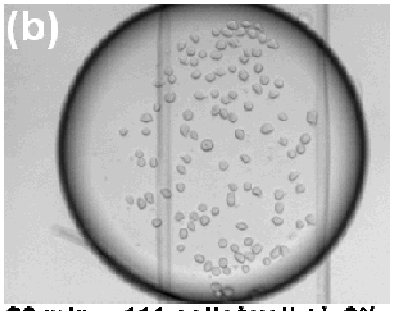
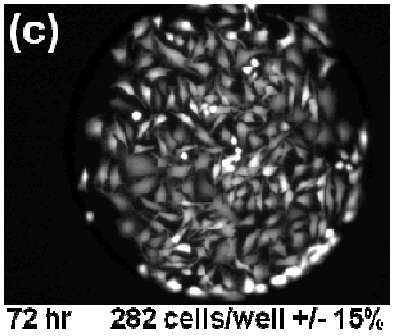
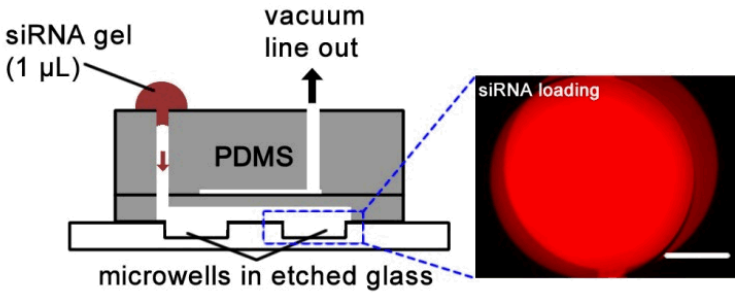
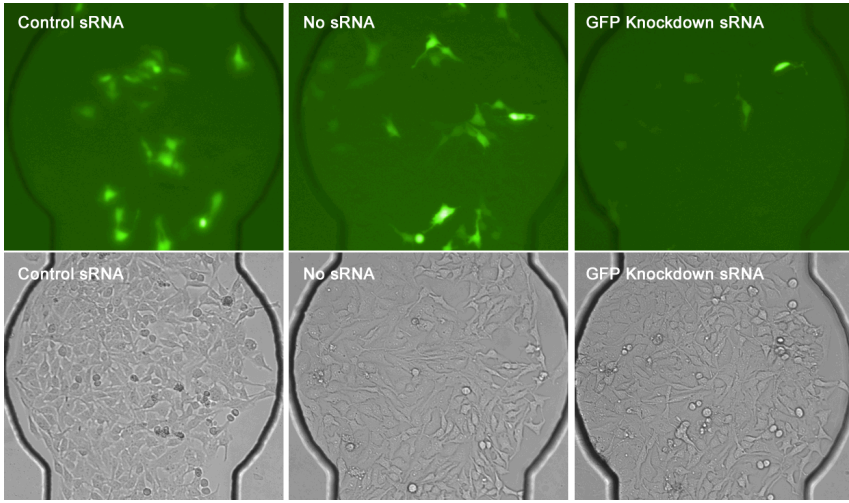
HeLa cells transfected with siRNA demonstrate knockdown properties of GFP associated siRNA analyzed on-chip











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Routes of Virus Entry

Driven by formation of clathrin coat, clathrin, AP2, EPS15, epsin 1 and cholesterol

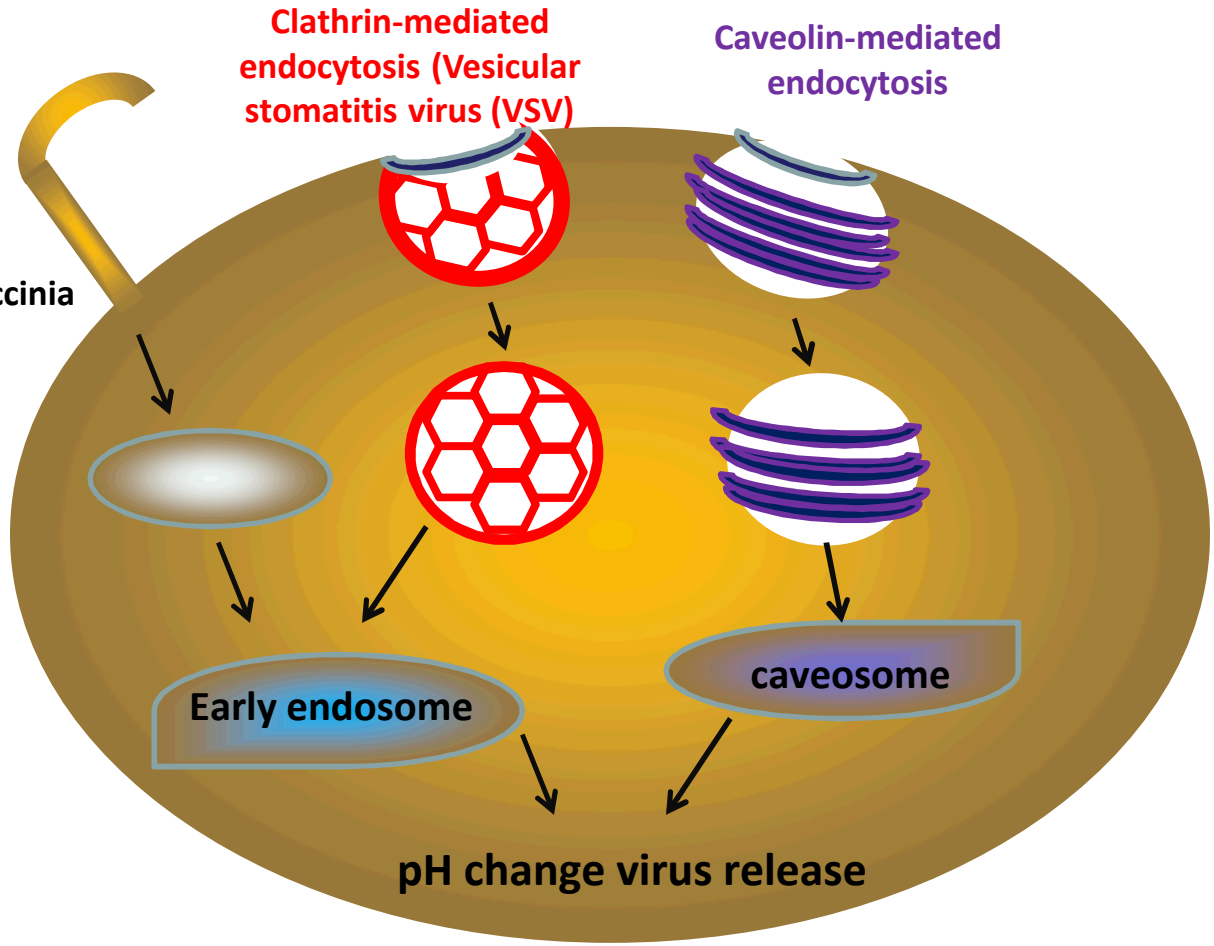
Dynamin Dependent

Coat protein: caveolin1, reg by tyr kinases, phosphatases, PKC, RhoA and cholesterol

Dynamin

Clathrin

caveolin



Actin mediated process with pm ruffles, regulated by Na+/H+ exchange—PI3K—Rac1, PAK1, and cholesterol

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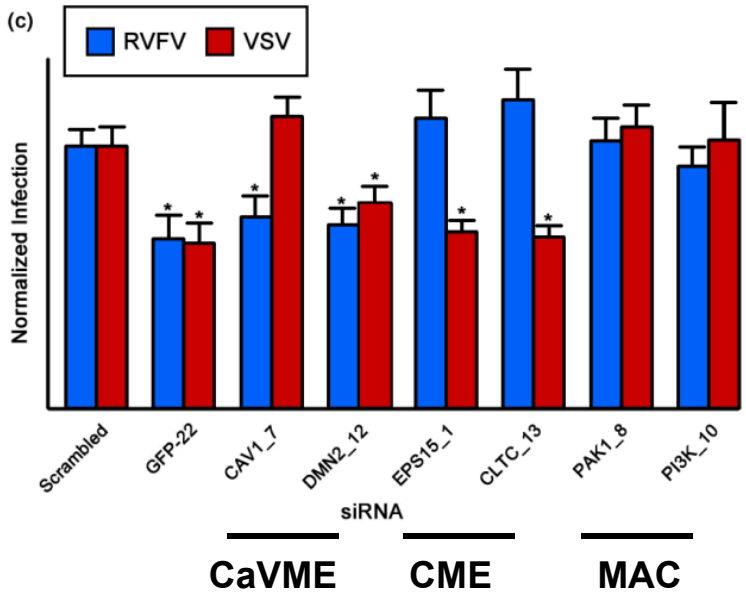
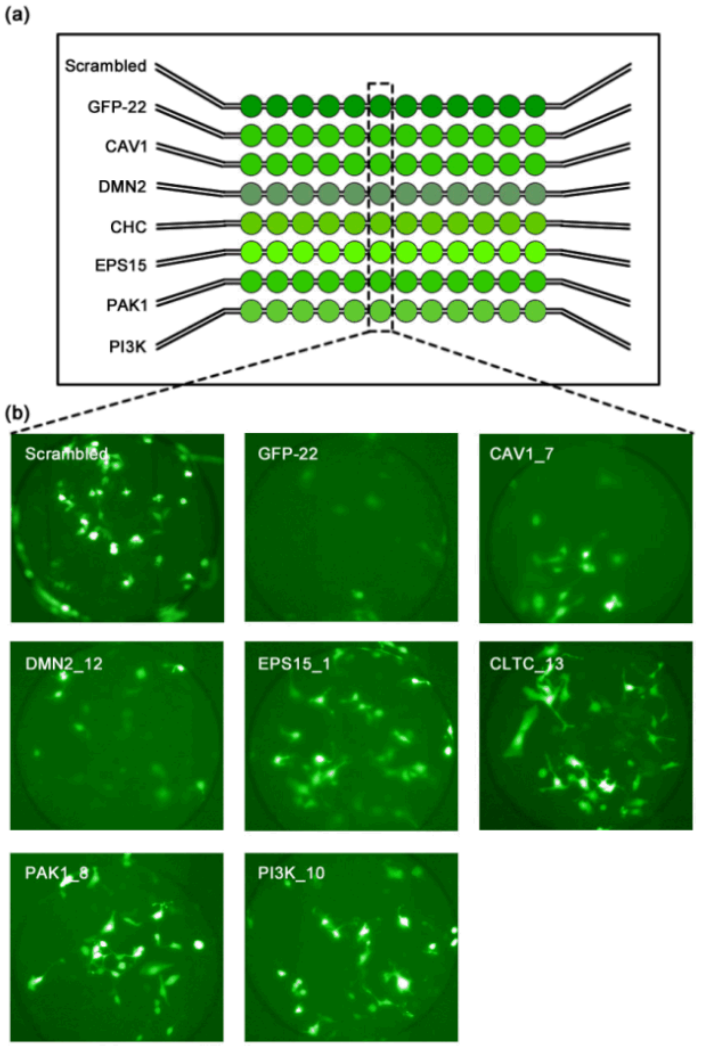
Virus Entry Screen: Multiple experiments per siRNA





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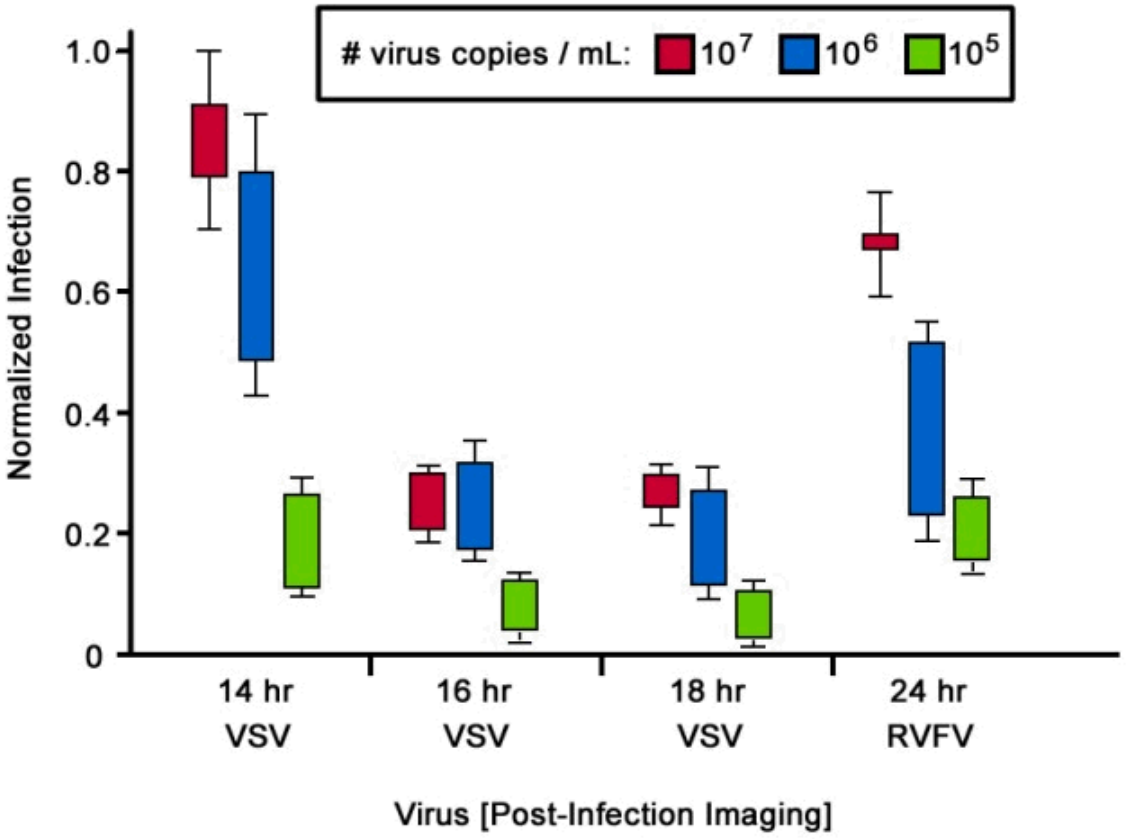
Sensitivity experiments – bandwidth of GFP response



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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

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