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Short communication

Development of generic Taqman PCR and RT-PCR assays for the detection of DNA and mRNA of β-actin-encoding sequences in a wide range of animal species

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A B S T R A C T

As a member of the European Virus Archive (EVA) consortium, our laboratory is developing and maintaining a large collection of viruses. This collection implies the use of a panel of cell lines originating from various animal species.

In order to make easier the handling of such a large panel of cell lines, wide spectrum real-time PCR and RT-PCR assays were developed to allow the detection and the quantification of DNA and mRNA of β-actin, one of the most commonly used eukaryotic housekeeping genes. By using two degenerated primers and a unique probe, these two assays were shown to detect nucleic acids of a panel of vertebrate and invertebrate cell lines commonly used in animal virology. This panel included human, monkey, rodent, dog, pig, fish, batrachian, mosquito and tick cell lines. Additionally, the two assays amplified successfully β-actin-encoding sequences of sandflies. Sensitivity evaluation performed on synthetic DNA and RNA sequences showed that the two assays were very sensitive and suitable for accurate quantification.

The two assays constitute together a convenient method suitable for multiple purposes. They can be used for instance to estimate the amount of contaminating cellular genetic material prior to sequence-independent amplification of viral genomes achieved before high-throughput sequencing, to evaluate the efficiency of DNase and/or RNase treatments performed on cellular extract and to check nucleic acid extraction by using β-actin-encoding sequences as endogenous control. This assay will constitute a precious tool for virologists working with multiple cell lines or animal models.

By allowing concomitant sequencing of thousands, and even millions, of DNA fragments, high-throughput sequencing methods have provided new perspectives in virology (Ambrose and Clewley, 2006). One of the most powerful applications of these methods is the sequencing of DNA fragments produced by sequence-independent nucleic acid amplification. Sequence-independent nucleic acid amplification consists in amplifying nucleic acids present in a given sample without using primers designed to target specifically sequences of interest. This amplification can be carried out by using different strategies, such as classical PCR or RT-PCR performed with random primers (Cheval et al., 2011) or Phi29 polymerase-based random amplification (Berthet et al., 2008). Contrary to assays designed to recover specifically a virus or a group of viruses, sequence-independent nucleic acid amplification avoids the bias towards any particular viral group, so allowing the sequencing of different viruses present simultaneously in a same sample. Thus, this method is used to conduct viral metagenomic studies, which aim to characterize virus communities present in environmental or biological samples (Delwart, 2007; Svraka et al., 2010). Besides, sequence-independent nucleic acid amplification is a helpful tool to discover novel viruses and to sequence genetically divergent viruses for which no sequence data are available, making impossible the design of specific primers (Bexfield and Kellam, 2011).

The main pitfall of sequence-independent nucleic acid amplification of viral genomes is the presence of host nucleic acids: they are also amplified during the random amplification steps, so decreasing the rate of viral sequences in the final product

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Table 1
Cell lines assessed for β-actin nucleic acids detection.

Cell line	ACTB mRNA accession No.	Culture medium[a]	Culture conditions
Primates			
Homo sapiens	BC016045		
SW13		RPMI, 10% FCS, 2 mM Gln	37 °C w/CO_2
HT-29		McCoy's 5A, 10% FCS, 2 mM Gln	37 °C w/CO_2
MRC-5		BME, 10% FCS, 2 mM Gln	37 °C w/CO_2
HuH-7		DMEM, 10% FCS, 2 mM Gln, NEAA	37 °C w/CO_2
Chlorocebus aethiops	AB004047		
Vero		EMEM, 7% FCS, 2 mM Gln	37 °C w/CO_2
MA-104		EMEM, 7% FCS, 2 mM Gln, NEAA	37 °C w/CO_2
BGM		EMEM, 5% FCS, 2 mM Gln	37 °C w/CO_2
Rodents			
Mus musculus	BC138614		
L929		EMEM, 7% FCS, 2 mM Gln	37 °C w/CO_2
Mesocricetus auratus	AF014363		
BHK-21		EMEM, 5% FCS, 5% tryptose phosphate, 2 mM Gln	37 °C w/CO_2
Other mammals			
Canis familiaris	AF021873		
MDCK		EMEM, 7% FCS, 2 mM Gln, NEAA	37 °C w/CO_2
Sus scrofa	AY550069		
PS		DMEM, 10% FCS, 2 mM Gln	37 °C w/CO_2
Fish			
Oncorhynchus tsawytscha	FJ546418		
CHSE-214		EMEM, 10% FCS, Hepes 25 mM, Gln 2 mM, sodium pyruvate 1 mM, NEAA	21 °C w/o CO_2
Batrachian			
Xenopus laevis	AF079161		
XTC-2		L-15, 10% FCS, 5% tryptose phosphate, 2 mM Gln	28 °C w/o CO_2
Arthropods			
Aedes albopictus	DQ647949		
C6/36		L-15, 10% FCS, 5% tryptose phosphate, 2 mM Gln	28 °C w/o CO_2
Aedes aegypti	XM_001655126		
AE		L-15, 10% FCS, 5% tryptose phosphate, 2 mM Gln	28 °C w/o CO_2
A20		L-15, 10% FCS, 5% tryptose phosphate, 2 mM Gln	28 °C w/o CO_2
Aedes pseudoscutellaris			
AP-61	Not available	L-15, 10% FCS, 5% tryptose phosphate, 2 mM Gln	28 °C w/o CO_2
Rhipicephalus appendiculatus			
RA-257	AY254899	L-15, 15% FCS, 5% tryptose phosphate, 2 mM Gln	28 °C w/o CO_2

[a] FCS: foetal calf serum; Gln: L-glutamine; NEAA: non-essential amino acids.

that is subsequently used for sequencing (Delwart, 2007). Different strategies can be used to eliminate unwanted nucleic acids. For instance, virions can be purified by ultracentrifugation. Additionally, host nucleic acids can be removed from the sample by enzymatic digestion (by DNases, RNases and/or benzonase) performed before extracting viral nucleic acids, while viral genetic material remains protected by the viral capsid. Therefore, detecting and quantifying contaminating cellular nucleic acids is crucial prior to sequence-independent amplification to evaluate the amount of contamination of a sample by eukaryotic nucleic acids and to evaluate the efficiency of the different methods used to reduce this level.

Complete degradation of host nucleic acids can be checked by PCR and/or RT-PCR assays that target cellular genetic sequences. The detection and the quantification of cellular nucleic acids generally target housekeeping gene sequences. Housekeeping genes are constitutive genes whose products are involved in basic cellular functions. They are expressed continuously in all cells of an organism, whatever the environmental conditions are (Bustin, 2000). Tens of housekeeping genes have been described (Zhu et al., 2008), including genes that encode ribosomal RNA, enzymes (cytochrome c oxydases, glyceraldehyde-3-phosphate dehydrogenase), structural proteins (actin, dynein), transcription factors and other kinds of proteins (ubiquitin, receptors).

Virologists commonly used numerous cell lines and animal models to isolate, cultivate and study viruses. Developing and maintaining collections of viruses also require the use of a panel of cell lines from various animal species (Gould et al., 2012). In order to avoid the requirement of different molecular systems, each being designed to target the nucleic acids of a particular host species, the aim of this study was to develop a generic system allowing the detection and the quantification of eukaryotic nucleic acids, whatever is the species they come from. One of the most commonly used housekeeping genes is ACTB, a member of the actin gene family, which encodes the isoform β of actin, a cytoplasmic protein involved in cytoskeleton maintenance and in cell motility that is expressed ubiquitously. This gene is highly conserved across eukaryotic species (Bunnell and Ervasti, 2011) and therefore constitutes a promising target for the purpose of designing a broad-spectrum molecular assay.

Various molecular assays were developed to detect and amplify β-actin mRNA of numerous species (Bjarnadottir and Jonsson, 2005; Brinkhof et al., 2006; Yang et al., 2013) but no generic assay targets ACTB sequences of a wide range of vertebrate and invertebrate species.

Sequences of β-actin-encoding mRNA of several animal species were retrieved from GenBank (see Table 1 for the corresponding accession numbers) and aligned with CLC Main Workbench 6 software (CLC bio). Two primers (Act.f and Act.r, Table 2) and one probe (Act.p) were designed to match with conserved nucleotide sequences within the exon V of ACTB gene (Nakajima-Iijima et al., 1985). The two primers were degenerated to match with all the analyzed sequences.

The primers and probe (Eurogentec) were assessed on nucleic acid extracts from a panel of vertebrate and invertebrate cell lines used commonly in animal virology (Table 1). This panel included human, monkey and rodent cell lines used for isolation and growing of most of the human and zoonotic viruses, dog MDCK cell line, which is the most widely used cell line for isolation and growing of Influenza A, B and C viruses (Amano and Cheng, 2005), and swine PS

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