Diagnosing latent EBV infection by in situ hybridization (ISH): Development of an easy and rapid method for producing EBERs RNA probes

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INTRODUCTION

Epstein-Barr virus (EBV), is a dsDNA virus that establishes a latent infection that lasts lifelong in memory B cells of about 95% of healthy individuals worldwide.

On the other hand, EBV is recognized as a class I carcinogenic agent by the IARC due to its etiopathogenic role in lymphomas (PTLD, Burkitt, T/NK and classical Hodgkin lymphomas) and carcinomas (i.e. nasopharyngeal and gastric), among others.

Detection of EBV within cancer cells is mandatory for establishing an association, which in turn is essential for some differential diagnosis, and for setting the conditions of therapy monitoring (i.e. qPCR of circulating virus), as well as for research purposes.

Currently, RNA in situ hybridization (RISH) targeting small non-coding EBV-RNA (EBERs 1 and 2) is the gold standard technique for diagnosing EBV latency.

RISH allows identification of specific RNAs through a reaction involving labeled complementary nucleotide probes which can be obtained from either commercial sources or in house-preparations.

Aim of this study: to develop a reproducible method to obtain RNA probes for efficient RISH EBV detection with reduced-cost and time-saving steps.

Strategy: To employ a special priming strategy based on the inclusion of both the T3 and T7 RNA polymerase promoter sequences in the 5' and 3' extremes of the primers, respectively.

MATERIAL AND METHODS

EBERs sequence was obtained from an +EBV cell lineage (Raji), cloned into a plasmid vector and sequenced for checking sequences and polymorphisms. PCR amplification of EBERs was carried utilizing special primers containing T3 and T7 RNA polymerase promoter sequences. Purified PCR product was utilized for RNA probe production through *in vitro* transcription. The study design is shown in Fig 1.

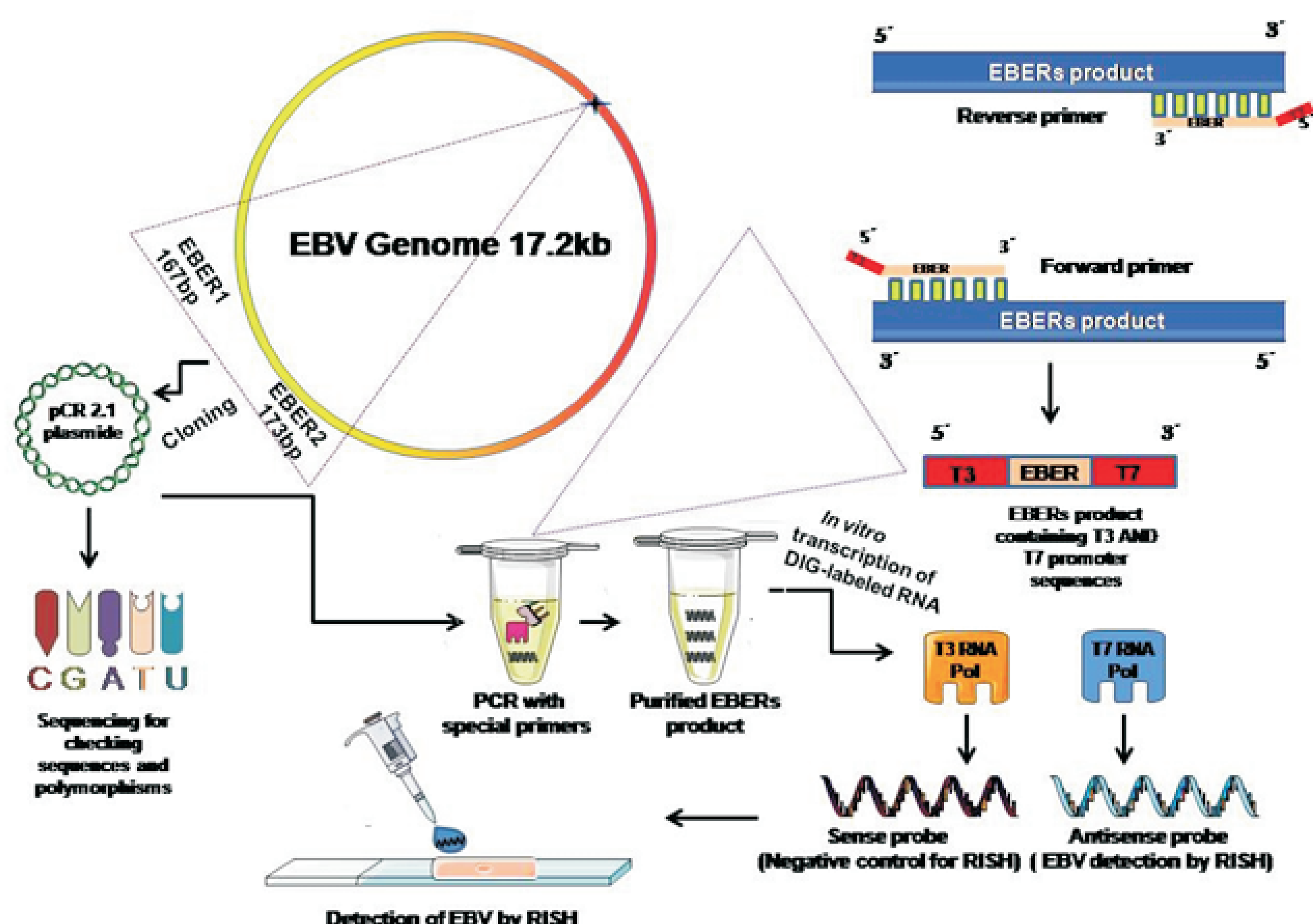

Figure 1. Study design.

RESULTS

EBER1 and EBER2 were amplified by specific PCR assays with regular primers, from a validated EBV+ DNA from cell lineage (Raji), cloned in a plasmid vector (pCR 2.1 TOPO) and expanded in DH5α *E.coli* . Direct sequencing allowed to check the sequences (Fig. 2).

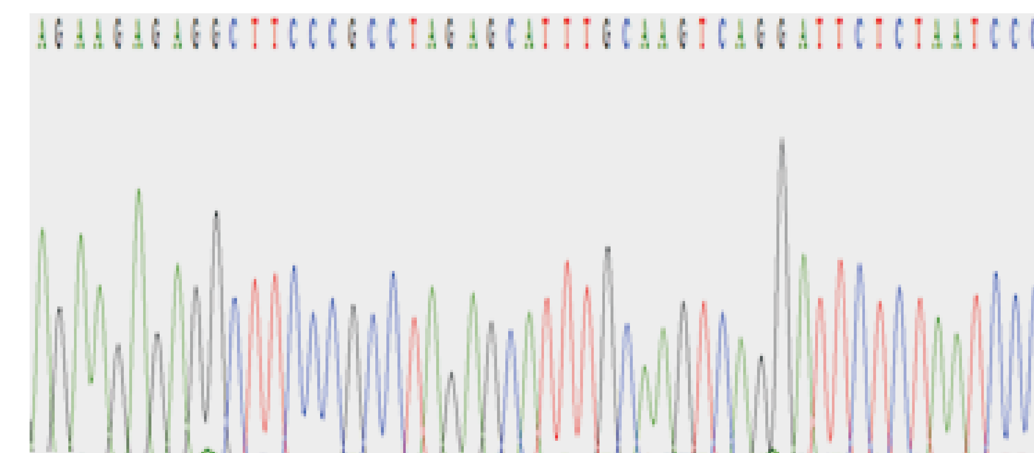

Fig 2: Sanger sequencing electroferogram of EBER1 fragments.

EBER 1 and 2 were amplified with special primers that included both the T3 and T7 RNA polymerase promoter sequences in the 5' and 3' extremes, respectively. PCR with long primers from a gDNA template is also possible (Fig. 3). After precipitation, the yield was approximately 600 ng/µL DNA from each EBERs.

Electrophoresis gel of EBERs PCR products

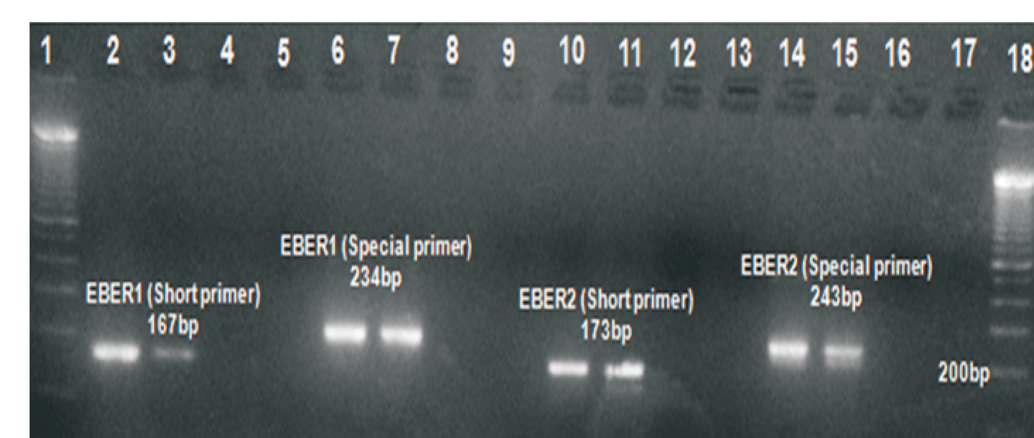

Fig 3: Electrophoresis of EBER1 and EBER2 PCR products, with primers specific for EBER 1 and 2. Lanes 1 and 18: MWM100 bp ladder (Life Tech); Lanes 2, 6, 10, and 14: EBERs product amplified from plasmid template; Lanes 3, 7, 11, and 15: EBERs product amplified from EBV+ Namalwa cell line; Lanes 4, 8,12, and 16: Negative control (EBV-Donor); Lanes 5, 9, 13, and 17: PCR control (H2O) (Agarose gel, 3%).

PCR products generated with special long primers were used as template for *in vitro* transcription, employing digoxigenin-UTP labeled and the respective T3 and T7 RNA polymerases . Average yield was ~200 ng/µL of DIG-labeled RNA probes (Fig.4).

Electrophoresis gel of RNA probes obtained from in vitro transcription

Fig. 4: Specific RNA obtained after in vitro transcription of EBER1 and EBER2 from a PCR product containing T3 and T7 promoter sequences. Lanes 1 and 6: MWM 100bp ladder (Life Tech.); Lanes 2 and 4: Sense

CONCLUSIONS

❖ We developed an easy and fast method to obtain efficient probes for EBV detection by RNA-ISH in a variety of tissue samples, which makes us independent from a costly commercial supply.

❖ According to titration experiments, one tube of transcribed probes can be used for 250 RISH assays.

❖ The method can easily be extended to produce probes for other applications, for instance, interleukin expression.

❖The probes are currently being tested concerning their reproducibility and stability for diagnostic usage.

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

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