PREPARATION OF NUCLEAR EXTRACTS FROM HeLa CELLS

Muhammad Tahman Shahid[a], Syeda Khair-ul-Bariyah[b]

Keywords: HeLa cells; nuclear extracts; immortal; cell lines; vaccines; polypeptide; RNA; DNA.

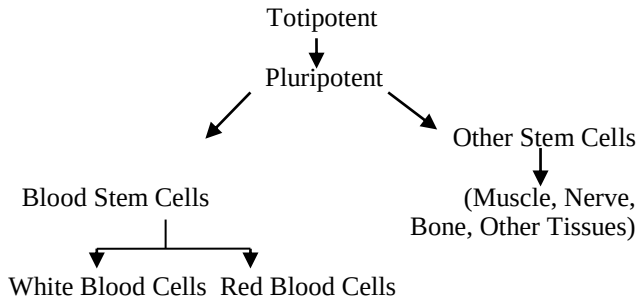
Stem cells are the powerful cells derived both from human and animal source. They have the ability to divide innumerably and differentiate into different cell types in the body. HeLa cells are the first immortal human cell lines grown in culture. These cell lines have proved beneficial in the development of polio vaccines and study of various viral cells. The present review aims to cover the studies done through nuclear extracts prepared from HeLa cells. Nuclear extracts from HeLa cells have been used to study the activation of ATM and ATR by double stranded DNA breaks, production of transcriptionally active extracts, analysis of RNA splicing in vitro using T7 RNA polymerase-derived splicing substrate RNAs and purification and characterization of proteins. By using these nuclear extracts, the mutational study of proteins still needs to be done to know exact reason of many unknown diseases. Wide variations of pH in extract preparation should be tested to get variations in extract obtained and methods should be modified for desired polypeptide length and to work with extracts at nano scale.

Corresponding Authors
E-Mail: skbariyah@gmail.com
[a] Center for Research in Molecular Medicine (CRIMM), The University of Lahore, Lahore, Pakistan.
[b] Department of Chemistry, Forman Christian College (A Chartered University), Lahore, Pakistan.

Introduction

Undifferentiated biological cells found in multicellular organisms are called stem cells. Through mitosis they differentiate into specialized cells and produce more stem cells. Stem cells have the property of self-renewal and the ability to differentiate into specialized cell types. In an adult organism, stem cells and progenitor cells make the repair system of the body and in a developing embryo differentiate into ectoderm, endoderm and mesoderm. According to potency, the stem cells may be totipotent, pluripotent, multipotent, oligopotent and unipotent.[1,2,3] Scheme 1 shows the hierarchy of stem cells.

Hierarchy of Stem Cells

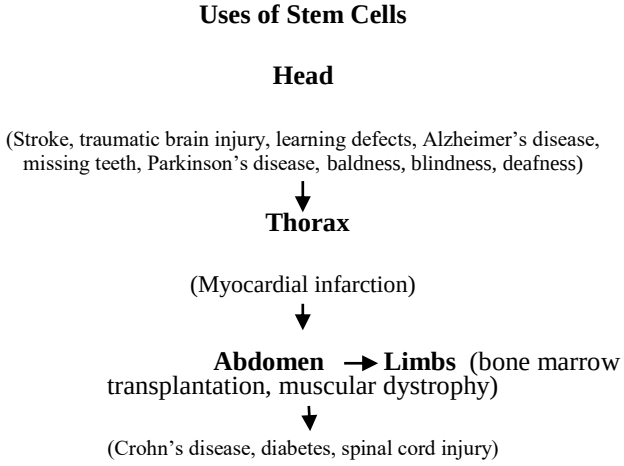


Scheme 1. Hierarchy of stem cells.

Two main types of stem cells are embryonic stem cells and adult stem cells. The embryonic stem cells are isolated from the inner cell mass of blastocysts whereas adult stem cells are found in various tissues and maintain and repair the tissue in which they are found. Three sources of adult stem cells are bone marrow, adipose tissue and blood. Embryonic stem cells are pluripotent and during development give rise to ectoderm, endoderm and mesoderm. The stem cells located in the organs of fetus are called fetal stem cells. They have two types: fetal proper stem cells and extraembryonic fetal stem cells. Fetal proper stem cells are multipotent as they come from fetus after abortion. They have a high level of division. Extraembryonic fetal stem cells cannot be distinguished from adult stem cells as they are obtained after birth. They are pluripotent and have a significant level of division.[4,5]

Stem cell treatment has proved to be promising in bone marrow transplants, Parkinson's disease, cancer, spinal cord injuries, Amyotrophic lateral sclerosis, muscle damage and multiple sclerosis. Many other diseases are still investigated where stem cells can prove to be potent and the risk of tumor formation due to multiple stem cells' division is also under study.[6,7,8] Scheme 2 shows the uses of stem cells.

Uses of Stem Cells



Scheme 2. Uses of stem cells.

The oldest and most commonly used cell line (stem cell) is HeLa cell, being an immortal cell line derived from cervical cancer cells of Henrietta Lacks, who died of cancer on February 8, 1951. The cells can divide innumerably as long as cell survival conditions are met, hence, they are called immortal. As these cells continue to mutate in cell culture, there are many strains of these. HeLa cells are the first successfully cloned human cells.[9,10,11] In 1950s, the first polio vaccine was tested by using HeLa cells.[12] Besides that, the effects of paro virus, oropouche virus and papillovirus were also studied by using these cell lines.[13,14] The ability of canine distemper virus to induce apoptosis in cancer cell lines is also revealed by HeLa cells.[15] The major research areas are cancer (apoptosis induction), gene mapping, effects of toxic substances and AIDS.[16] Figure 1 shows electron micrograph of apoptotic HeLa cells.

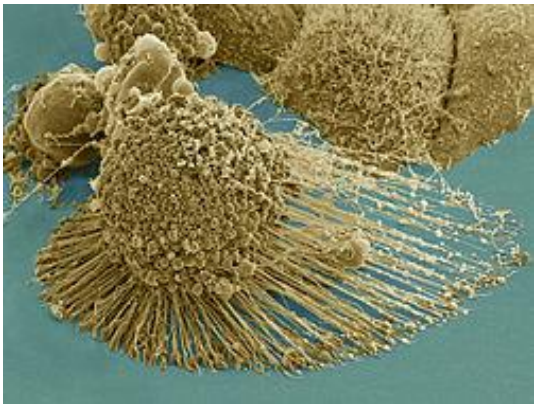


Figure 1. Electron Micrograph of HeLa Cells (http://en.wikipedia.org/wiki/File:HeLa-IV.jpg)

Nuclear extracts are prepared from HeLa cells since long to determine the mechanisms of splicing and polyadenylation. Characterization of these extracts has been done extensively.[17] The present review aims to cover the studies done so far on the nuclear extract preparation from HeLa cells and their uses.

Preparation of Nuclear Extracts

The general method for the preparation of nuclear extracts involves collecting cells from culture and microfuging them at 500 rpm for 10 minutes. The supernatant is discarded and the packed cell volume (pcv) is measured. The cell pellets are resuspended in a hypotonic buffer which is about 5 times in volume to pcv and centrifuged for 5 minutes at 5000 rpm. The supernatant is discarded and the packed cells are resuspended in a hypotonic buffer which is 3 times in volume to pcv. They are then allowed to swell for 10 minutes on ice. The cells are transferred to a glass Dounce homogenizer and homogenized by using 10 up and down strokes via type B pestle. The cells are again centrifuged for 15 minutes at 6500 rpm to collect nuclei. The packed nuclear volume (pnv) is measured and they are suspended in a low-salt buffer the volume of which is ½ pnv. In it high-salt buffer is added the volume of which is equal to ½ pnv. With continuous gentle mixing the nuclei are allowed to extract. By centrifuging the extracted nuclei for 30 minutes at 25000×g, the nuclei are pelleted. If the final volume is less than 100µL then it is made up to 100 µL by a solution containing equal volumes of high and low salt buffers. After dialyzing for 45 minutes, the dialysate is centrifuged for 20 minutes at 14500 rpm. The pellet is discarded and 5 µL of supernatant is removed for assay and the rest of it is cooled to –80 °C.

Preparation of Nuclear Extracts from HeLa Cells

The master regulators of DNA damage signalling pathway responding to a wide variety of DNA damage are Ataxia telangiectasia-mutated (ATM) and ATM-Rad3-related (ATR) kinases. An in vitro biochemical assay to study the activation of ATM and ATR by double stranded DNA breaks involves preparation of nuclear extracts from cultured HeLa cells followed by generation of DNA fragments using DNA oligonucleotides. Incubation of DNA fragments in extracts is then done along with analysis of the phosphorylation of ATM or ATR substrates.[18]

Many different DNA base lesions are corrected by base excision repair (BER) pathway. The BER activity of nuclear cell extracts from HeLa cells was investigated and the substrate used was a circular DNA molecule with either uracil or an AP-site in a defined position. From the same batch of cells, the BER activity of nuclear extracts varies inversely with the volume of nuclear extraction buffer relative to nuclei volume. Negative correlation was seen of the uracil-DNA glycosylase activity with the volume of extraction buffer. The method for the preparation of nuclear extracts was demonstrated to be an important factor along with conditions used in analysis.[19]

Many protocols for the preparation of splicing-competent extracts from whole cells, nuclei and cytoplasmic fractions and optimized production of transcriptionally active extracts from HeLa cells have also been reported.[20,21] A number of methods have been reported for the preparation of effective nuclear and cytoplasmic extracts from adenovirus-infected HeLa cells. Preparation of extracts from the infected cells along with analysis of RNA splicing in vitro using T7 RNA polymerase-derived splicing substrate RNAs has been investigated successfully.[22] For in vitro end joining using plasmid DNA as substrate, a protocol was reported which involved preparation of nuclear extracts from HeLa cells, plasmid substrate DNA preparation, in vitro DNA repair mechanisms and gel electrophoresis of the product. Overall the assay seemed powerful, but, it didn't consider the in vivo assembly of DNA into chromatin.[23]

In nuclear extracts of HeLa cells, 95-kDa CYP 450 2E1 promoter binding protein was found by bioaffinity mass spectrometry.[24] The protein was purified and characterized functionally from HeLa cells with the final preparation containing two polypeptides of 70 and 86 kDa mass, respectively. Interaction of both polypeptides was noted with a GC-stretch adjacent to the binding site of transcription termination factor 1. Electrophoretic mobility shift assay analysed specificity of binding to the barrier DNA. The biochemical properties of the protein resembled Ku antigen. In Ku depleted HeLa cell extracts, the recombinant Ku protein partially rescued the barrier activity, proving the synergic act of transcription termination factor 1 and Ku to prevent head-on-collision between the replication and transcription machinery.[25]

A high performance affinity purification technique was developed for cisplatin (CDDP)-damaged DNA binding proteins directly from HeLaS3 cell. Submicron beads made of styrene and glycidyl methacrylate (GMA) were used. Nonspecific protein adsorption was found to decrease. To prepare the beads for purification, immobilization of telomeric repeats (TTAGGG) (n) was done. Higher affinity to CDDP-DNA was shown by 9 proteins. These proteins were identified by amino acid sequence analysis including HMGB (high mobility group), hUBF (human upstream binding factor) and Ku autoantigen.[26] The electrophoretic mobility shift assay (EMSA) is for the study of the interaction of transcription factors to specific DNA sequences. The most important step in this assay is the preparation of high quality nuclear extracts. DNA-binding proteins were isolated from cultured cell lines and autopsy tissue samples from the human brain. Rapid method (RM) was used which followed low salt detergent lysis steps along with high salt extraction of nuclei. Three oligonucleotide probes (AP1, NF-κB and URE) were used to test the ability of nuclear extracts to form DNA-protein complex. The nuclear extracts taken by standard and RM methods showed similar capacity to form DNA-protein complex. Competition experiment was used to check the specificity of each nuclear extract forming the complex. Whether the nuclear extract was prepared by standard method or RM, unspecific bands were observed from both preparations. Hence, rapid method being simple allows preparation of nuclear extracts from several cell lines and tissue samples at the same time at much shorter time without affecting the DNA-binding activity. Cell type or tissue specificity can be determined efficiently and economically by using RM.[27] In mRNA decay, poly(A) tail removal is the rate limiting step, being responsible for translational silencing of maternal mRNA during oocyte maturation. Deadenylation in HeLa cell extracts and by PARN (purified mammalian poly (A)-specific exoribonuclease) was found to be stimulated by m(7)-guanosine cap on substrate RNAs. In the enzyme preparation, cap-binding proteins were not detectable and PARN was found to bind to m(7)GTP-Sepharose being eluted with the cap analogue m(7)GTP. During oocyte maturation, xenopus PARN catalyzed mRNA deadenylation. From oocyte extract, the enzyme was depleted with m(7)GTP-Sepharose. It could be linked to m(7)GpppG cap and could deadenylate m(7)GpppG-capped RNAs effectively than ApppG-capped RNAs. It proved PARN to be responsible for deadenylation during oocyte maturation.[28] For in vitro splicing, HeLa cell nuclear and cytosolic S100 extracts have also been prepared.[29] Chromatin degradation into oligonucleosomal and 30-50 Kb fragments is the basis of apoptosis. Both types of fragments can be recapitulated by crude nuclear extract from apoptotic rat thymocytes. The assay used HeLa cell nuclei as an exogenous substrate. By using size exclusion chromatography, a novel activity was identified producing 30-50 Kb DNA fragments and another 25 Kb activity that produced 30-50 Kb and oligonucleosomal fragments. DNA fragments with 3'-OH termini were produced by both activities. Inhibition of the activities was shown by serine protease inhibitors. By incubating the extracts with naked linear DNA, both activities turned insensitive to protease inhibitors. It indicated the presence of nuclease and protease activities in the preparation. Involvement of non-caspase proteases in apoptosis was proved and their function was supposed to be altering chromatin substructure and exposing it to nucleolytic attack.[30]

Modification of the preparation of crude nuclear extracts made it possible to establish an assay forming link between weak late and strong very late viral promoters. In case of both promoters, the virus-induced RNA polymerase initiates at a TAAG sequence motif. Investigation was done on the sequences responsible for functional TAAG motif and their role with respect to the strength of very late promoters. Hybrid promoters were constructed between the early pe38 and the very late polyhedrin promoters. The replacement of 7 nucleotides upstream of the non-functional TAAG sequences in the pe38 promoter with the corresponding sequences of the polyhedrin promoter was investigated to be sufficient for recognition by the virus-induced RNA polymerase. Replacement of the 5' untranslated sequences of the pe38 promoter by polyhedrin promoter and 7 nucleotides upstream of the TAAG motif established the strength of very late polyhedrin promoter.[31] A group of clones was isolated from a single cell line HeLa. They were methylated over the GPH alpha gene and showed a 400-fold range in its expression.[32] Various cellular components like mitochondrion, nuclear envelope, centromere etc are recognized by sera from patients with primary biliary cirrhosis. In these sera, a novel antibody reacts with a particular protein. By using rat liver cytoplasmic antigens, the antigen was identified by immunoprecipitation of [35S] methionine labelled HeLa cell extracts and by immunoblot using disrupted HeLa cell extracts.[33] Basic proteins which were normally lost by cathodic drift of carrier ampholyte focusing were separated from HeLa cells by 2D-electrophoresis using pH 12 with IPGs 8-12, 9-12 and 10-12. Due to IPG 9-12 the hectic procedure of nuclei preparation prior to histone extraction could be omitted.[34]Immunoaffinity purification on two types of affinity columns was used to prepare an antibody specific for human O6-methylguanine-DNA methyltransferase. The affinity columns had purified human and mouse methyltransferase proteins as ligands. The antibodies were used in Western blotting analysis of fractioned cell extracts and above 90% of the methyltransferase protein was recovered in the cytoplasmic fractions with MR-M cells and human HeLa S3 cells.[35]

Immunostaining confirmed the cytoplasmic localization of methyltransferase in HeLa S3 cells. The fractioned cell extracts from HeLa S3 cells treated with alkylating agents were analyzed by Western blotting analysis. Amount of the enzyme was found to decrease rapidly in the nuclear fraction than in the cytoplasmic fraction and slower recovery of the enzyme level was seen in the cytoplasmic fraction. It is therefore seen that after repair reaction methyltransferase protein is degraded in the nucleus and cytoplasmic enzyme is transported in it.[36] From nuclear extracts of HeLa cells, small nuclear ribonucleoproteins i.e snRNPs (U1, U2, U4, U6) were removed by antisense affinity depletion. Splicing activity was fully restored, after addition of a highly purified preparation of SR proteins, in reactions depleted of U1 snRNP. No reconstitution of splicing in reactions depleted of other snRNPs was noted. Reconstitution with SR proteins was seen of spliceosomes formed in the U1 snRNP-depleted reactions. The assembly of precursor messenger RNA (pre-mRNA) into a spliceosome is facilitated by high concentrations of SR proteins when there are no interactions with U1 snRNP.[37] The circular ribozyme has shown enhanced resistance to nuclear degradation as compared to linear form in nuclear and cytoplasmic extracts from HeLa cells.[38] Sterol regulatory element binding protein (SREBP)

was purified from nuclear extracts of human HeLa cells by ion exchange, gel filtration and DNA-affinity chromatography. A cluster of bands at 59-60 kDa were seen by sodium dodecyl sulfate gel electrophoresis of the purified preparation. As seen by cross-linking experiments, each band was bound to SRE-1 element. SREBP was found to work in coherrence with Sp1 nuclear factor to gain high level, sterol suppressible transcription of the gene for the LDL receptor.[39]

Characterization and differentiation of Su antigen has been carried out by Western immunoblotting and immunoprecipitation employing DNase CTNE and extracts of 35S-methionine labelled HeLa cells.[40] Nuclear extracts from various monkey and rat cell lines were yielded that could allow splicing of a model adenovirus pre-mRNA substrate.[41] A nuclear protein was identified from extracts of HeLa cells that could bind to the TAR element RNA in a sequence specific manner and moderated TAR activity in vivo.[42] By using antibodies raised against isolated rat liver nuclear matrix and cross-reactive with a 65-kDa HeLa cell nuclear matrix protein IGA-65, the role of nuclear matrix proteins in premessenger RNA splicing was investigated.[43]Incubation of SP6 generated mouse histone H4 mRNA precursors in nuclear extracts of HeLa cells yielded processed mRNA species ending on the 3' adenosine of the conserved terminal ACCA sequence.[44] By using HeLa nuclear extracts or ribonucleoproteins (RNPs) from rat liver nuclei as antigens, a monospecific anti-(U1)RNP serum was recognized in each preparation i.e. one polypeptide of 68 or 70 kD, respectively. HeLa nuclear extracts showed three additional antigenic polypeptides of 29, 28, and 16 kD with a serum of combined anti-Sm/(U1)RNP specificity, whereas, only two additional polypeptides of 27 and 16 kD were observed in rat liver RNPs.[45] By lysing mitotic HeLa cells in low-salt hypotonic buffer, cytoplasmic extracts were prepared and chromosomes were separated by centrifuging. The mitotic factors were extracted with high-salt (0.2 M-NaCl) buffer and both the protein fractions were evaluated for their maturation-promoting activity (MPA) in the Xenopus oocytes. The results showed that both the cytoplasmic and chromosomal fractions were identical in many respects, including their ability to induce GVBD, but the specific activity of the chromosomal fraction was at least 3-fold greater than that of the cytoplasmic fractions.[46] An enzyme was purified from uninfected HeLa cells that could cleave the 5'-terminal protein (VPg) from poliovirus RNA by rapid phenol extraction assay.[47]

Extracts were prepared from M cells which were as active in protein synthesis as S cell extracts. Binding of Met-tRNAf to 40S ribosomal subunits and binding of mRNA to ribosomes also exhibited similar activity in both extracts. In the preparation of cell-free systems, the difference in protein synthesizing activity was eliminated. The ribosomes of M cells had small molecular weight RNA, which inhibited protein synthesis in vitro and had possibly a nuclear origin causing reduction in the rate of protein synthesis in M cells.[48] A method for the covalent attachment of poly A and other nucleic acids to a methylene dianiline derivative of starch was described and its properties and use for the recovery of poly U sequences from both nuclear and cytoplasmic extracts of HeLa cells was also investigated.[49]

Conclusion

HeLa cells are the oldest stem cells, being the first human cell line to be isolated and cultured. The nuclear extracts from these cell lines have proved beneficial in the extraction of many proteins and enzymes. Moreover, RNA and DNA splicing and protein binding has been clarified. By culturing these cell lines in a variety of pH and temperature conditions (where the cells remain viable) and then taking extracts can uncover many unknown cellular and nuclear components. The area of genetic mutations should be focussed and methods still need to be modified to splice the DNA/RNA at the desired point and to get polypeptides of desired length. Moreover, by extracting nuclear extracts from HeLa cells of patients suffering from sarcoma of various types and diseases like HIV Aids, many unknown factors still unknown, can be explored and direction of studies can be shifted to success.

References

[1]Schöler, H. R., *Human Biotechnology as Social Challenge*, **2007**, 28.

[2]Mitalipov, S. and Wolf, D., *Adv. Biochem. Eng. Biotechnol.*, **2009**, *114*, 185-99.

[3]Ulloa-Montoya, F., Verfaillie, C. M. and Hu, W. S., *J. Biosci. Bioeng.*, **2005**, *100(1)*, 12-27.

[4]Ariff, B. and Eng, L. H., *Stem Cells: From Benchtop to Bedside*, **2005**, 5.

[5]Moore, K. L., Persaud, T. V. N. and Torchia, A. G., Philadelphia, PA: Saunders, Elsevier, **2013**.

[6]Lindvall, O., *Pharmacol. Res.*, **2003**, *47(4)*, 279-87.

[7]Goldman, S. and Windrem, M., *Phil. Trans. Roy. Soc. London B., Biol. Sci.*, **2006**, *361(1473)*, 1463-75.

[8]*Consumer Reports on Health*, **2005**, 8-9.

[9]Rahbari, R., Sheahan, T. and Badge, R.M., *BioTechniques*, **2009**, *46(4)*, 277-84.

[10]Scherer, W. F., Syverton, J. T. and Gey, G. O., *J. Exp. Med.*, **1953**, *97(5)*, 695-710.

[11]Puck, T. T. and Marcus, P. I., *Proc. Natl. Acad. Sci., USA*, **1955**, *41(7)*, 432-7.

[12]Scherer, W. F., Syverton, J. T. and Gey, G. O., *J. Exp. Med.*, **1953**, *97(5)*, 695-710.

[13]Acrani, G. O., Gomes, R. and Silva, M. L., V*irus Res.*, **2010**, *149(1)*, 56-63.

[14]Hou, S. Y., Wu, S. and Chiang, C., *J. Biol. Chem.*, **2002**, *277(47)*, 45619-29.

[15]Del Puerto, H. L., Martins, A. S. and Vasconcelos, A. C., *Virol. J.*, **2011**, *8*, 334.

[16]Smith and Van, *Baltimore City Paper*, **2002**.

[17]Nilsen, T. W., *Cold Spring Harb. Protoc.*, **2013**, *6*, 579-83.

[18]Shiotani, B. and Zou, L., *Methods Mol. Biol*., **2011**, *782*, 181-91.

[19]Akbari, M. and Krokan, H. E., *Mutat. Res*., **2011**, *736(1-2)*, 33-8.

[20]Kataoka, N. and Dreyfuss, G., *Methods Mol. Biol.*, **2008**, *488*, 357-65.

[21]Abmayr, S. M., Yao, T. and Parmely, T., *Curr. Protoc. Mol. Biol.*, **2006**.

[22]Mühlemann, O. and Akusjärvi, G., *Methods Mol. Med*., **2007**, *131*, 33-46.

[23]Iliakis, G., Rosidi, B. and Wang, M., *Methods Mol. Biol.*, **2006**, *314*, 123-31.

[24]Zhu, Y.I., Valdes, R.J. and Linder, M.W., *Clin. Chim. Acta*, **2006**, *371(1-2)*, 71-8.

[25]Wallisch, M., Kunkel, E. and Grummt, F., *Biol. Chem.*, **2002**, *83(5)*, 765-71.

[26]Tomohiro, T., Sawada, J. J. and Okuno, H., *Bioconjug. Chem*., **2002**, *13(2)*, 163-6.

[27]Lahiri, D. K. and Ge, Y., *Brain Res. Brain Res. Protoc*., **2000**, *5(3)*, 257-65.

[28]Dehlin, E. and Wahle, E., *EMBO J*, **2000**, *19(5)*, 1079-86.

[29]Mayeda, A. and Krainer, A. R., Methods Mol. Biol., **1999**, *118*, 309-14.

[30]Hughes, F. M. and Cidlowski, J. A., *Cell Death Differ*., **1998**, *5(12)*, 1017-27.

[31]Mans, R. M. and Knebel, D. M., *J. Virol.*, **1998**, *72(4*), 2991-8.

[32]Cox, G. S., Gutkin, D. W. and Cosgrove, D. E., *Biochim. Biophys. Acta*, **1998**, *1396(1)*, 67-87.

[33]Miyakawa, H., Kako, M. and Ueno, U., *Scand. J. Immunol*., **1998**, *47(1)*, 63-8.

[34]Görg, A., Obermaier, C. and Madjar, J. J., *Electrophoresis*, **1997**, *18(3-4)*, 328-37.

[35]Ishibashi, T., Nakabeppu, Y. and Sekiguchi, M., *Mutat. Res.*, **1994**, *315(3)*, 199-212.

[36]Ishibashi, T., Nakabeppu, Y. and Sekiguchi, M., *Mutat. Res.*, **1994**, *315(3)*, 199-212.

[37]Crispino, J. D., Blencowe, B. J. and Sharp, P. A., *Science*, **1994**, *265(5180)*, 1866-9.

[38]Puttaraju, M., Perrotta, A. T. and Been, M. D., *Nucleic Acids Res*., **1993**, *21(18)*, 4253-8.

[39]Wang, X., Briggs, M. R. and Brown, M. S., *J. Biol. Chem*., **1993**, *268(19)*, 14497-504.

[40]Treadwell, E. L., Müller, U. R. and Volkman, A., *J. Immunol. Methods*, **1991**, *142(2)*, 157-67.

[41]La Branche, H., Frappier, D. and Chabot, B., *Nucleic Acids Res.*, **1991**, *19(16)*, 4509-14.

[42]Marciniak. R. A., Gracia-Blanco, M. A. and Sharp, P. A., *Proc. Natl. Acad. Sci*., **1990**, *87(9)*, 3624-8.

[43]Smith, H. C., Harris, S. G. and Berget, S. M., *Exp. Cell Res.*, **1989**, *182(2)*, 521-33.

[44]Gick, O., Krämer, A. and Birnstiel, M. L., *EMBO*, **1986**, *5(6)*, 1319-26.

[45]Guldner, H. H., Lakomek, H. J. and Bautz, F. A., *J. Immunol. Methods*, **1983**, *64(1-2)*, 45-59.

[46]Adlakha, R. C., Sahasrabuddhe, C. G. and Rao, P. N., *J. Cell Sci.*, **1982**, *54*, 193-206.

[47]Ambros, V. and Baltimore, D., *J. Biol. Chem*., **1980**, *255(14)*, 6739-44.

[48]Tarnowka, M. A. and Baglioni, C., *J. Cell Physiol*., **1979**, *99(3)*, 359-67.

[49]Venkatesan, S., Nakazato, H. and Edmonds, M., *Nucleic Acids Res*., **1976**, *3(8)*, 1925-36.

Received: 12.03.2014.
Accepted: 19.03.2014.