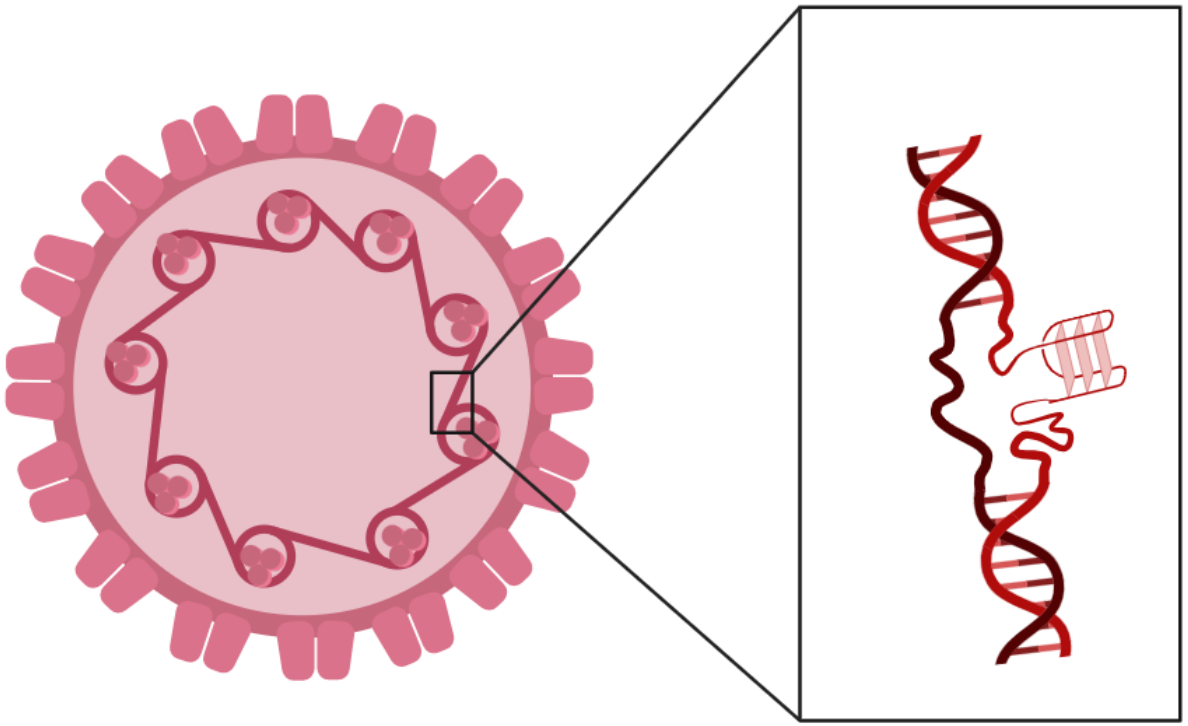
REVEALING G-QUADRUPLEX DNA STRUCTURES IN HPV16 USING QUANTITATIVE PCR STOP AND LIGANDS



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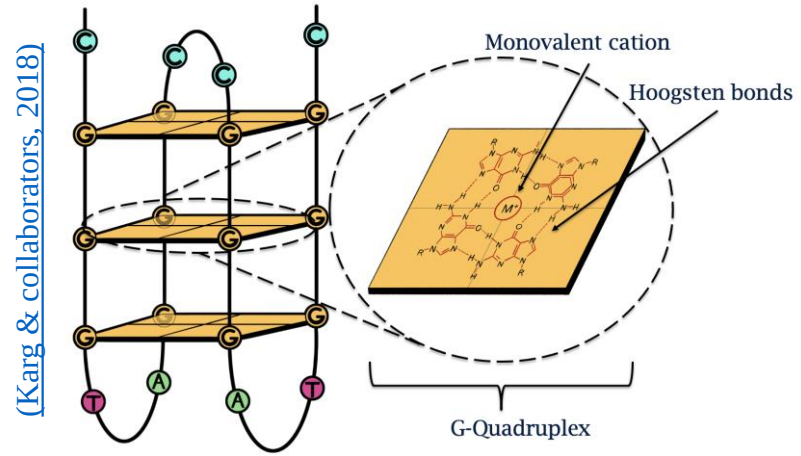
INTRODUCTION

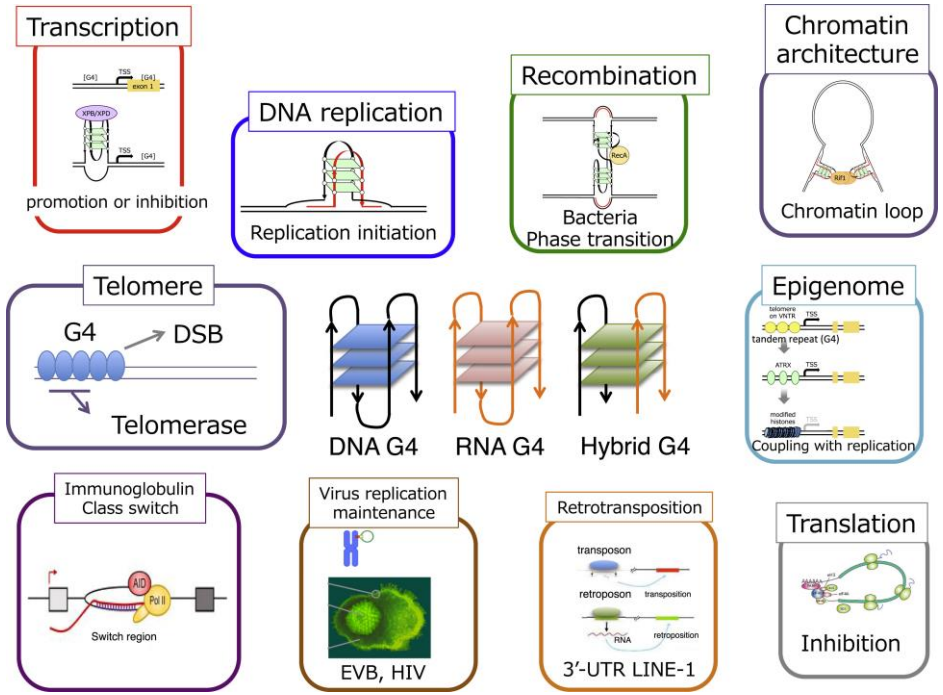
G-Quadruplexes are non-canonical conformations that can emerge in guanine-rich regions of nucleic acids under specific intracellular conditions, such as the presence of monovalent cations, pH fluctuations, temperature changes, and cellular crowding. Additionally, they can be modulated by small molecules, also known as G4 ligands.



(Karg & collaborators, 2018)

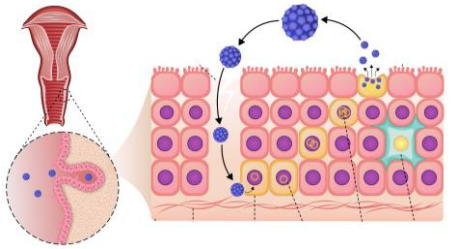
The identification of G4 structures in diverse viruses medical importance suggests their relevance in infectious mechanisms, making them promising therapeutic targets. Recently, over 100 putative G-quadruplex-forming sequences (PQS) have been predicted in the genomes of three high-risk Human Papillomaviruses using bioinformatic tools

Biological functions of G-Quadruplexes



(Masai & Tanaka, 2020)

Human Papillomavirus 16



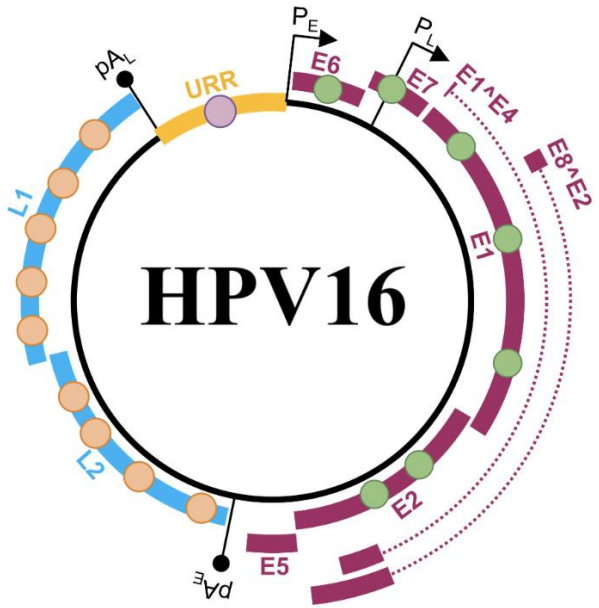
HPV cause the most prevalent sexually transmitted infection worldwide. Genotype 16 is highlighted due to its high risk for oncogenicity; however, it lacks direct antiviral treatments. Accordingly, exploring the structural features of the HPV 16 genome and understanding their role could reveal strategies for designing antiviral treatments against this infection.

OBJECTIVES

- Confirmation of the *in vitro* presence of DNA G-quadruplex structures in Human Papillomavirus type 16
- Foundation laying for further studies regarding viral G4-DNA function and application
- Generation of translational knowledge in virology

MATERIALS & METHODS

1. G4-Forming Sequences Classification & Selection

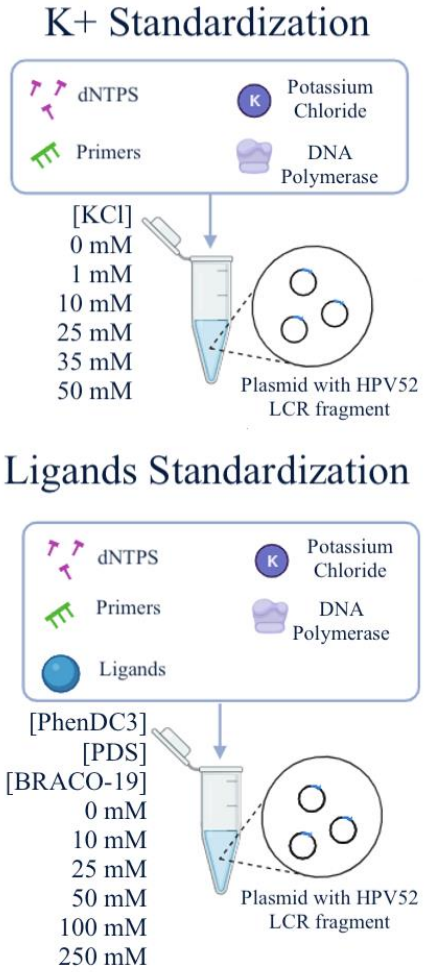


Late Region G4 zones
- 1L2 (47 PQS)
- L2A (7 PQS)
- L2B (3 PQS)
- L2C (5 PQS)
- L1A (2 PQS)
- 1L1 (17 PQS)
- L1B (9 PQS)
- L1C (1 PQS)
- L1D (6 PQS)

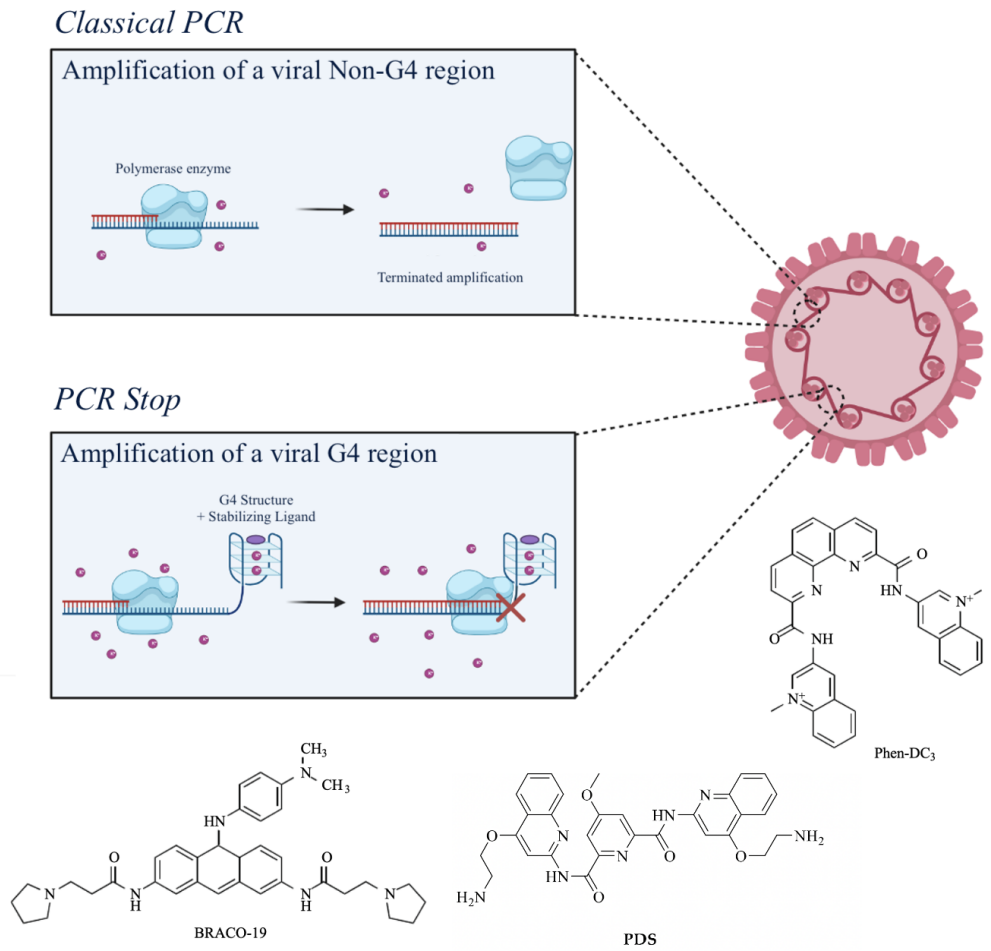
Early Region G4 zones
- E6 (4 PQS)
- E7 (5 PQS)
- 1E1 (26 PQS)
- E1A (6 PQS)
- E1B (2 PQS)
- E2M (34 PQS)
- E2M1 (13 PQS)

LCR G4 zone
- 13 PQS

2. qPCR Standardization



3. qPCR Stop Assays and Ct Data Collection



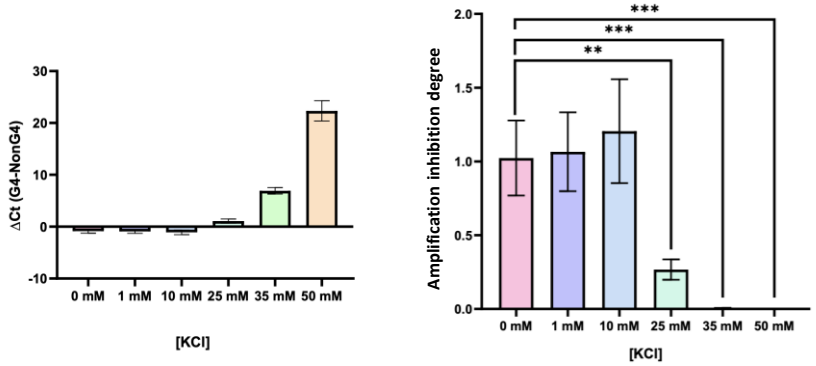
RESULTS

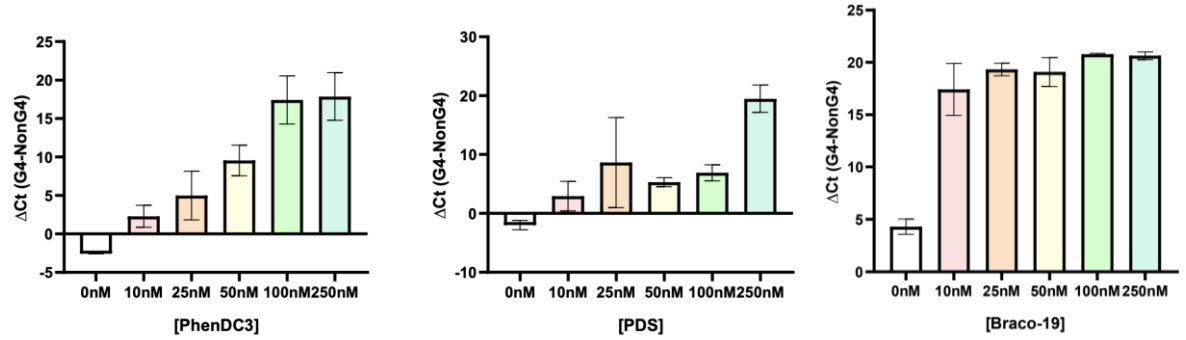
1. Predicted G4-Forming Sequences Classification & Selection

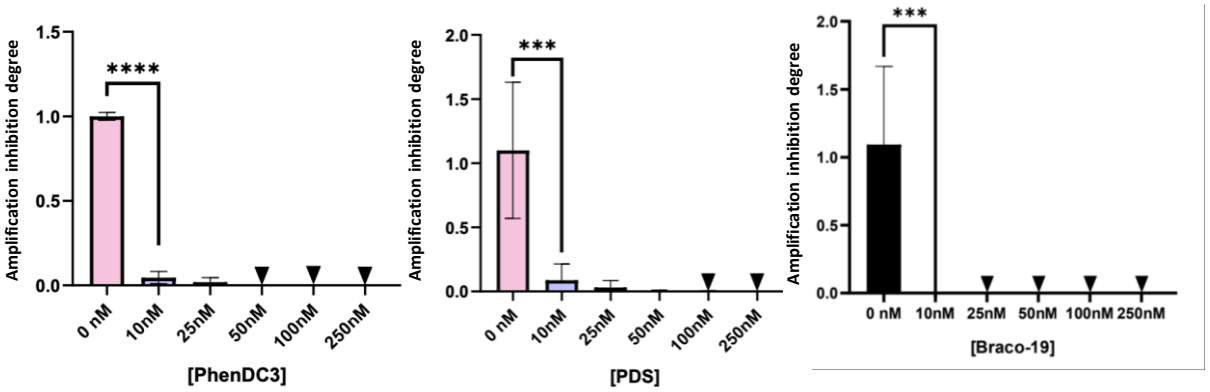
Gene	Cluster[1]	Zones	G4 Forming Sequences	Mean G4catchall Score	Domains
E6	G4-E6(+)	G4-E6	4 (0%, 75%)	1.097	Zinc finger 2-E6
E7	G4-E7(+)		5 (60%, 0%)	0.447	Zinc finger-E7
E1	G4-E1-1(-)	G4-E1	1 (100%, 0%)	0.273	H1 Histone Binding Domain
	G4-E1-2(+)		26 (35%, 4%)	0.863	
	G4-E1-3(+)		7 (0%, 42%)	2.023	
	G4-E1-4(+)		2 (50%, 50%)	0.9535	
E2	G4-E2-1(+)	G4-E2M	2 (0%, 100%)	0.956	Transactivation Domain
	G4-E2-2(-)		32 (28%, 3%)	0.796	Hinge
	G4-E2-3(-)		13 (15%, 15%)	0.88	
L2	G4-L2-1(+)	G4-L2	47 (30%, 0%)	0.878	
	G4-L2-2(-)		7 (29%, 14%)	1.634	
	G4-L2-3(-)		2 (100%, 0%)	0.376	
	G4-L2-4(+)		1 (0%, 100%)	0.484	
	G4-L2-5(-)		2 (0%, 0%)	*	
	G4-L2-6(-)		3 (67%, 0%)	0.363	
L1	G4-L1-1(-)		2 (0%, 50%)	0.84	
	G4-L1-2(+)	G4-L1	17 (4%, 6%)	0.714	
	G4-L1-3(+)		4 (0%, 0%)	*	
	G4-L1-4(+)		5 (20%, 40%)	0.599	
	G4-L1-5(+)		1 (0%, 100%)	0.833	
	G4-L1-6(-)		4 (0%, 25%)	1.04	
	G4-L1-7(-)		2 (0%, 100%)	1.168	

Region	Cluster	Zones	G4 Forming Sequences	Mean G4catchall Score	Domains
LCR	G4-LCR-16(-)	G4-LCR-16	13 (38%, 23%)	0.655	N/A

2. qPCR Standardization







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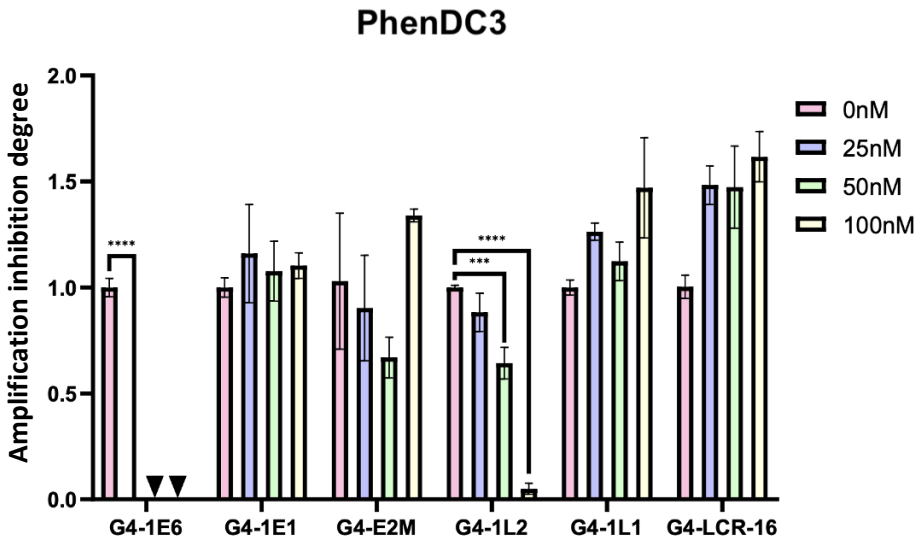
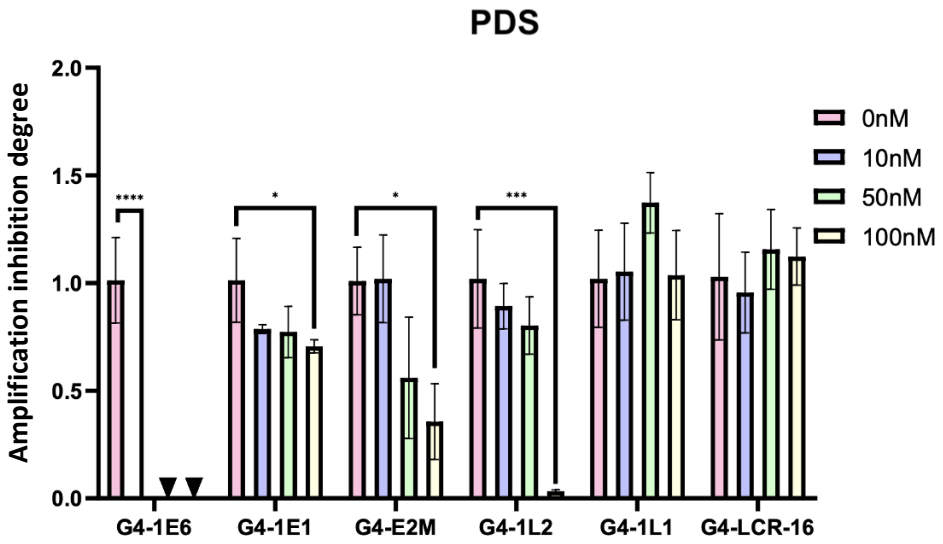
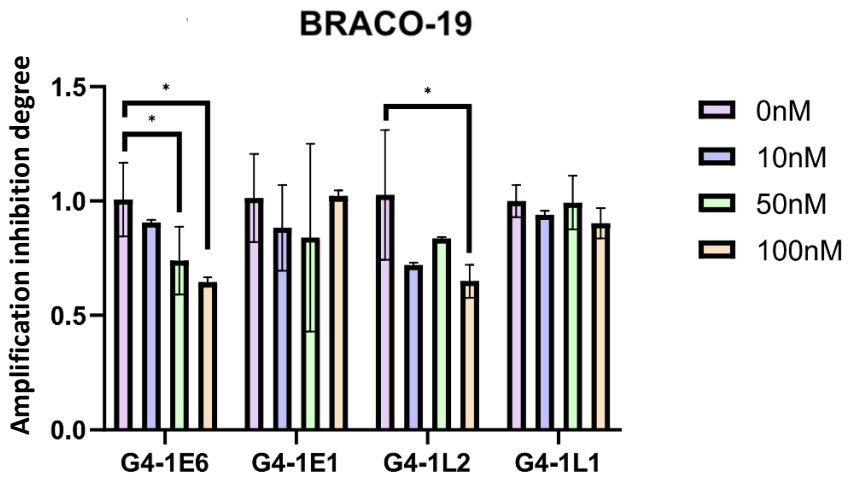




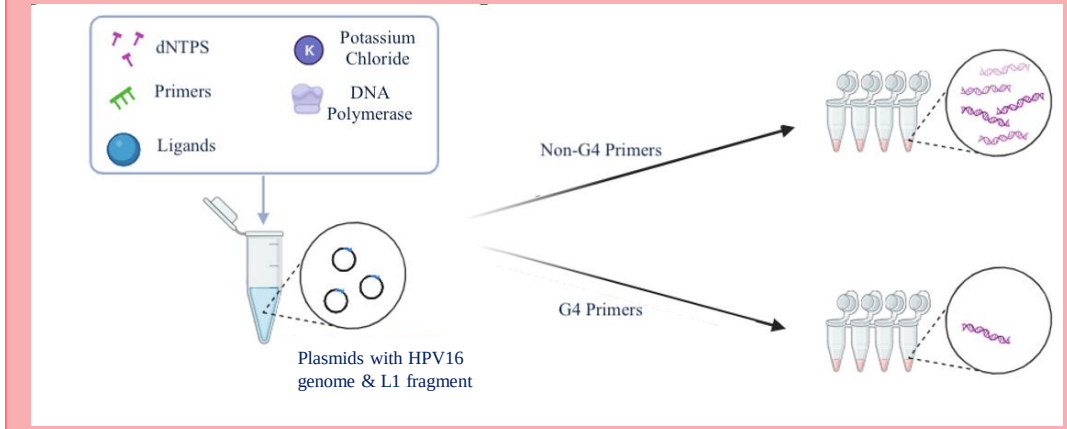


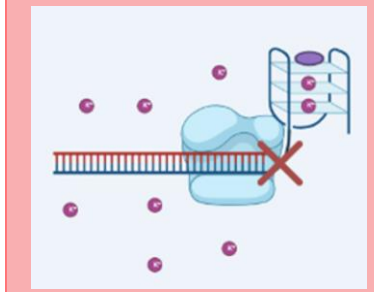
The inhibition degree for each zone was calculated through the modified $2^{-\Delta\Delta Ct}$ ecuation. Ranging from 0 to 1, the degree value is proportional to the amplification.

Data were processed by a one-way variance analysis (ANOVA).

CONCLUSIONS

A qPCR Stop assay was standardized and succesfully employed for the confirmation of G4-DNA structures in HPV16, proving its eficiency for viral G4 detection.

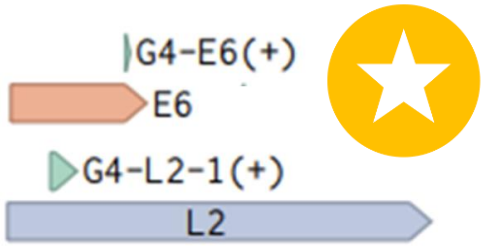




[KCl] & [Ligands] for qPCR Stop Assay

- [KCl] → 35 mM
- [PhenDC3] → 0, 25, 50, 100 nM
- [PDS] → 0, 10, 50, 100 nM
- [BRACO-19] → 0, 10, 50, 100 nM

Zones **1E6** and **1L2** demonstrated a higher sensibility, indicating the presence and likelihood of biologically relevant G-quadruplexes. Nuclear Magnetic Resonance (NMR) studies are suggested for further 3D structure characterization.



PERSPECTIVES

G4-DNA has translational potential for therapeutic applications as molecular targets

G4 structures may explain certain pathogenic mechanisms of infection in HPV and other viruses

Characterizing the 3D structure qPCR Stop-detected G4s could aid in the development of antiviral therapies against HPV16

Ultimately, the study of G-quadruplexes may unlock groundbreaking advancements in molecular diagnostics and therapies