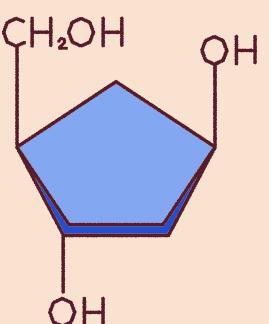
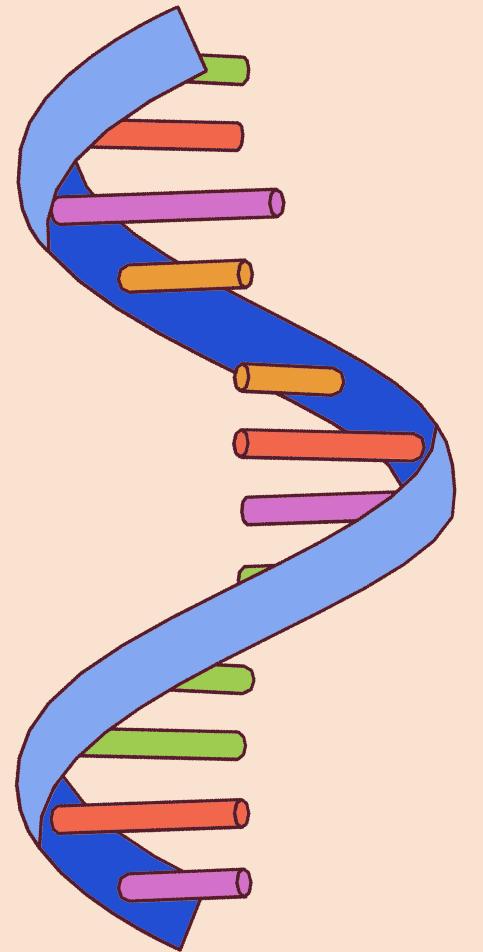
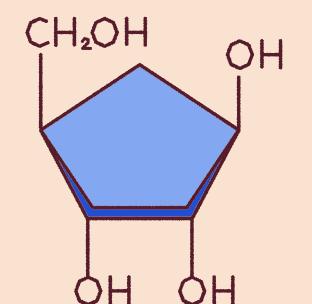


# **CRISPR-BASED DIAGNOSTIC GUIDE RNA DESIGN FOR HPV E6 GENE**

By Abhishikta Pradhan

# Abstract

- This project focuses on designing, validating, and optimizing gRNAs targeting the E6 gene of HPV for use in CRISPR-based diagnostics. Tools used include CRISPER for gRNA selection, NCBI BLAST for off-target analysis, Clustal Omega for strain comparison, and ViennaRNA (RNAfold) for secondary structure prediction.



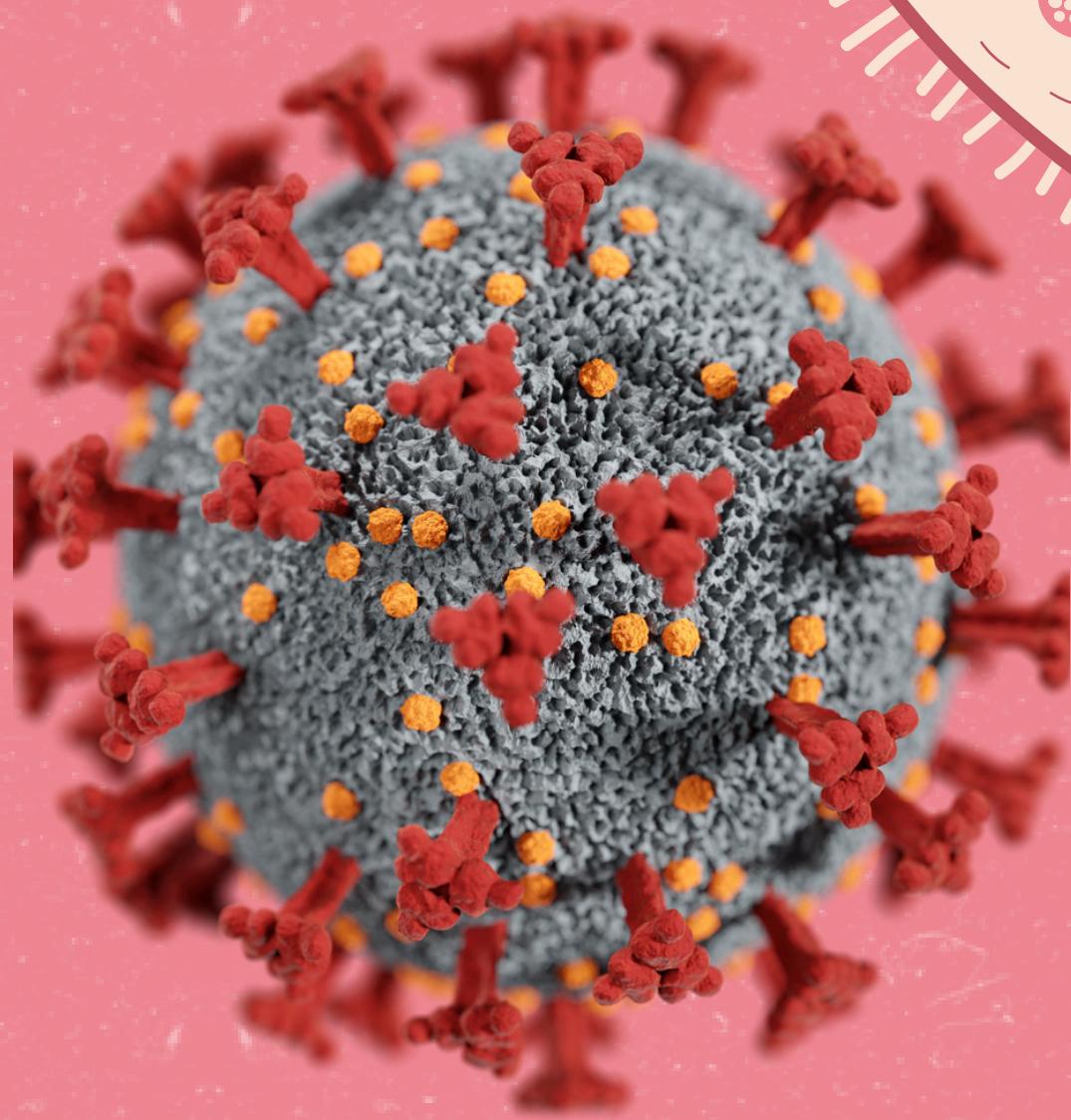
## Background & Target Info

### *Why E6 for CRISPR diagnostics?*

- HPV types 16 and 18 are high-risk strains linked to cervical cancer. The E6 gene is highly conserved and a promising target for molecular diagnostics.

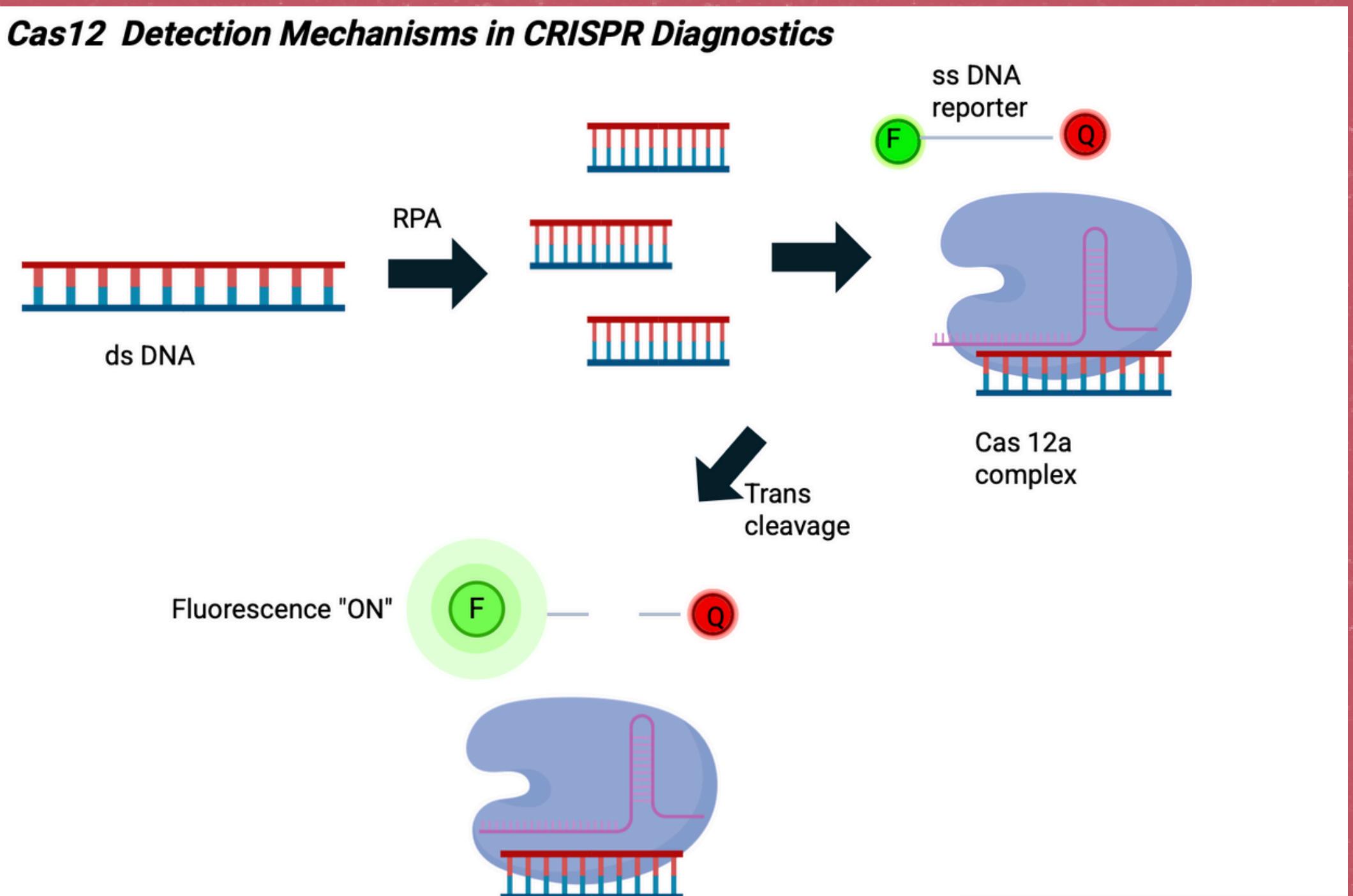
### *Why CRISPR?*

- CRISPR diagnostics like SHERLOCK and DETECTR offer high specificity, rapid detection, and work with isothermal amplification, removing the need for thermal cyclers. They enable visual or fluorescent readouts, ideal for point-of-care use. However, effective gRNA design is key .It must ensure strain specificity, tolerate mutations, and avoid problematic secondary structures.



# Cas Detection Logic

1. Target DNA (dsDNA) is first amplified using recombinase polymerase amplification (RPA) to generate sufficient template.
2. The Cas12a enzyme, guided by a custom gRNA, binds to the amplified target DNA with sequence specificity.
3. Upon binding, Cas12a undergoes conformational activation and acquires non-specific trans-cleavage activity.
4. A fluorescent reporter molecule (ssDNA tagged with a fluorophore and quencher) is introduced into the system.
5. Cas12a cleaves the reporter, separating the fluorophore (F) from the quencher (Q).
6. This cleavage results in a visible fluorescence signal, indicating the presence of the target sequence.



# gRNA Design Tool Outputs

Tool Used: CRISPOR

Target: HPV16 E6 gene

Output: 6 high-confidence candidate gRNAs selected based on on-target score, specificity, and position within the gene.

gRNA no	#guideId	targetSeq	mitSpecScore	cfdSpecScore	offtargetCount	targetGenomeGeneLocus	DeepCpf1-Score
gRNA 1	75forw	TTTCAGGACC CACAGGAGC GACCCAGA	-1	-1	0	intergenic:NP_04 1331.2/NP_04133 2.2-NP_041326.1	78.313156
gRNA 2	218forw	TTTATGCATA GTATATAGA GATGGGAA	-1	-1	0	intergenic:NP_04 1331.2/NP_04133 2.2-NP_041326.1	73.385025
gRNA 3	129rev	TTTGCAGCTC TGTGCATAAAC TGTGGTA	-1	-1	0	intergenic:NP_04 1331.2/NP_04133 2.2-NP_041326.1	72.28891
gRNA 4	317forw	TTTGTATGGA ACAACATTAG AACAGCA	-1	-1	0	intergenic:NP_04 1331.2/NP_04133 2.2-NP_041326.1	72.00835
gRNA 5	263rev	TTTATCACAT ACAGCATATG GATTCCC	-1	-1	0	intergenic:NP_04 1331.2/NP_04133 2.2-NP_041326.1	68.71315

# BLAST Validation

- All gRNAs show high conservation within HPV-16, with no off-target hits in human or other viral genomes.
- Ensures strong diagnostic potential for HPV-16 specific CRISPR detection.

gRNA ID	Sequence	BLAST Hits	Organism(s)	% Identity
gRNA_1	AGGACCCACAGGAG CGACCCAGA	100+	HPV-16 isolates only	100%
gRNA_2	TGCATAGTATATAG AGATGGGAA	100+	HPV-16 isolates only	100%
gRNA_3	CAGCTCTGTGCATAA CTGTGGTA	100+	HPV-16 isolates only	100%
gRNA_4	TATGGAACAAACATT AGAACAGCA	100+	HPV-16 isolates only	100%
gRNA_5	TCACATACAGCATAT GGATTCCC	100+	HPV-16 isolates only	100%
gRNA_6	ACAGTTAATACACCT AATTAACA	100+	HPV-16 isolates only	100%

# Mutation Analysis

A mutation resistance check was performed using NCBI dbSNP databases. No reported single nucleotide polymorphisms (SNPs) were found overlapping the gRNA binding regions in the HPV16 E6 gene. This suggests high potential stability, but it's important to note that novel or unreported variants may still exist.

# Strain Comparison

# Results:

- gRNA2 & gRNA4 = some similarity across strains
  - gRNA1,3,5,6 = no matches (type-specific)

# gRNA 2 alignment

# gRNA 4 alignment

# RNA Secondary Structure

- Used RNAfold (ViennaRNA)
- Predicted structure of each gRNA
- Minimum Free Energy (MFE) value per gRNA
- Ideal gRNAs: mostly linear (minimal hairpins)

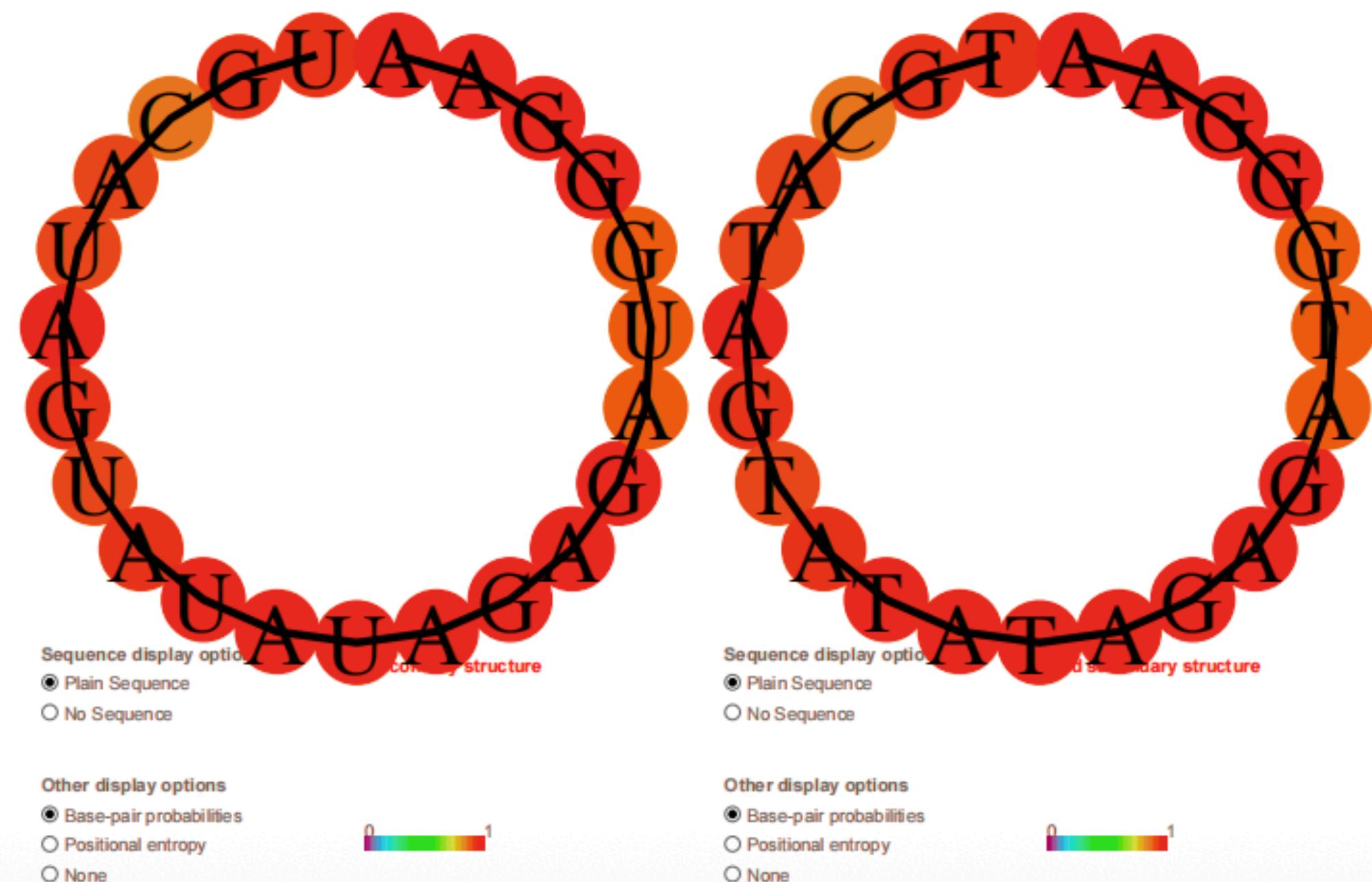
gRNA ID	Sequence (RNA)	MFE (kcal/mol)	Structure	Interpretation	Verdict
gRNA1	AGGACCCACAGG AGCGACCCAGA	-0.90	Small loop	Mild folding, mostly linear	Keep
gRNA2	AGGACCCACAGG AGCGACCCAGA	0	Fully linear	Ideal structure	Keep
gRNA3	AGGACCCACAGG AGCGACCCAGA	-1.30	Hairpin at 5'	Some folding near seed region	Caution
gRNA4	AGGACCCACAGG AGCGACCCAGA	-0.30	Small hairpin	Very light folding, tolerable	Keep
gRNA5	CACAUACAGCAU AUGGAUUCCC	-0.50	Mid-sequence stem	Moderate fold, possible interference	Caution
gRNA6	ACAGUUAAUACA CCUAAUUAACA	-2.40	Large hairpin	Seed region folded, may disrupt binding	Discard

# RNA Secondary Structure

Ideal gRNAs: mostly linear (minimal hairpins)

## Graphical output

You may look at the interactive drawing of the MFE structure below. If you do not see the interactive drawing and you are using Internet Explorer, please install the [Adobe SVG plugin](#). A note on base-pairing probabilities: The structure below is colored by base-pairing probabilities. For unpaired regions the color denotes the probability of being unpaired.



# Conclusions

## Conclusion 01

gRNA1, gRNA2, and gRNA4 passed all major validation steps, including BLAST specificity, SNP screening, and RNA structural feasibility.

## Conclusion 02

These three gRNAs are recommended for downstream Cas12-based detection assays targeting HPV-16.

## Conclusion 03

gRNA 3, 5, 6 folded unfavorably but passed Blast,Strain specificity.

# Thank you!



# Resources page

## Tools & Web Servers

- CRISPR Guide Design: [CRISPOR](#)
- BLASTN (short sequences): [NCBI BLAST](#)
- Strain Alignment: [Clustal Omega](#)
- Mutation Check: [dbSNP](#)
- RNA Secondary Structure: [RNAfold \(ViennaRNA\)](#)

## Databases

- HPV Sequences: NCBI Nucleotide database
- E6 Gene Reference: HPV-16 RefSeq (e.g., NC\_001526.4)
- SNPs in HPV: NCBI dbSNP