NON-HUMAN PRIMATE BIOMEDICAL RESEARCH FOR TACKLING EMERGING INFECTIOUS DISEASES (II): ZIKA VIRUS

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CONFIRMATION OF ATTENDANCE

Name: **Surname:**

Date	Title	Speaker	Duration	Organizer signature
10.01.2017	Genetic humanization and the generation of human antibodies from transgenic animals	Dr. Susy Senatore	1h	
07.02.2017	Tissue engineering by bio-printing: current state of futuristic technology	Dr. Vijay Chandrasekar	1h	
07.03.2017	Non-human primate biomedical research to study neurological diseases	Dr. Yingjun Liu	1h	
04.04.2017	Non-human primate biomedical research for tackling emerging infectious diseases (I): Ebola virus	Dr. Regina Reimann	1h	
02.05.2017	Non-human primate biomedical research for tackling emerging infectious diseases (II): Zika virus	Dr. Karl Frontzek	1h	
06.06.2017	Recent development in microbiome research: effect of diet and microbiota composition on the development of diseases	Dr. Katrin Frauenknecht	1h	

Organizers: Prof. A. Aguzzi, Dr. Silvia Sorce

Signature by
Adriano Aguzzi
Karl Frontzek
Silvia Sorce

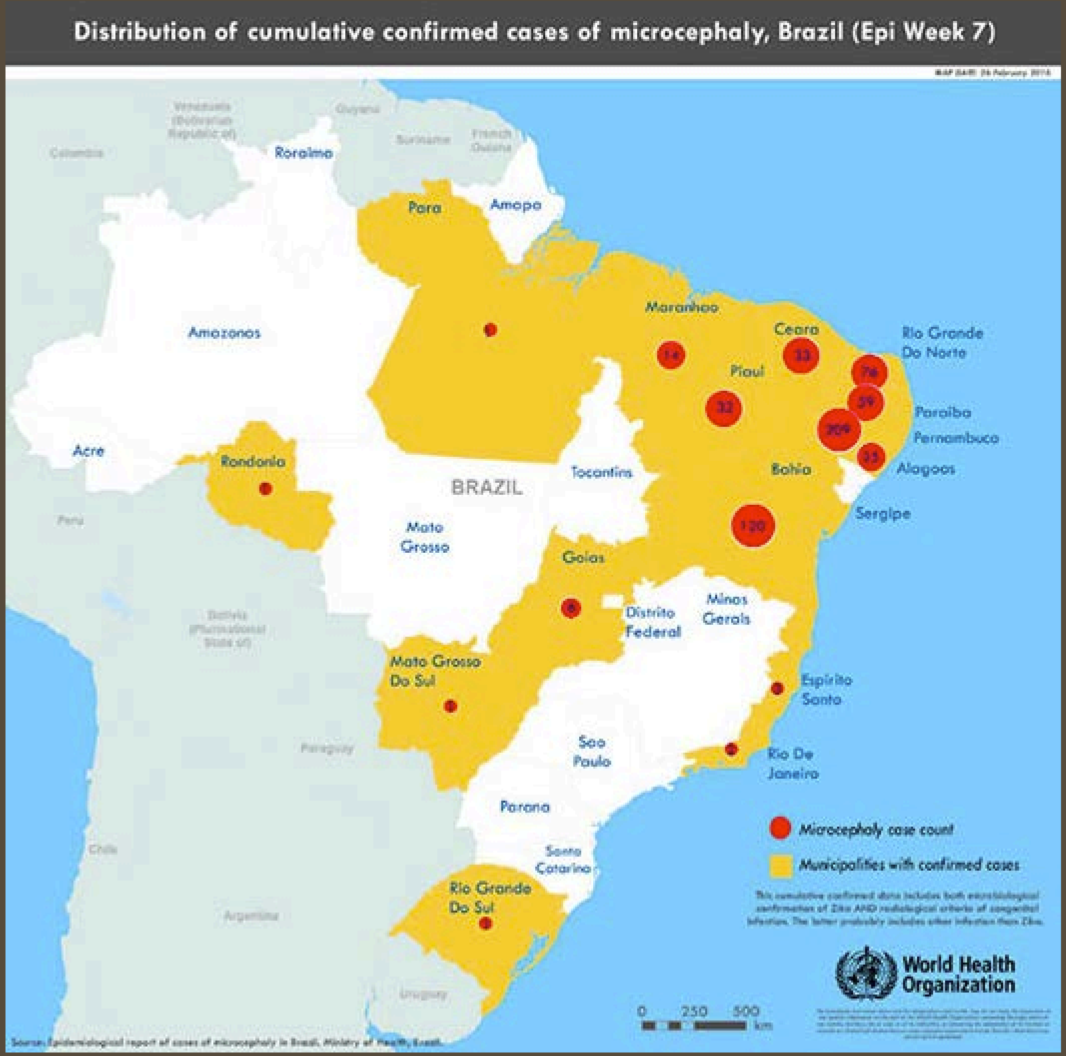
NON-HUMAN PRIMATE BIOMEDICAL RESEARCH FOR TACKLING EMERGING INFECTIOUS DISEASES (II): ZIKA VIRUS

- Introduction to Zika virus
- Rapid development of a DNA vaccine for Zika virus
- Rapid, Low-Cost Detection of Zika Virus Using Programmable Biomolecular Components

INTRODUCTION TO ZIKA VIRUS

THE ZIKA VIRUS

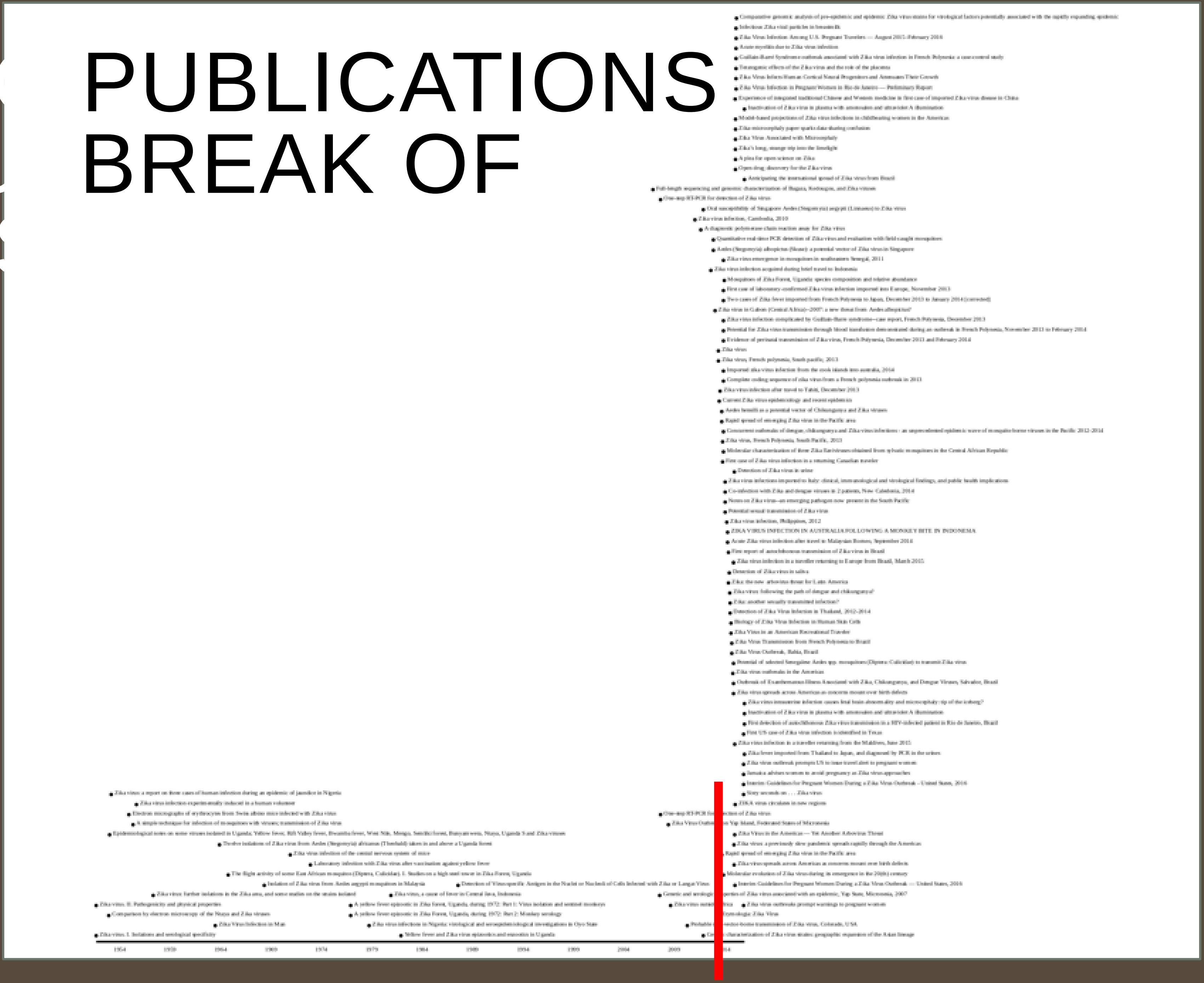
Dick et al., Trans R Soc Trop Med Hyg (1952)



http://origin.who.int/test/timelines/zika-virus-storymap/images/30-October-2015.jpg

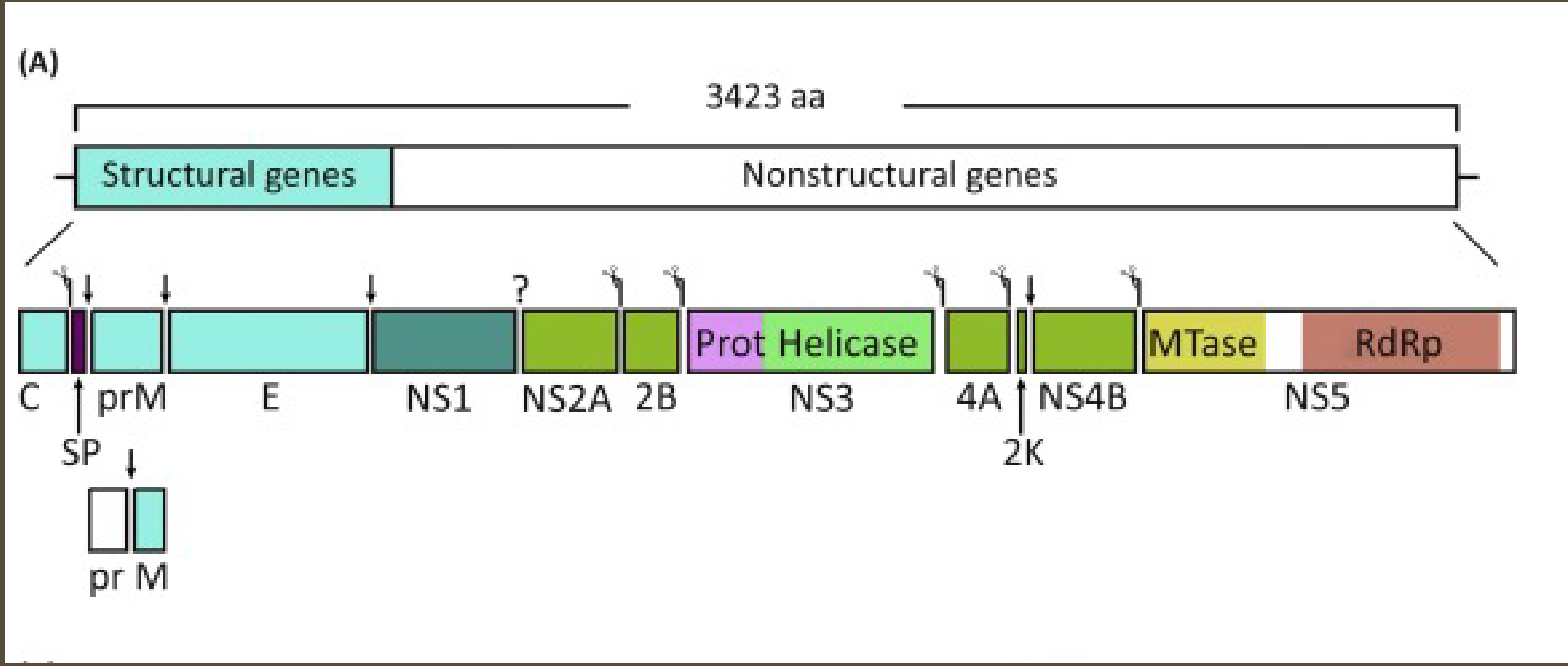
https://www.cdc.gov/ncbddd/birthdefects/microcephaly.html

SCIENTIFI PUBLICATIONS AT THE OU BREAK OF ZIKA VIRUS



https://upload.wikimedia.org/wikipedia/commons/thumb/a/a4/Timeline_of_scientific_publications_about_Zika_virus%2C_based_on_Wikidata.svg/2000px-Timeline_of_scientific_publications_about_Zika_virus%2C_based_on_Wikidata.svg.png

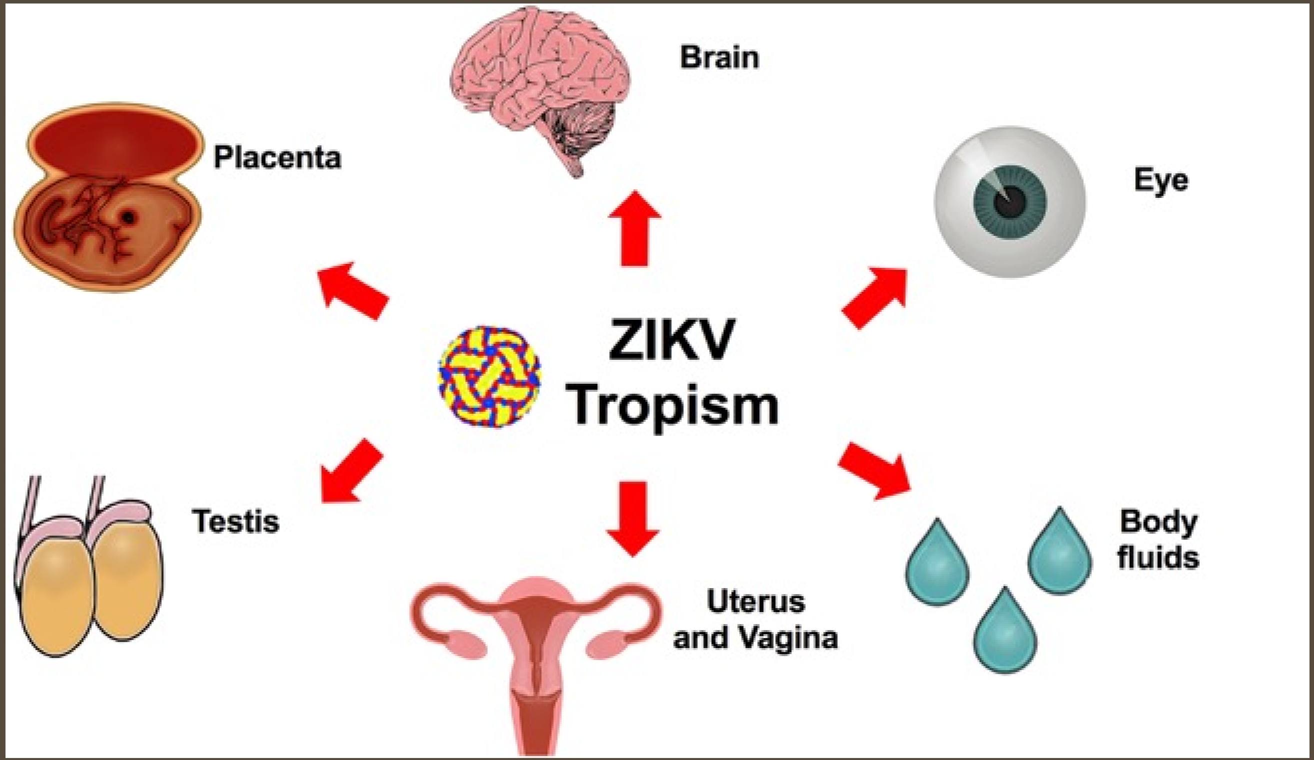
GENOMIC ORGANIZATION OF ZIKA VIRUS



Shi & Gao, Trends Biochem Sci 2017

ZIKA VIRUS

- Flavivirus (West Nile Virus, Dengue), genome of ~ 11 kb (+)-stranded RNA
- virion has a diameter of ~ 50 nm and contains a nucleoplasmid surrounded by a lipid bilayer with membranal proteins



Modified from Miner & Diamond, Cell Host Microbe 2017

RAPID DEVELOPMENT OF A DNA VACCINE FOR ZIKA VIRUS

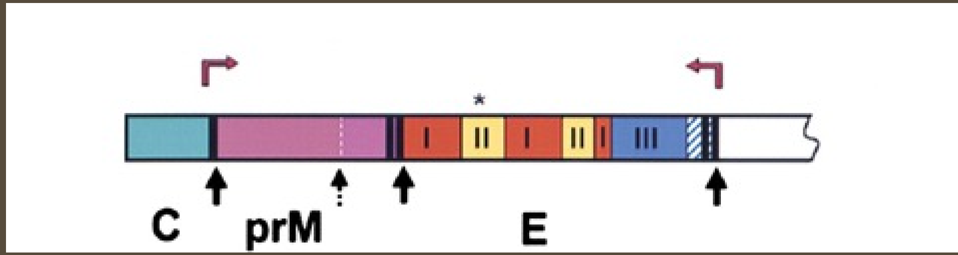
Dowd et al., Science 2016

GOAL OF THE STUDY

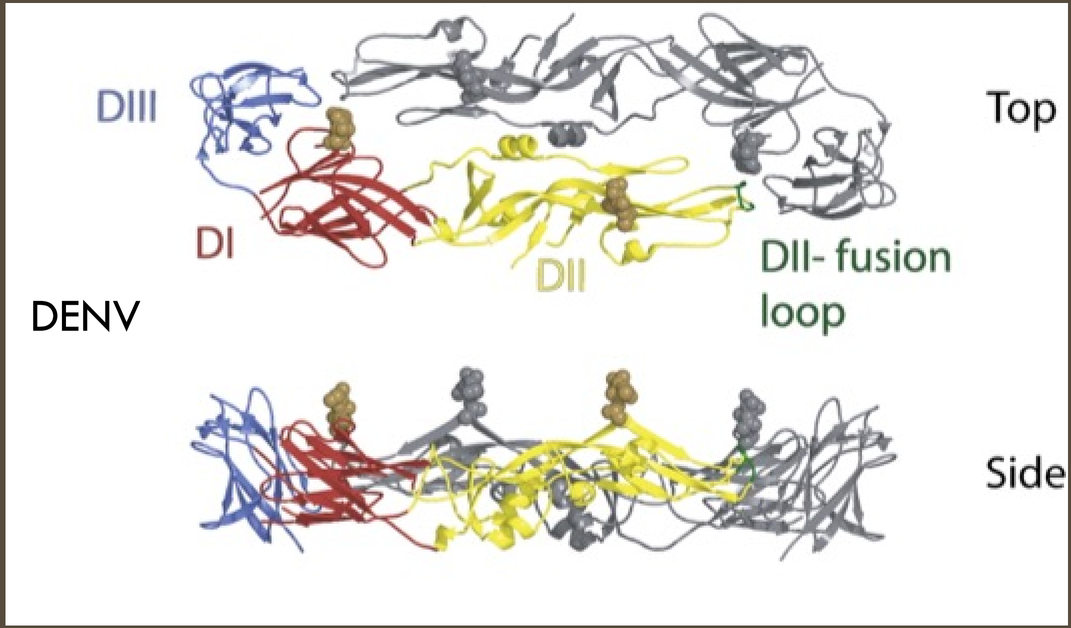
- To create & test a DNA vaccine against the current strains of the Zika virus epidemic
- Advantages of DNA-based vaccines:
  - rapid testing of a variety of batches
  - rapid production of GMP material
  - good tolerability in humans
  - established path in regulatory approval

ANTIGEN DESIGN

Subviral particles with similar antigenic properties compared to virions by expression of prM-E



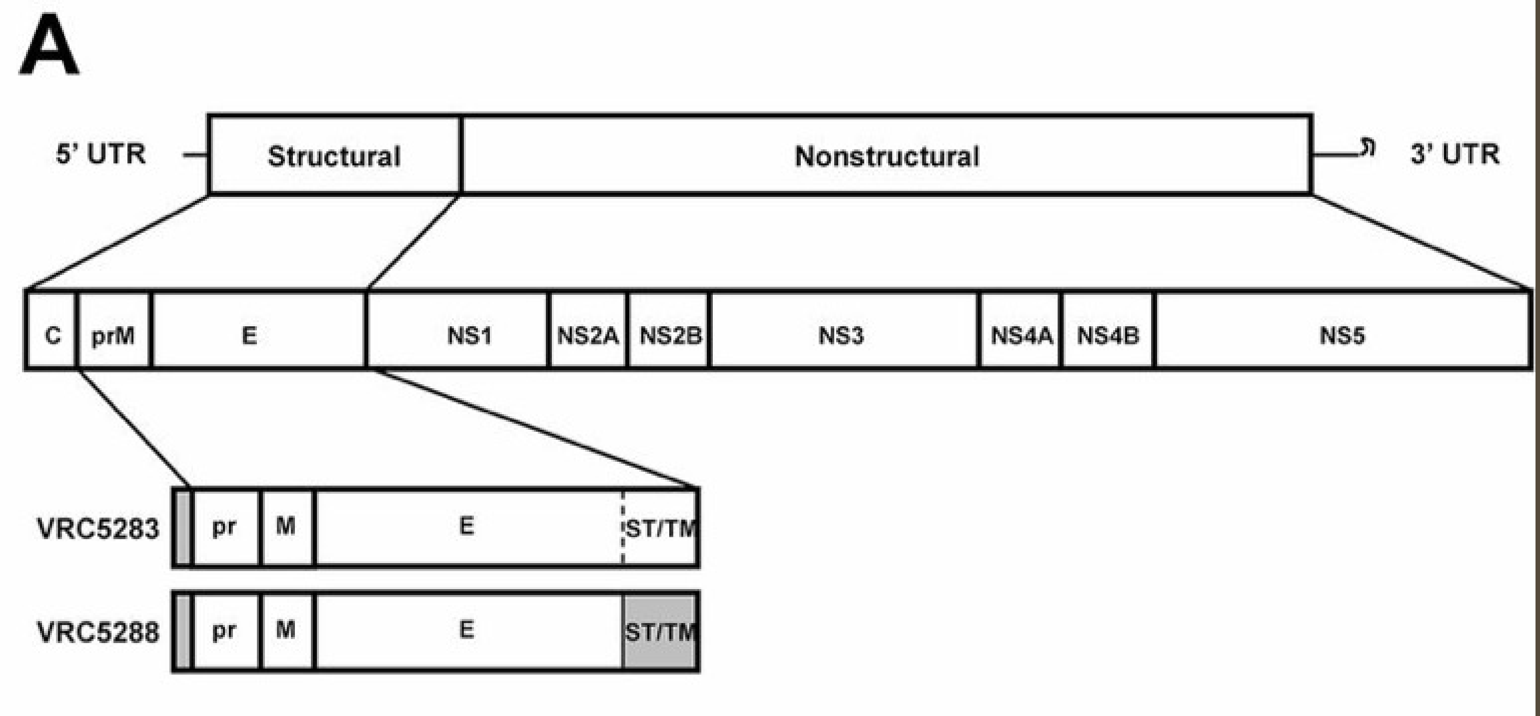
Ferlenghi et al., Molecular Cell 2001



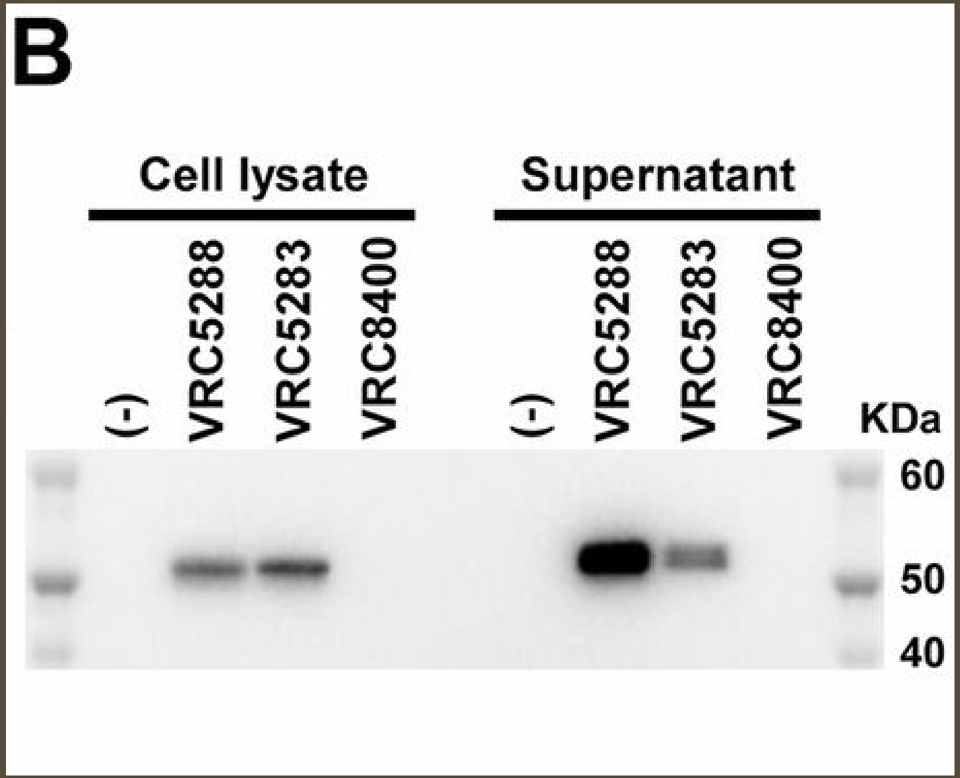
Pierson et al., Cell Host Microbe 2008

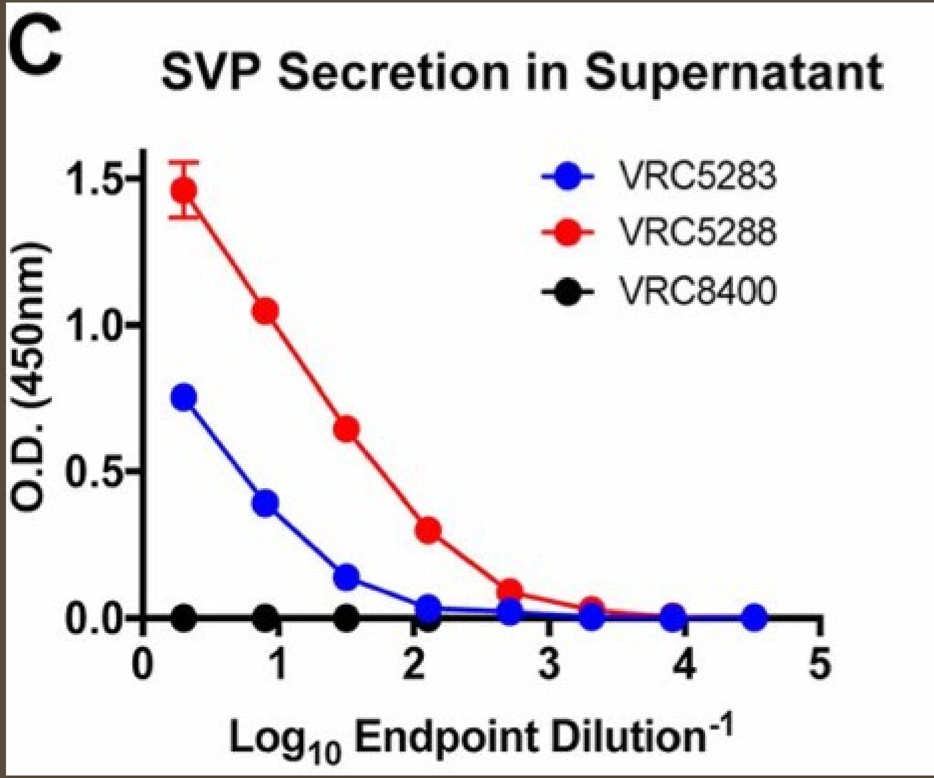
ANTIGEN DESIGN

- strain H/PF/2013 from French Polynesia similar to the one circulating in the Americas

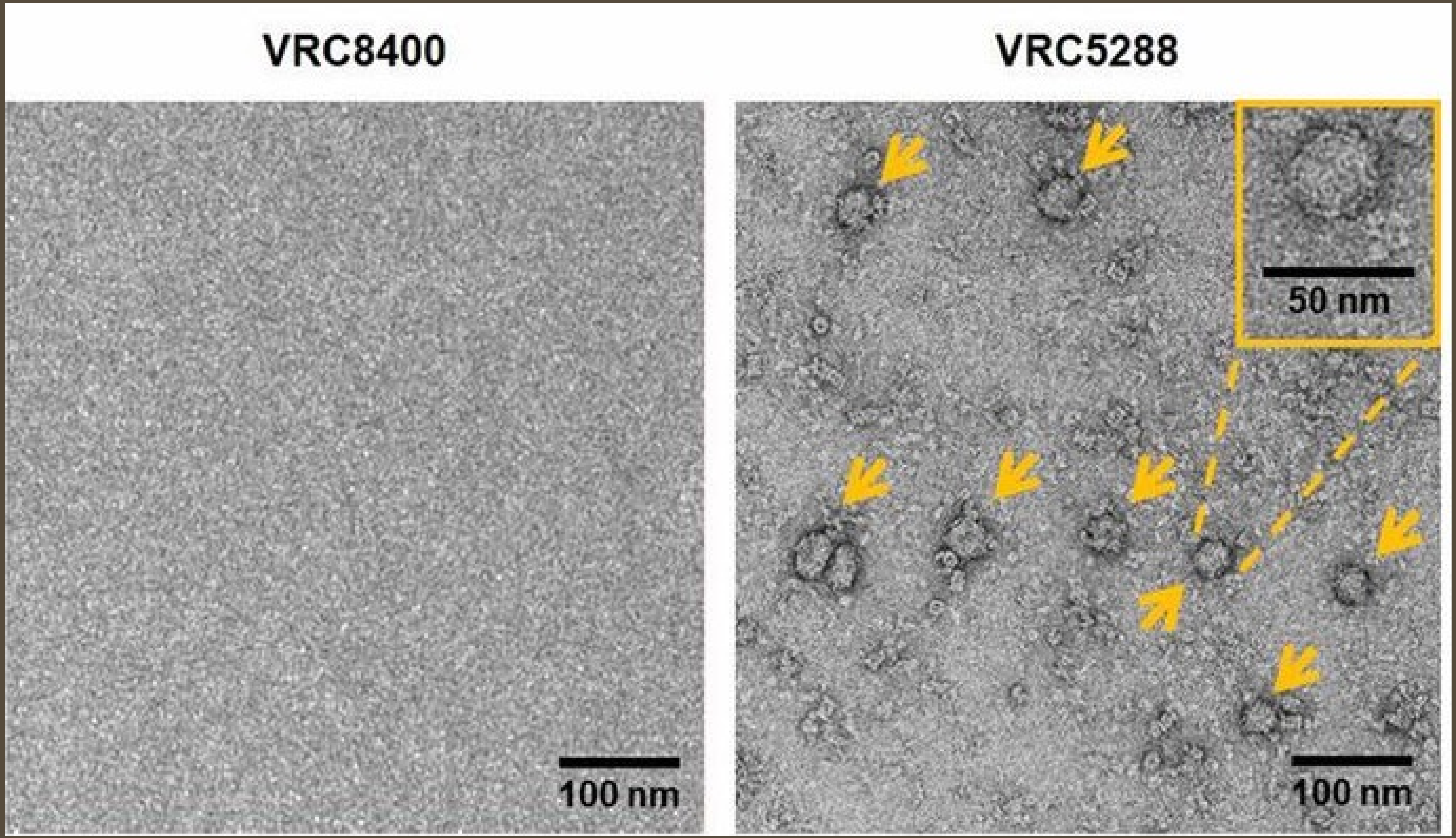


EFFICIENT SECRETION OF THE ANTIGEN FROM CELS

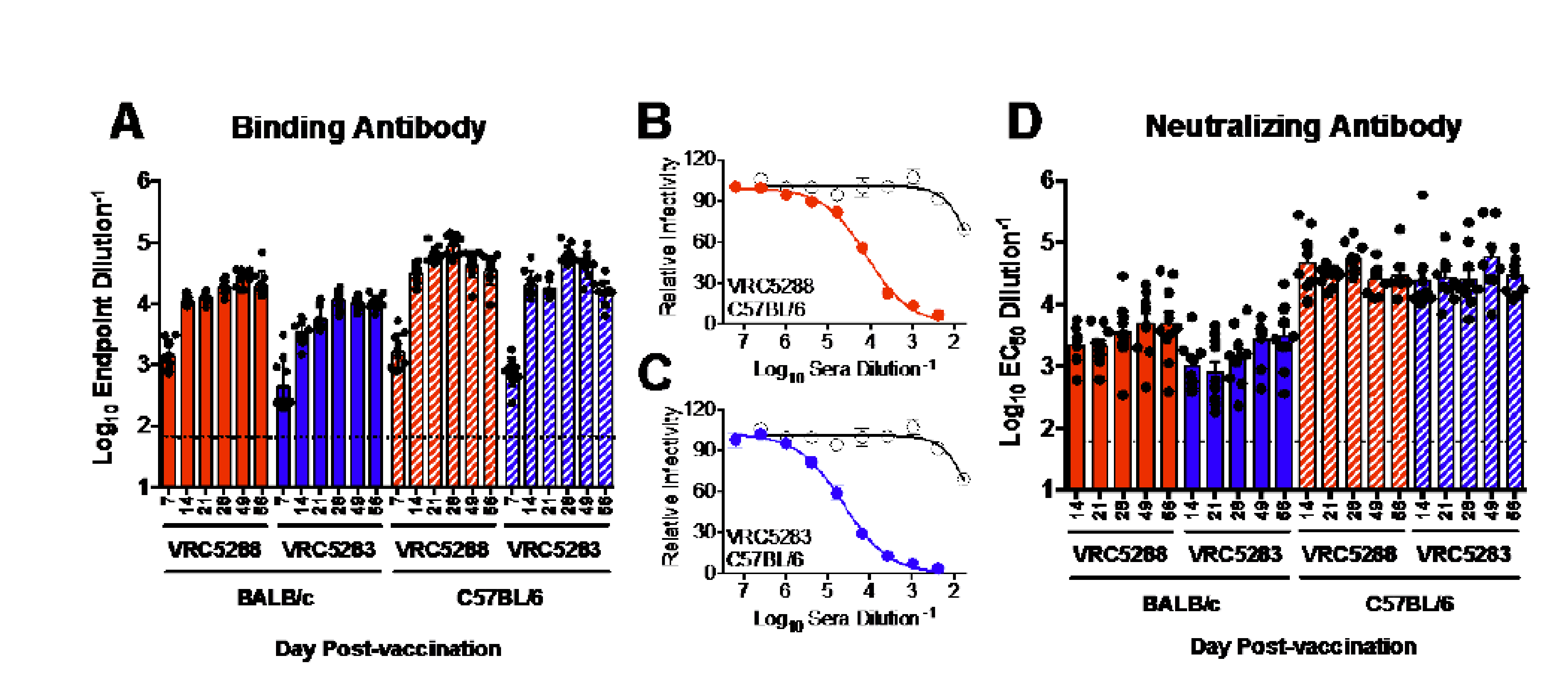




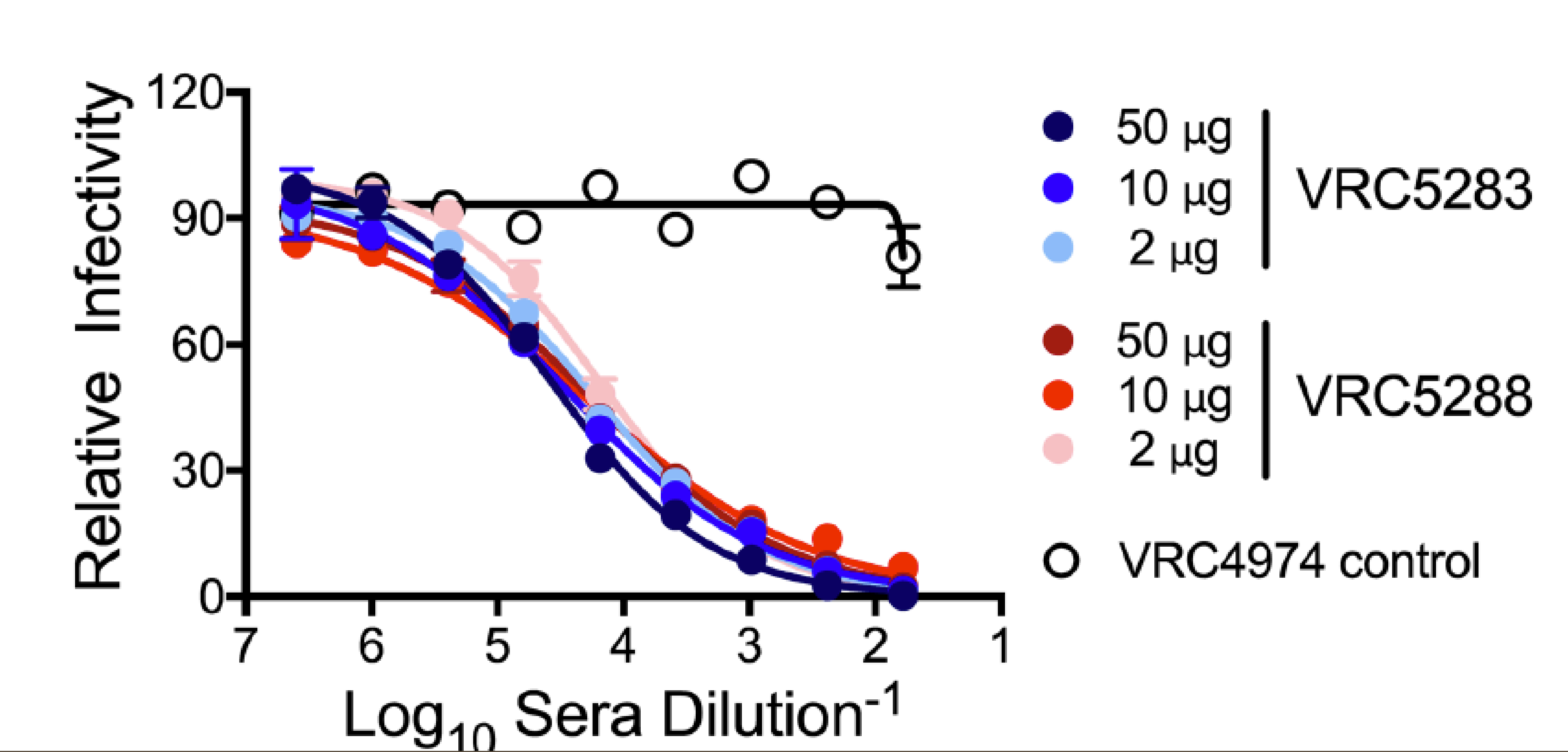
ELECTRON MICROSCOPY OF SECRETED PARTICLES SHOWS FLAVIVIRUS-LIKE SPHERES



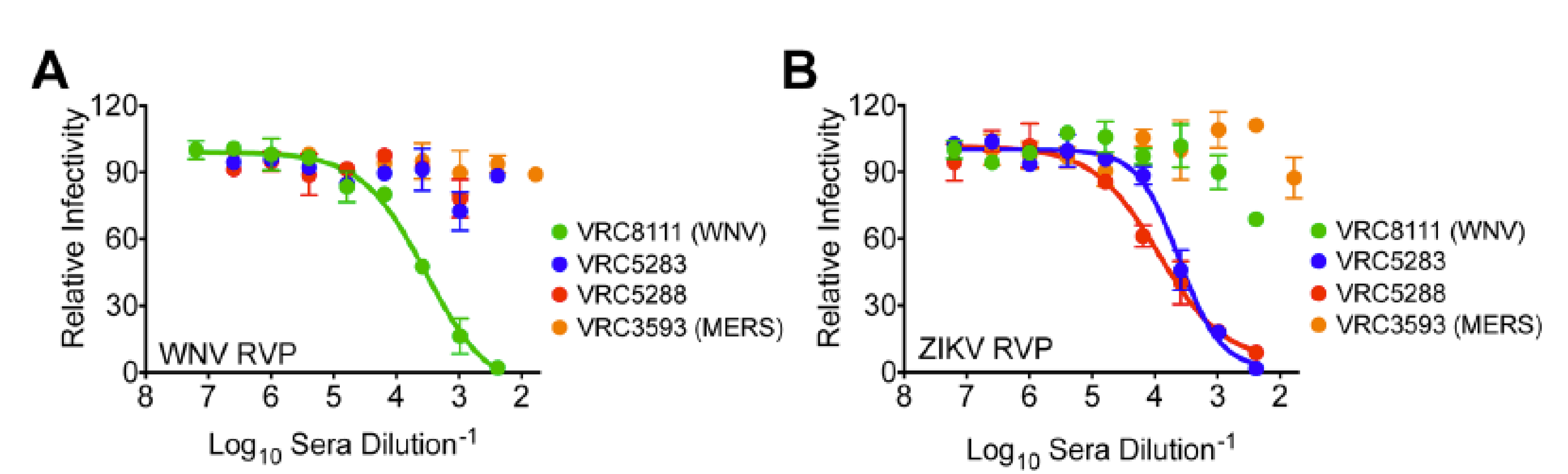
EFFICIENT SECRETION OF NEUTRALIZING ANTIBODIES AFTER VACCINATION



EFFICIENT VACCINATION THROUGH INJECTION OF 50, 10 AND 2 µg

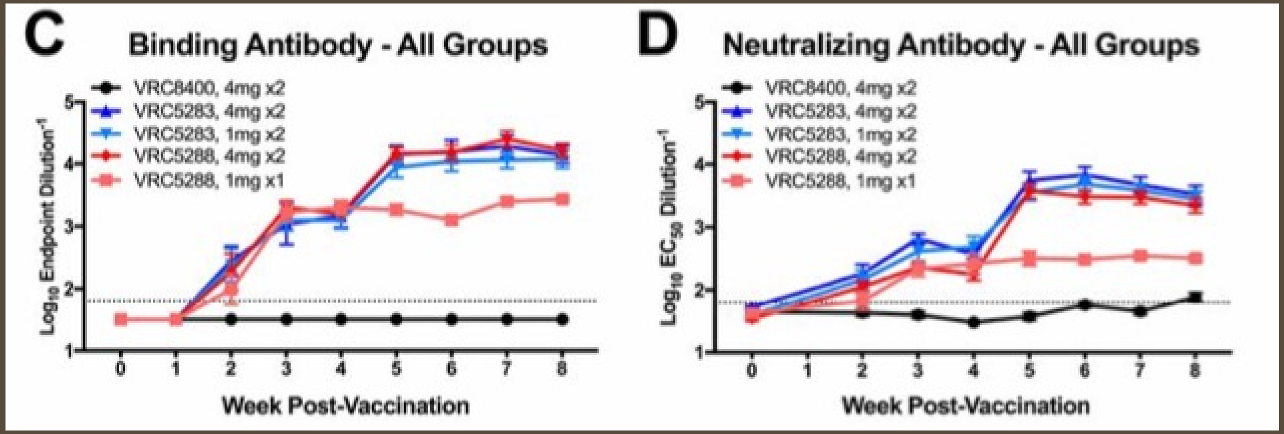


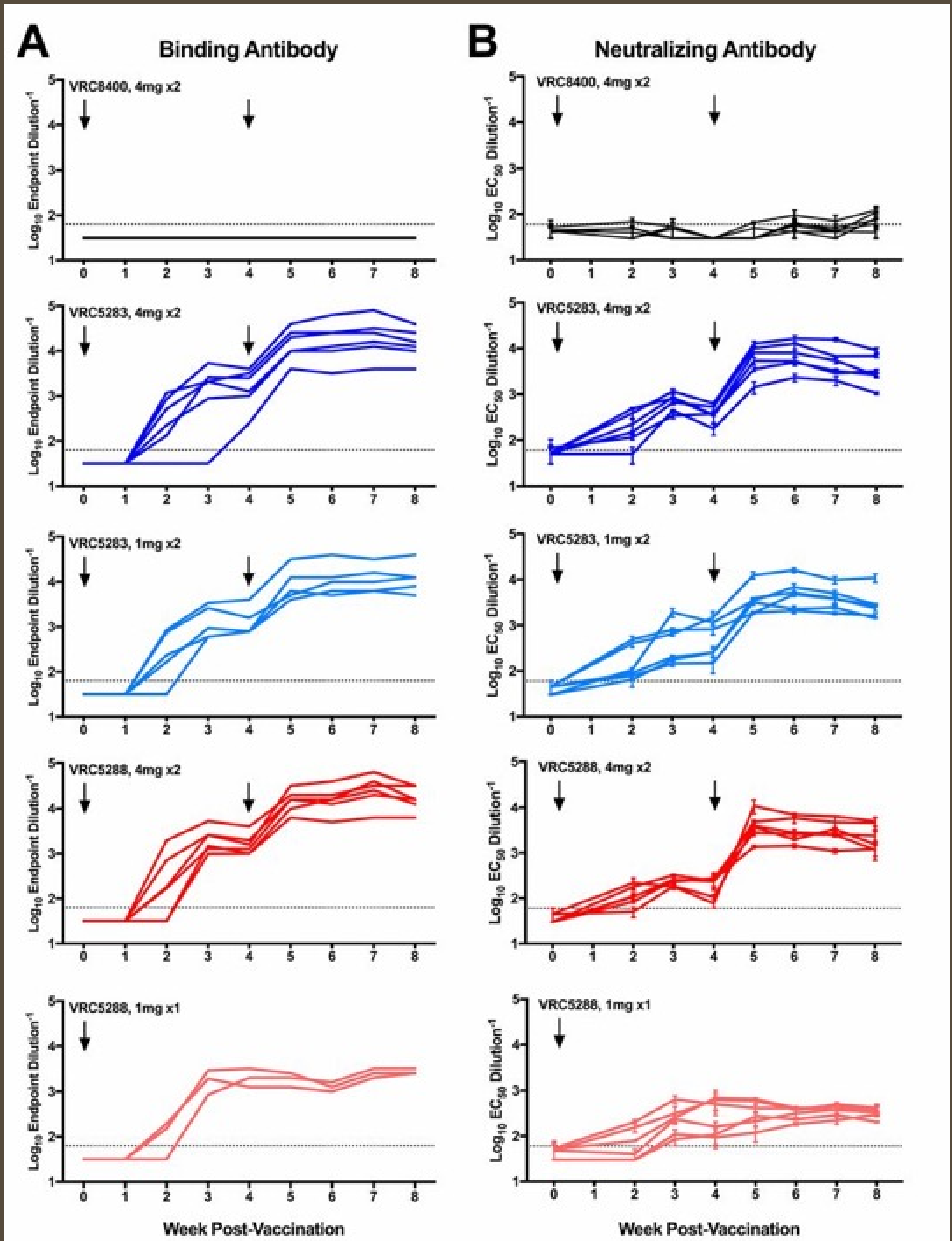
SIMILAR VACCINATION EFFICIACY OF ZIKA VACCINE WHEN COMPARED TO WNV VACCINE



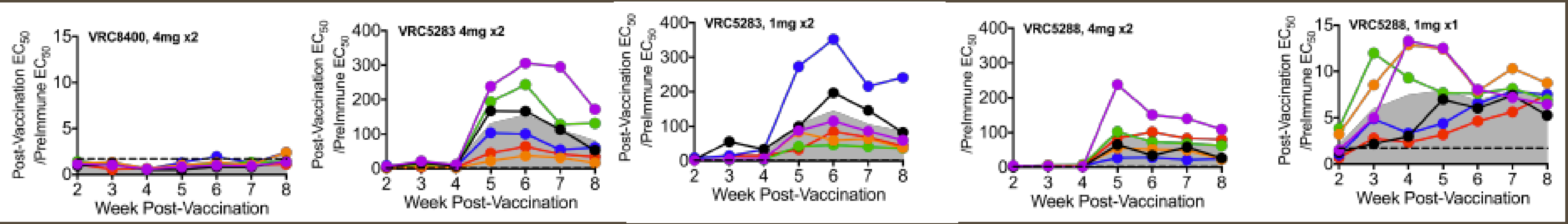
ASSESSING IMMUNOGENICITY IN NON-HUMAN PRIMATES

- treatment groups (n = 6 rhesus macaques per group)
  - 2 x 1 mg (VRC5283)
  - 2 x 4 mg (VRC5283+VRC5288)
  - 1 x 1 mg (VRC5288)

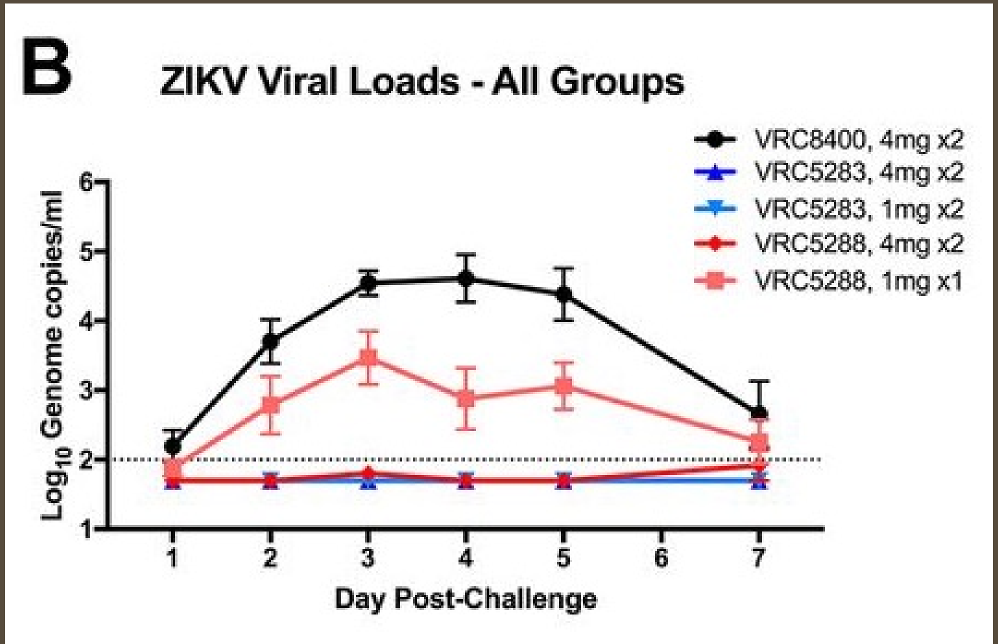


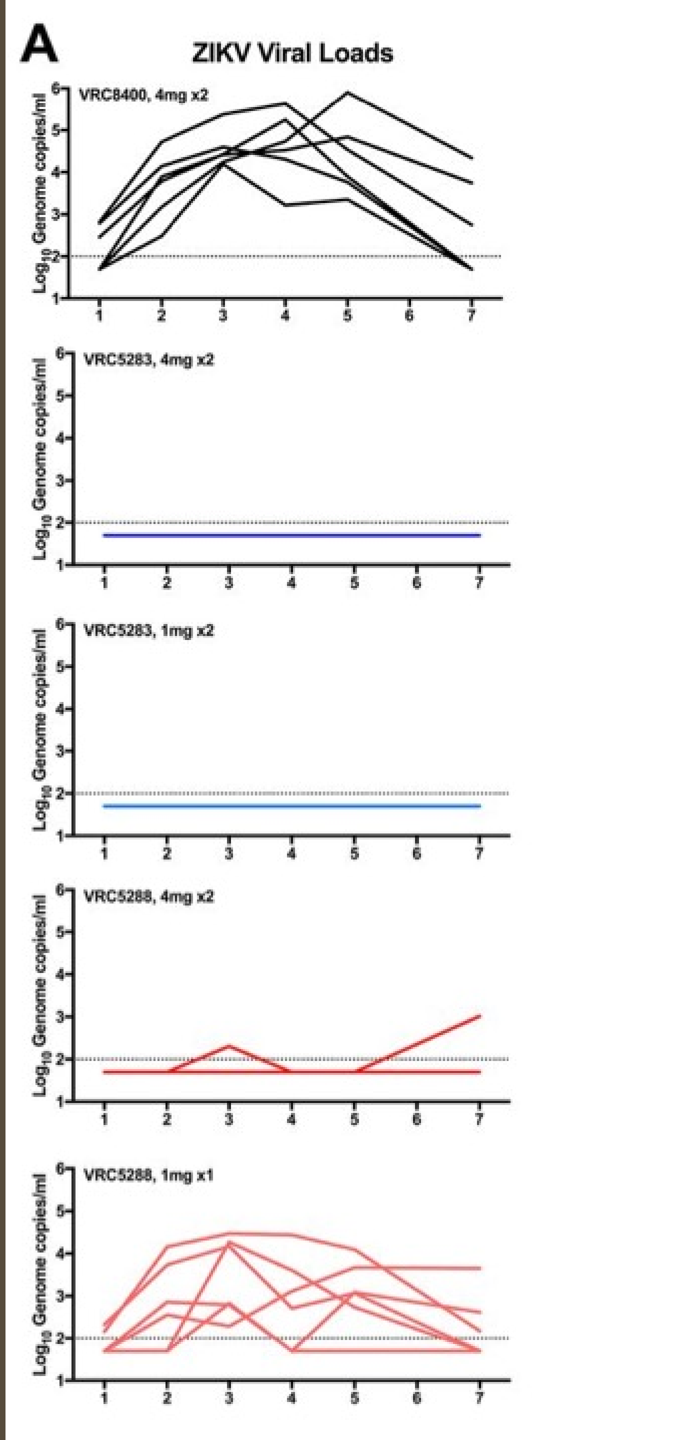


IMMUNOGENICITY OF DNA VACCINE IN NON-HUMAN PRIMATES

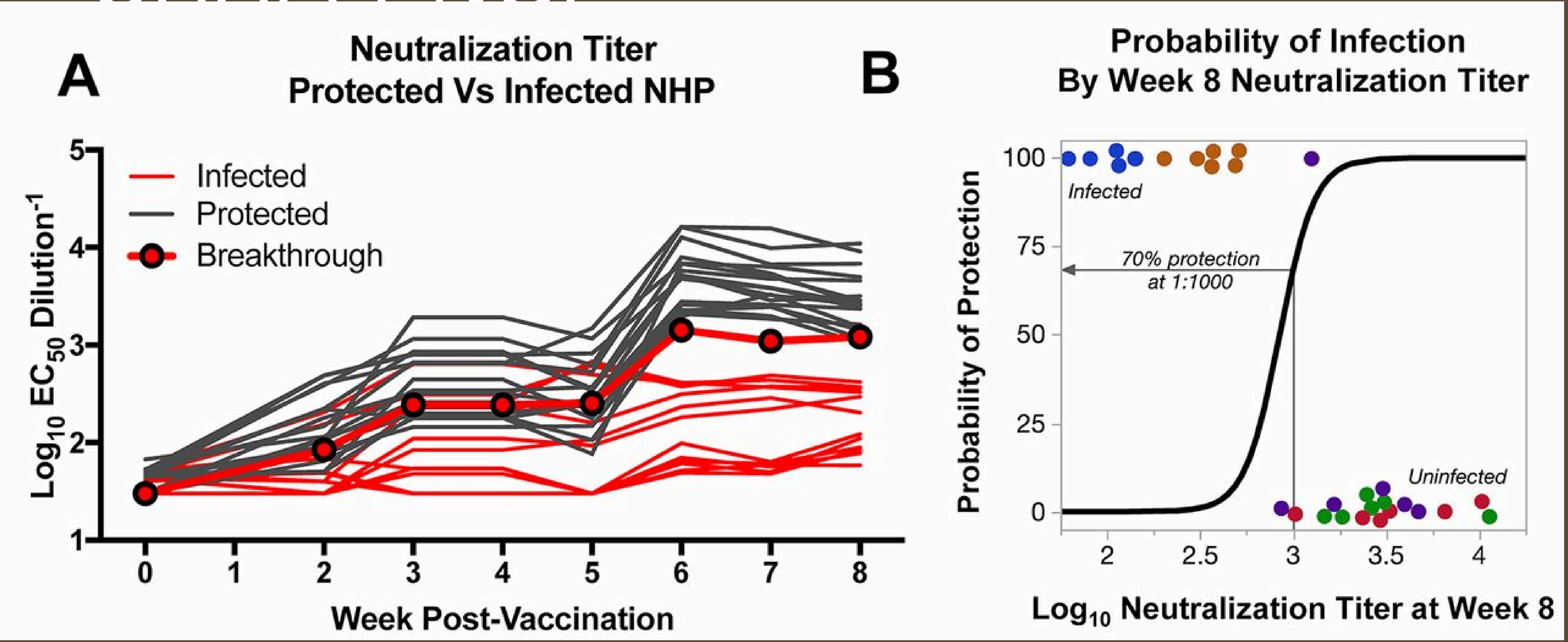


SUCCESSFUL ABLATION OF ZIKA VIREMIA THROUGH VACCINATION



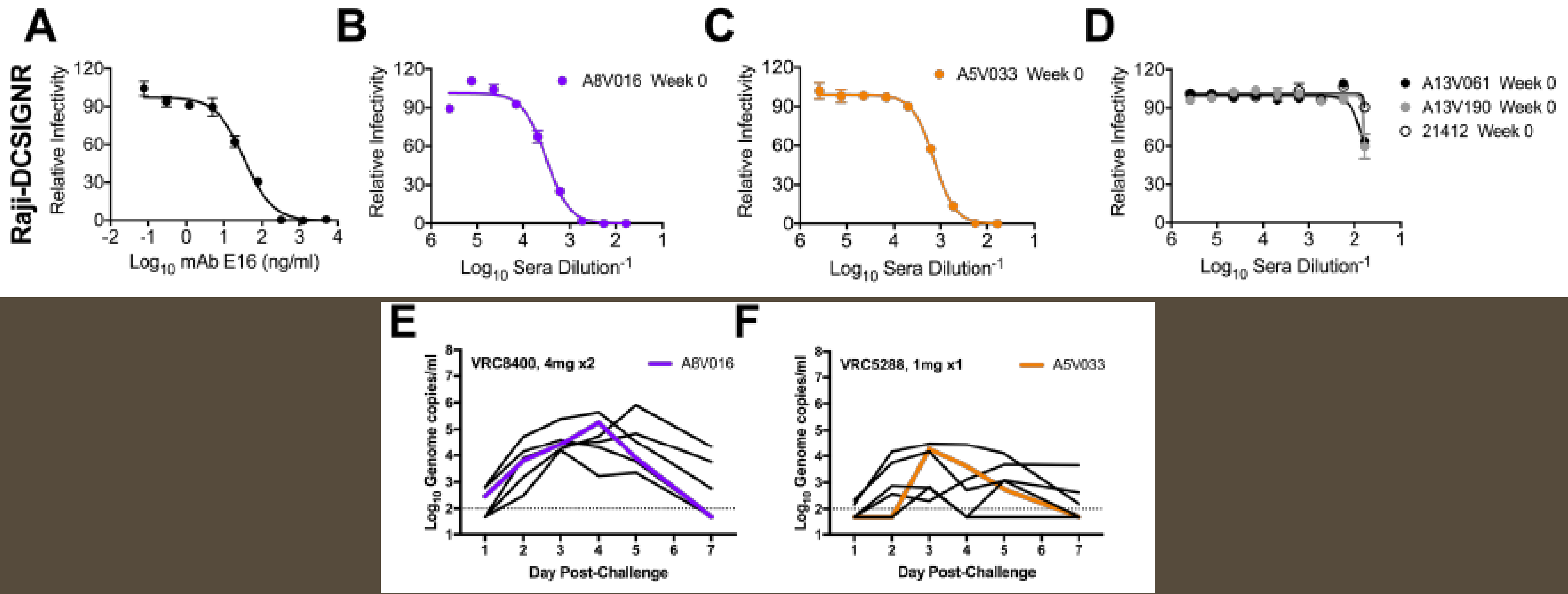


PROTECTION FROM ZIKV CHALLENGE CORRELATES WITH NAb TITERS PRESENT AT CHALLENGE



CAVEATS

- in Dengue virus (also a flavivirus), waning immunity was associated with a heightened risk for secondary infections and severe disease



https://clinicaltrials.gov/ct2/show/NCT02840487?term=NCT02840487&rank=1

Safety and Immunogenicity of a Zika Virus DNA Vaccine, VRC-ZKADNA085-00-VP, in Healthy Adults

This study is ongoing, but not recruiting participants.

Sponsor:
National Institute of Allergy and Infectious Diseases (NIAID)

Information provided by (Responsible Party):
National Institutes of Health Clinical Center (CC) (National Institute of Allergy and Infectious Diseases (NIAID))

ClinicalTrials.gov Identifier:
NCT02840487

First received: July 19, 2016
Last updated: April 20, 2017
Last verified: April 11, 2017
History of Changes

CONCLUSIONS

- a Phase I trial with VRC5288 was initiated
  - > to test a variety of regimens and doses for safety and immunogenicity

Estimated Enrollment:	120
Study Start Date:	July 11, 2016
Estimated Study Completion Date:	December 28, 2018
Estimated Primary Completion Date:	December 29, 2017 (Final data collection date for primary outcome measure)

RAPID, LOW-COST DETECTION OF ZIKA VIRUS USING PROGRAMMABLE BIOMOLECULAR COMPONENTS

Pardee et al., Cell 2016

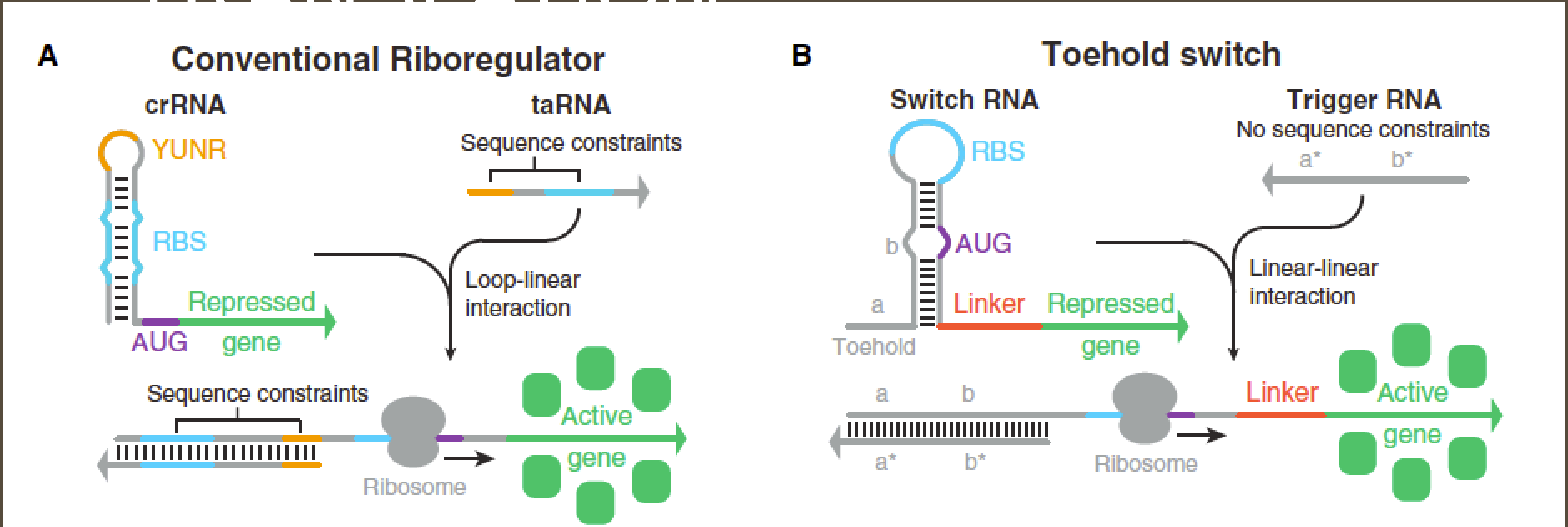
GOAL OF THE STUDY

- to create a cell-free, paper-based, low-cost RNA sensor for the Zika virus genome

DETECTION OF ZIKA VIRUS

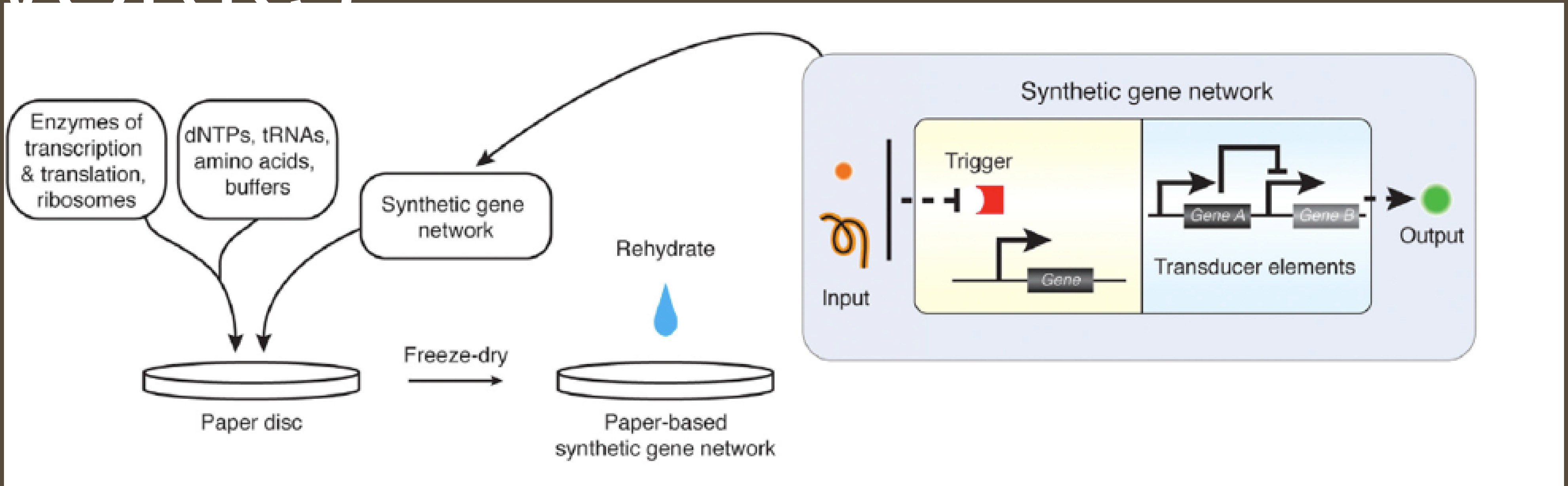
- serological approaches (e.g. antibodies) are flawed by cross-reactivity with infections through other flaviviruses
- nucleic acid based detection methods have to be employed to specifically detect the infectious agent, such as PCR or isothermal nucleic acid amplification
- however, NA-based detection methods are costly, need trained personnel and equipment

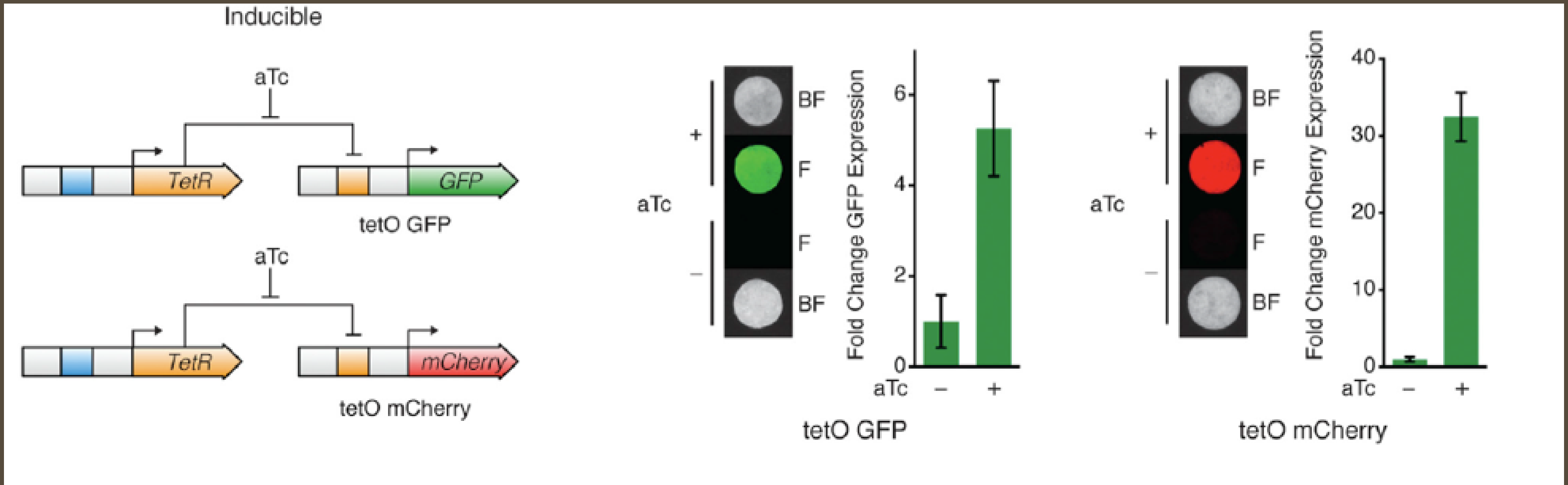
TOEHOLD SWITCHES FOR CONTROL OF RNA TRANSLATION



Green et al., Cell 2014

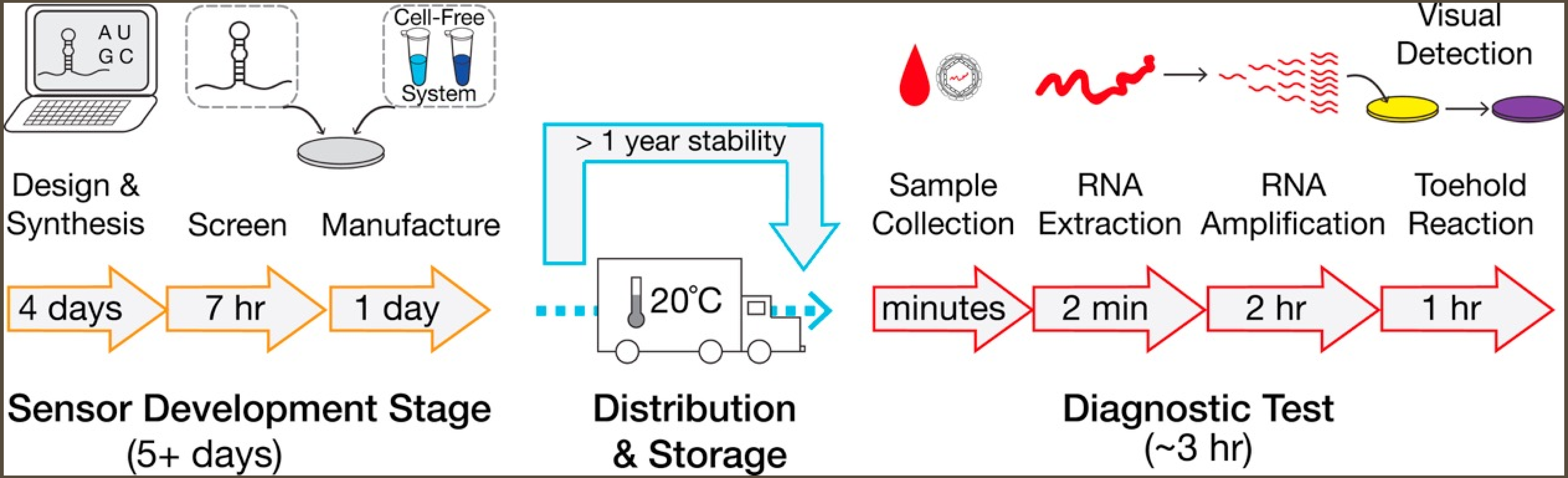
PAPER-BASED GENE NETWORKS



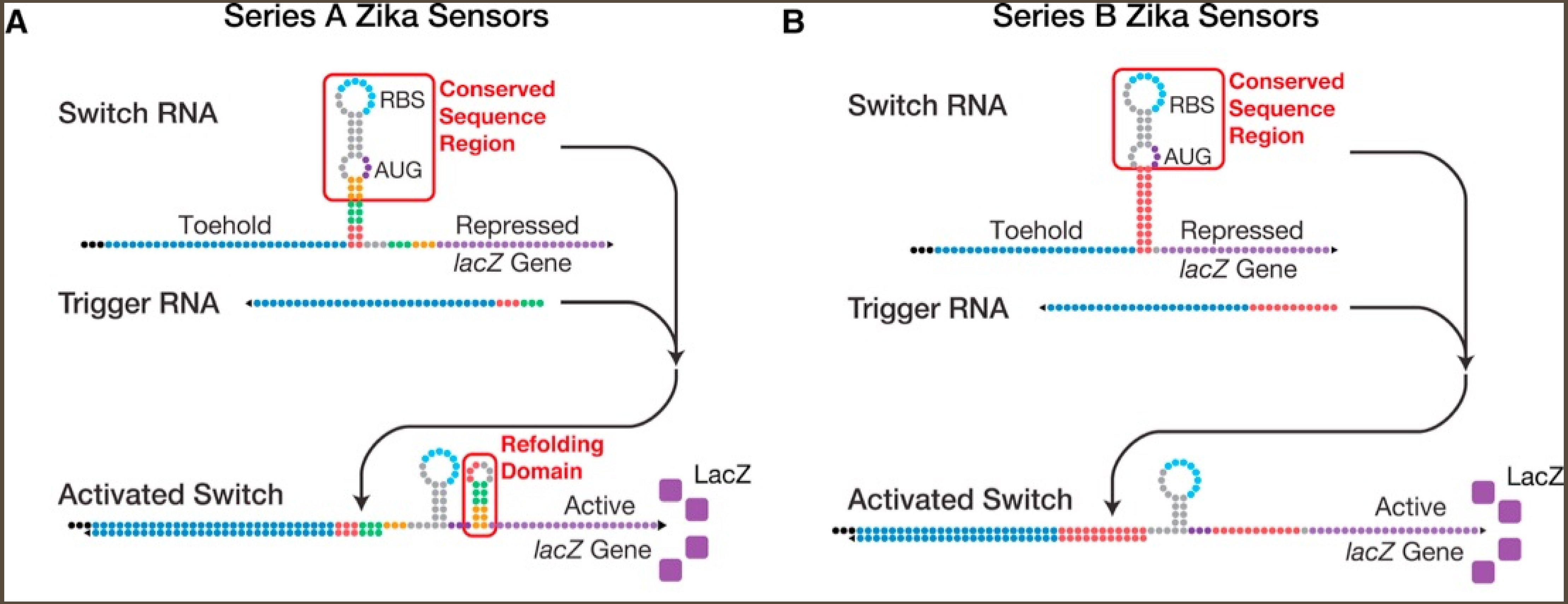


Pardee et al., Cell 2014

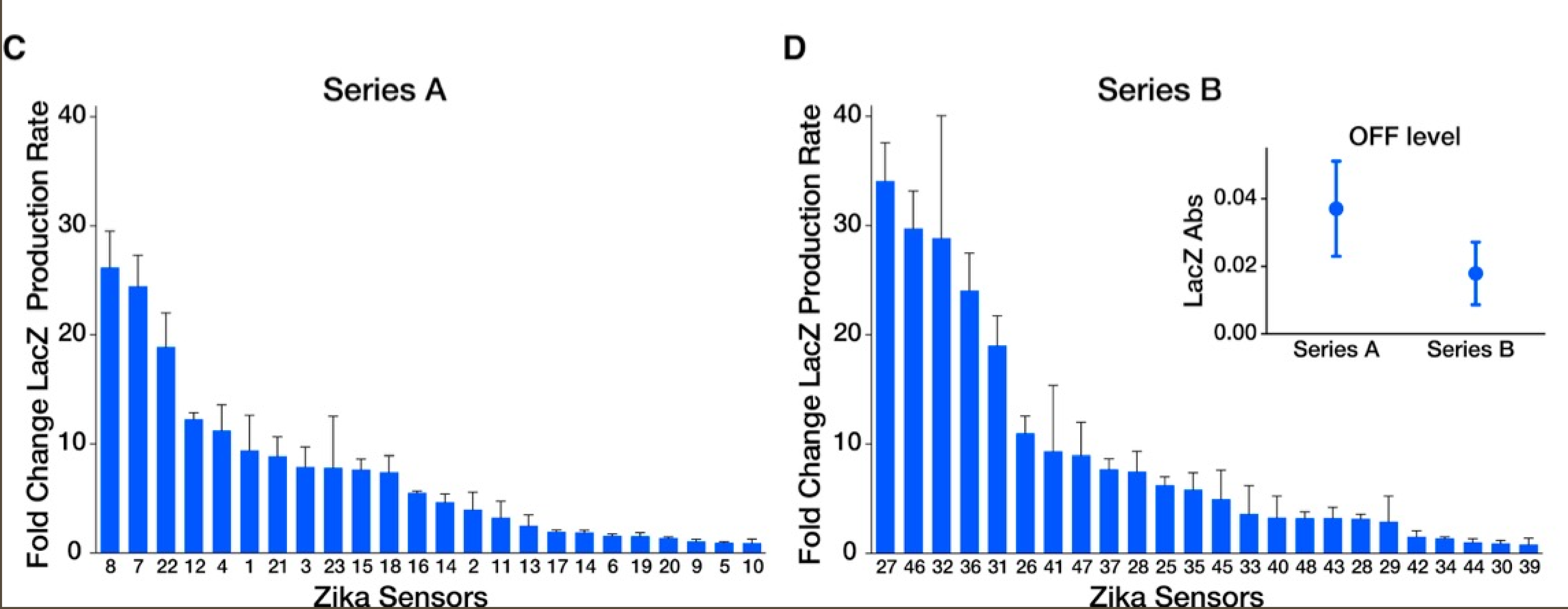
WORKFLOW FOR SYNTHESIS, VALIDATION AND APPLICATION OF ZIKA VIRUS TOEHOLD SENSORS



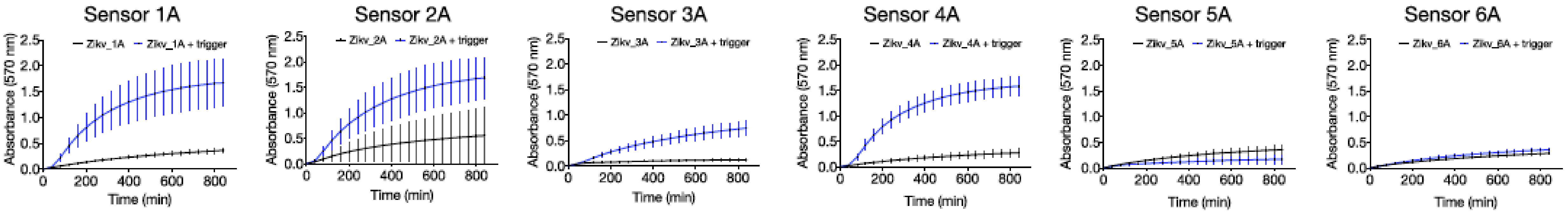
2 VERSIONS OF ZIKA VIRUS TOEHOLD SWITCHES



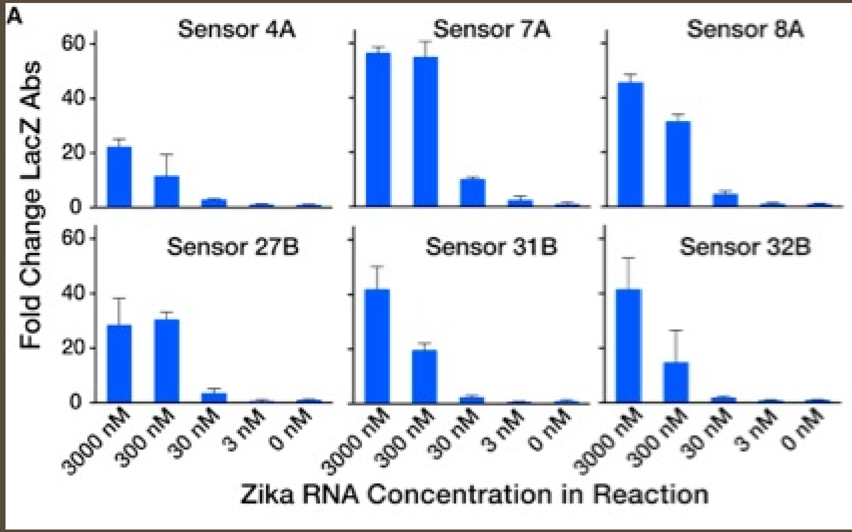
REDUCED lacZ LEAKAGE IN THE OFF STATE THROUGH LACK OF REFOLDING DOMAIN IN VERSION B

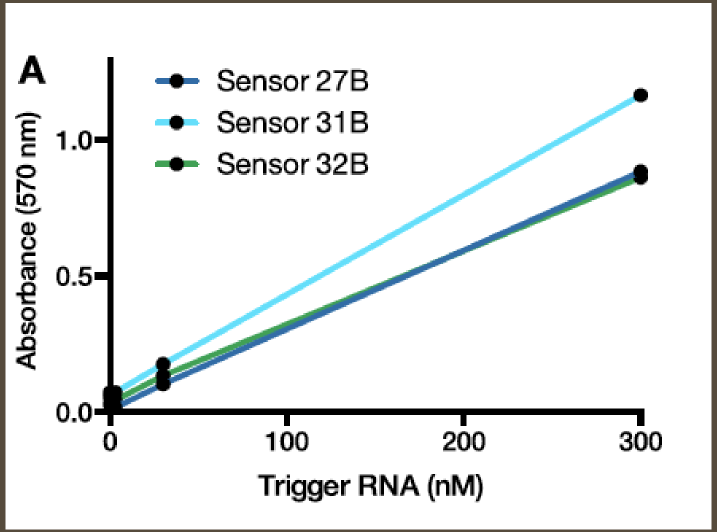


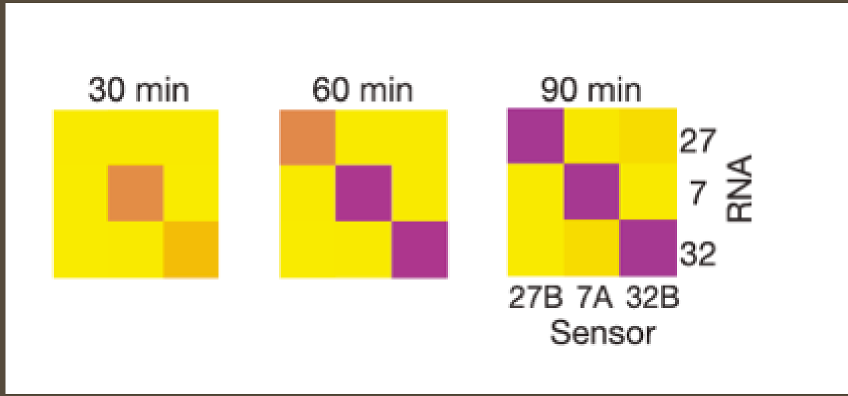
OVER 50% OF ALL SENSORS TESTED SHOWED A 5-FOLD INCREASE BY ADDITION OF TRIGGER+SENSOR



SENSORS ARE ACTIVATED FROM c(RNA)=30 nM ON AND THE RESPONSE IS LINEAR AND ORTHOGONAL





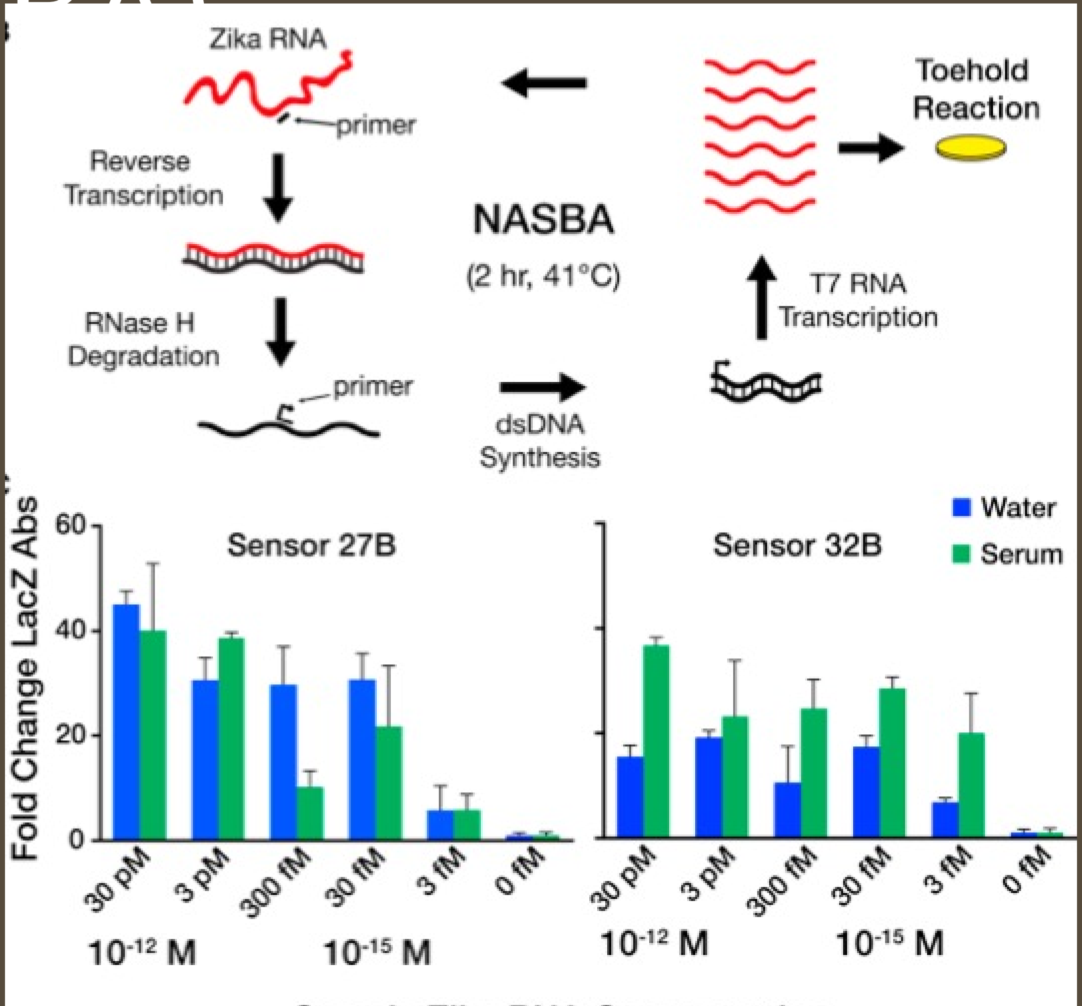


30 nM IS NOT ENOUGH

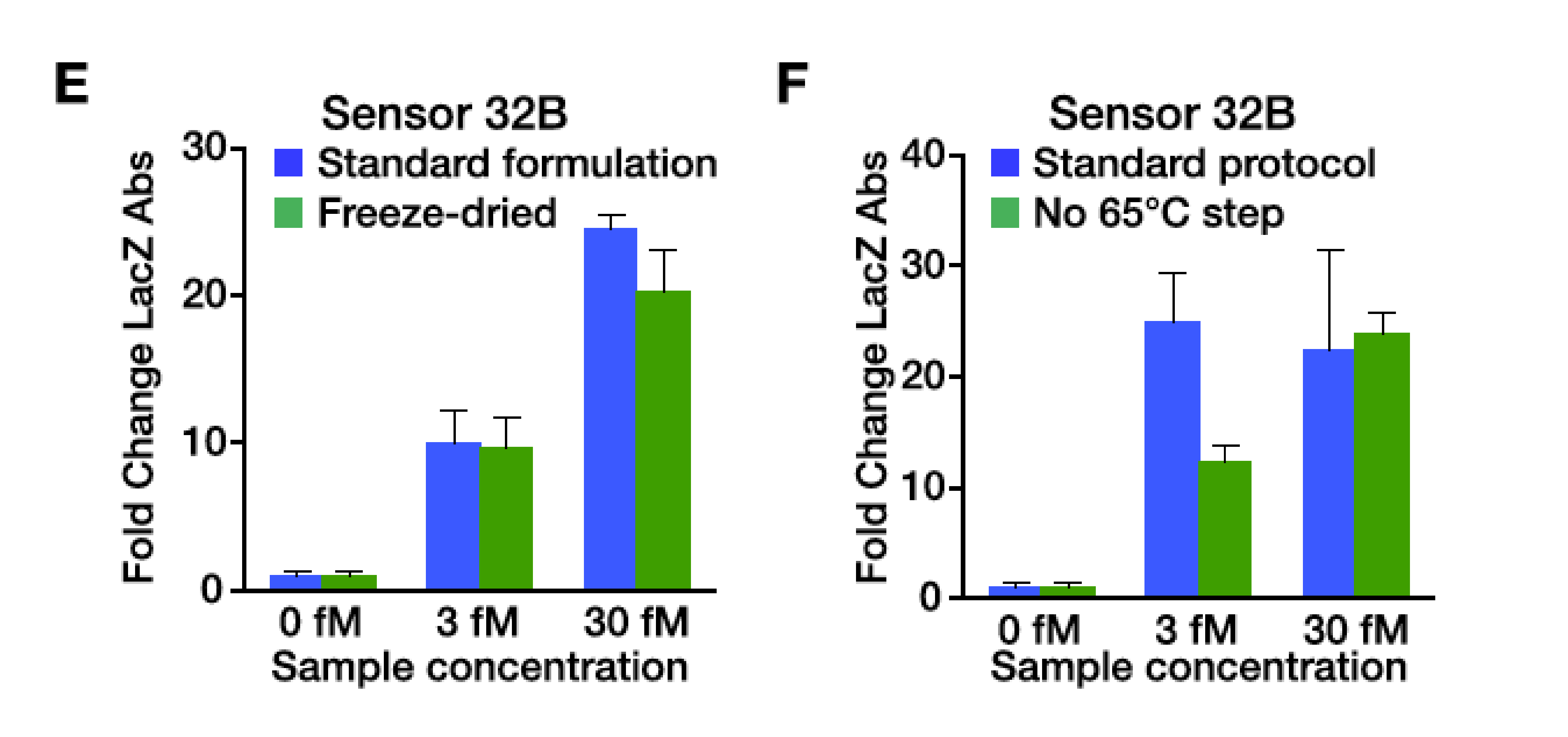
Concentrations of Zika virus in body fluids:

- urine (human) – 365 fM (Gourinat et al., Emerg Infect Dis 2015)
- saliva (human) – 4.9 fM (Barzon et al., Euro Surveill 2016)
- serum (non-human primate) – 4.1 fM (Zika Experimental Science Team. 2016)
- serum (human) – 1.2 fM (Lanciotti et al., Emerg Infect Dis 2008)

GAINING SENSITIVITY THROUGH IMPLEMENTATION OF NUCLEIC ACID SEQUENCE BASED AMPLIFICATION (NASBA)



NASBA REACTION KIT CAN BE FREEZE DRIED AND IS FUNCTIONAL IN THE ABSENCE OF INITIAL 65°C HEATING STEP



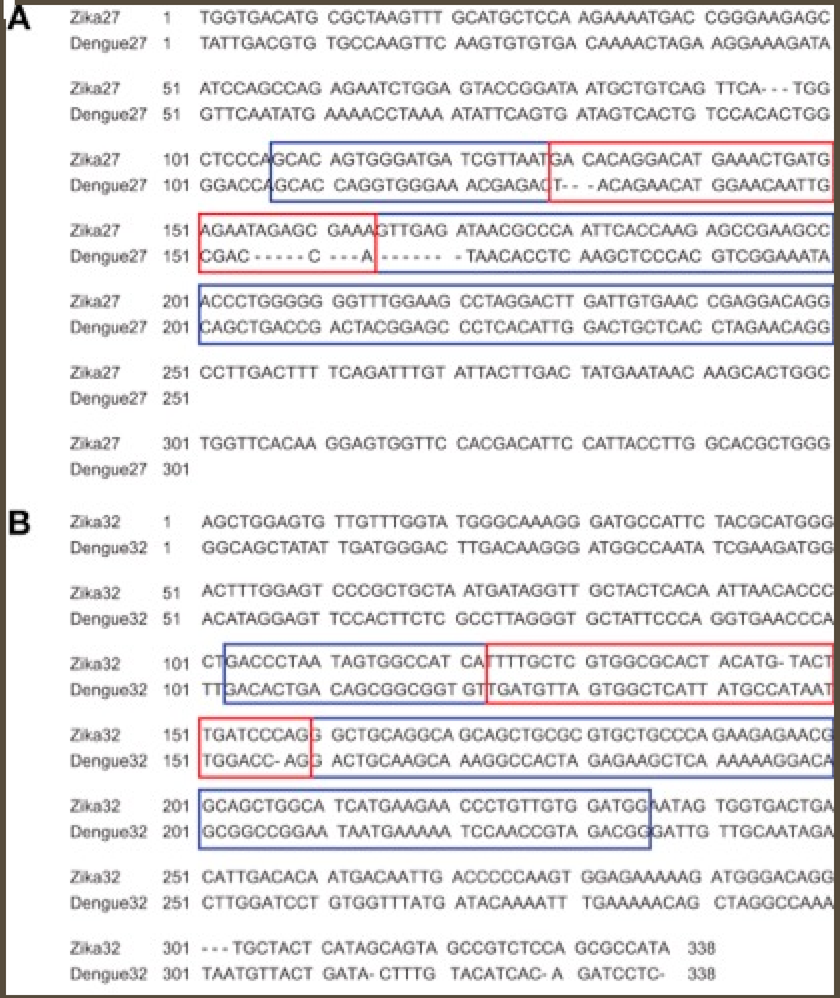
MOVING TOWARD A FIELD-READY DIAGNOSTIC PLATFORM

1) testing sensor specificity against related viruses that share clinical symptoms, partial homology, and geographic range with Zika virus

2) building a second-generation portable, battery-powered reader to provide lab-quality results in low-resource environments

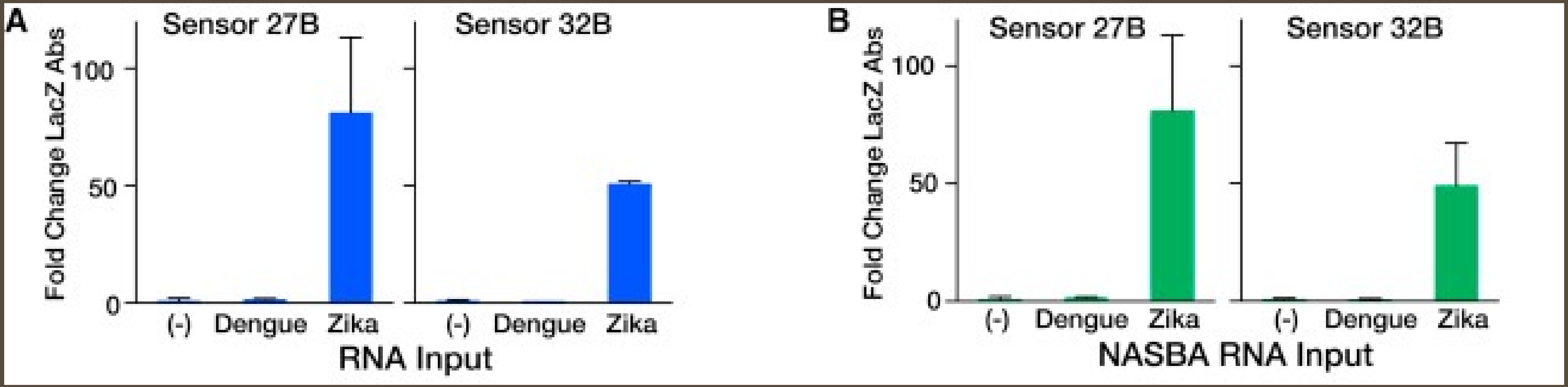
3) developing a low-cost and tractable method for viral RNA extraction.

DENGUE AND ZIKA SHARE AROUND 50% SEQUENCE HOMOLOGY



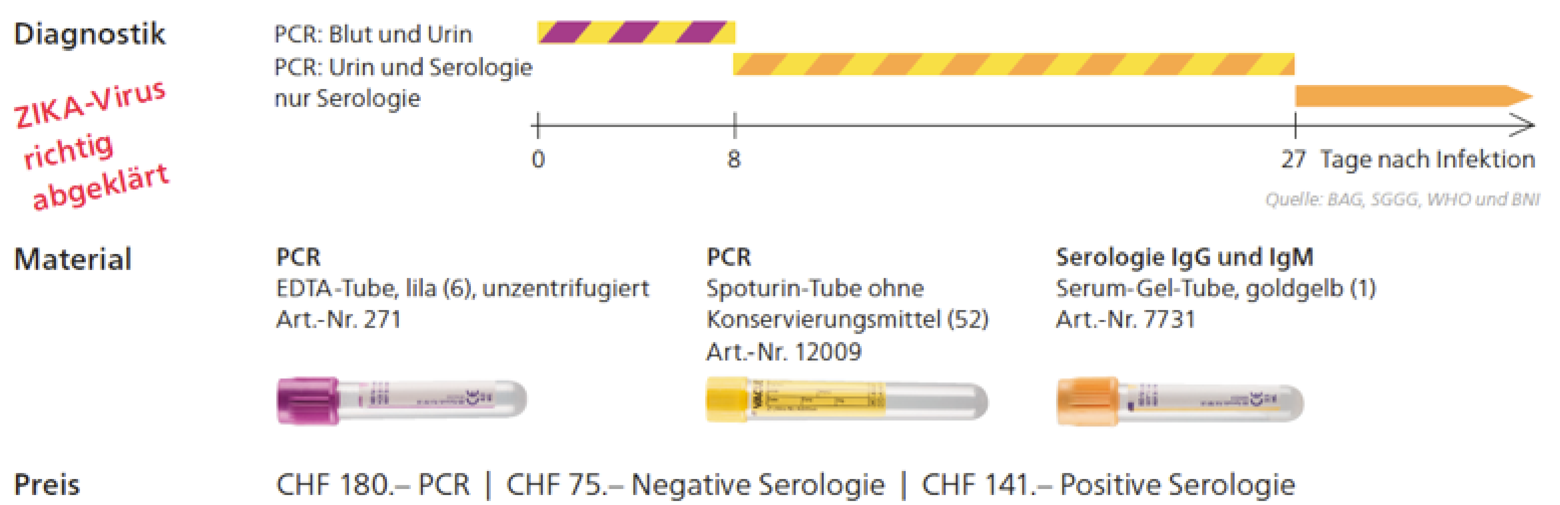
Red boxes: trigger RNA
Blue boxes: NASBA sequences

DENGUE RNA AND NASBA-AMPLIFIED DENGUE RNA DOES NOT ACTIVATE TOEHOLD SENSORS



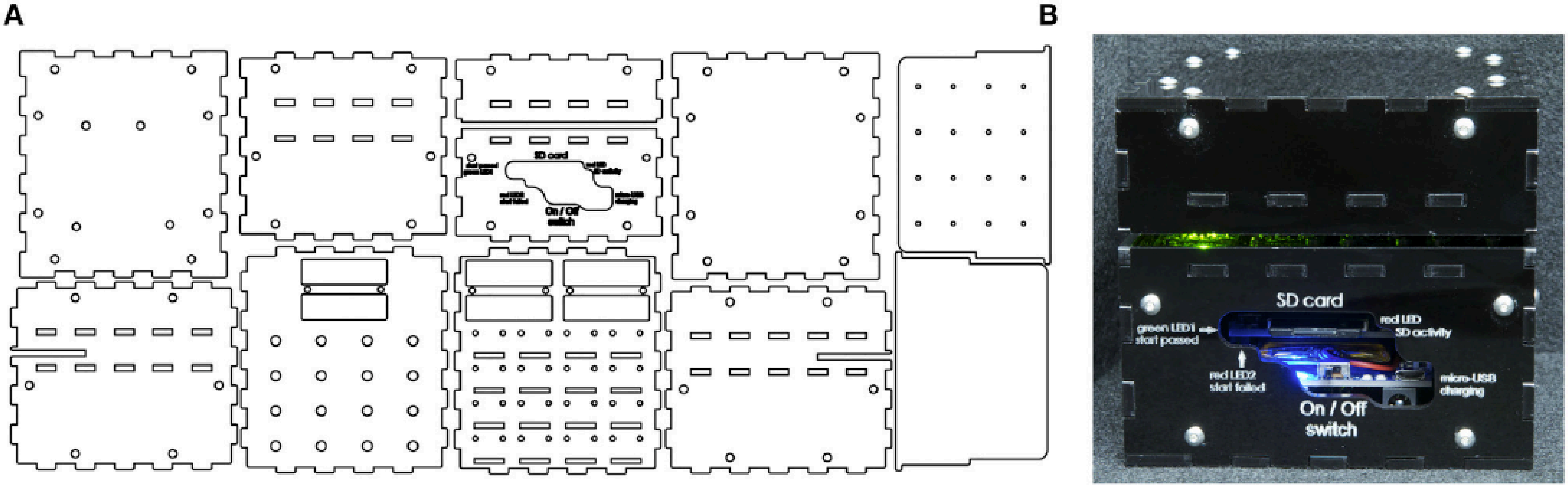
BUILDING AN ELECTRONIC READER

- readily available consumer components
- low-cost companion technology
- open-source software
- laser-cut acrylic housing
- Li-Ion battery, rechargable
- 4 GB on-board storage
- total cost < 250$

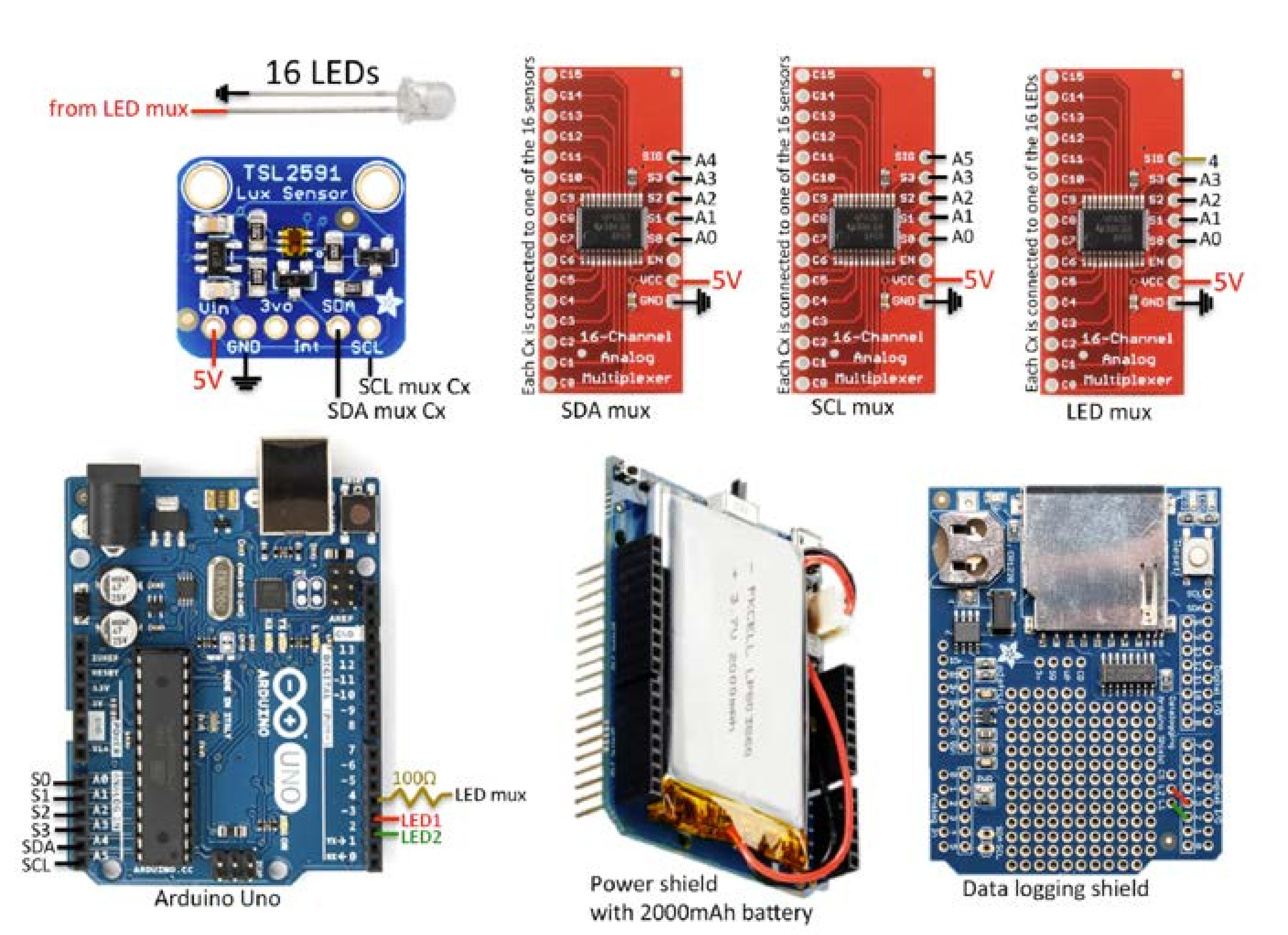


From: viollier.ch

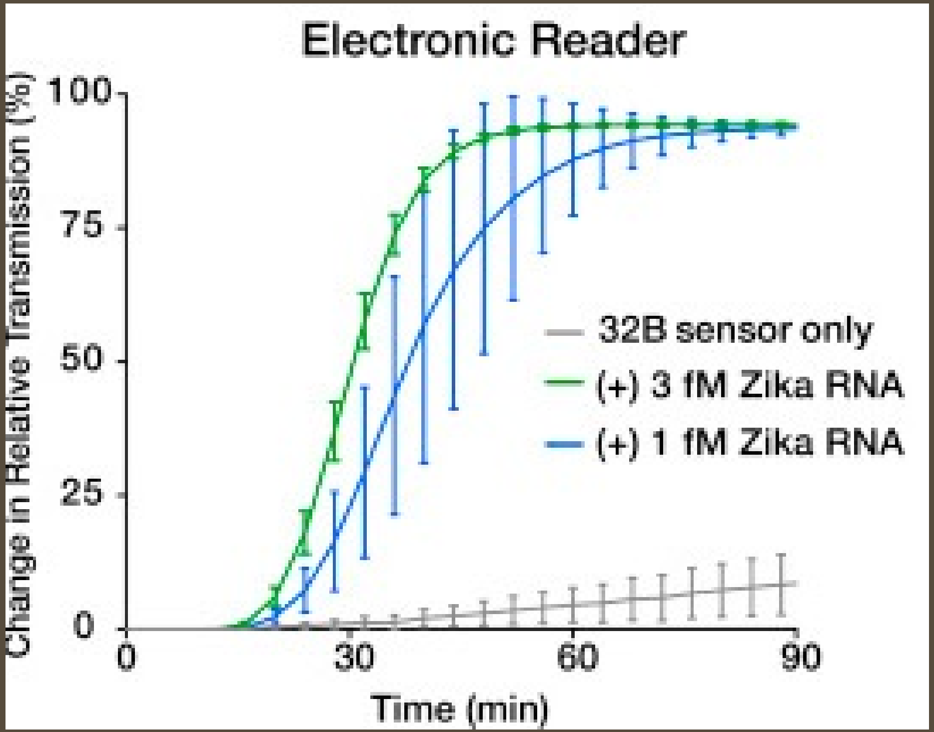
PORTABLE ELECTRONIC READER



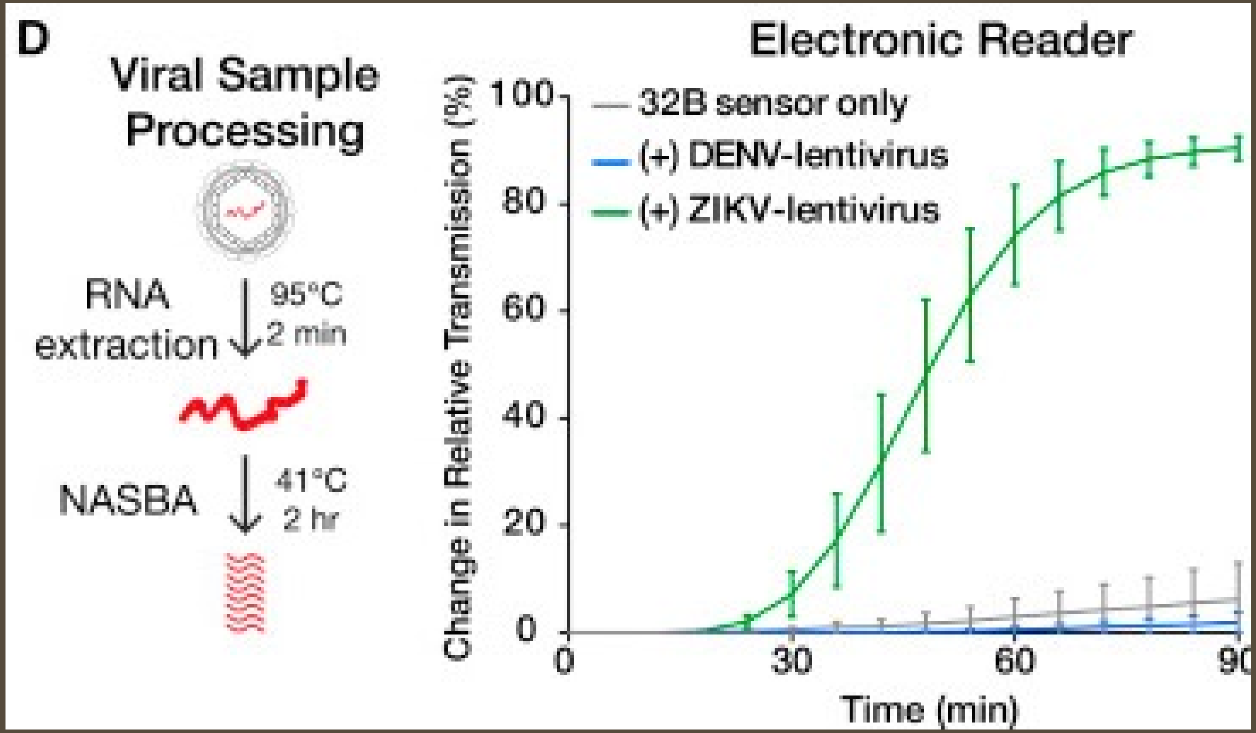
PORTABLE ELECTRONIC READER

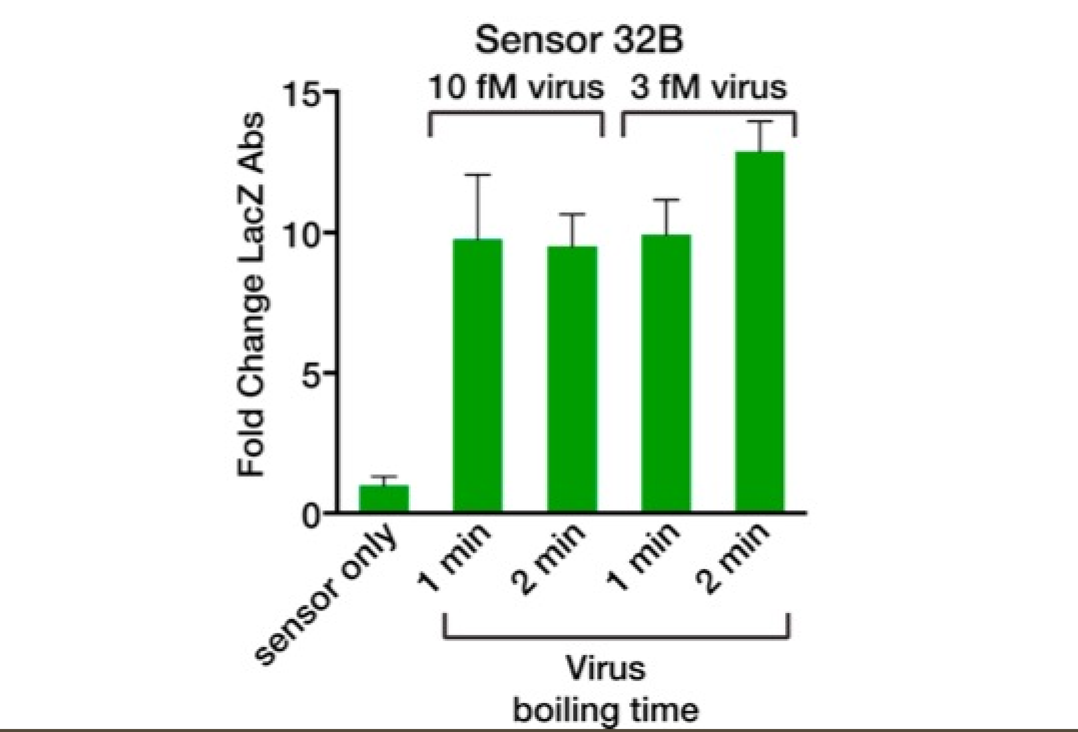


DETECTION OF CLINICALLY RELEVANT RNA CONCENTRATIONS AFTER A FEW MINUTES



INCORPORATION OF A VIRUS EXTRACTION PROTOCOL



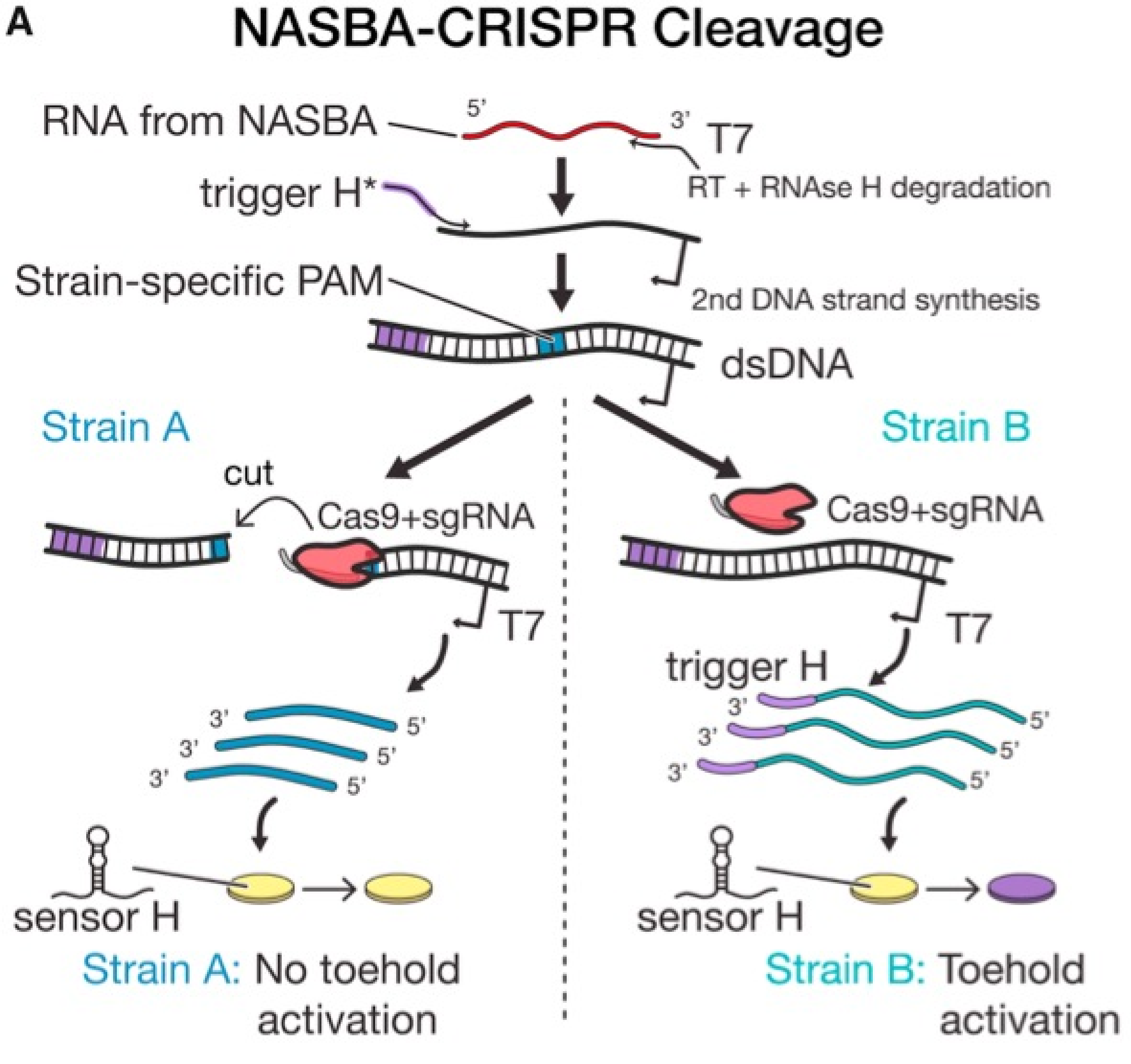


NASBA-CRISPR BASED CLEAVAGE (NASBACC) ASSAY

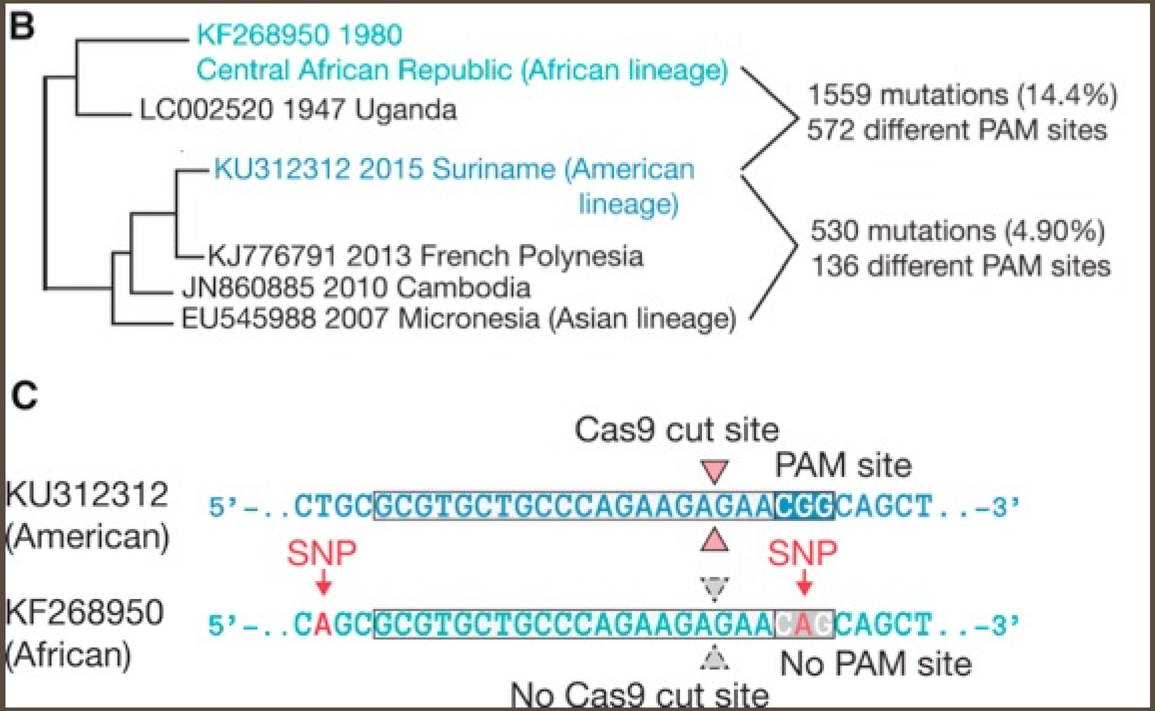
- development of a CRISPR-based cleavage assay that can distinguish different strains on a single-base resolution

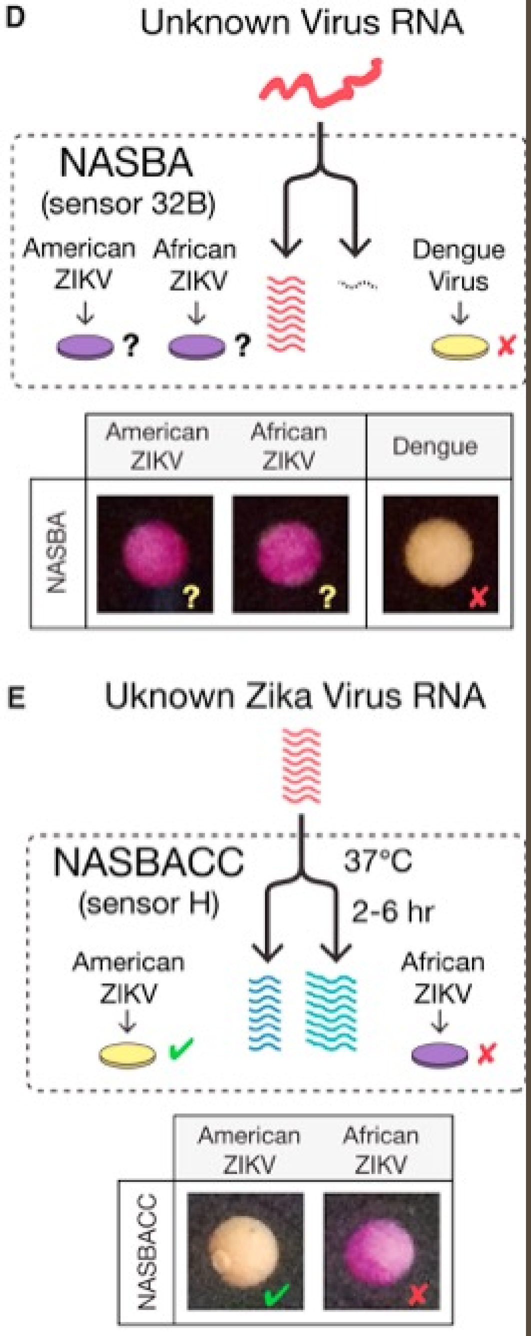
Each mutation has a 23% probability (**44/192**) of creating a new PAM site, a 23% probability (**44/192**) of destroying and existing PAM site, and a 2% probability (**4/192**) of inverting the orientation of an existing PAM site.
Overall, any given point mutations has a 48% probability (**92/192**) of disrupting an existing PAM site.

WORKFLOW OF NASBACC

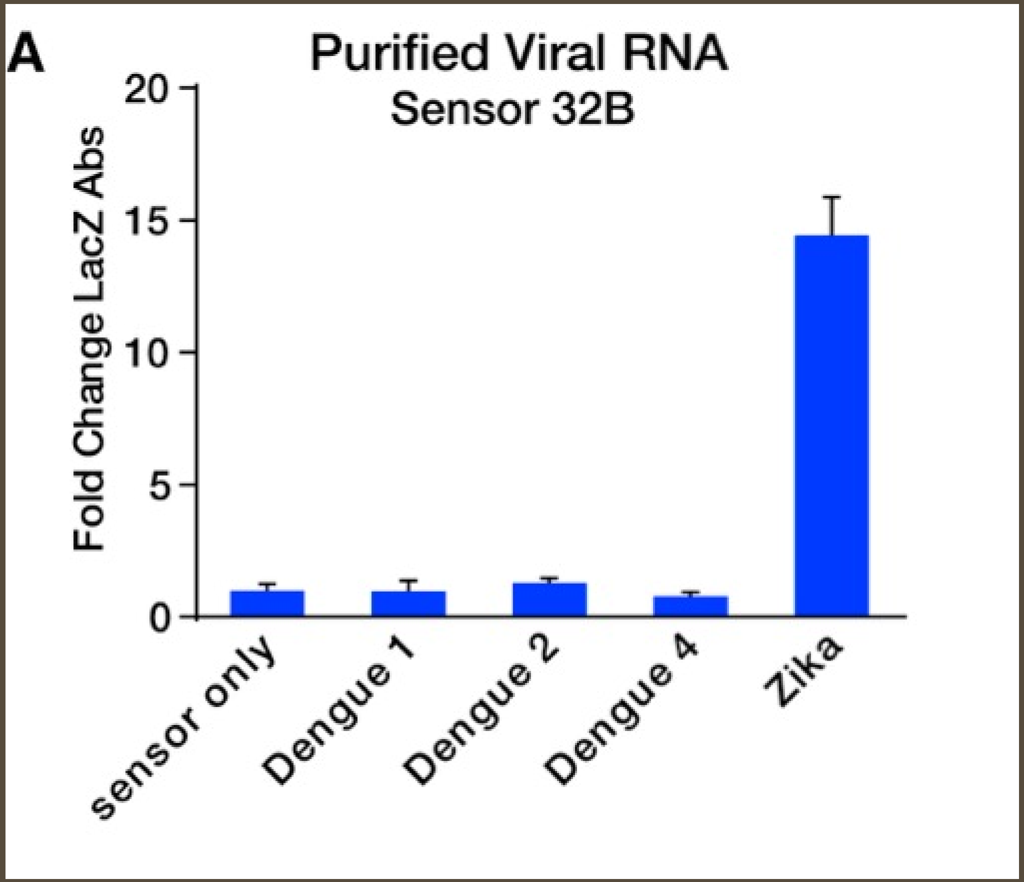


DETECTION OF DIFFERENT STRAINS USING NASBACC

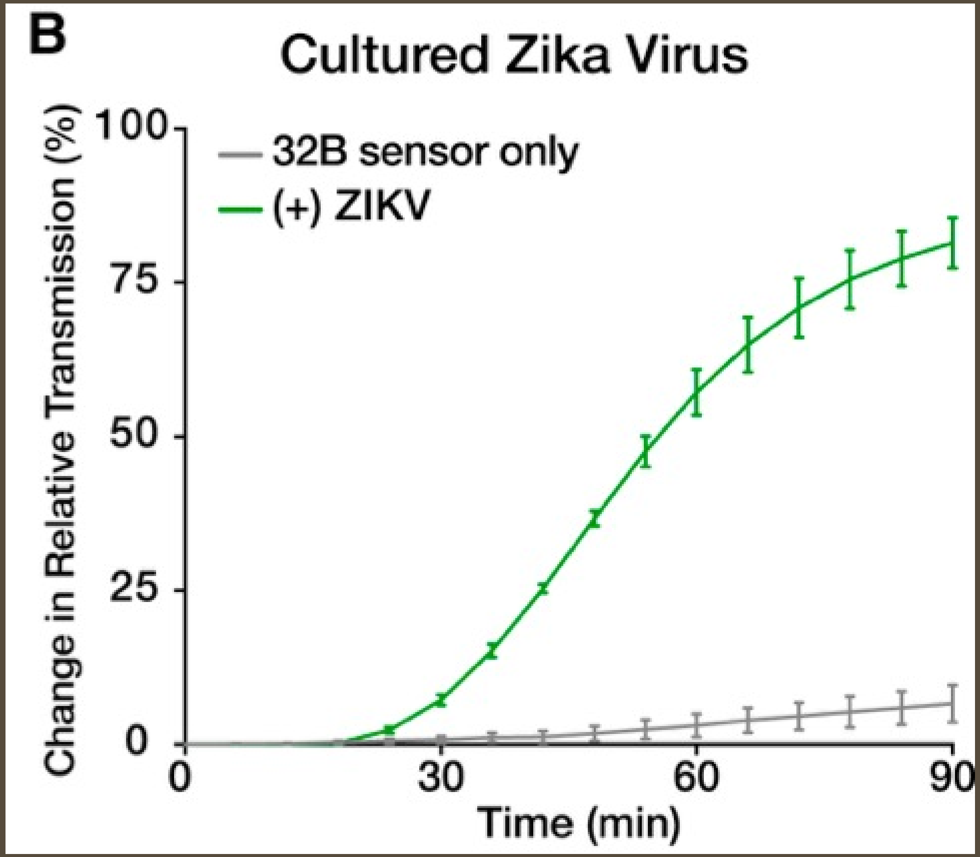




VALIDATION OF DIAGNOSTIC WORKFLOW ON PURIFIED ZIKA VIRUS RNA

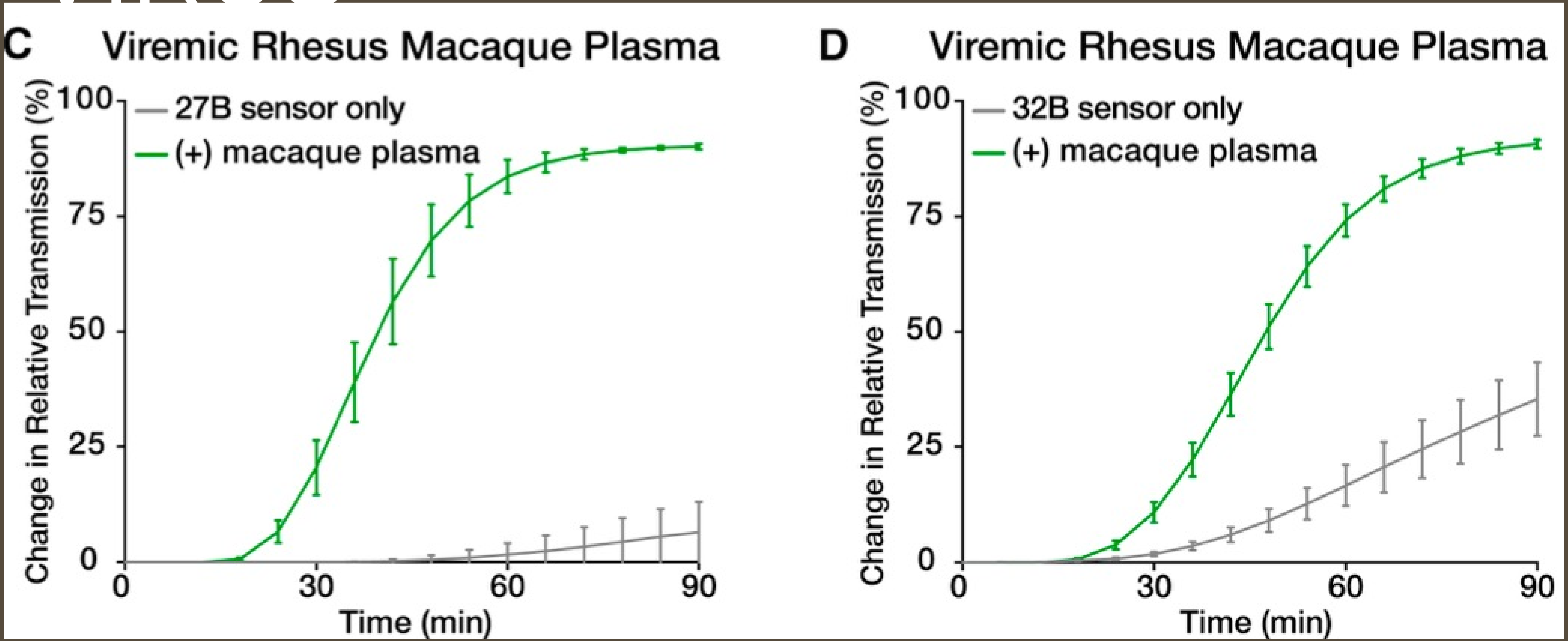


VALIDATION OF DIAGNOSTIC WORKFLOW ON LIVE ZIKA VIRUS



Active Zika virus was cultured in the laboratory and spiked into human serum (7%) at a final concentration of 10 fM, to mimic a clinical sample

VALIDATION OF DIAGNOSTIC WORKFLOW ON LIVE ZIKA VIRUS



1:10 diluted viremic macaque plasma with an undiluted titer of 2.8 fM

CONCLUSIONS

PROs

- cheap, reliable detection method for fast (and strain-specific detection) of several Zika, but not Dengue strains

CONTRAs

- sensitivity is on the edge of what is described in the literature (3 fM, one report suggests 1.8 fM RNA viral load in one human)
- test is just established with blood, but not with saliva, urine etc. (maybe more readily accessible in the field)

Thank you