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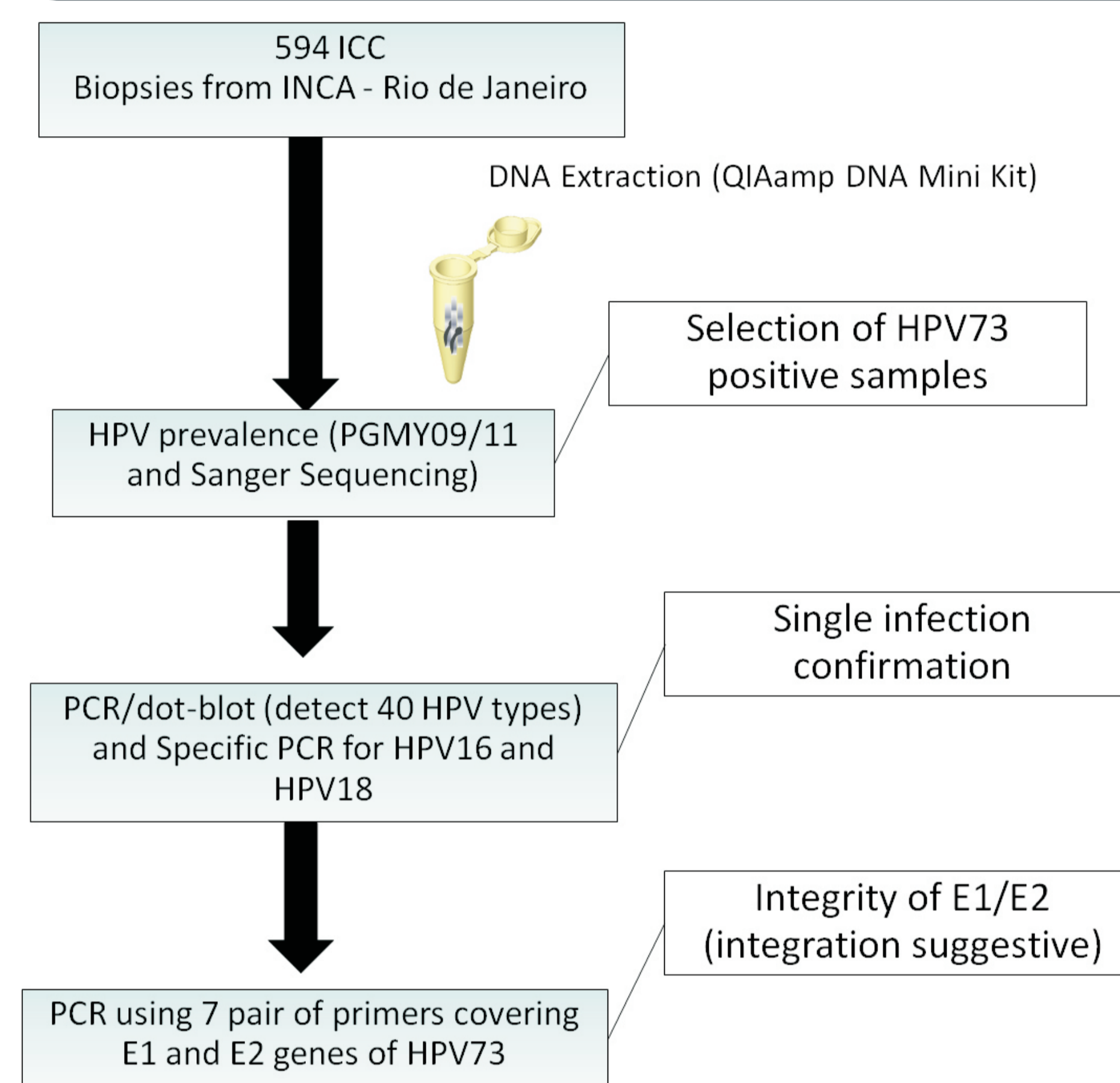
INTRODUCTION

More than 40 genotypes of HPV have been found to infect the genital mucosa and 12 (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) are characterized as high-risk types (HR-HPV) for invasive cervical cancer (ICC) according to the International Agency for Research on Cancer. However, due to their low prevalence in ICC and lack of evidence of biological data, eight HPV types have been classified as probably (HPV68) or possibly (HPV26, 53, 66, 67, 70, 73, and 82) HR-HPV. Identification and characterization of the biology of rare carcinogenic HPV types is important to support the programs of HPV screening and vaccination, and to understanding the basis of HPV carcinogenicity.

GOALS

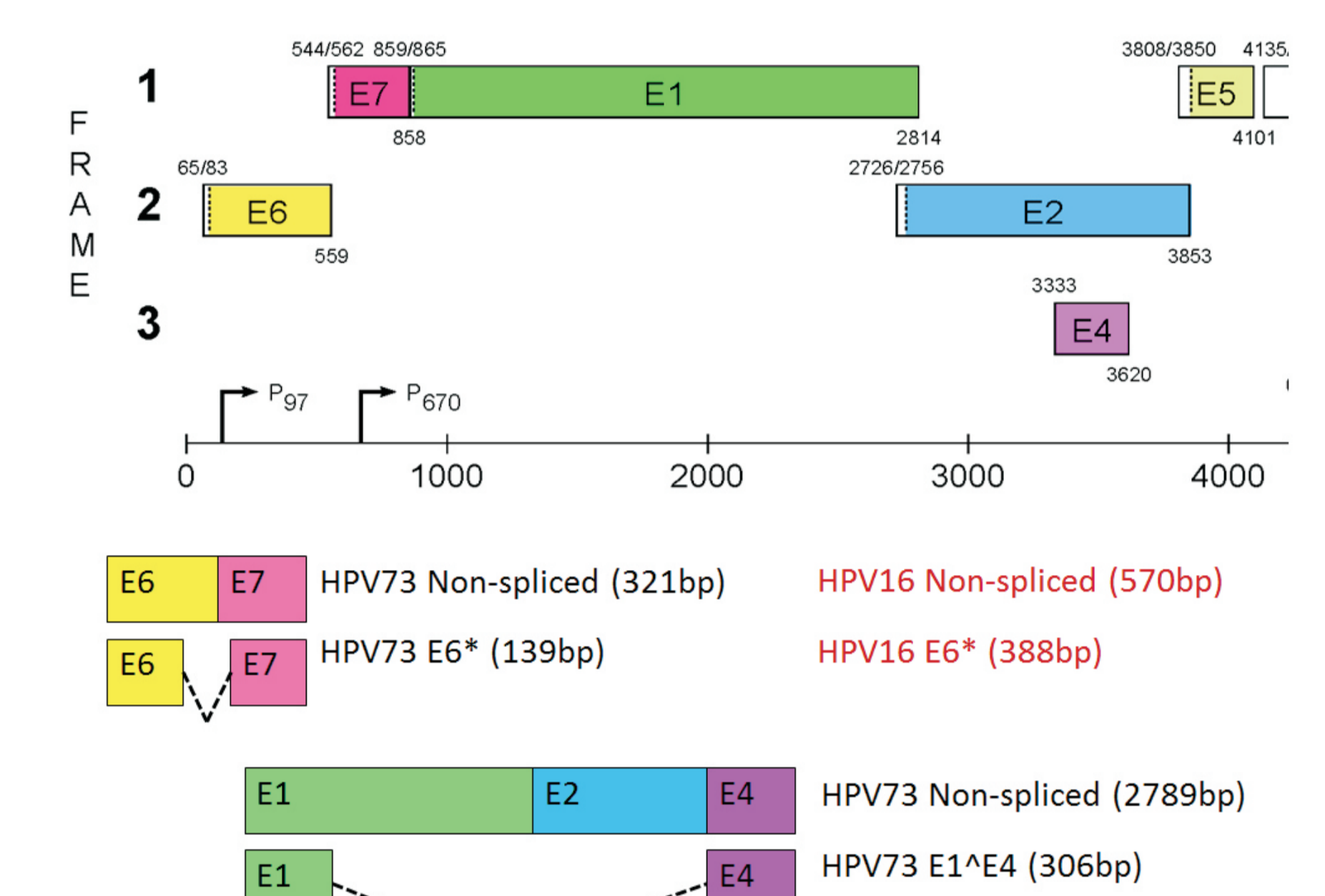
To provide molecular evidence for the carcinogenic role of HPV73 in invasive cervical cancer (ICC). HPV73 is neither routinely evaluated in HPV screening, nor included in any of the prophylactic vaccines.

METHODS



Transcriptional activity

RNA -> cDNA -> assessing transcripts by PCR: Size & Sequencing



<http://pave.niaid.nih.gov/images/transcript/HPV16.png>

RESULTS

Table 1. HPV prevalence in ICC in a Brazilian cohort from Rio de Janeiro.

HPV type	N	%
16*	370	62.3
18*	77	13.0
45*	33	5.6
35	12	2.0
58*	11	1.9
52*	8	1.3
73	8	1.3
31*	7	1.2
33*	7	1.2
39	6	1.0
59	6	1.0
26	2	0.3
51	2	0.3
56	2	0.3
68	2	0.3
83	1	0.2
Co-infection	20	3.4
Undetermined	20	3.4
Total	594	100.0

*Gardasil 9 FDA-approved HPV vaccine to HPV16, 18, 31, 33, 45, 52, 58, 6 and 11. In red, HPV73 positive samples selected to analysis

Single infection confirmation

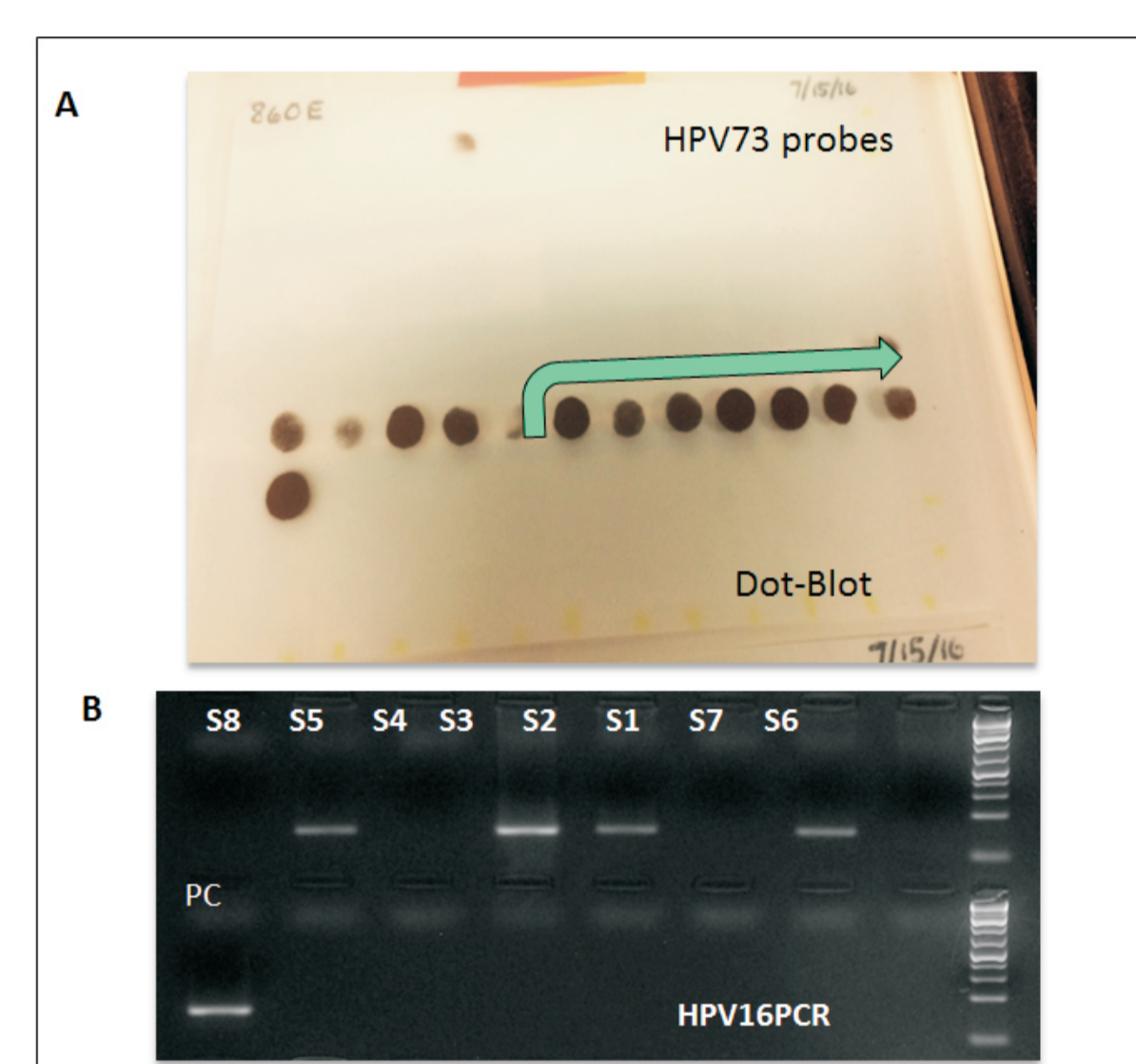


Figure 1. Single infection confirmation was carried out by two different methods. A, shows dots of hybridization of PCR product and HPV73 probes. All the samples displayed a strong intensity for HPV73 hybridization. B, agarose gel shows PCR amplification with PCR specific for HPV16, which 04 of 08 samples displayed presence of HPV16 DNA.

Table 2. Summary of additional methods for confirmation of HPV73 single infection. Four of eight samples single infection was confirmed.

Samples	Dot - Blot	DNA	
		HPV16	HPV18
S1	73	-	-
S2	73	+	-
S3	73, 16	+	-
S4	73	-	-
S5	73, 58	+	-
S6	73	-	-
S7	73	+	-
S8	73	-	-

In red, samples with confirmation of single infection by all methodologies.

HPV73 positive in ICC (N=8)

Transcriptional activity

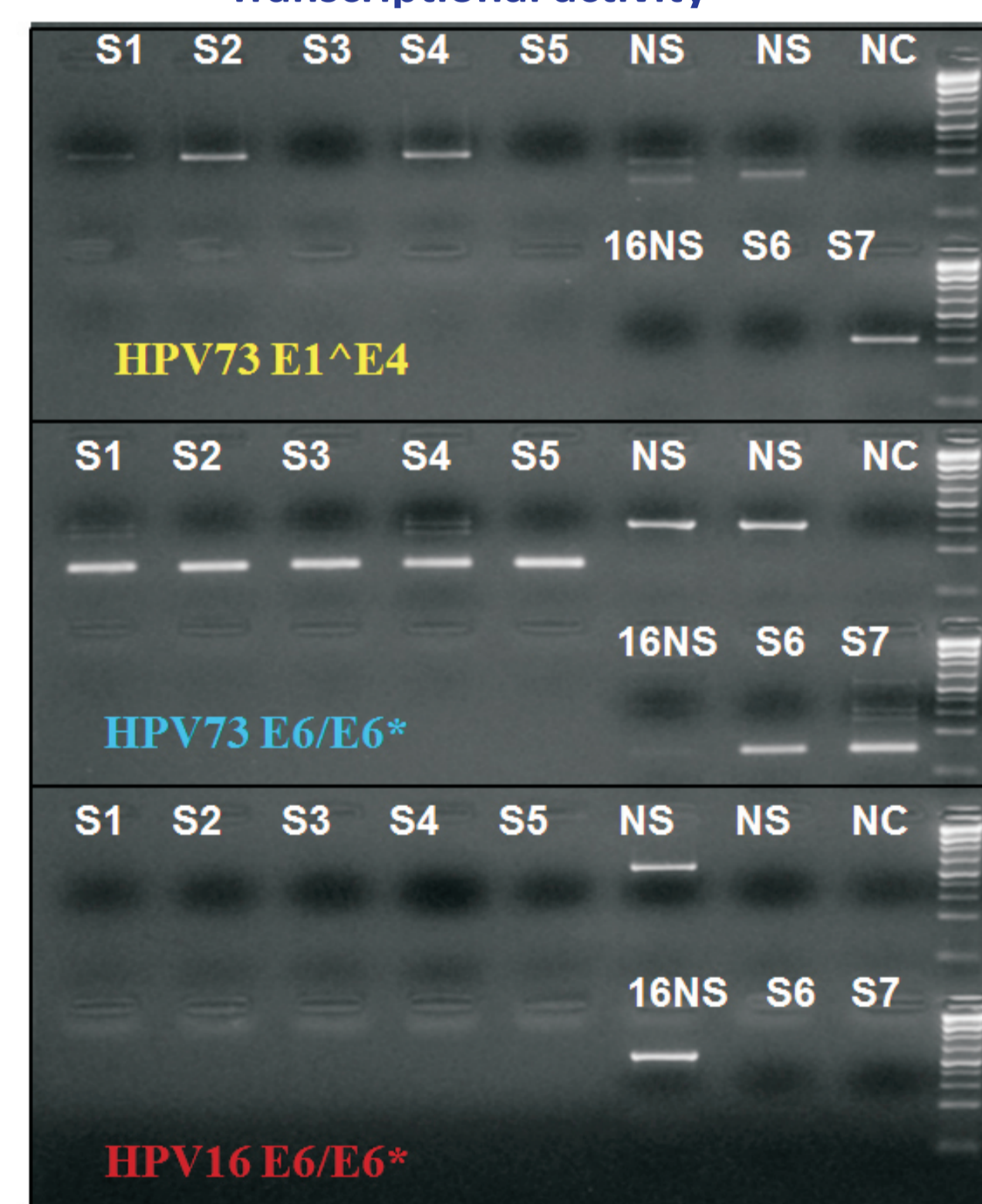


Figure 2. Transcriptional activity of HPV73 (E1^E4 and E6*) and HPV16 (E6*) was assessed by PCR of cDNA. Presence of HPV73 E1^E4 was observed in 04/08 samples (upper agarose gel), HPV73 E6* was observed in all samples (middle agarose gel). No transcripts of HPV16 (E6*) was observed (lower agarose gel). NC: negative control, NS: non-spliced form with HPV73 positive DNA.

Table 3. Summary of transcriptional activity of HPV73 and HPV16

Sample	DNA		HPV73RNA		HPV16RNA
	Dot - Blot	HPV16	E1^E4	E6*	E6*
S1	73	-	+	+	-
S2	73	+	+	+	-
S3	73, 16	+	-	+	-
S4	73	-	+	+	-
S5	73, 58	+	-	+	-
S6	73	-	-	+	-
S7	73	+	+	+	-
S8	73	-	*	*	*

*No RNA available. In red, samples with presence of transcripts. No HPV16 transcripts were observed

E1/E2 integrity

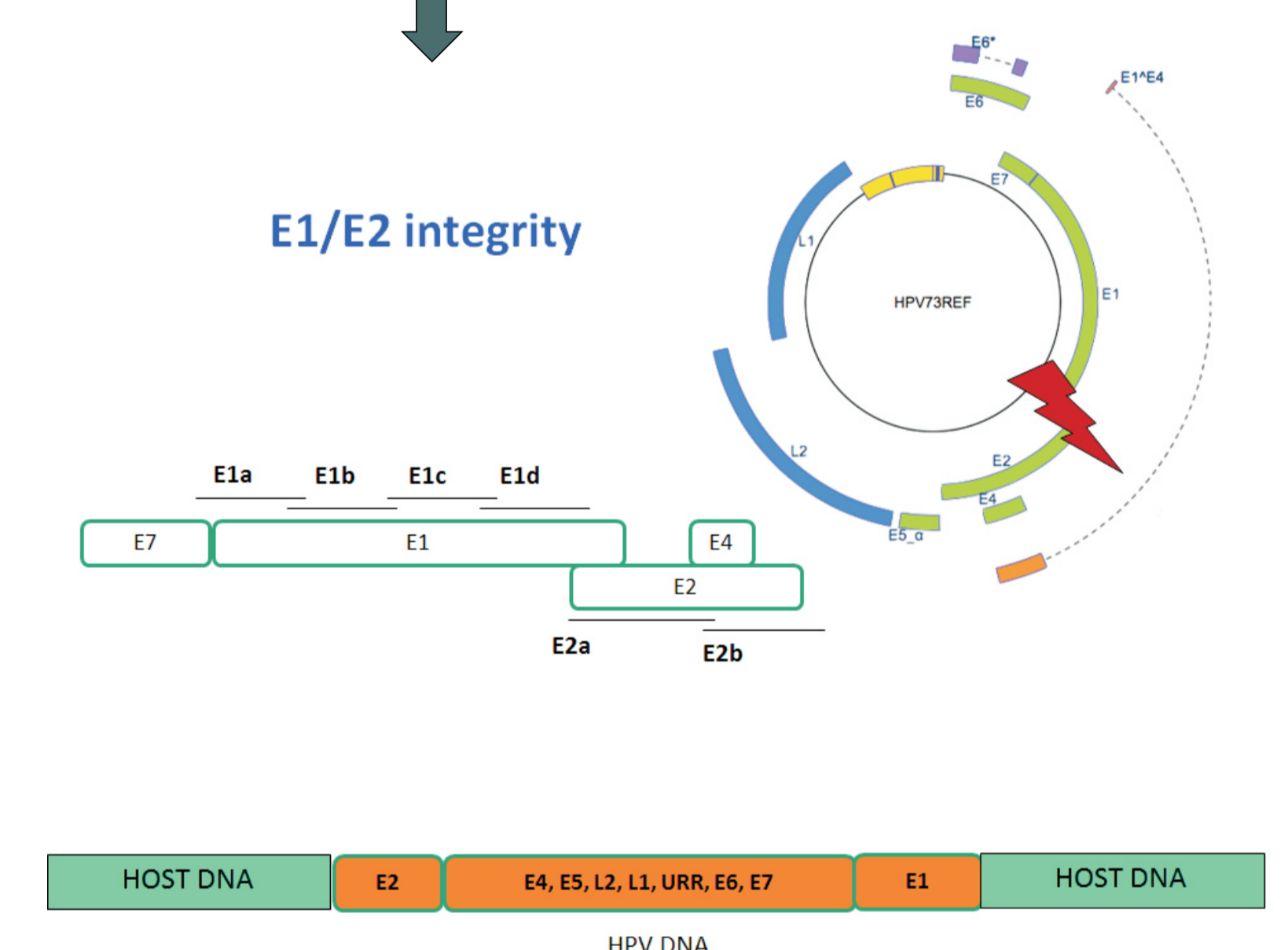


Table 4. Summary of integrity of E1 and E2, and HPV73 E1^E4 transcript. Disruption of E1/E2 ORFs were present in 04 of 08 samples HPV73 positives. This data is in agreement with our transcripts activity data, which E1^E4 wasn't seen when disruption is present

Samples	HPV73RNA		DNA				
	E1^E4	E1a	E1b	E1c	E1d	E2a	E2b
S1	+	+	+	+	+	+	+
S2	+	+	+	+	+	+	+
S3	-	+	+	+	-	-	-
S4	+	+	+	+	+	+	+
S5	-	+	-	-	-	-	-
S6	-	+	+	+	+	+	+
S7	+	+	+	+	+	+	+
S8	-	+	+	-	-	-	+

In red are samples with disruption in E1/E2.

CONCLUSION

These data provide compelling evidence for the association of HPV73 and invasive cervix cancer, including:

- Single HPV73 type presence in cancer tissues
- Active HPV73 E6/E6* transcription in tumor tissue
- Disruption of HPV73 genome suggestive of integration

These results suggest that HPV73 should be considered carcinogenic. Additional studies in tumor tissues and screening specimens are warranted.

SUPPORT



NCI, NIH

Projeto Gráfico: Serviço de Edição e Informação Técnico-Científica / INCA

