CRISPR-Based Disease Detection Strategies

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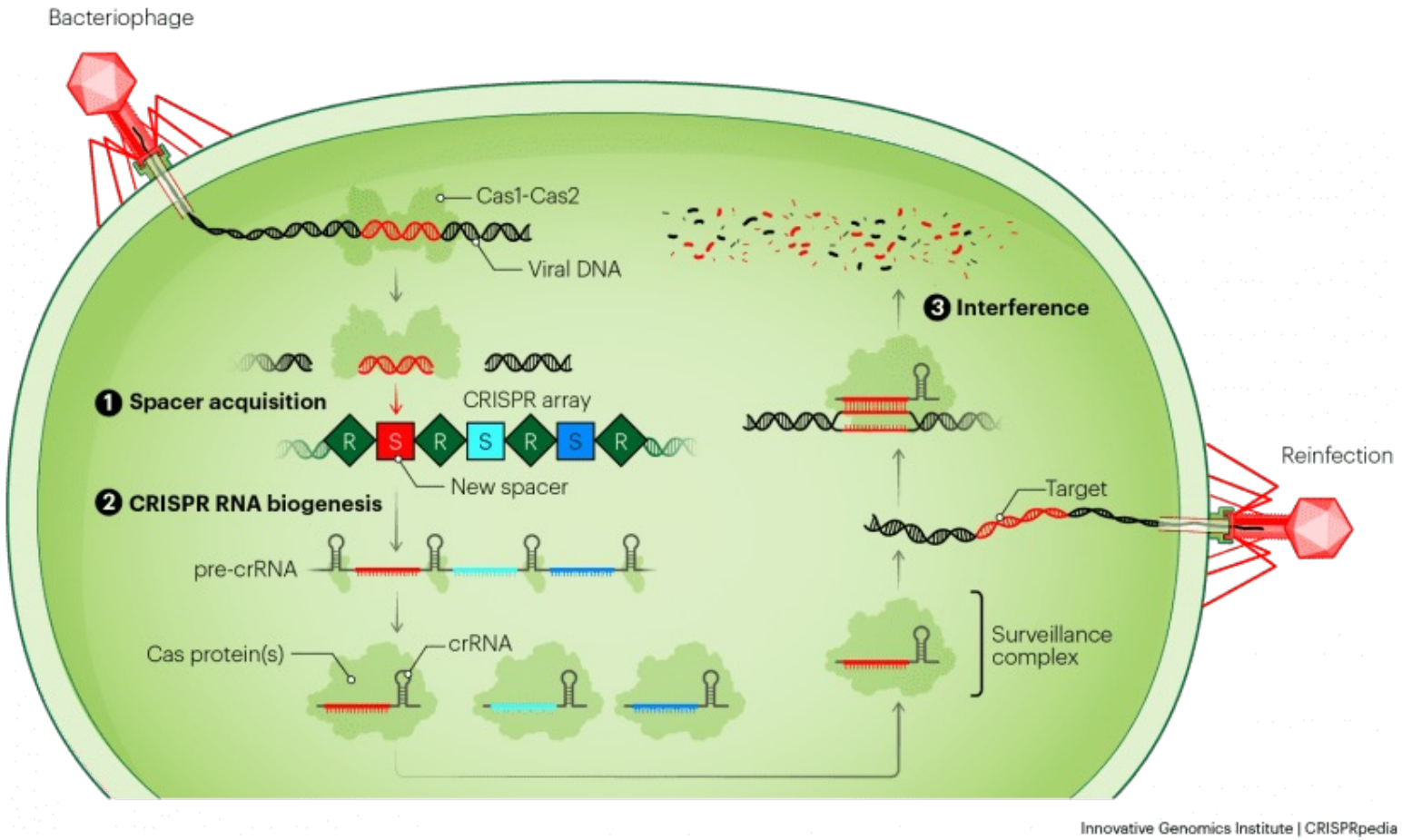
What is CRISPR?

- **C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats
- Cas- **C**RISPR-**as**sociated protein



History of CRISPR

- **1987**- Dr. Yoshizumi Ishino and his team noticed an odd series of repeated, interspersed sequences when cloning a gene in *E. coli*
- **1993-** J.D. van Embden discovered different strains of *M. tuberculosis* had unique spacer sequences between repeats. Mojica and Jansen first coined CRISPRs
- **2007-** Rodolphe Barrangou and others at Danisco found that spacers were integrated viral genes and removing or adding these spacers changed bacterial resistance to phage attacks

(Asian Scientist Magazine, Oct. 2020)

(RiffReporter, Mar. 2020)

(Bruce McCabe, Nov. 2022)

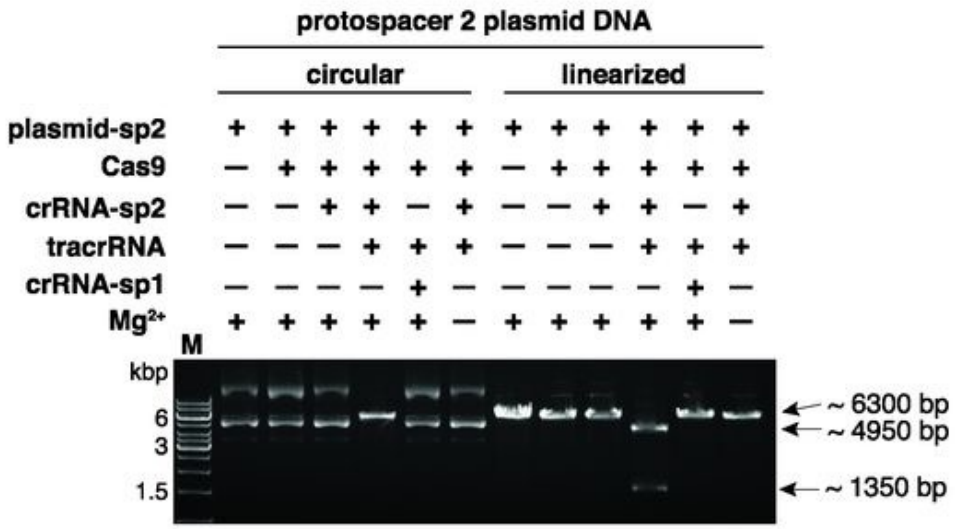
The Gel that Changed the World

RESEARCH ARTICLE

A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity

MARTIN JINEK, KRZYSZTOF CHYLINSKI, INES FONFARA, MICHAEL HAUER, JENNIFER A. DOUDNA , AND EMMANUELLE CHARPENTIER Authors Info & Affiliations

SCIENCE • 28 Jun 2012 • Vol 337, Issue 6096 • pp. 816-821 • DOI: 10.1126/science.1225829

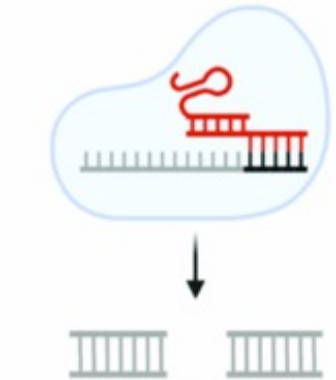
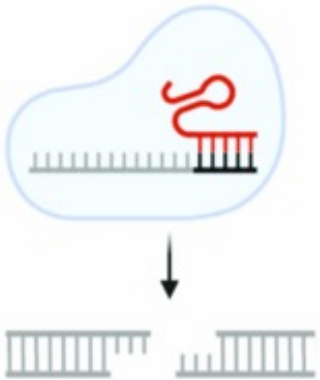
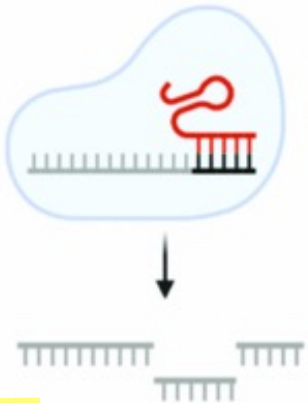


(Jinek, M., et al. 2012)

("Jennifer Doudna – Academy of Achievement," 2023)

Common CRISPR Terms

- crRNA- **CR**ISPR **RNA**;
  - Entire RNA that associates with Cas-protein, includes scaffold and spacer region
- gRNA- **G**uide **RNA**
  - Spacer region on crRNA that ‘guides’ Cas-protein where to cut based on complementarity to target
- PAM- **P**rotospacer **A**djacent **M**otif
  - Short sequence Cas-proteins search for first before checking target region for complementarity
- PFS- **P**rotospacer **F**lanking **S**equence
  - Similar to PAM but for Cas13, not every Cas13 protein variant has a PFS
- Collateral Cleavage a.k.a *trans*-Cleavage
  - Non-discriminate cleavage caused by activated Cas-proteins in the Type V family after finding their target sequence

	Cas9	Cas12	Cas13
	 dsDNA	 dsDNA	 ssRNA
Target	DNA	DNA	RNA
Protospacer restrictions	PAM	PAM	PFS
Cut	Blunt-ended DSB	Sticky-ended DSB	Degraded RNA
Spacer size	16-20 nt	16-25 nt	25-35 nt
Characteristics	No collateral cleavage No secondary structure restrictions	Collateral cleavage No secondary structure restrictions	Collateral cleavage Secondary structure restrictions
Use	Gene editing Nucleic acid detection	Gene editing Nucleic acid detection	RNA knockdown Nucleic acid detection

(Puig-Serra, P.; et al. 2022)

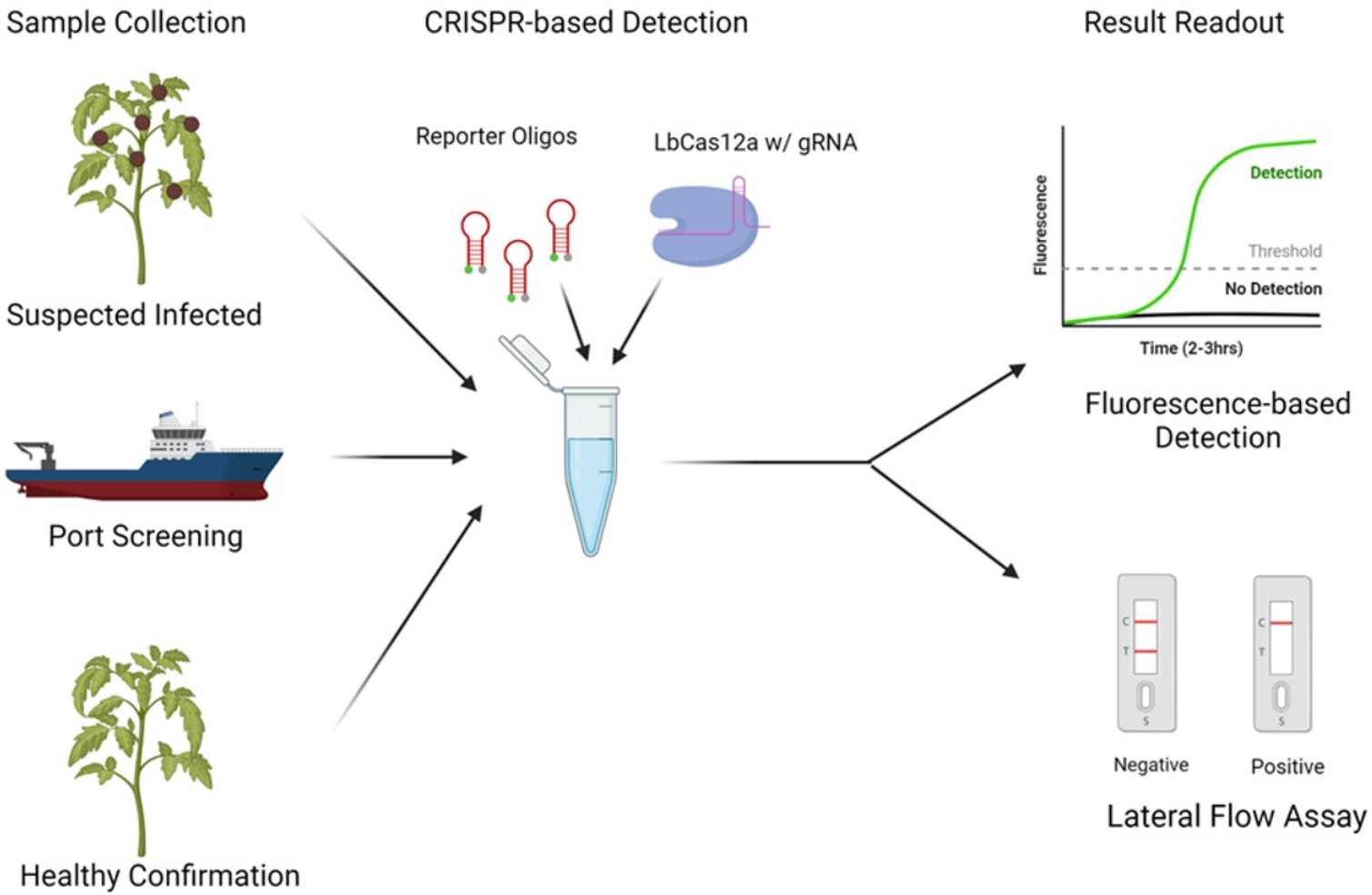
Fellowship with USDA-APHIS, PPCDL

Dr. Eric Newberry

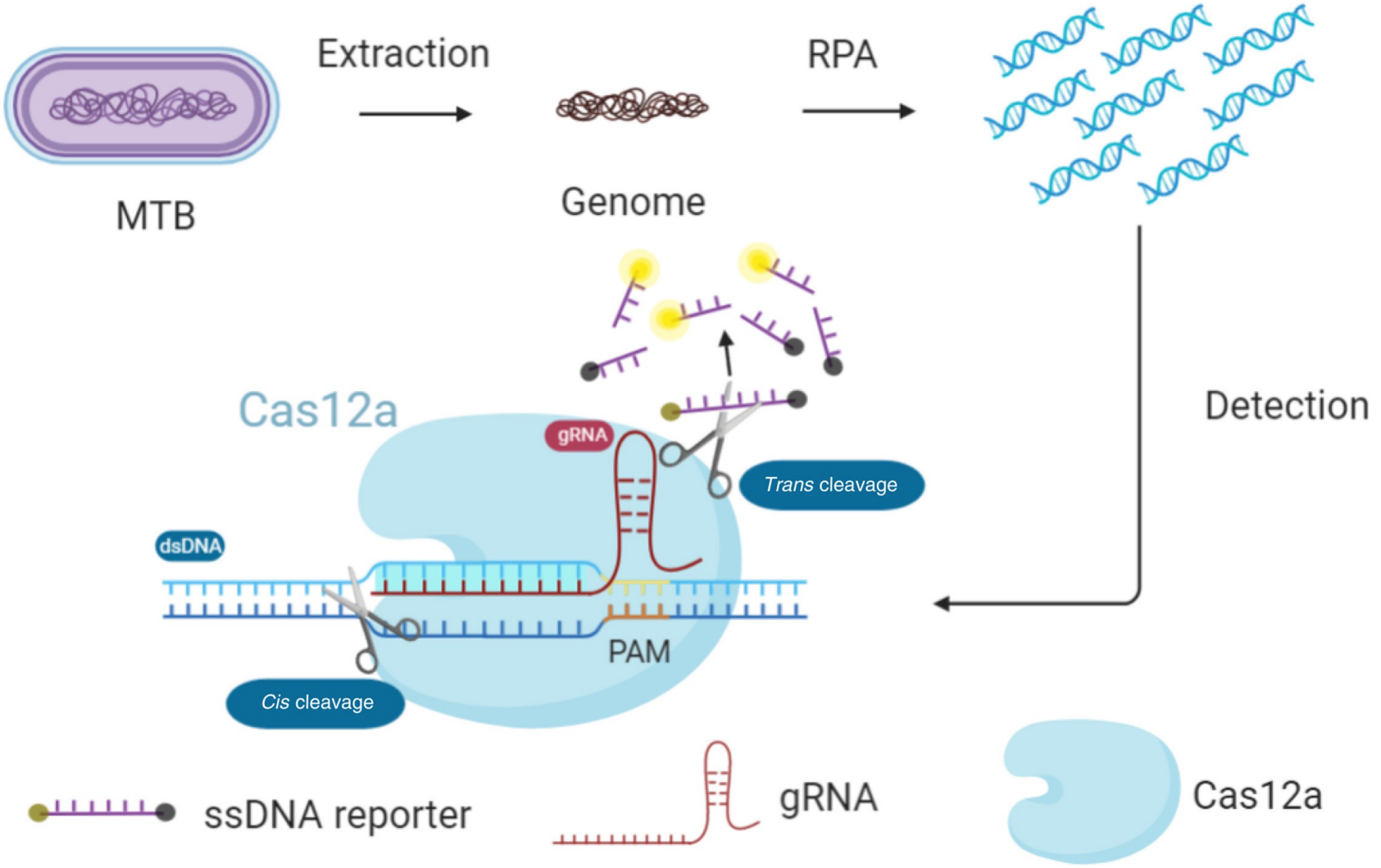
Dr. Yazmin Rivera

Dr. Stefano Costanzo

United States Department of Agriculture



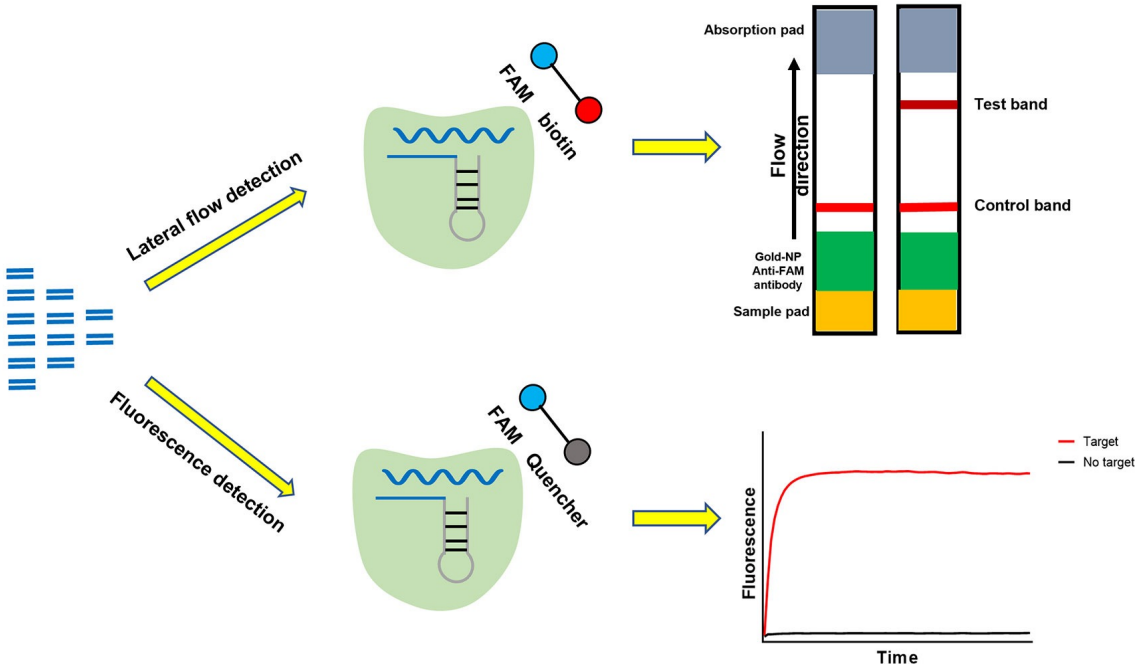
Cas12a DETECTR- **D**NA **E**ndonuclease-**T**argeted **C**RISPR **T**rans-**R**eporter



(Xu, H; et al. 2020)

ssDNA Reporter Oligos- ”Reporters” (Cas12a)

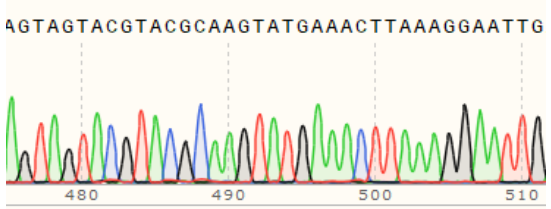
- Fluorescence-based Detection
  - **F**luorescence resonance **e**nergy **t**ransfer (FRET)-based reporter
  - Fluorophore bound to a quencher on a short oligo
  - When separated by *trans*-cleavage, emitted fluorescence increases
  - *Example: /56-FAM/ATTTA /3BHQ_1/*
- Lateral Flow Assay (LFA)
  - Paper test strip
  - Streptavidin-biotinylation
  - Portable, rapid
  - *Example: /56-FAM/ATTTA /3Bio/*

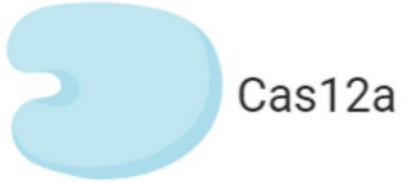


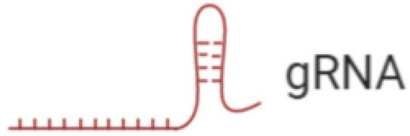
Yin, D., et al. 2022

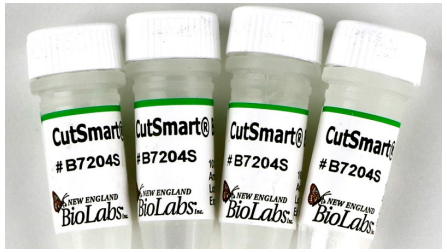
Let’s Build a CRISPR-based Detection Assay

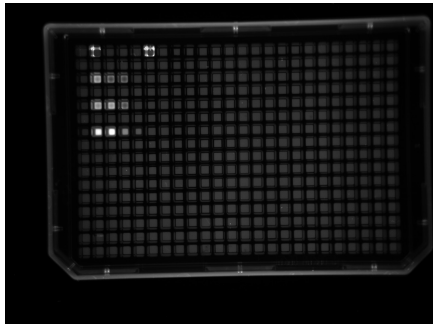
- What you will need:
  - High-quality sequence data for species of interest
  - A CRISPR/Cas-protein
    - Cas12a- DNA
    - Cas13- ssRNA
  - crRNA
    - Scaffold compatible with Cas-protein
    - gRNA sequence specific to
  - Reporter oligos
    - FAM-quencher for fluorescence
    - FAM-biotin for LFA
  - Buffer containing Mg
    - NEB-CutSmart
  - Result Readout
    - Fluorometer plate reader
    - LFA test strips

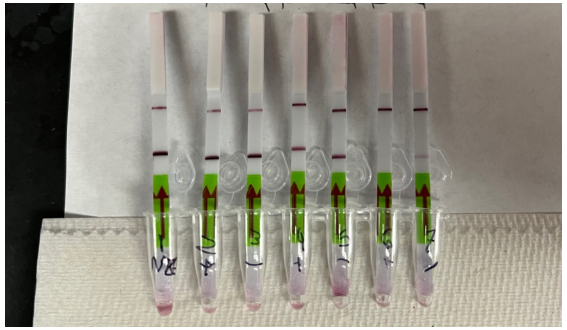








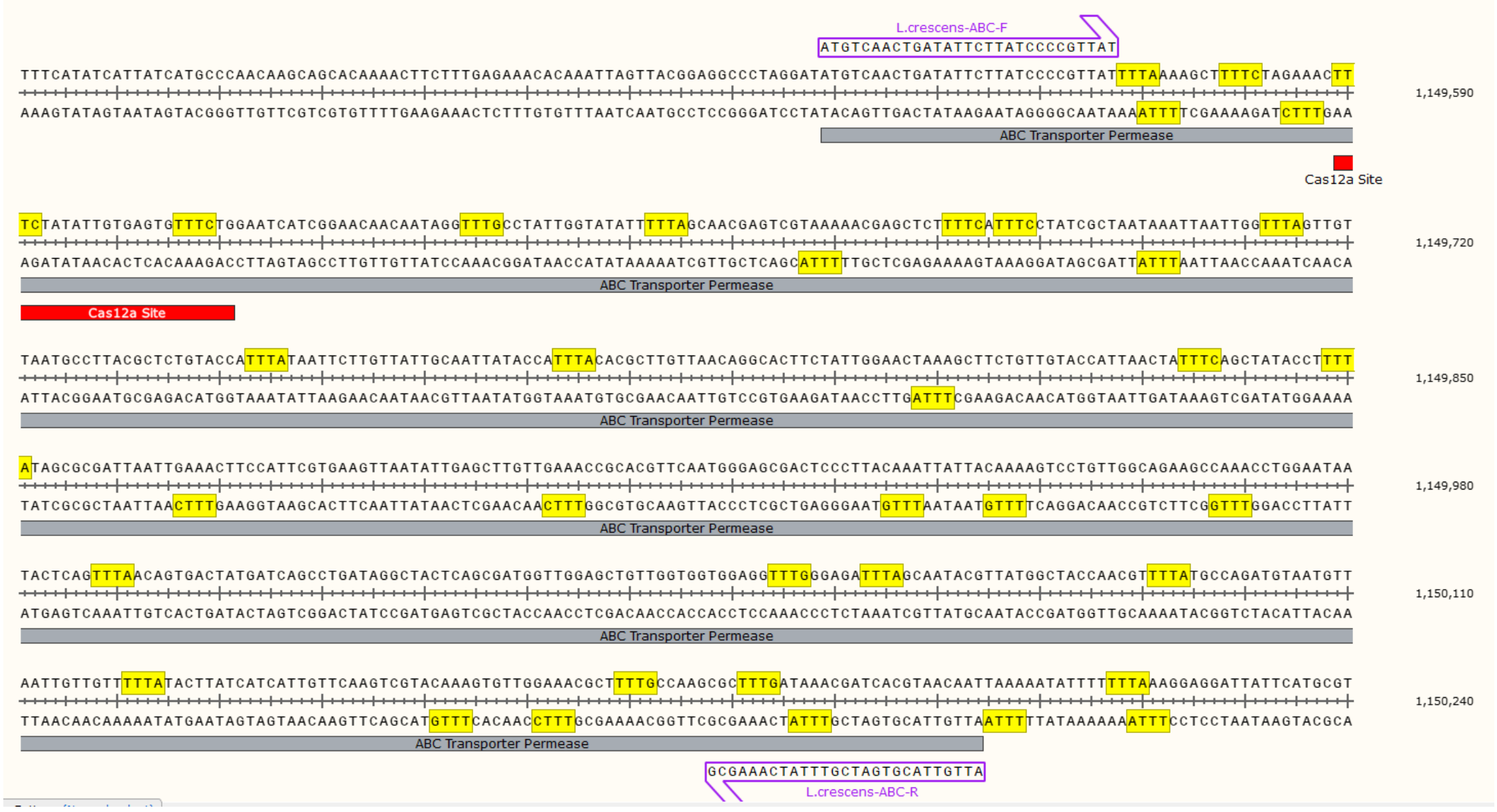




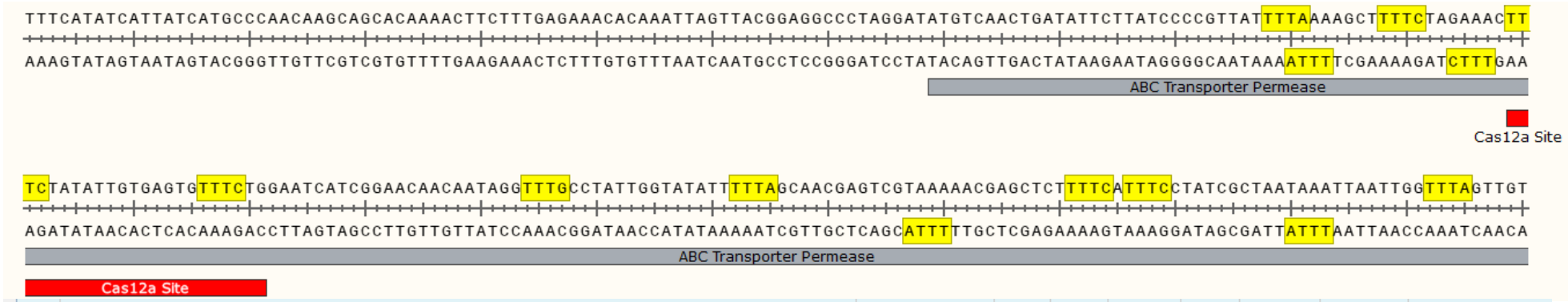
How to Find a Cas12a Target Site

- *Liberibacter crescens*
  - Culturable close-relative to *Candidatus liberibacter spp. asiaticus*
    - Casual pathogen for Huanglongbing- “Citrus Greening Disease”
  - Gram-negative bacteria, plant pathogen
    - Prokaryotic genome=DNA
- Cas12a
  - LbCas12a isolated from *Lachnospiraceae bacterium*
    - Most common Cas12a protein used in CRISPR-based DNA detection
  - PAM site requirement is TTTV (V=any N, not T)
  - 20-23nt spacer region size
  - Tolerates 1-2 mismatches depending on location to PAM

L. crescens Target Site Selection

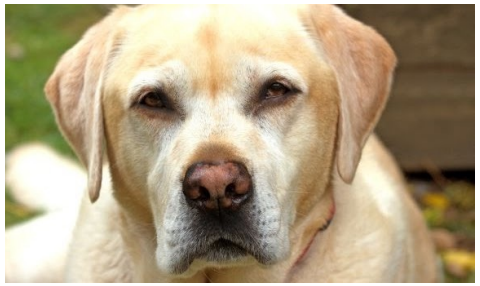


L. crescens Off-Target Screening



	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	Liberibacter crescens strain BT-0, complete genome	Liberibacter cre...	46.1	46.1	100%	0.016	100.00%	1522119	CP010522.1
☑	Liberibacter crescens BT-1, complete genome	Liberibacter cre...	46.1	46.1	100%	0.016	100.00%	1504659	CP003789.1
☑	Cervus elaphus genome assembly, chromosome: 13	Cervus elaphus	40.1	136	100%	1.0	100.00%	72175023	OU343090.1
☑	Canis lupus familiaris breed Labrador retriever chromosome 10a	Canis lupus fa...	38.2	132	100%	4.0	100.00%	69938001	CP050591.1
☑	Canis lupus familiaris breed Labrador retriever chromosome 10b	Canis lupus fa...	38.2	132	100%	4.0	100.00%	69942321	CP050611.1
☑	Salarias fasciatus genome assembly, chromosome: 10	Salarias fasciatus	38.2	38.2	100%	4.0	95.65%	28124721	LR597445.1
☑	Canis lupus genome assembly, chromosome: 10	Canis lupus	38.2	132	100%	4.0	100.00%	71927348	HG994393.1
☑	Cottoperca gobio genome assembly, chromosome: 1	Cottoperca gobio	38.2	98.6	100%	4.0	100.00%	27055867	LR131916.1
☑	Apoderus coryli genome assembly, chromosome: 2	Apoderus coryli	38.2	370	100%	4.0	100.00%	45504066	OU452199.1

Red Deer



Domesticated dog

Lawnmower blenny

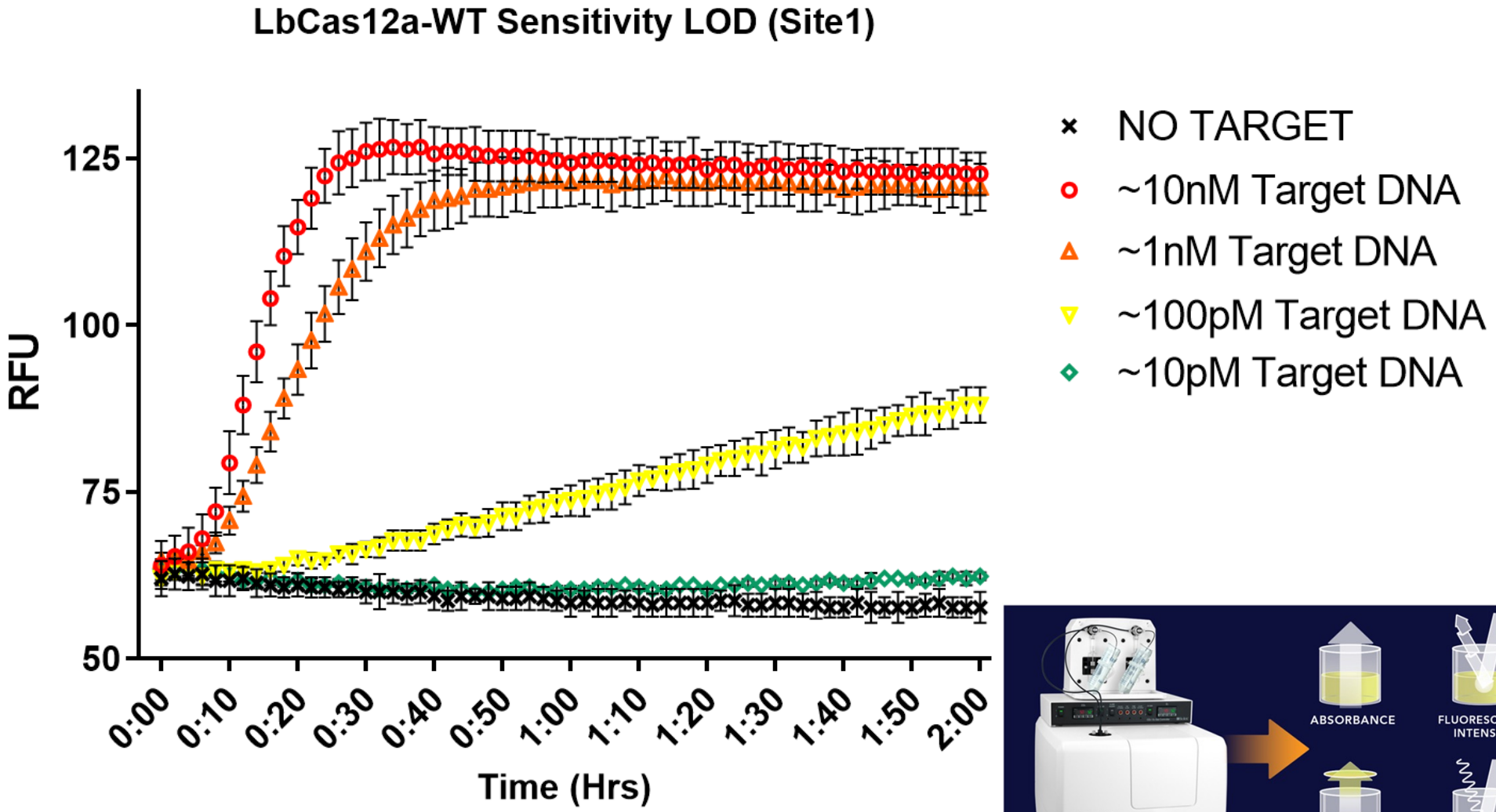
Hazel-leaf roller weevil

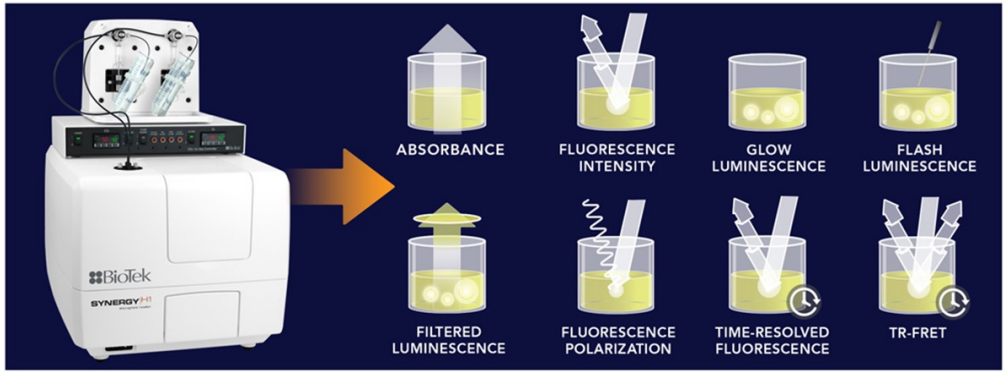
Black-eyed peas

Optimal Target Sites

- Maximal on-targeting for species of interest
- Minimal off-targeting of species that could generate false positives
  - Screen heavily for other species captured by designed gRNAs
- Region with little to no variability or rapid change
- PAM/PFS site compatible with Cas-protein being used
- Regions of higher copy number may produce better results

Establishment of WT-Cas12a Detection Assay

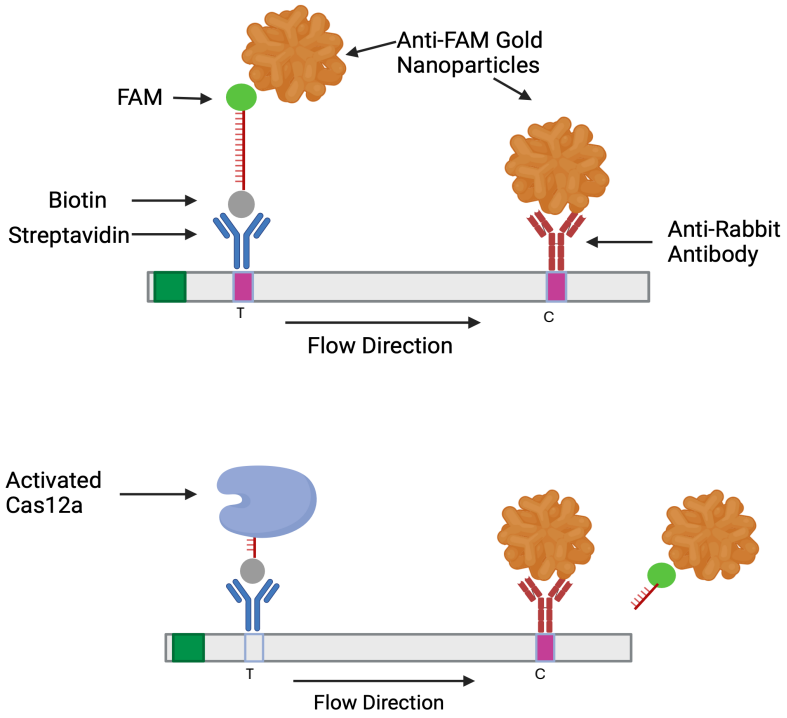




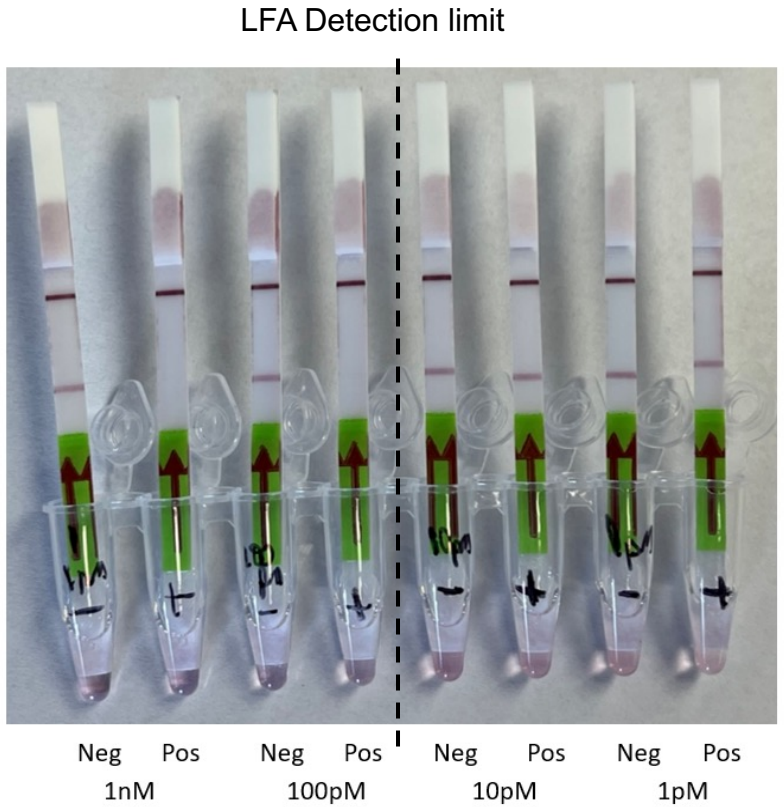
(unpublished)

Lateral Flow Assay

A



B



(unpublished)

Integration with RPA vs LAMP

- RPA
  - **R**ecombinase **P**olymerase **A**mplification
  - Isothermal at ~39°C and amplification time required is ~20min
  - Cons: Long primer (~30-35nt) very high off targeting rate, 9-10 mismatch tolerance
- LAMP
  - **L**oop-mediated isothermal **amp**lification
  - Isothermal at ~65°C and cost-effective
  - Cons: Complex primer design and isothermal reaction is too hot for LbCas12a

Summary of CRISPR-based Detection

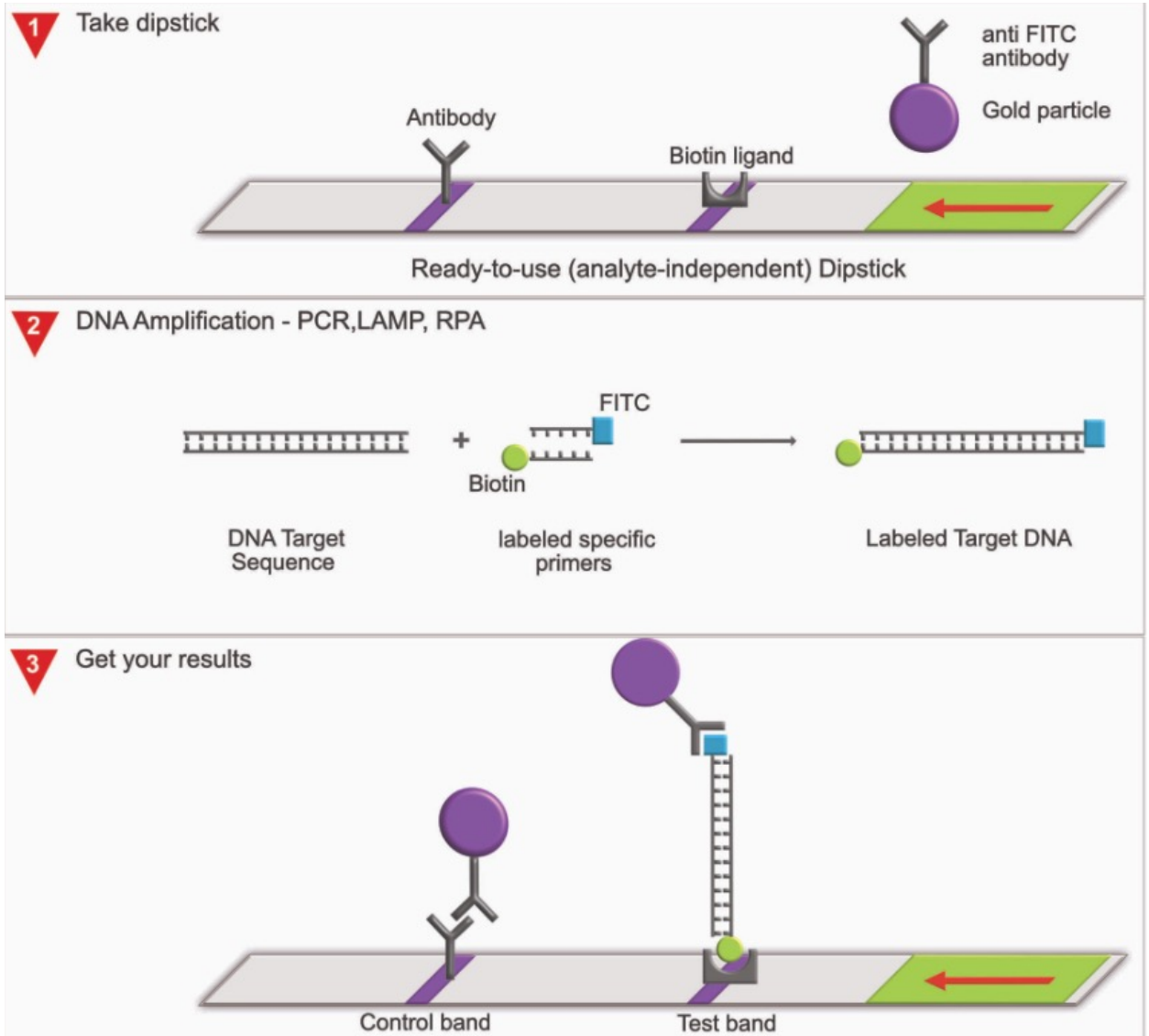
- DNA/RNA-target identifier that breaks apart reporter oligos after finding complimentary match to gRNA
- Pros:
  - Easy to design target sites with simple guidelines
  - Customizable and modular to fit needs of your assay
  - New advancements happening daily
  - Potential for in-field, on-site testing
- Cons:
  - Potential for off-targeting a similar sequence
  - CRISPR alone is not as sensitive as established technology like qPCR or HTS
  - Still a developing technology

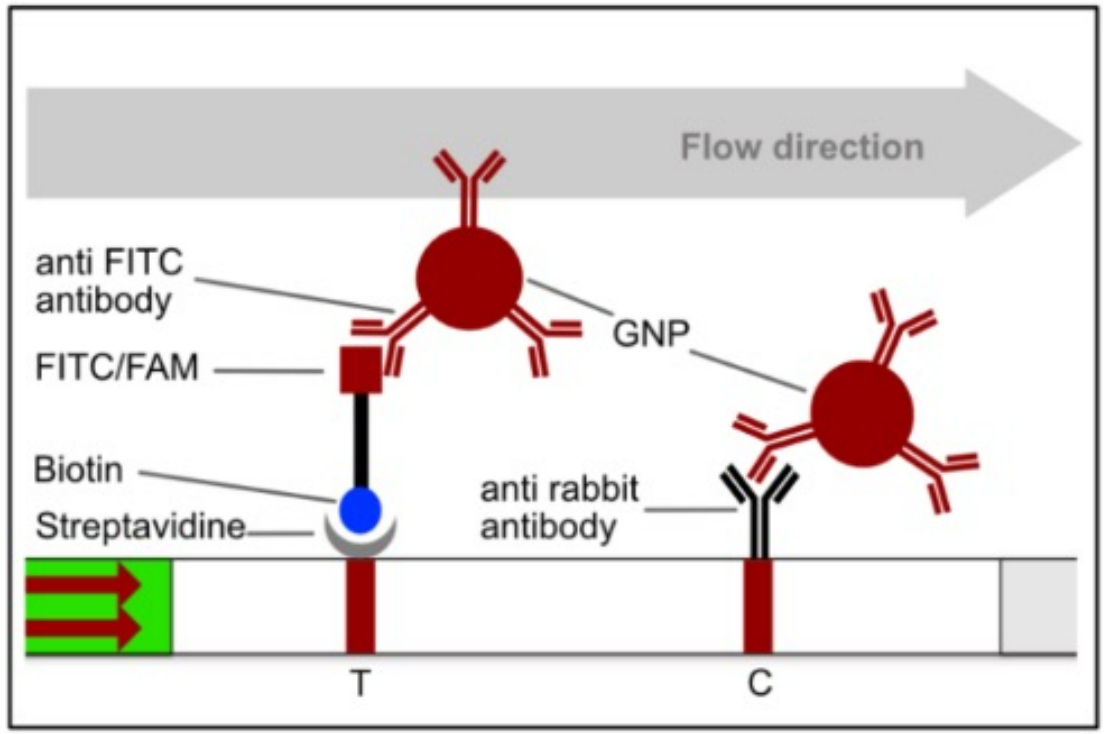
Thank you- Q&A?

The Q Lab

USDA United States Department of Agriculture

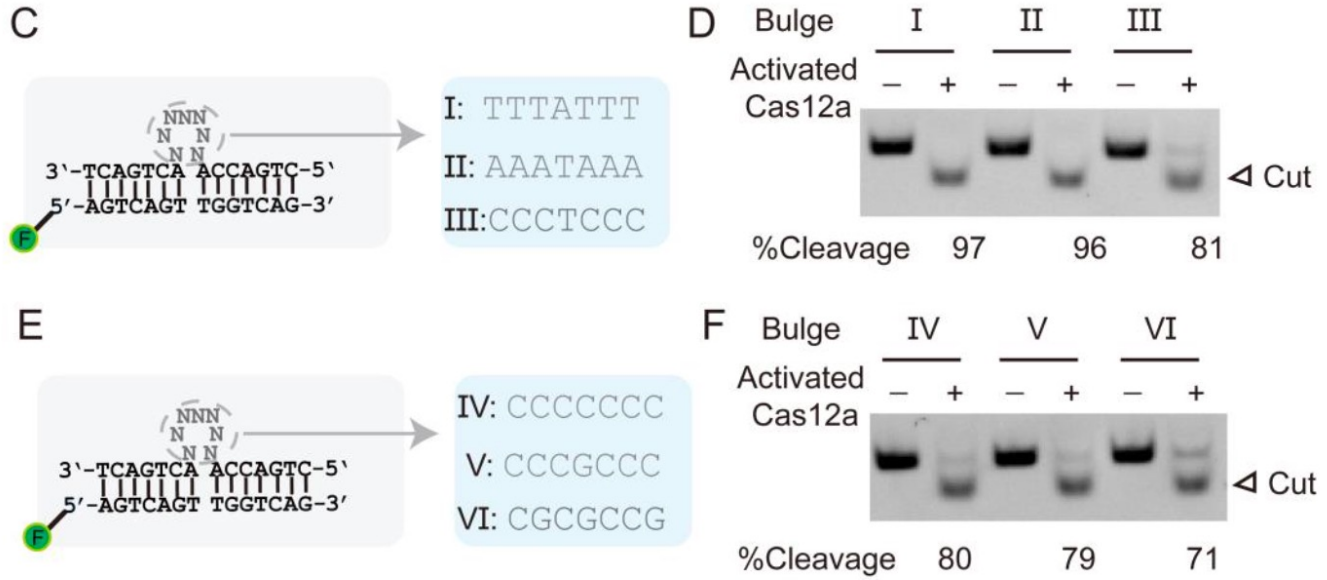
Lateral Flow Assay (LFA) Explained





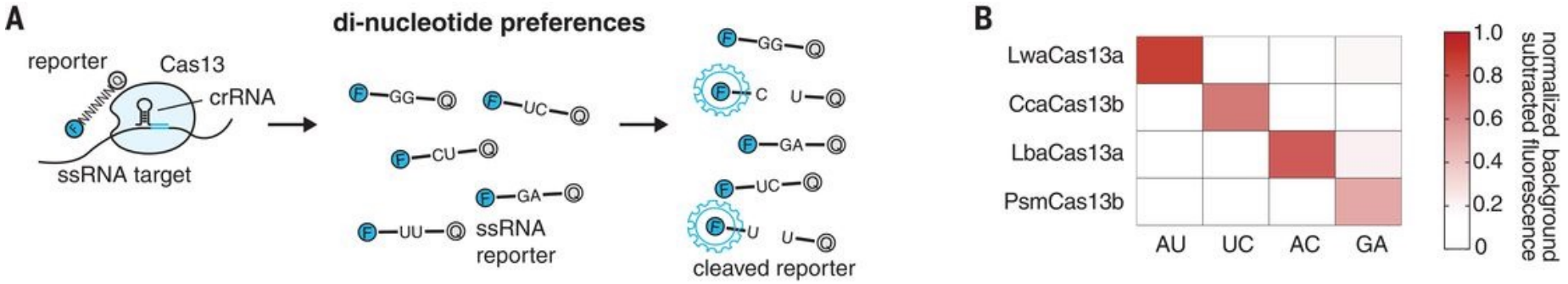
Optimal *trans*-cleavage

Cas12a



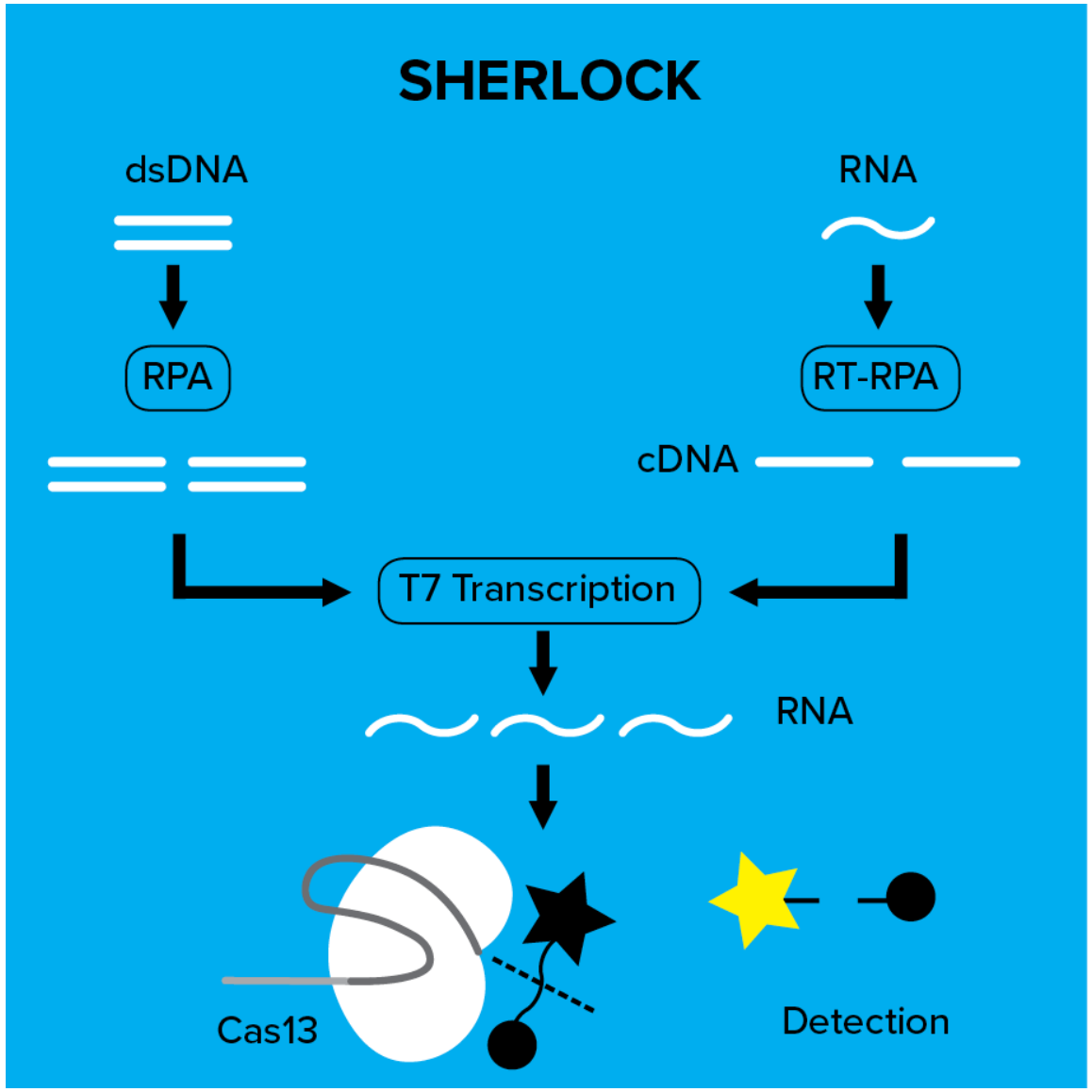
(Shi., K., et al 2021)

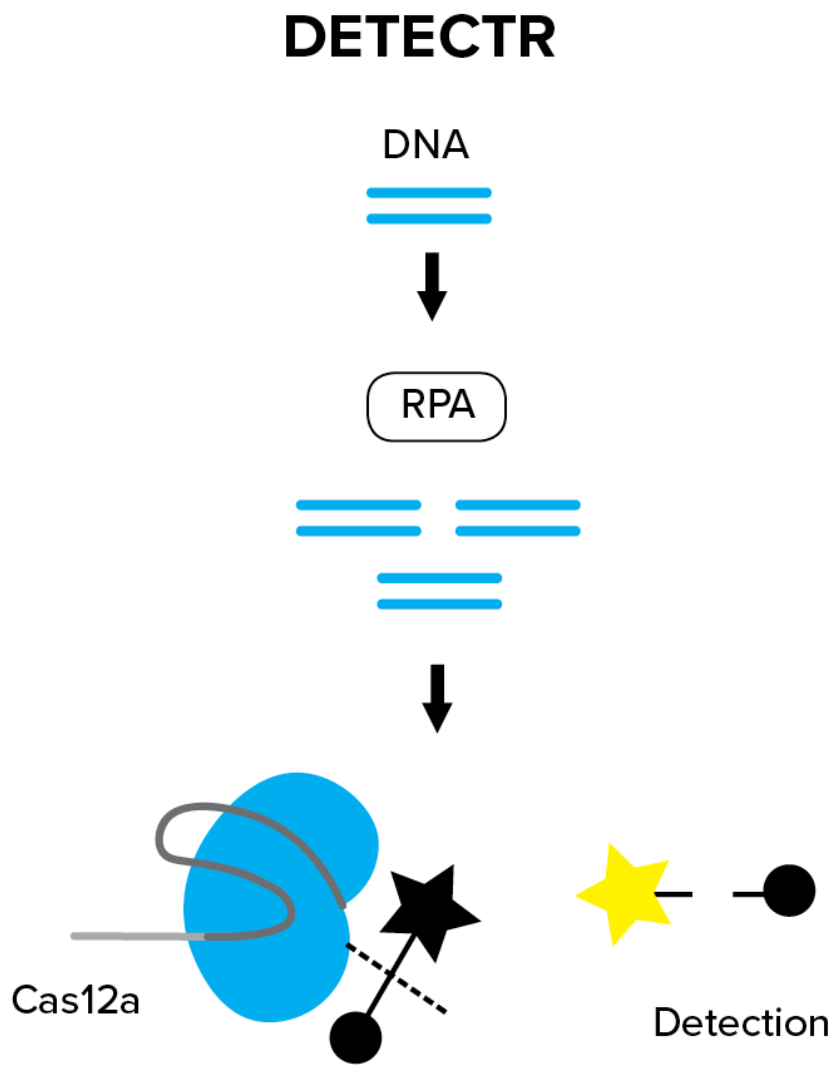
Cas13



(Gootenberg, J., et al. 2018)

Cas13 vs. Cas12a Detection





https://blog.addgene.org/finding-nucleic-acids-with-sherlock-and-