









NATIONAL RESEARCH, DEVELOPMENT AND INNOVATION FUND

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Development of novel Hot-Start (HS) polymerase technologies for the Polymerase Chain Reaction (PCR)

There has been a trend to move away from chemically modified HS methods to antibody HS, with no alternative technology available to replace this. qPCR requires more complete HS than traditional end-point PCR. Endpoint PCR results are traditionally analysed, visually by agarose gel electrophoresis. Non-specific products and primer dimers do not influence the presence/absence of a correct product. In qPCR, quantitation is a measurement of PCR efficiency; non-specific products will affect this and also affect fluorescent measurements. This explains why all qPCR kit manufacturers' favour antibody HS rather than other inadequately performing chemically modified HS technologies. Recombinant antibody fragment HS would be an attractive alternative to HS antibody on the market.

The goal of the project was to create a new quality class of PCR reagents. The project has accomplished with great success.

The project has combined the skill sets of two SMEs, the Polymerase Chain Reaction (PCR) reagents development (PCR Biosystems) and protein biochemistry (TargetEx). TargetEx has developed Hot-Start (HS) antibodies and novel HS antibody fragments. PCR Biosystems has developed a novel HS assay, which formed the basis of an automated screen. The screen then was used to measure HS properties of candidate proteins that had developed by TargetEx. This has led to high quality HS PCR and qPCR mixes that are now available on the market worldwide.

In former studies, we have used qPCR to measure mouse genomic DNA in several key manufacturers' antibody Hot-Start (HS) kits. In all the kits, we have tested there was a trace level of mouse genomic DNA. This trace level could negatively influence the development of diagnostic and research assays especially if for example a particular human disease gene has high sequence identity, to that of a mouse gene. A 100% mouse DNA free antibody based HS molecule, which is one of the main outcomes of the project, will become a breakthrough on the market, we think. PCR Biosystems will incorporate new HS reagents into its existing product portfolio of more than 30 HS PCR kits. The kits will benefit from improved HS qualities and also reduced productions costs. PCR Biosystems and TargetEx will jointly own any IP resulting from this project. Furthermore, we make available the license of this technology to other PCR companies. The extent of our ambition is that this method of HS becomes an industry standard.

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Contractors

TargetEx Ltd.

Headquarters: Madách Imre utca 31/2, Dunakeszi, Hungary, H-2120

Tel: +361-279-3153 Fax: +361-279-3154

E-mail: info@targetex.com web: http://www.targetex.com/

Supporter

National Research, Development and Innovation Fund Hungarian Government Kéthly Anna tér 1., Budapest, Hungary, H-1077

E-mail: nkfialap@nkfih.gov.hu

Tel: +36 1 795-9500

web: http://nkfia.kormany.hu/