A *C. elegans*-Based Foam for Rapid On-Site Detection of Residual Live Virus

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LDRD Day

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Sandia National Laboratories

Clearance Sampling

In the response to and recovery from deliberate or accidental release of biological agents, initial remediation efforts are necessarily followed by tests for presence of residual live bacteria or virus.

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Need for Improved Clearance Sampling Methods

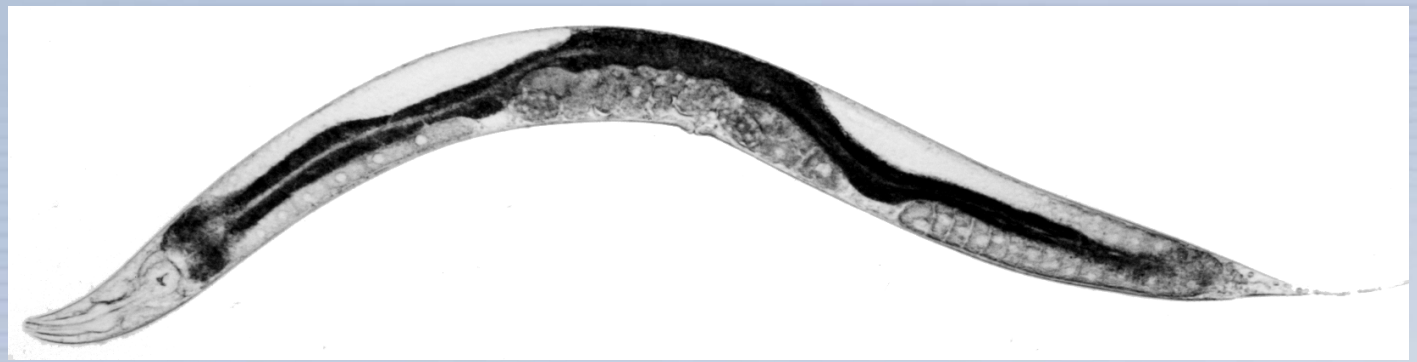
Problems with Current Protocol:

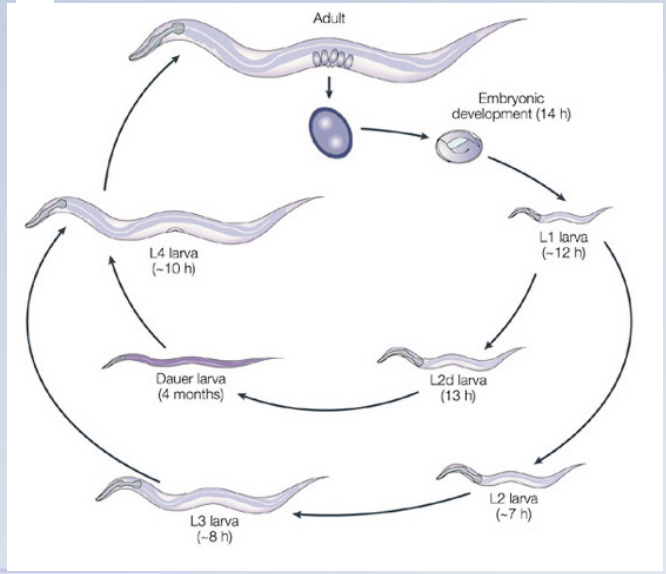
- **Time-intensive, costly, and requires significant laboratory capacity and space**
- **Large numbers of samples may be required to achieve a high degree of statistical certainty in results**
- **Potential loss of sample during collection, transportation to, and processing at laboratory erodes confidence in accuracy of results**
- **Detection of live virus requires evaluation of replication potential within a eukaryotic host**

Proposed novel, on-site method

- Eliminates requirement of taking swab samples and risk of sample loss
- Eliminates requirement for tracking samples
- Reduces time from days to hours
- Reduces cost significantly
- Provides high-degree of statistical certainty in results - cover entire contaminated area
- Amplification mechanism improves detection

Caenorhabditis elegans





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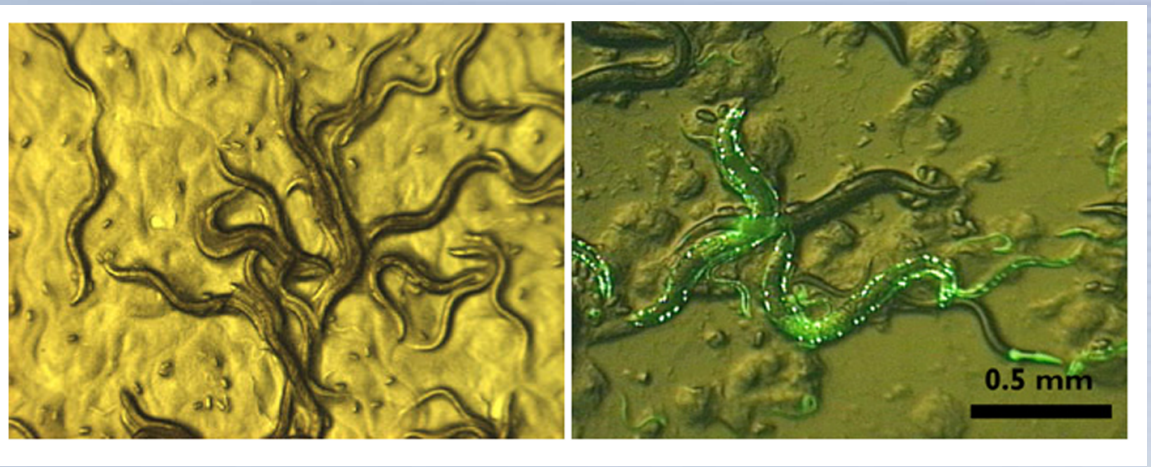
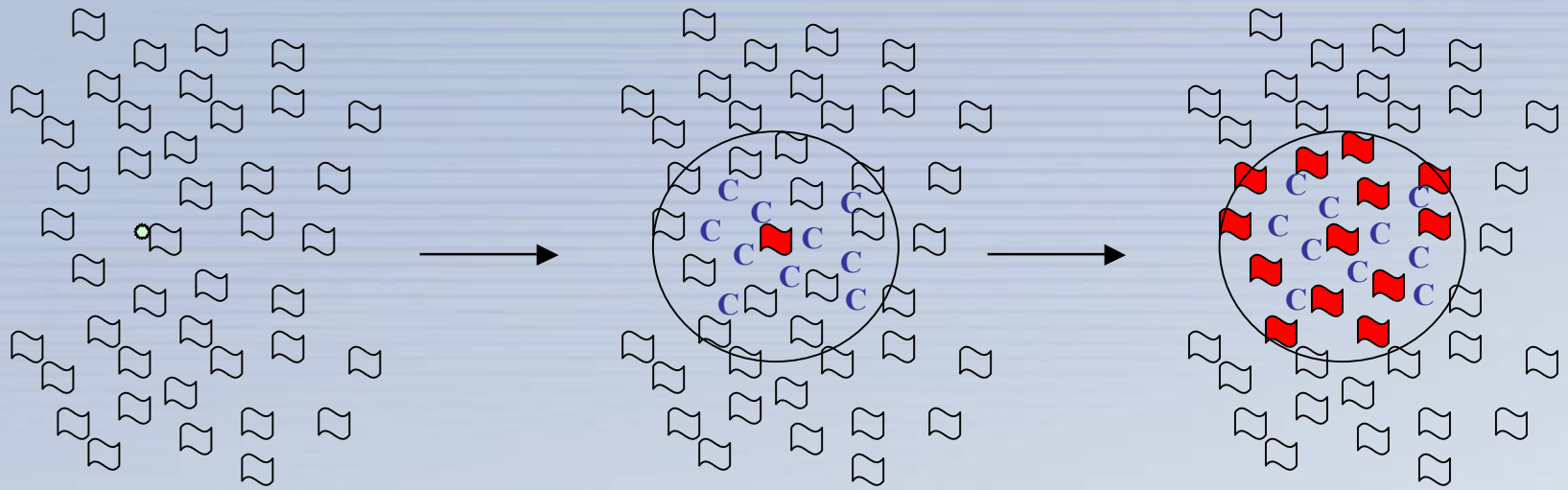
C. elegans – ideal eukaryotic host for on-site clearance sampling



- Non-parasitic soil nematodes, susceptible to viral and bacterial infection
- Fully transparent at all stages of development, such that internal fluorescence can be detected easily
- Easily cultured on either agar plates or in large fluid volumes, and could be suspended in an aqueous-based, oxygen-permeable gel
- Genetically well-characterized, with a plethora of genetic tools available to facilitate generation of fluorescent reporter strains

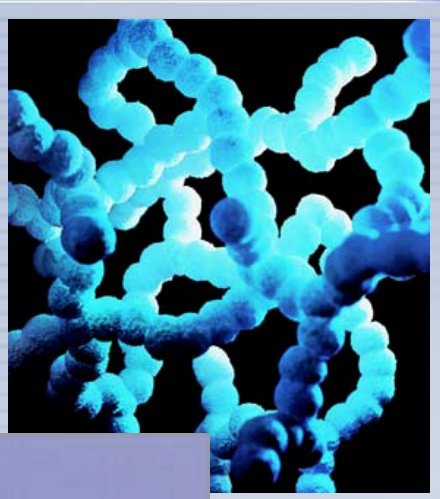
Overall Strategy

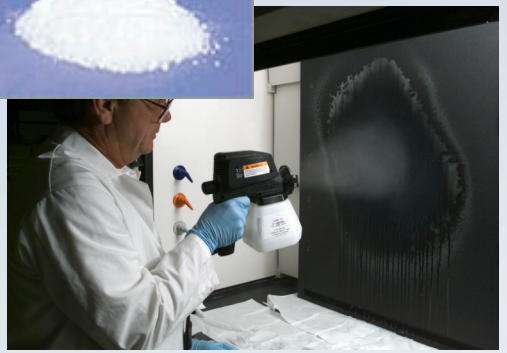


1. *C. elegans* ( ) encounters live virus ( )
2. Viral infection triggers production of RFP and Cre recombinase
3. RFP signal amplification

C. elegans Embedded in Gel

- Gel will be constructed with non-toxic, non-corrosive ingredients (silica gel, polymers, etc.)
- Gel will be deployed through off-the-shelf equipment (e.g., paint sprayers)
- Gel will contain wetting agents to help penetrate into cracks and crevices
- Gel will remain in position on all surfaces (horizontal, downward facing, vertical) for several hours
- Gel will be easy to clean-up by vacuuming, brushing, or drying up
  - Will incorporate crystallizing polymers (similar to carpet cleaners) that will cause the gel to dry to small, non-sticky particles that will not adhere to a surface





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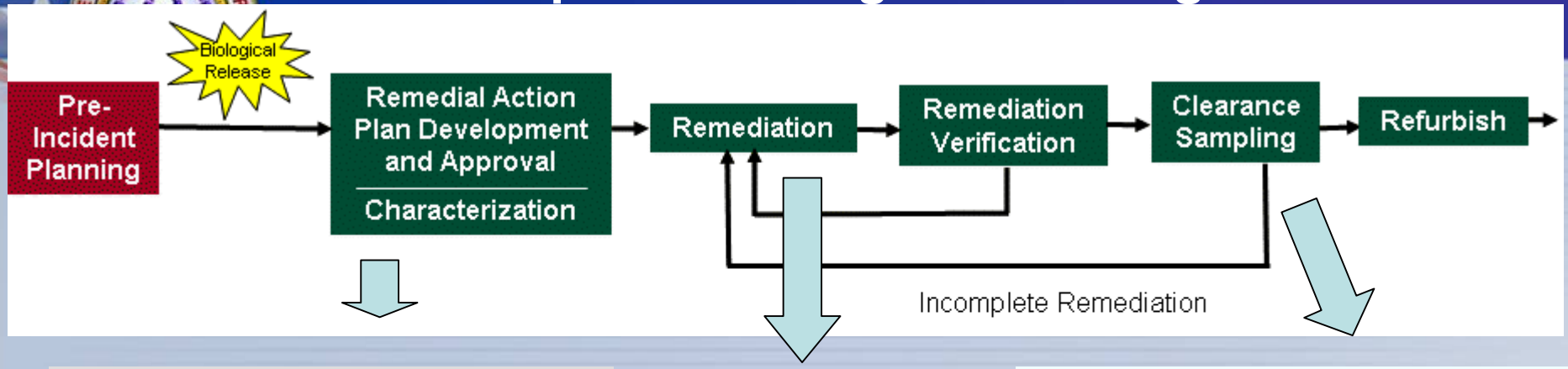
Con-Ops for *C. elegans*-based gel



In-situ approach

- *C. elegans*-based gel sprayed onto surfaces
- Wait defined number of hours
- Scan surface for fluorescent signal
- Clean-up gel

Ex-situ approach

- Samples collected from surface and taken to lab
- Samples exposed to *C. elegans*-based gel
- Scan sample for fluorescent signal

Decontamination may or may not be required following the release of a virus

In-situ approach

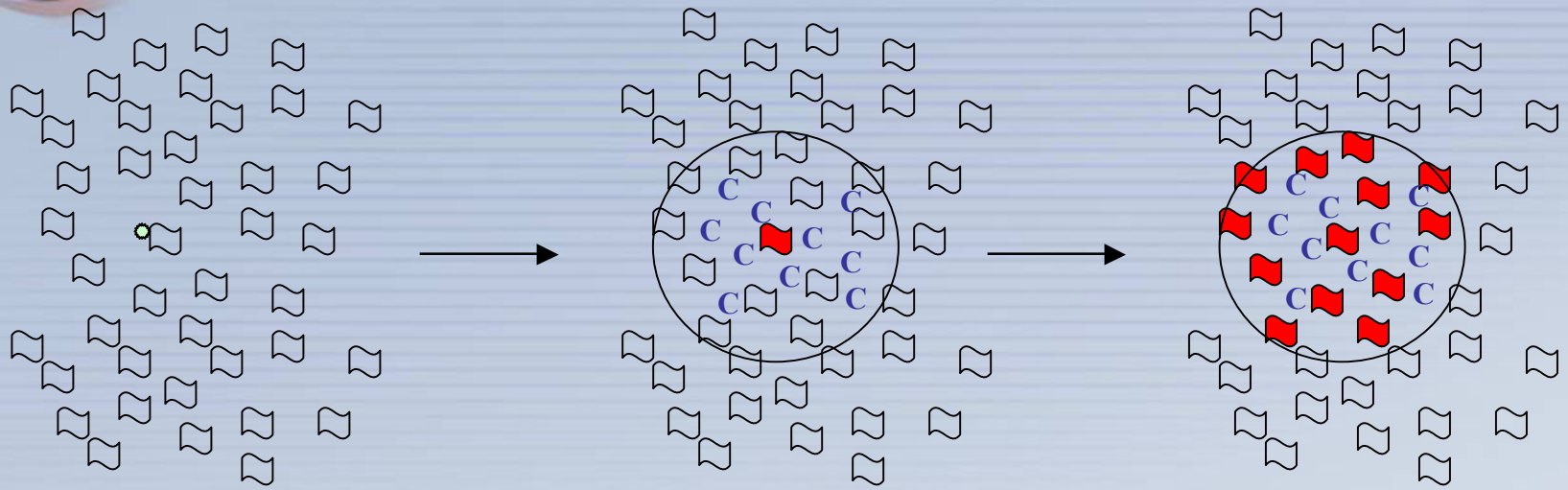
- *C. elegans*-based gel sprayed onto surfaces
- Wait defined number of hours
- Scan surface for fluorescent signal
- Clean-up gel

Ex-situ approach

- Samples collected from surface and taken to lab
- Samples exposed to *C. elegans*-based gel
- Scan sample for fluorescent signal

The *C. elegans*-based gel could be utilized in both the characterization and clearance phases of the restoration process even if remediation is not required.

Project Requirements



1. Strains of *C. elegans* susceptible to viral infection
   - Increase general susceptibility to viral infection
   - Express exogenous viral receptors for specific susceptibility
2. Mechanism for cellular detection of viral infection
3. Mechanism to amplify signal to adjacent cells and nematodes
4. Gel in which to embed *C. elegans*

NIAID Category A Viral Pathogens

Table 1: NIAID Category A Viral Pathogens

Disease	Family	Virus	Type	Replication Site in Host	BSL2 Strain	Tagged Strain
Hemorrhagic fever	Arenaviridea	LCM	ssRNA	Cytoplasm	Yes	No
Hemorrhagic fever	Arenaviridea	Junin	ssRNA	Cytoplasm	Yes	No
Hemorrhagic fever	Arenaviridea	Machupo	ssRNA	Cytoplasm	No	No
Hemorrhagic fever	Arenaviridea	Guanarito	ssRNA	Cytoplasm	No	No
Hemorrhagic fever	Arenaviridea	Lassa	ssRNA	Cytoplasm	No	No
Hemorrhagic fever	Bunyaviridea	Hantaviruses	ssRNA	Cytoplasm	No	No
Hemorrhagic fever	Bunyaviridea	Rift Valley Fever	ssRNA	Cytoplasm	Yes	Yes
Hemorrhagic fever	Flaviviridae	Dengue	ssRNA	Cytoplasm	Yes	No
Hemorrhagic fever	Filoviridae	Ebola	ssRNA	Cytoplasm	No	No
Hemorrhagic fever	Filoviridae	Marburg	ssRNA	Cytoplasm	No	No
Smallpox	Poxviridae	Variola major	dsDNA	Cytoplasm	Yes	Yes

Selected Viral Pathogens for Study

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Rift Valley Fever Virus (RVFV) MP12

Vaccinia Virus (VacV)

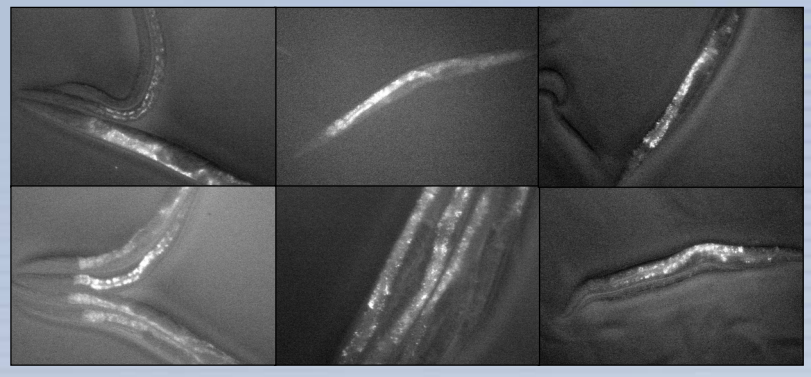
Vesicular Stomatitis Virus (VSV)

Need for Red Fluorescent Virus Due to High Levels of Autofluorescence

rVSV-GFP

-

+



Fluorescence emission from *C. elegans* homogenate

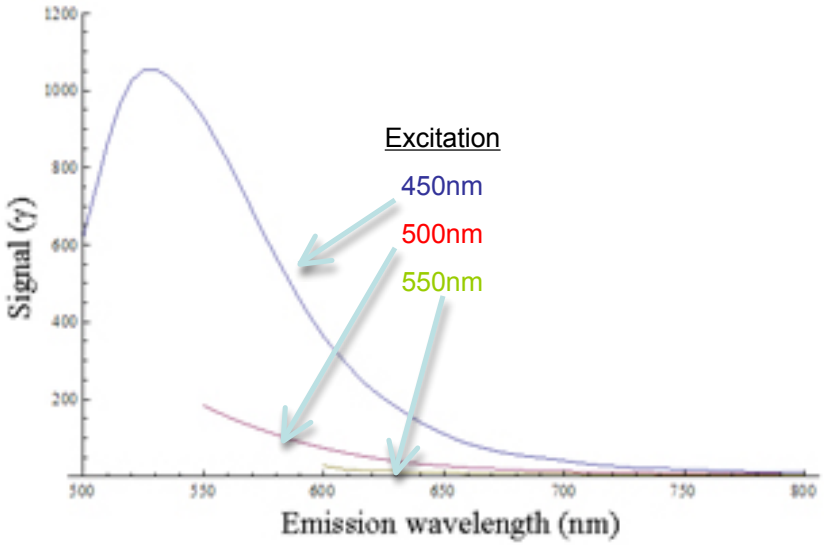
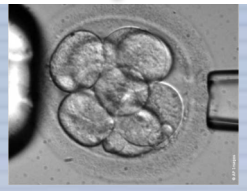


Figure 2: Fluorescence of *C. elegans* homogenate: Excitation at 450 (blue line), 500 (red line) and 550 (yellow line) nm showing reduction in signal 600nm and longer.

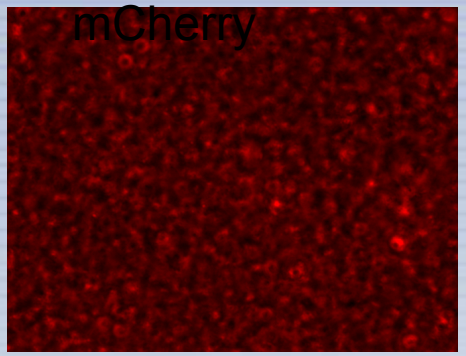
Recombinant Viruses Generated from Genomic Elements

Virus	Type	Single cycle or Infectious	Abbreviation
Vesicular stomatitis virus	RNA	Infectious	rVSV-mCherry
Vesicular stomatitis virus	RNA	Single cycle	rVSVΔG-mCherry
Vesicular stomatitis virus	RNA	Single cycle	rVSVΔG-NiVFG-mCherry
Vaccinia virus	DNA	Infectious	rVacV-mCherry
Rift Valley Fever Virus	RNA	Infectious	rRVFV-MP12-mCherry

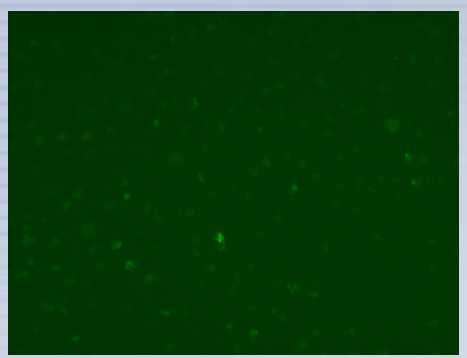
RFVF-MP12, VacV and VSV All Infect *C. elegans* Cells



rVacV-mCherry

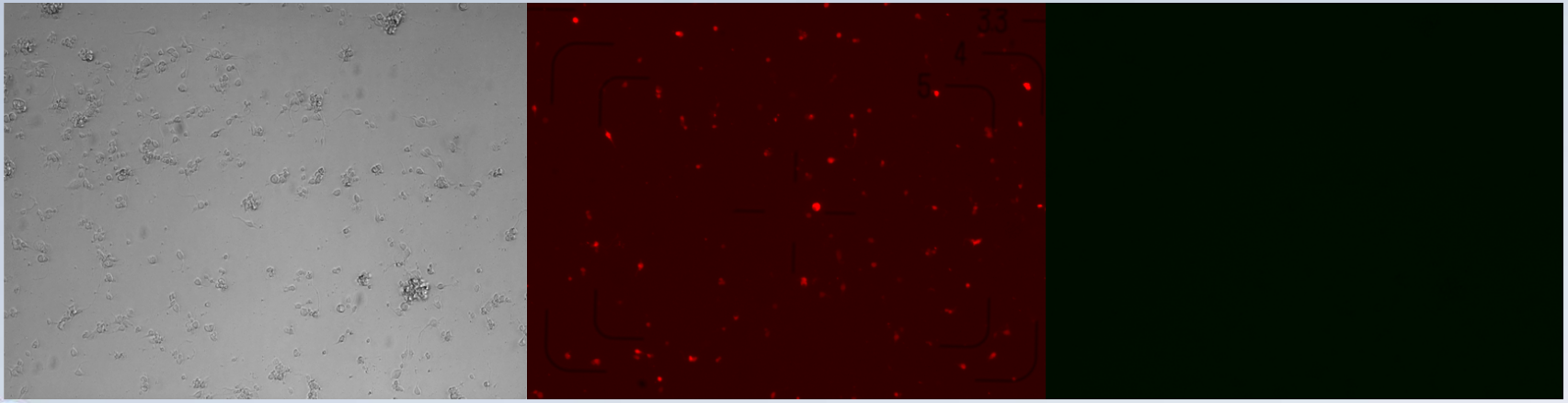


rRFVF-MP12-GFP



rVSV-mCherry

uninfected



C. elegans Mutants Screened with rVSV-mCherry

DC strains from Creg Darby, UCSF

strain	genotype
DC1	bah-1(br1)
DC1032	bus-4(br4);him-5(e1490)
DC1033	bus-12(br5);him-5(e1490)
DC1043	bah-2(br7);him-5(e1490)
DC1045	bah-1(br1);him-5(e1490)
DC1046	srf-2(br10);him-5(e1490)
DC1048	srf-3(br6);him-5(e1490)
DC1062	bah-3(br9);him-5(e1490)
DC1156	bah-4(br25);him-5(e1490)
DC19	bus-5(br19)
DC2	bus-17(br2)
DC20	br20
DC23	br23ts
DC24	br24dm
DC7	bah-2(br7)
DC9	bah-3(br9)
CB6055	bus-8(e2698) X
MT1522	ced-3(n717)
MT2405	ced-3(n717) unc-26(e205)
MT2547	ced-4(n1162)
MT2550	unc-79(e1068) ced-4(n1162)
N2	wild type
WM27	rde-1(ne219)
WM49	rde-4(ne301) III
MT2495	lin-15B (n744)
MT8189	lin-15B (n765)
CB6430	sqt-3(e2924)
BE3	sqt-2(sc3)
BE1	sqt-1(sc1)
CB24	sqt-3(e24)
CB1350	sqt-1(e1350)
BE16	bli-6 (sc16)
CB1255	vab-11 (e1255)
CB3241	clr-1 (e1745) 15C temp sens
CB518	bli-5 (e518)
CB767	bli-3 (e767)
CB769	bli-1 (e769)
ML514	che-14 (ok193)
CB3687	che-14 (e1960)

***rde* - mutations in RNAi genes**

***sqt* - mutations in collagen genes**

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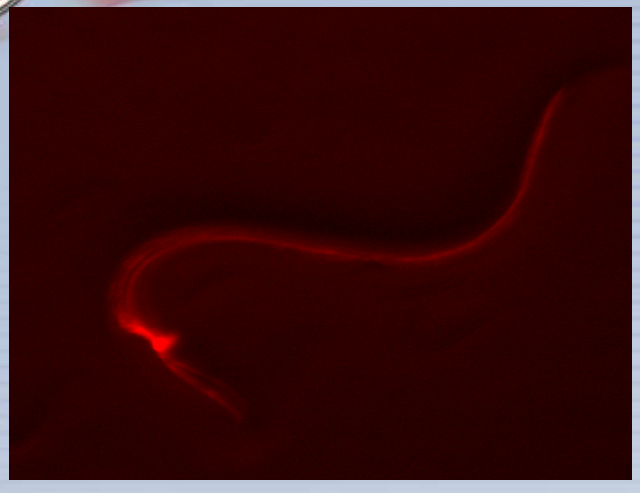
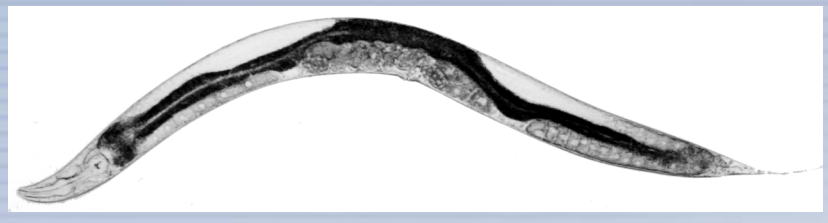
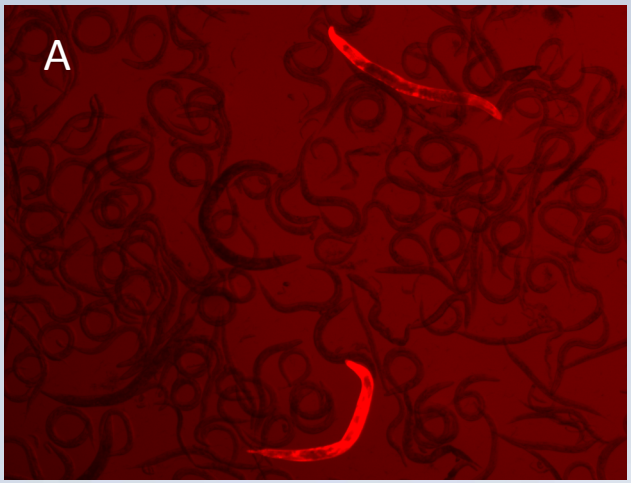
rVSV-mCherry Infects Intact *C. elegans*

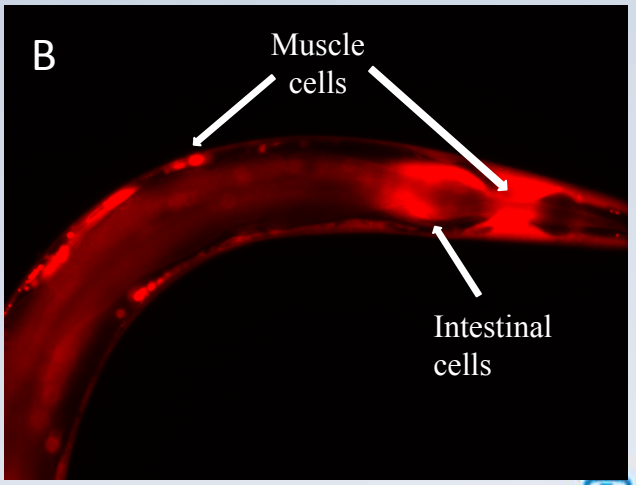




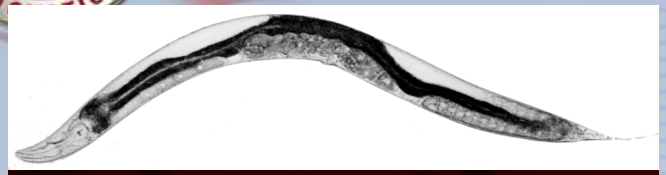
Chitinase/collagenase treatment 2 hrs 26o,10KPsi
Virus added with 2% DMSO
First screen 16 hrs. post infection

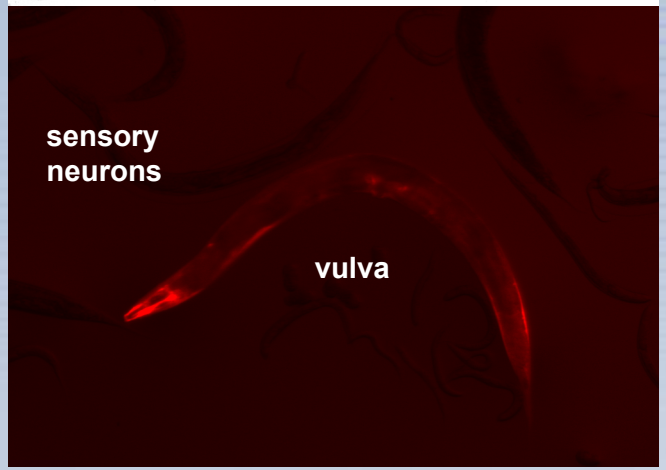
24 hours

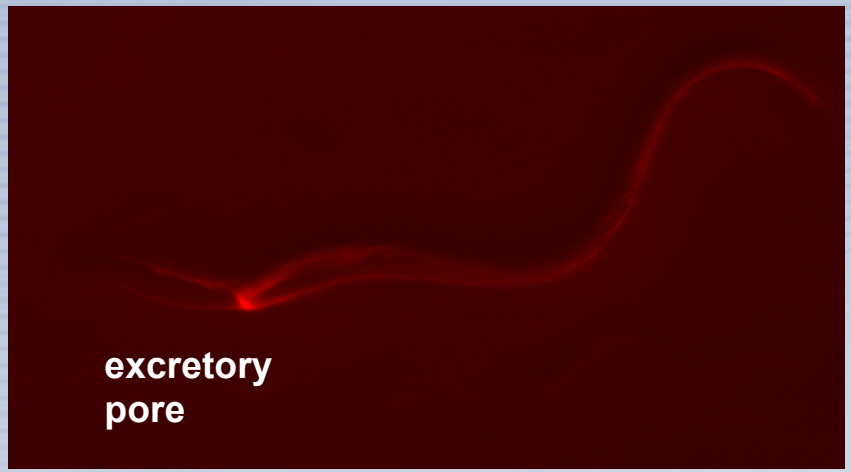


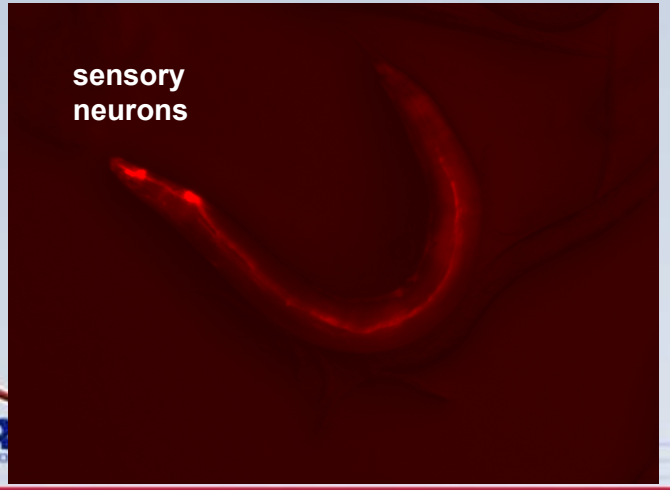


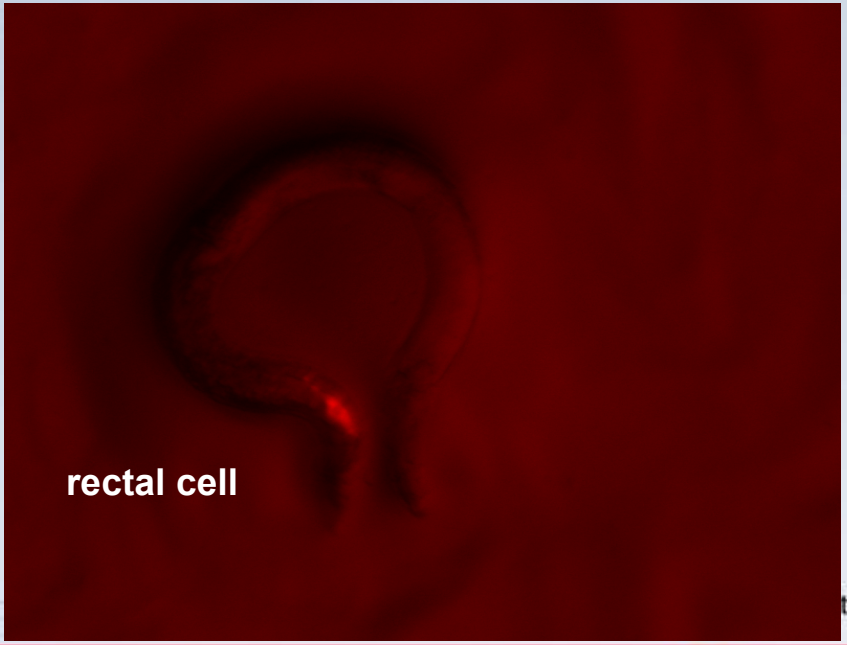
Initial Viral Infection Observed in Specific Cells



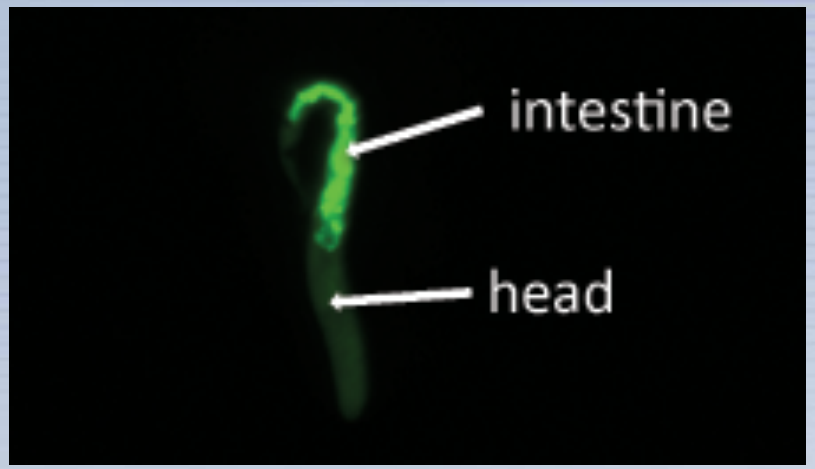






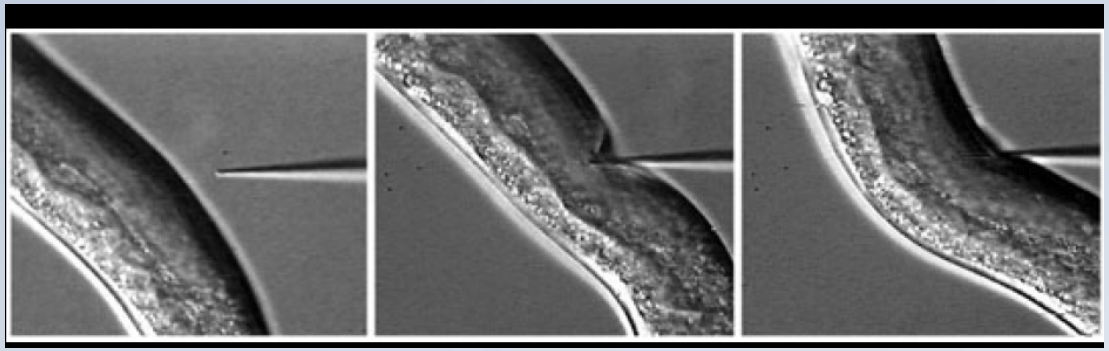


C. elegans expressing EphB2 (mammalian receptor for Nipah virus)



act-5::*ephB2-GFP*

(*act-5* intestine-specific promoter)



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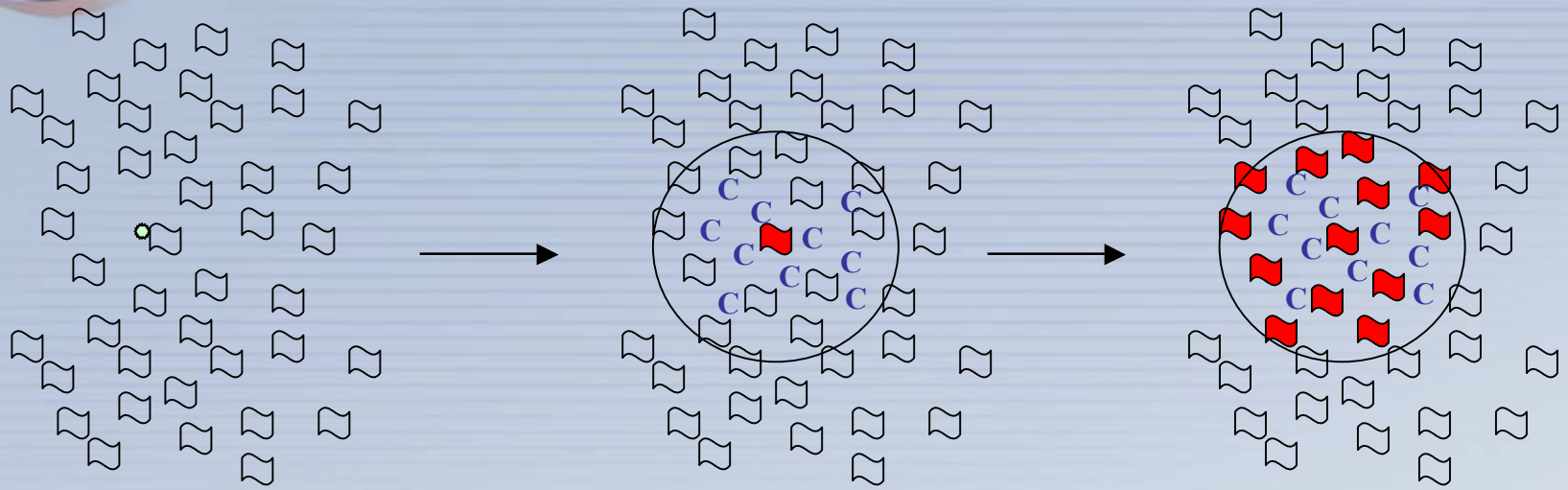
Project Requirements



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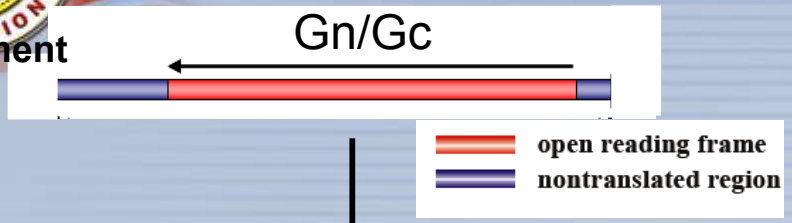
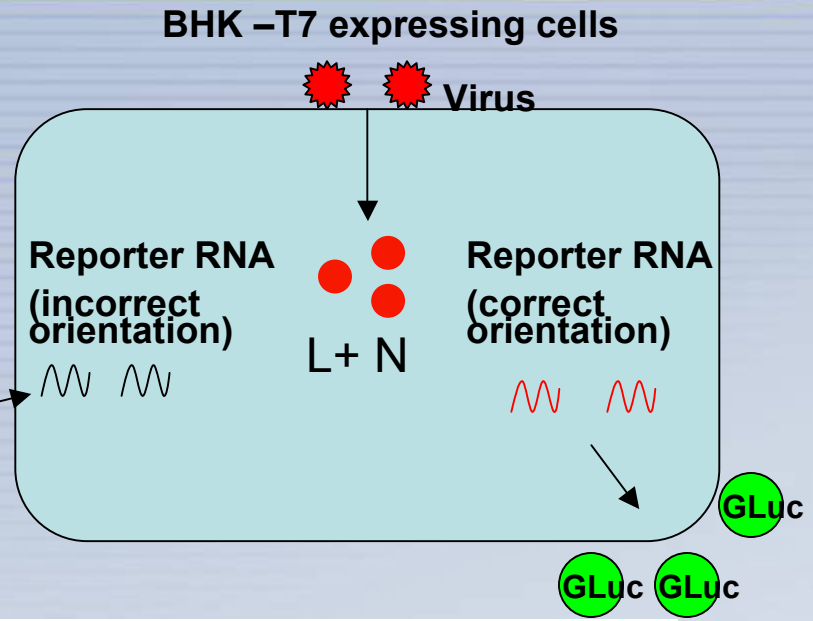
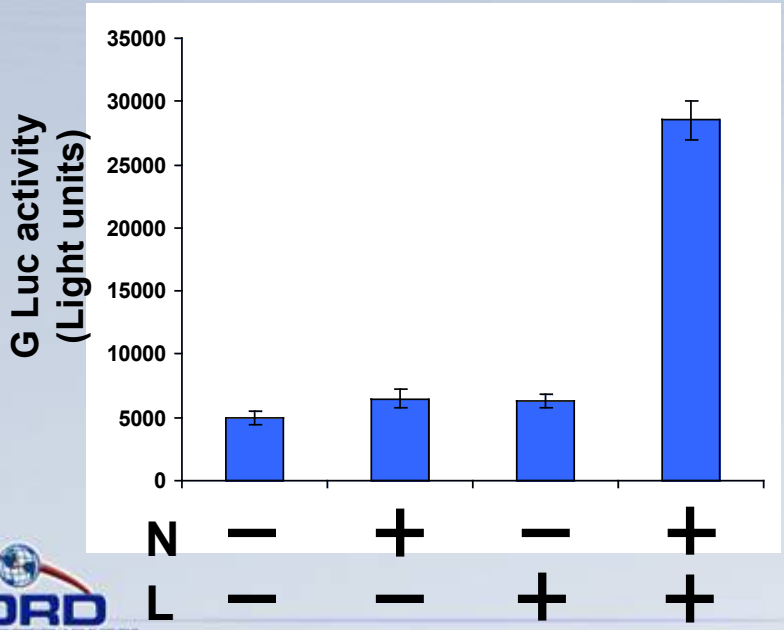
RVFV Sensor Design and Testing

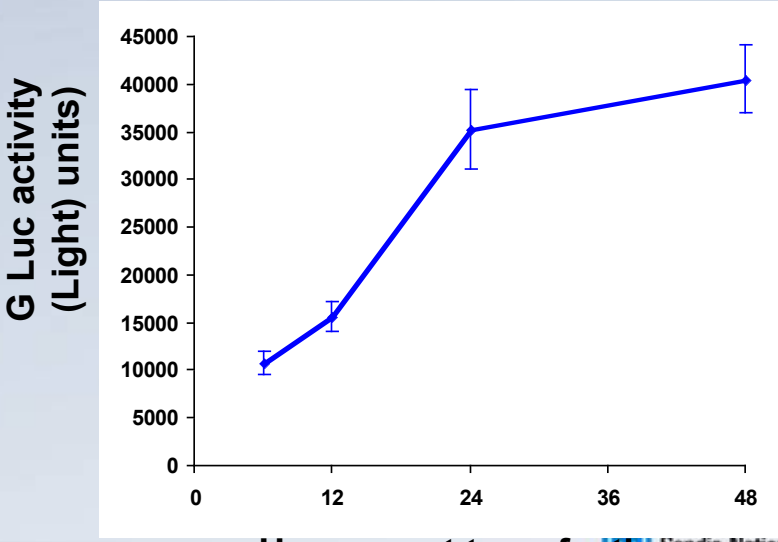
RVFV M segment genome



RVFV sensor design







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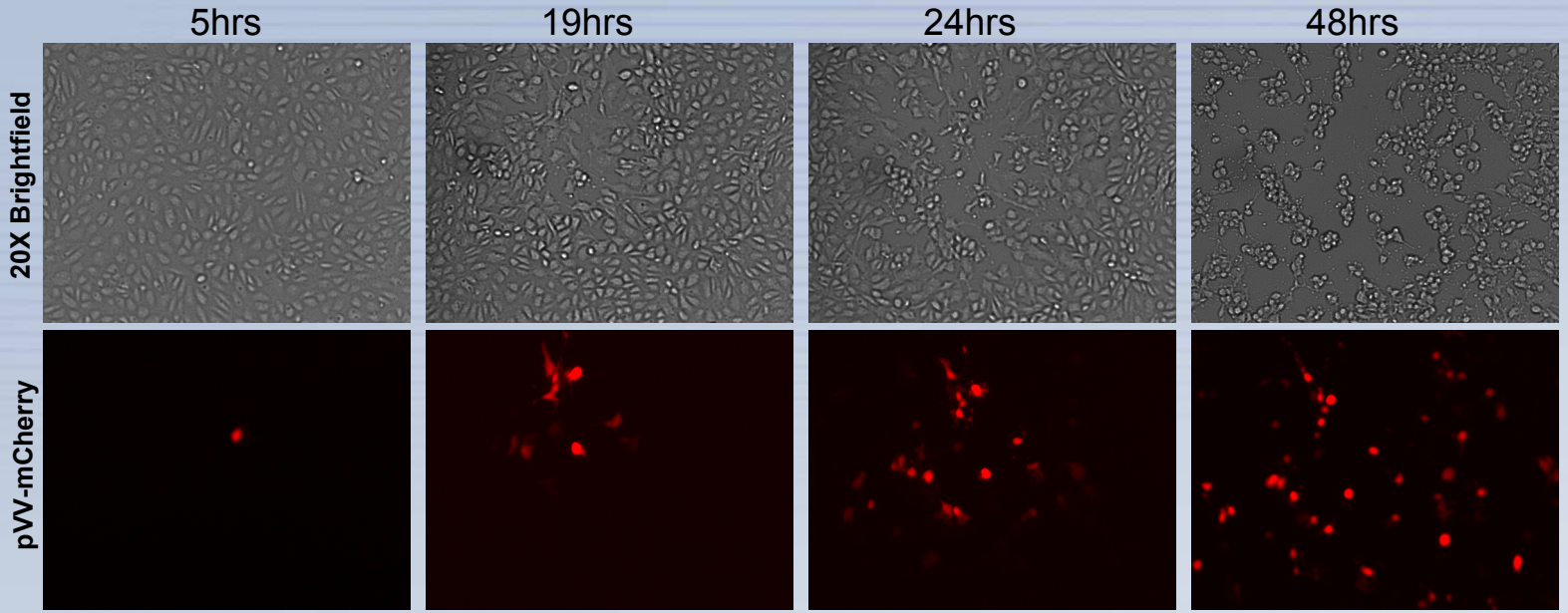
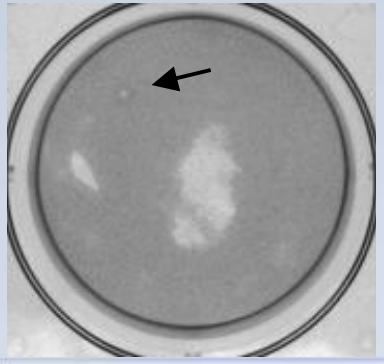
VacV Sensor Design and Testing

A

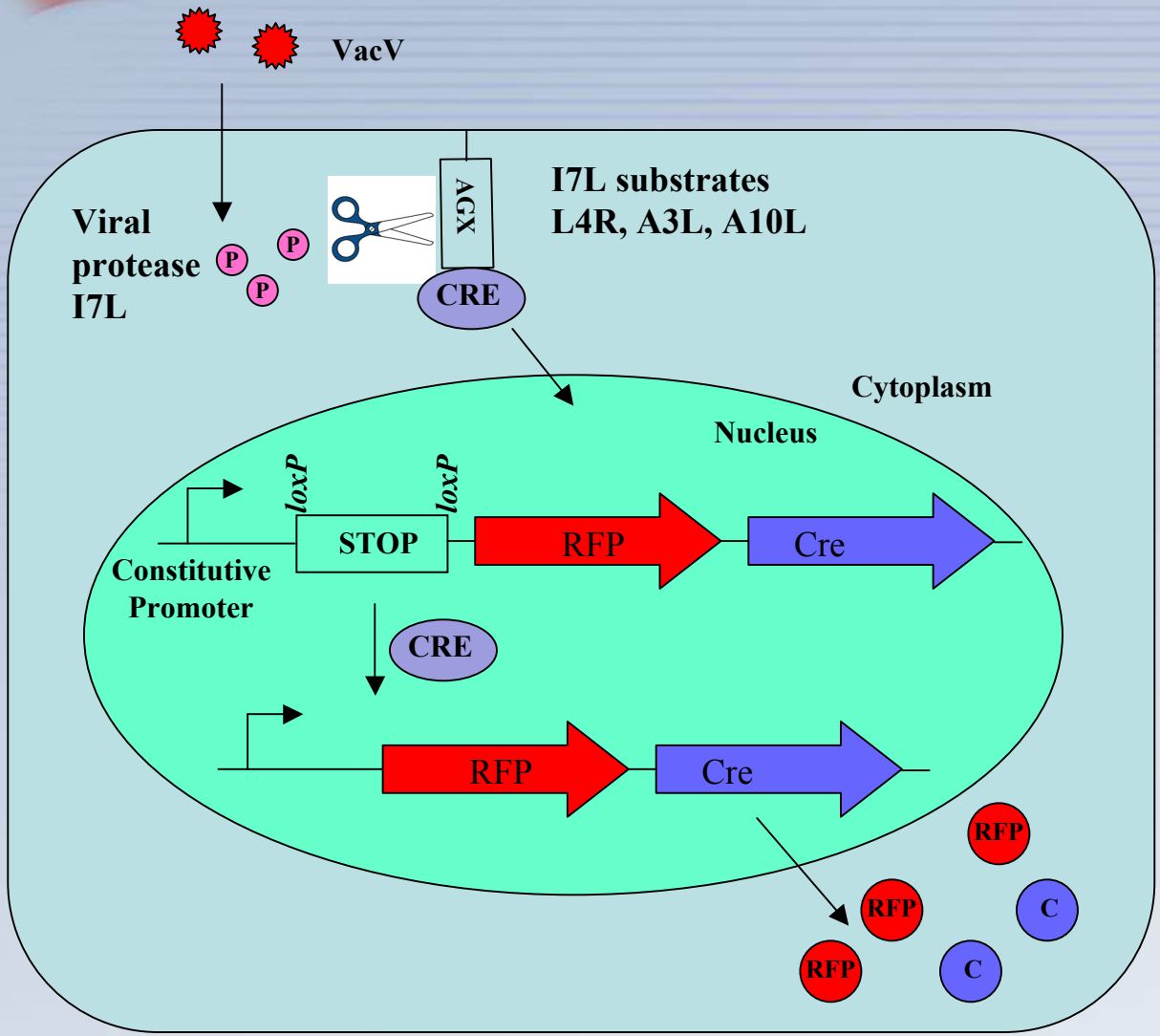


B

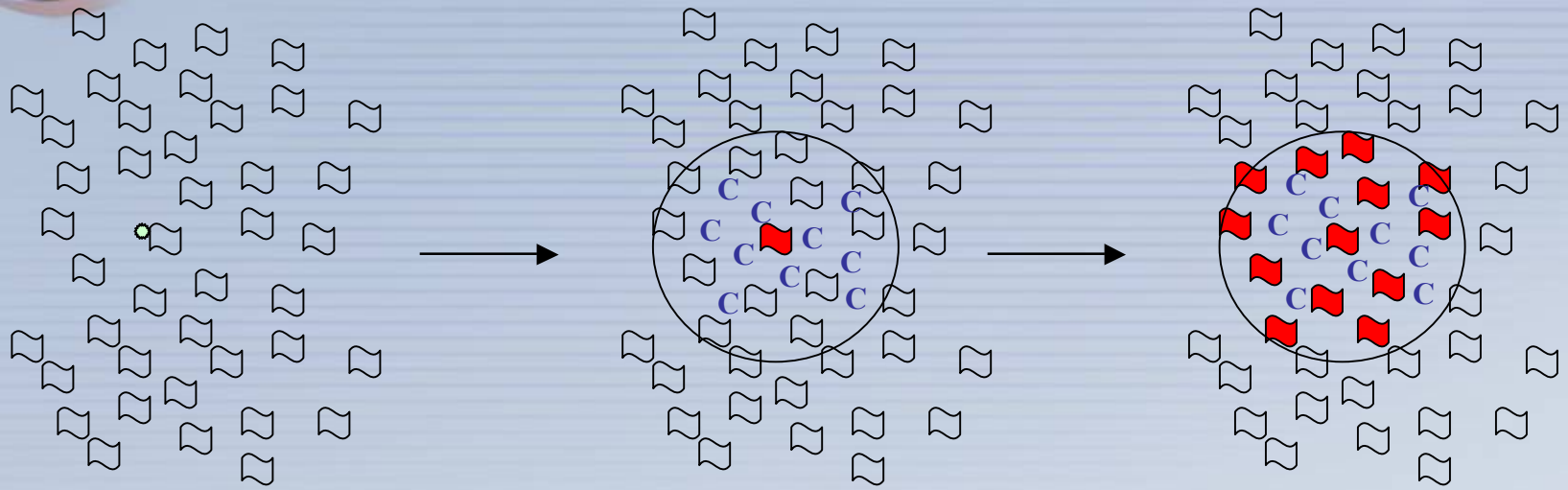


48hrs

Novel Sensor for *VacV*



Project Requirements



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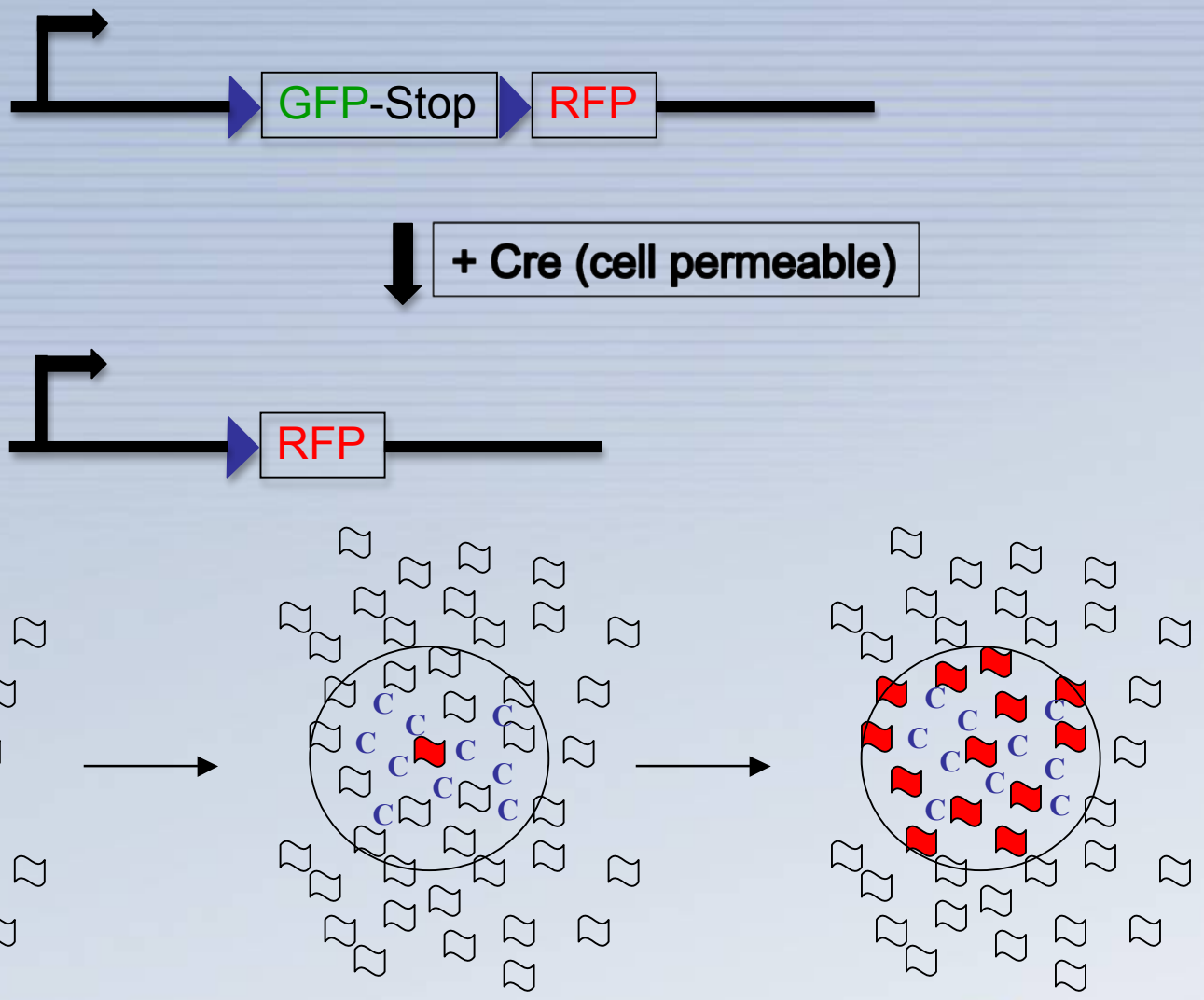
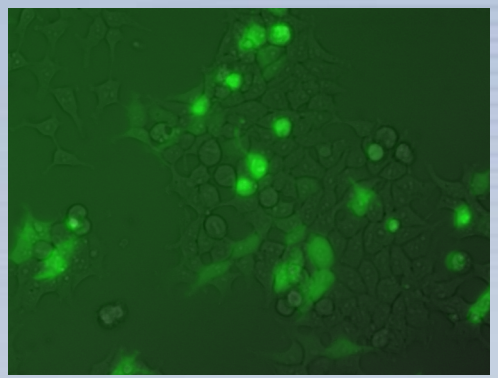
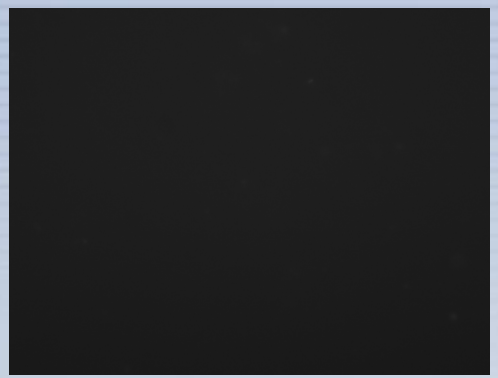
Concept for Amplification Strategy Employing Cre Recombination

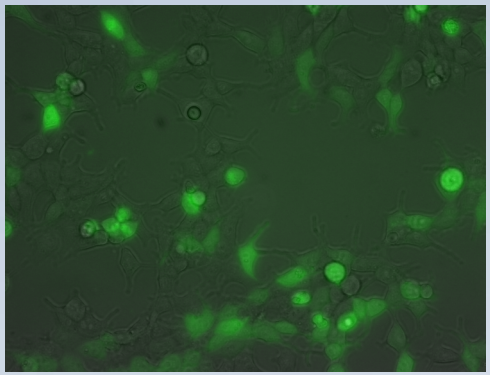


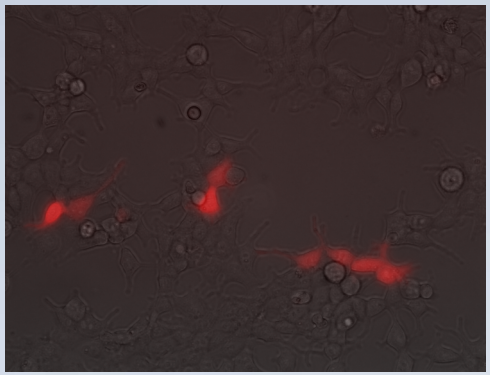
CMV-lox-GFPstop-lox-mKate2 in HEK293 cells





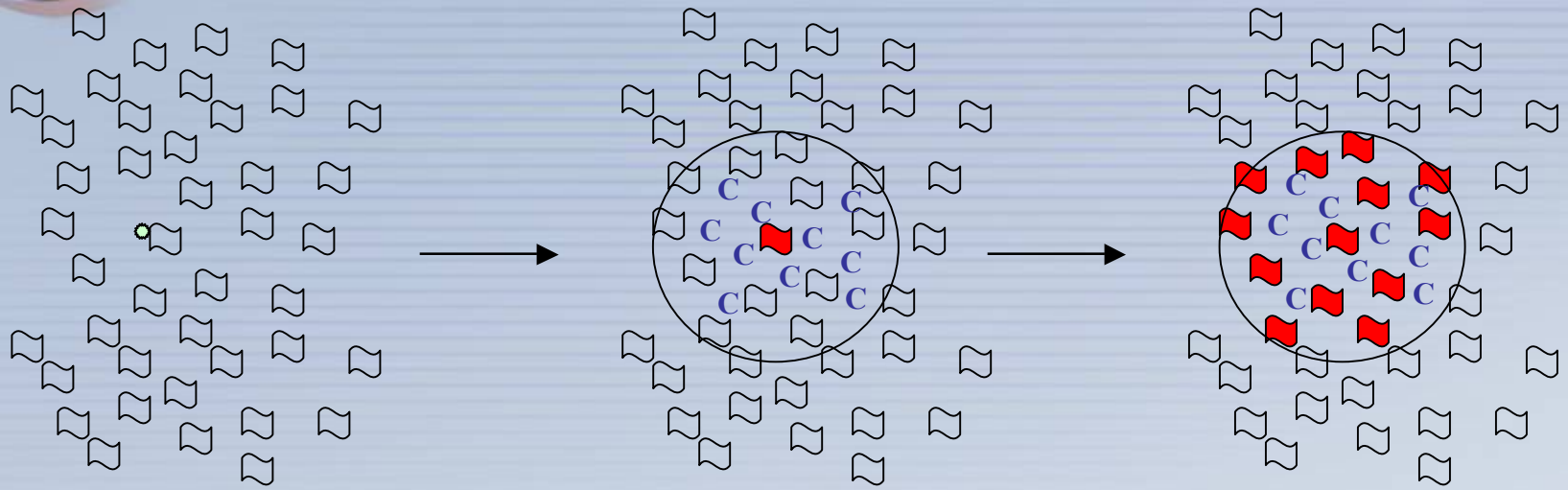
CMV::lox-GFP-STOP-lox-mKate2





CMV::lox-GFP-STOP-lox-mKate2 +
2 hours after addition of 1:2000 dil
membrane-permeable Cre protein

Project Requirements

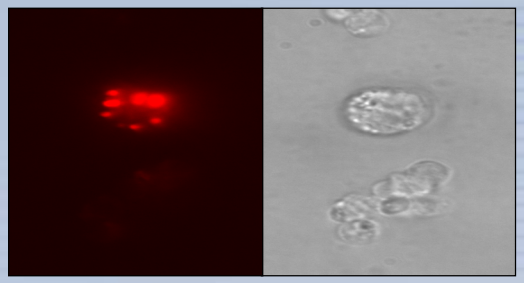


1. Strains of *C. elegans* susceptible to viral infection
   - Increased susceptibility to viral infection
   - Express exogenous viral receptors
2. Mechanism of cellular detection of viral infection
3. Mechanism to amplify signal to adjacent cells and nematodes
4. Gel in which to embed *C. elegans*

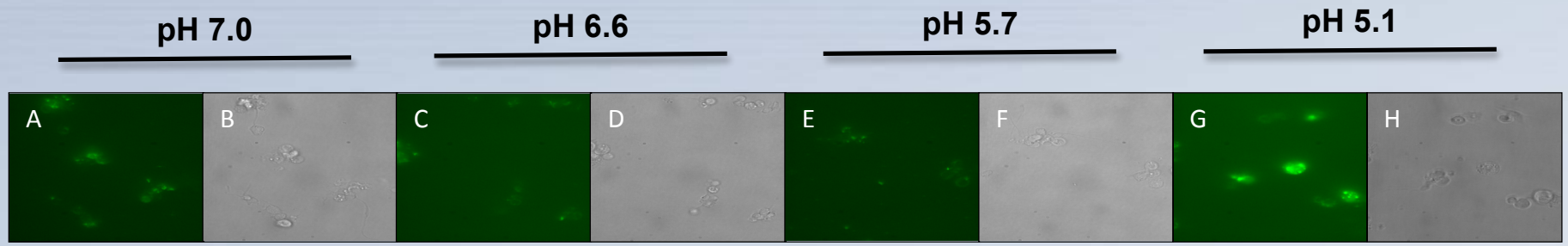
LDRD LABORATORY DIRECTED RESEARCH & DEVELOPMENT

Sandia National Laboratories

Low pH Shock Appears to Enhance Viral Entry



Lysotracker red staining of *C. elegans* embryonic cells indicates the presence of low pH vesicles within the cells.

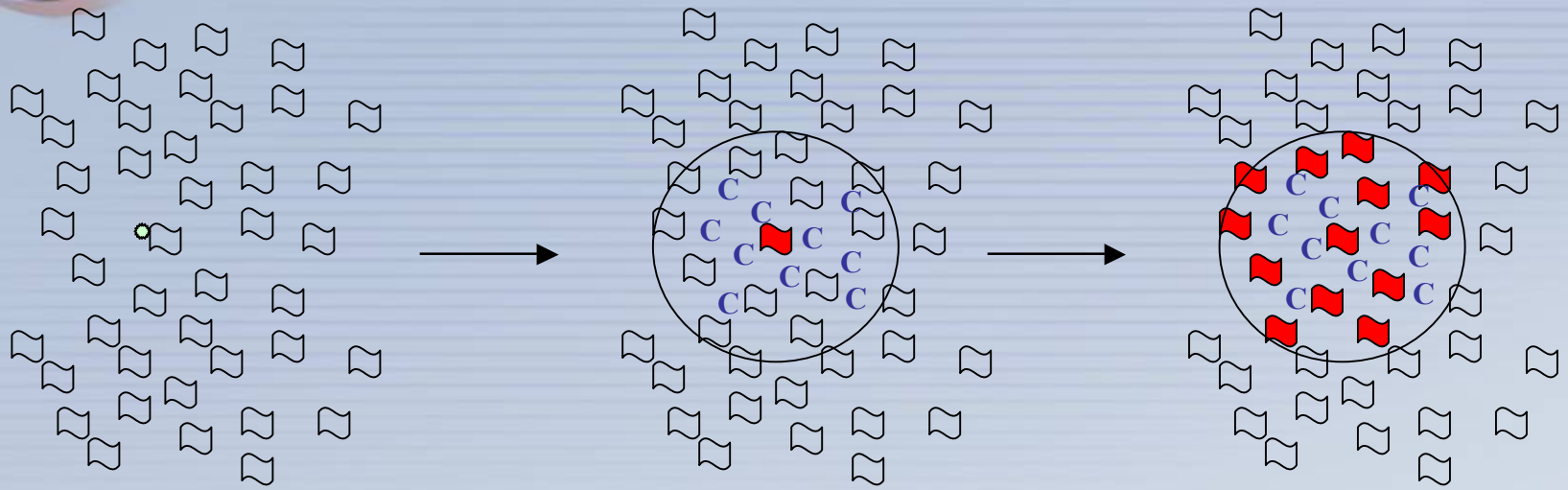


Embryonic stem cells pulsed with media at specified pH for 1 min following addition of rVSV-GFP

Requirements of the Gel

- Oxygen permeable
- Promote suspension of virus (with a surfactant?)
- Contain chemicals to weaken cuticle (collagenase/chitinase)
- low pH?

Ongoing work



- Optimize protocol for improving *C. elegans* susceptibility to viral infection
- Generate *C.elegans* expressing Nipah viral receptor in cells likely to see virus
- Optimize RVFV cellular sensor and test VacV cellular sensor
- Optimize Cre-based system to amplify signal from cellular sensors to adjacent nematodes
- Test gel compositions in which to embed *C. elegans*

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