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PREPARATION OF RABBIT POLYCLONAL ANTIBODIES AGAINST GFP AND MCHERRY PROTEINS

A. Turgimbayeva, M. Baltabekova, A. Yelyubay, A. Ibrayeva, A. Mussakhmetov, S. Abeldenov, B. Khassenov

National Center for Biotechnology, Valikhanov str. 13/1, Astana, 010000, Kazakhstan
aigerim6119@gmail.com

ABSTRACT

The aim of this study is to get polyclonal antibodies against GFP and mCherry recombinant proteins. Bacterial strain producers of fluorescent proteins were obtained due to transformation of *Escherichia coli* with the prepared expression vectors: pET-28c/gfp and pET-28c/mcherry. After 16 hours of induction GFP and mCherry were extracted by method of metal-chelate affinity chromatography from bacterial cultures. The extracted proteins possessed 98% of electrophoretic degree of purity, as well as the high concentration of the proteins in fractions provides intense glow of the proteins under ultraviolet light. Rabbits were immunized with the recombinant proteins GFP and mCherry, from serum of which corresponding immunoglobulins were purified by 35% of ammonium sulfate precipitation. The concentration of antibodies after purification was 4.4 μg/μl. The optimal dilution and the specificity of anti-GFP and anti-mCherry antibodies were confirmed by total proteins extracts of bacterial, yeast-derived and HEK293 cells.

Keywords: GFP, mCherry, recombinant proteins, polyclonal antibodies, Western-blotting.

INTRODUCTION

In the beginning of the 1960s during the research on bioluminescent properties of jellyfish *Aequorea victoria* Osamu Shimomura isolated aequorin protein,which was bound to another protein, accidentally called as green fluorescent protein (GFP) [1]. Aequorin is a photoprotein (21 kDa) composed of two separate subunits and apoaequorin, coelenterazine and luceferin. Aequorin by itself emits blue color. Specific luminescence of luminous organs is due to the cooperative working of aequorin and GFP, which converts Ca^{2+} -induced luminescent signals into green luminescence [2]. Douglas Prasher was able to identify the nucleotide and amino acid sequences of the gene and protein respectively [3]. GFP protein consists of 238 amino acid residues (26,9 kDa) and its maximum excitement and emission comprises 490 nm and 510 nm, respectively. Fluorescent proteins and chromoproteins of the GFP family are formed by three unique, subjected to post-translational modification, amino acid residues in 65-67 positions of the helix (numbered according to *Aequorea victoria* GFP). As the result, chromophore is located at the very center of β-barrel. Through such protein membrane the protection from contact with the solvent is provided. In addition, fluorescent proteins are stabilized by several non-covalent bonds that prevent thermal and chemical denaturation [4]. Determination of its primary structure allowed scientific society to investigate the properties of GFP in more details and to find use in various areas of molecular biology. In 2008 Osamu Shimomura, Martin Chalfie and Roger Tsien were awarded the Nobel Prize in chemistry with formulation “for the discovery and development of the green fluorescent protein GFP”. After GFP cloning it was first used as a gene expression marker in bacteria and sensory neurons in nematodes *C.elegans* [5]. Based on the information about isolated form jellyfish GFP protein a broad spectrum of blue, light blue and yellow mutant proteins, as well as just identified fluorescent proteins of other colors, were constructed, which allowed a color palette to widen the spectrum of red and orange color [6-8].

Besides GFP, in scientific research one more member of the family of fluorescent proteins is used – mCherry (28,8kDa). mCherry and the majority of red fluorescent proteins appear from a protein, isolated from *Discosoma sp*. mCherry in comparison with other monomers is very fast-

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growing that makes it possible to observe the results quickly after activation of transcription. Moreover, this protein is quite photostable and resistant to photobleaching. Amongst red fluorescent proteins mCherry offers the longest lengths of waves, the highest photostability and excellent pH-resistance. The maximum excitement of the protein is achieved at 587 nm, the maximum emission – at 610 nm. “m” in the name stands for its configuration- monomer, which can have a significance in experimental development work (other variants may be with prefix “td” for the tandem –dimer).

From 1994 it became possible to clone gene that is fused with the gene of fluorescent protein, after that a cell or an organism is transfected with the obtained genetic construction [9]. Nowadays, this method of labelling the target protein allows defining expression, localization, translocation, interaction and degradation of the target protein in a living system in real time. Despite the proteins, it is possible to label mRNA molecules to detect their localization in the cell and synthesis dynamics [10]. Such data possess high value for the cell biology investigation. Another area of fluorescent proteins application – photobleaching techniques. Photobleaching is considered undesirable effect, because it complicates visualization of fluorescent label. In spite of this, fluorescent proteins (FP) are used in photobleaching techniques for protein motion detection in living cells and for studying the effect of external factors on its motility [11]. Similar data are extremely important in case when movement of protein inside the cell is closely related to its functional activity and interaction with other molecules. Moreover, FP allow to determine activity of interest promoter taking into account its genetic surrounding, time and impact of external conditions and also type of the cell and tissue [5, 12]. Unlike enzyme-based analyses, this method has low sensitivity, but it gives opportunity to observe intracellular processes *in vivo* without external interference and to get multicolored fluorescent images of several promoter using FP-reporters. A series of possibilities that remain undiscovered are ensured by so-called FP-timers, which change its fluorescent color with time and thus allow detection of their temporary expression in retrospective. In FP-timers various rates of maturation (from several minutes up to several hours or even days) are suitable for studying processes in different timescales [13]. Under the control of a specific promoter in an appropriate genetic construction FP facilitate visualization of specific cell types in the whole organism, organs, tissues and cell cultures. This ability is valuable in immunology, neurobiology, developmental biology, transplantology and in carcinogenesis. A mixture of several FP, obtained by random recombination, allows marking cell using more than 100 hues distinguishable by fluorescent microscopy. Marking with fluorescent proteins is used in FUCCI (Fluorescent Ubiquitination-based Cell Cycle Indicator) [14] method to monitor changes in molecular complexes of living cells.

Having marked the target molecule with fluorescent protein the expression of FP in cell culture can be analyzed quantitatively and qualitatively by using antibodies. For this purpose, cell lysate is analyzed using Western-blotting. In addition to Western-blotting the range of application of antibodies in laboratory work is extremely wide: immunoprecipitation, enzyme immunoassay, ELISPOT, antibody microarray, X-ray crystallography, flow cytometry, immunofluorescence, and immunocytochemistry [15]. To conduct Western-blotting, immunocytochemistry, immunofluorescence, immunoprecipitation and affinity chromatography polyclonal antibodies are perfectly suitable being superior to monoclonal antibodies in terms of their cheapness and time of production. Monoclonal antibodies are required for therapeutic and diagnostic purposes that need large amount of antibodies specifically identical to a single epitope. Polyclonal antibodies enhance signal detection of the target protein with low expression because the molecules of these antibodies bind to several protein epitopes. Polyclonal antibodies in comparison to monoclonal antibodies are less specific to small changes of antigen (polymorphism, heterogeneous glycosylation, mild denaturation) and are applicable in case of unknown nature of antigen. However, due to the features of polyclonal antibodies, production technique purity of the antigen and absence of any impurities are of a high importance. Thereby,

in the immunization of animals it is preferable to use recombinant protein, as in this case it is possible to achieve very high purity.

The purpose of this research is to produce rabbit polyclonal antibodies against fluorescent GFP and mCherry proteins. There were performed following objectives: production of recombinant GFP and mCherry proteins in bacteria; immunization of rabbits with the obtained recombinant proteins; extraction and purification of polyclonal antibodies; verification of sensitivity and specificity of the produced polyclonal anti-GFP and anti-mCherry antibodies.

MATERIALS AND METHODS

Strains and vectors

In this experiment JM109, TOP10F', ArcticExpress (DE3)RP, BL21(DE3), Rosetta(DE3), Rosseta(DE3)pLysS and Rosetta2(DE3) strains of *Escherichia coli* were used.

Plasmids pVEE/gfp and pRS/mcherry, as donors of genes GFP and mCherry respectively, and commercial expression vector pET-28c (+) (Novagen) were used.

Oligonucleotides

5'-*Nde*I-gfp	5'-GGGAATTCCATATGGTGAGCAAGGGC-3'
3'-*BamH*I-gfp	5'-CGCGGATCCGTTACTTGTACAGCTCGTCC-3'
M13fr	5`-GTAAAACGACGGCCAG-3`
M13rv	5`-CAGGAAACAGCTATGAC-3`
T7fw	5'-TAATACGACTCACTATAGGG-3`
T7rv	5'-GCTAGTTATTGCTCAGCGG-3'

Mediums

Law salt Luria-Bertani broth medium (1% tryptone, 0,5% yeast extract, 0,5% NaCl) for *E.coli* cell culturing was used. SOC medium (2% tryptone, 0,5% yeast extract, 0,05% NaCl, 2,5 mM of KCl, 20 mM of MgSO4, 20 mM of glucose, pH 7,5) was used for incubation of cells after transformation. The concentration of antibiotic kanamycin in the medium composed 50 μg/μl. The preparation of mediums was carried out according to the protocol of Maniatis [16].

Reagents

All reagents used in the research were of Sigma-Aldrich, AppliChem, Promega, Amresco production with category of purity "for molecular biology".

Cloning of *gfp* and *mcherry*

Genes of fluorescent proteins *gfp* and *mcherry* were amplified by polymerase chain reaction, using primers 5'-*Nde*I-gfp and 3'-*BamH*I-gfp and pVEE/gfp and pRS/mcherry plasmids as templates. Primers were designed in such a way that the expressed recombinant proteins contained hexahistidine tag at the N-terminus. PCR amplified *gfp* and *mcherry* genes were cloned into expression vector pET-28c (+) at the restriction sites *Nde*I and *BamH*I. The nucleotide sequences from 5' and 3' ends for *gfp* and *mcherry* genes are identical for 20 bp, and therefore the same pair of primers was used for independent cloning of two genes.

Hydrolytic restriction of plasmid DNA, dephosphorylation and ligation were carried out using *Nde*I, *BamH*I, FastAP, T4 ligase enzymes with the corresponding buffers for them: Tango, T4 Ligase Buffer of ThermoScientific production. Experiment with them was conducted in accordance to the manufacturer's recommendations.

Plasmid DNA extraction was done using MiniPrep kit (Promega, USA) according to the manufacturer's recommendations.

As the result, the resulting expression vectors pET-28c/gfp and pET-28c/mcherry were produced. Detection of direct nucleotide sequence was done by Sanger method using "Big Dye Terminator v 3.1 Cycle Sequencing Kit" and commercial primers T7fw/T7rv.

Expression of *gfp* and *mcherry*

Electrocompetent cells of *Escherichia coli* BL21(DE3), BL21(DE3)pLysS, Rosetta(DE3), Rosetta(DE3)pLysS, ArcticExpress(DE3)RP strains were transformed with vectors pET-28c/gfp

и pET-28c/mcherry by electroporation method. Selection of transformant colonies was done on a solid medium containing kanamycin. For detection of producing abilities of transformant colonies transformed cells were inoculated on selective media, containing 0.5 mM isopropyl-β-D-1-thiogalactopyranoside (IPTG) for induction of GFP and mCherry proteins.

Production of protein was carried out using recombinant strains Rosetta (DE3)/pET-28c/gfp and Rosetta(DE3)/pET-28c/mcherry. The culture of recombinant strains was produced in 400 ml of Luria Bertani broth medium with kanamycin antibiotic at +37°C and by shaking at 150 rpm. Upon reaching an optical density OD_{600}=0,6, which corresponds to the mid-log growth phase of *Escherichia coli*, 0,5 mM of isopropyl-β-D-1-thiogalactopyranoside was added for induction and incubating was continued at +37°C. The culture with IPTG was incubated for 16 hours, after that the cells were collected by centrifugation at 6000g for 7 minutes at +4°C.

Cell lysis and Chromatographic Purification of GFP and mCherry proteins

Cells were resuspended in the buffer (20 мM NaCl, 20 мM Hepes-NaOH (pH 7,5) from calculation 5 ml of buffer per 1 gram of wet cells and then lysed by ultrasonication. Cell lysis was accomplished using lysozyme (3mg/ml) followed by ultrasonication using disintegrator UP200S at 24 kHz pulsing regime (10 pulses, 10sec/pulse) on ice. Separation of soluble and insoluble fractions was carried out by high-speed centrifugation at angular acceleration of 40000g for 1 hour at temperature of +4°C.

Purification of GFP protein from water- soluble fraction was carried out by metal-chelate affinity chromatography with nickel (Ni^{2+}) ions using HiTrap Chelating HP 1 ml columns (GE Healthcare). HisTrap HP 1ml (GE Healthcare) column was used for mCherry protein purification. Cell lysate was loaded according to the manufacturer's protocol. Linear imidazole gradient from 20 mM to 500 mM in buffer (500 мM NaCl, 20 мM Hepes-NaOH (pH7,5)) was used for elution of target proteins. In the experiment for fast protein purification liquid chromatography FPLC (General Electric, AKTA Purifier10 with Frac920 fraction collector) was used. Eluates were fractioned by 500 µl. Protein detection was carried out at a wavelength of 260 nm. Fractions, corresponding to the peaks on chromatogram were subjected to electrophoresis in 12% polyacrylamide gel under denaturing condition according to the Laemmli method [17]. Quantitative determination of the protein concentration in lysate and fractions was done by Bradford method using bovine serum albumin as a standard [18].

Immunization of animals and polyclonal antibodies production

In order to determine the immunogenicity of GFP and mCherry proteins, immunization parameters and immune doses of antigen for mice immunization with GFP recombinant protein were worked and calculated. A group of test animals was formed from 4 male outbred mice at age 14-16 days. From tail vein 700 µl of blood was taken, serum as a negative control in ELISA was obtained by centrifugation (3200g, 10 minutes, room temperature). Recombinant protein was diluted in PBS buffer of 50 µg/ml and 100 µg/ml concentrations and was injected intraperitoneally to all four mice: two mice with 50 µg and two mice with 100 µg. Next immunization was done 7 days after first immunization. Third immunization was done 4 days after the second immunization. Forth immunization was done on the next day and fifth immunization- the day after the preceding immunization. In all cases the amount of injected protein was the same as in the primary immunization. Upon completion of the immunization 700 µl of blood was collected from tail vein and serum was separated by centrifugation. Later on, serum of control and experimental blood were used in ELISA.

Using the obtained results of calculated dose for mice, recalculation of the amount of GFP and mCherry proteins for rabbit immunization was done. In rabbit immunization 2 rabbits at the age of 6 months were involved.

From ear vein 1 ml of blood was taken, the serum of which then served as a negative control in ELISA. Recombinant GFP and mCherry proteins, diluted in PBS buffer of 300 µg/ml concentration, were once injected subcutaneously into the back of the rabbits in the amount of 1 ml. The next immunization was administered 13 days after the first one, and rabbits were injected with 150 µg of GFP and mCherry. The third immunization was done 15 days after the

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second one. The fourth and fifth immunizations were done 7 days after the preceding immunization and the amount of GFP and mCherry proteins injected composed 150 μg. Upon completion of the immunization, 10 ml of blood was collected from ear vein and the serum was separated by centrifugation.

Later on, serum of control and experimental blood were used in ELISA and Western-blotting. ELISA results were recorded at wavelength of 490 nm. For Western-blotting 1 μg of GFP was taken and serum was diluted from 1:1000 to 1:3000 times. In case of mCherry protein 2 μg was taken for Western-blotting and serum dilutions from 1:100 to 1:5000 were tested.

Antibody purification from serum was done by precipitating immunoglobulins at 35% ammonium sulfate. After incubation of serum with ammonium sulfate, the solution was centrifuged at 40000g for 30 minutes at temperature of +4°C. The supernatant was removed and the pellet was suspended in PBS buffer. Produced antibodies were aliquoted to 1 ml and samples of antibodies that were stored at various temperature regimes +4°C and -20°C were prepared.

RESULTS AND DISCUSSION

As the result of the cloning, expression vectors pET-28c/gfp and pET-28c/mcherry were obtained with the corresponding genes *gfp* and *mcherry*, which are under the control of T7 phage RNA polymerase promoter that maintains high-level synthesis in bacterial cell. Transformation with these vectors of several strains of *Escherichia coli* having various genotype: BL21(DE3), ArcticExpress(DE3)RP and Rosetta(DE3)pLysS with inoculation on agarose medium with IPTG showed that fluorescence of all colonies was observed only in strain Rosetta(DE3)pLysS/pET-28c/gfp (figure 1).

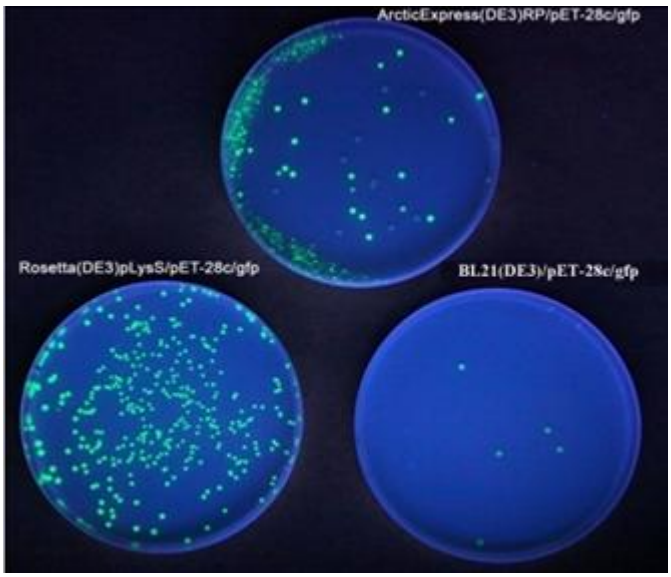

Fig. 1. *Escherichia coli* transformant colonies that express *gfp* gene

In other strains selective fluorescence (about 75%) was observed for ArcticExpress(DE3)RP/pET-28c/gfp and 40% for BL21(DE3)/pET-28c/gfp. Appearingly, the absence of fluorescence in several colonies can be explained by low expression level of eukaryotic *gfp* in recombinant for it surrounding due to features of genotypes in consequence of deficiency of specific tRNA. Unlike these strains, Rosetta(DE3)pLysS strain has additional plasmid pLysSRAR, that contains AGG, AGA, AUA, CUA, CCC and GGA codons that are frequently used by eukaryotic cells for Arg, Ile, Leu, Pro and Gly amino acids synthesis, which maintained sufficient amount of GFP recombinant protein in bacterial cells. Another possible explanation is damage in GFP protein's tertiary structure, due to improper packing of

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polypeptide chains in the protein, which leads to the disruption in protein "maturation" and fluorophore formation.

The use of strong inducible T7 phage promoter ensured intracellular accumulation of fluorescent proteins in *Escherichia coli* from 20% to 40% of the total protein. Research on accumulation of GFP heterologous protein in cells of clones of recombinant strains, positive for expression of *gfp* gene, depending on induction time, showed that all examined clones express *gfp* gene after 2 hours of induction of culture with IPTG (figure 2). Moreover, GFP protein accumulates maximally in cells of strain ArcticExpress (DE3) RP after 16 hours of induction. This fact can be explained by the presence of additional chaperones Cnp10 and Cnp60 in the strain, which provides more compact accumulation of heterologous protein in cells. But further research on the stability of recombinant strains in terms of productivity of GFP protein showed that the more preferred strains are Rosetta family strains.

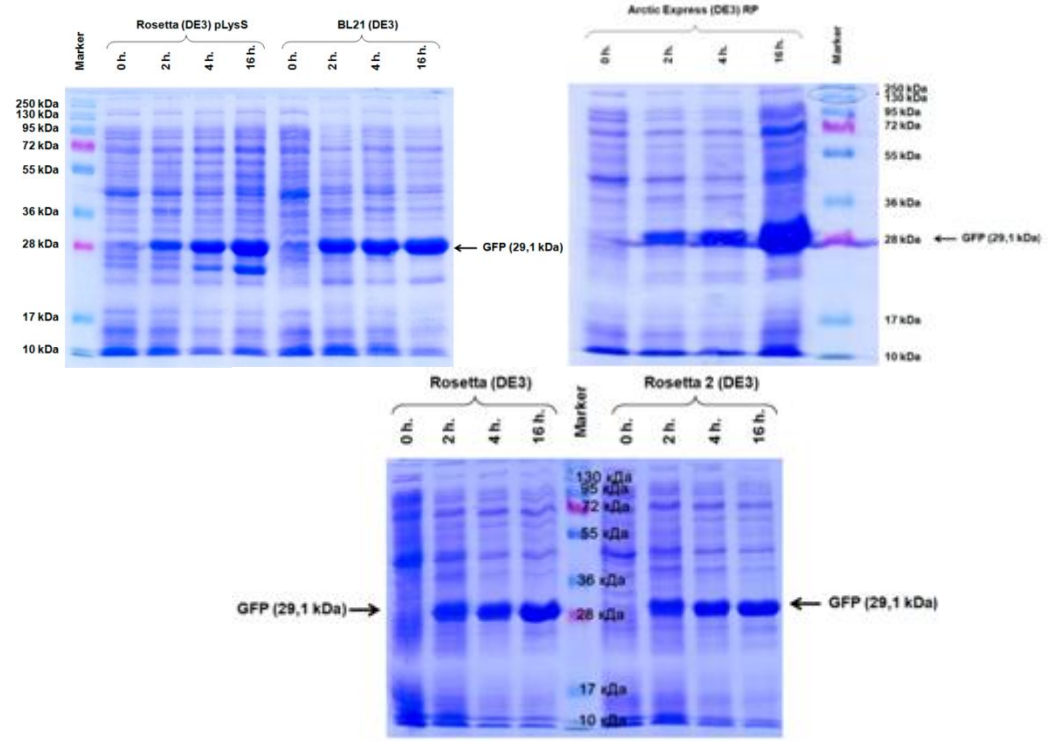

Fig. 2. GFP recombinant protein accumulation in cells of BL21(DE3), ArcticExpress(DE3)RP, Rosetta(DE3), Rosetta(DE3)pLysS, Rosetta2(DE3) strains after 2,4 and 16 hours of culture incubation with IPTG

Taking into consideration the data on optimization of induction of GFP protein the same conditions for mCherry protein were used: 16 hours of incubation of Rosetta(DE3)/pET-28c/mcherry strain.

Purification of GFP and mCherry proteins from water-soluble lysates of Rosetta(DE3)/pET-28c/gfp, Rosetta(DE3)/pET-28c/cherry recombinant strains by metal-chelate affinity chromatography showed that proteins start to come off at initial concentration of imidazole in buffer of 200 mM. At low concentration of imidazole proteins that have low affinity to Ni^{2+} ions elute. Typically, these proteins include *Escherichia coli* proteins that contain no more than three consecutively located histidine residues.

Electrophoresis of protein fractions showed that the expression of *gfp* and *cherry* genes in cells is high and proteins are presented in high quantity in lysate. Due to polyhistidine tag, proteins exhibit high affinity for Ni^{2+} ions, fractioning in linear gradient of imidazole allowed producing proteins in preparative quantities and with high purity (figure 3).

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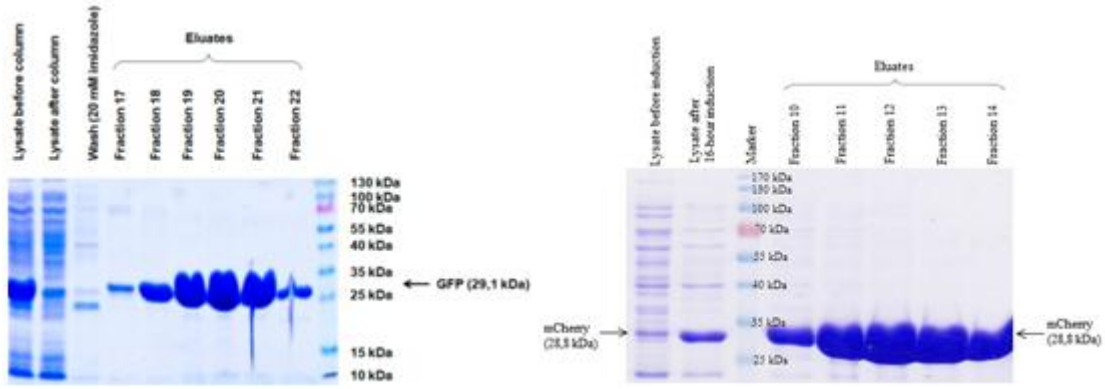

Fig. 3. Results of electrophoresis of GFP and Cherry proteins

Measurement of protein concentration in lysate and fractions allowed making a rough estimate of protein yield. From 400 ml of Rosetta(DE3)/pET-28c/gfp bacterial culture 67.7 mg of highly purified GFP protein was extracted. From the same amount of Rosetta(DE3)/pET-28c/mcherry culture 25.0 mg of Cherry protein was extracted. The extracted proteins possessed 98% of electrophoretic purity. This indicator is very high for proteins obtained by method of plasmid gene expression in heterologous surrounding.

High concentration of proteins in fractions provides intense luminescence of GFP and mCherry proteins upon ultraviolet radiation at a wavelength of 312 nm (figure 4).

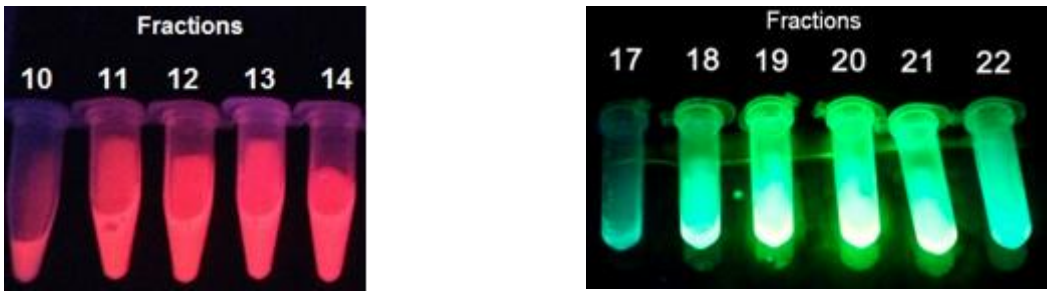

Fig. 4. Luminescence of GFP and Cherry at different concentrations under the UV light
A - Luminescence of Cherry, B - Luminescence of GFP

As can be seen from figure 4, the luminescence intensity depends on the concentration of protein in fractions, also it can be noticed that maturation of GFP and mCherry proteins in bacterial cells was successful. The most intense luminescence has fraction 20 in GFP and fractions 12, 13, 14 in mCherry. GFP and mCherry proteins produced in a preparative quantity were used in the experiments of animal immunization.

The results of mice immunization showed that experimental animals produced antibodies after the immunization with GFP at a 100 μg and 50 μg quantities. The developed scheme of immunization was also successful in case of rabbits as well. In Western-blotting using GFP protein the serum was titrated with dilution of 1:100, 1:500, 1:1000, 1:1500, 1:2000, 1:2500 and 1:3000. Rabbit serum obtained after 5-fold immunization with recombinant protein contains large number of immunoglobulins, interacting with GFP. Even at a dilution of 300 times positive reaction was observed.

Rabbit immunization scheme used in GFP injection was as effective in case of mCherry protein. Tested polyclonal antibodies against GFP and mCherry were precipitated with 35% ammonium sulfate solution. After purification, the concentration of proteins in antibodies was measured by Bradford method and composed 4.4 μg/μl.

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Next, Western-blotting against GFP and mCherry proteins was conducted to determine the optimal dilution of polyclonal antibodies, in which the serum was titrated with dilutions: 1:100, 1:500, 1:1000,1:1500,1:2000,1:2500,1:3000,1:4000,1:5000 (figure 5).

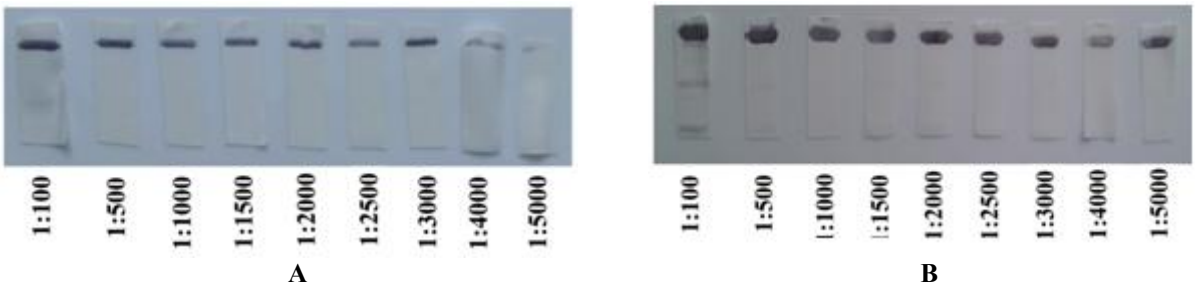
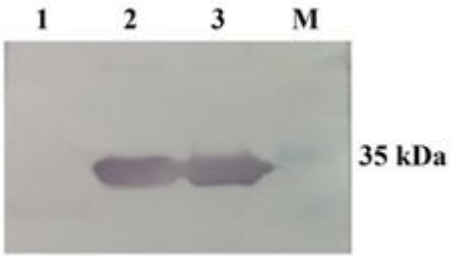


Fig. 5. Western-blotting of polyclonal antibodies against GFP (A) and mCherry (B) proteins with different dilution
A - western-blotting of polyclonal anti-GFP antibodies
B - western-blotting of polyclonal anti-mCherry antibodies

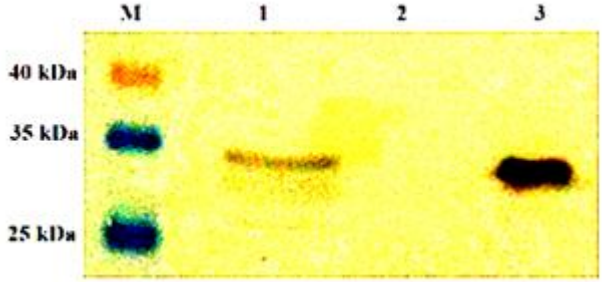
Specificity of anti-mCherry antibodies was confirmed by Western-blotting using GFP and mCherry. It is found that anti-mCherry antibodies don't react to "antigen-antibody" interaction with the related GFP protein. Obtained anti-mCherry showed high specificity. The specificity of these antibodies was also confirmed in Western-blotting on bacterial lysate containing own proteins of *Escherichia coli* and mCherry (figure 6).



1 – GFP (negative control); 2 – mCherry in bacterial lysate; 3 – mCherry (positive control); M – protein molecular mass marker

Fig. 6. Western-blotting of polyclonal anti-mCherry antibodies in bacterial lysates

Anti-mCherry antibodies were used to evaluate the level of *mcherry* gene expression in human HEK293T cells, transfected with eukaryotic pBudCE4.1/gfp/mcherry vector, in which *mcherry* gene was under the control of human elongation factor subunit 1α (EF-1α) promoter. Full cell lysate was analyzed on the 7th day after calcium phosphate transfection (figure 7).



1 – GFP (negative control); 2 – mCherry inbacterial lysate; 3 – mCherry (positive control); M – protein molecular mass marker

Fig. 7. Western-blotting of polyclonal anti-mCherry antibodies on cell lysate of HEK293T cell line

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Anti-GFP antibodies were used to evaluate the effect of methanol induction time on the level of secretory GFP gene expression in *Pichia pastoris* X33 yeast with integrated GFP gene under the control of alcohol-inducible promoter (AOX1) (figure 8).

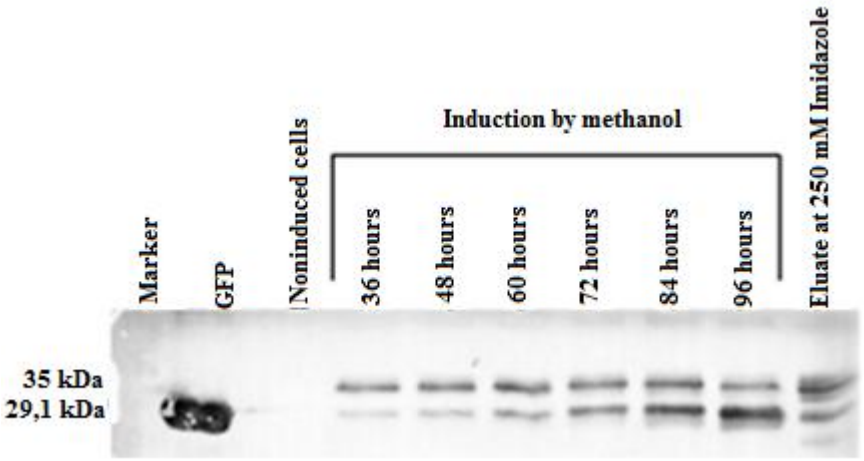

Fig. 8. Evaluation of secretory GFP gene expression in *Pichia pastoris* X33 yeasts under the effect of the induction time

As seen from the presented data, antibodies worked well with the fluorescent protein secreted into culture medium by yeasts. It is noteworthy that GFP is glycosylated in yeasts, which is illustrated as additional bands on the Western-blotting.

CONCLUSION

Expression vectors pET-28c/gfp and pET-28c/mcherry were designed, with which transformation of Rosetta (DE3) family strain allowed getting super producers of recombinant fluorescent proteins. Highly purified proteins were produced and extracted in 67,7 mg and 25,0 mg for GFP and mCherry proteins, respectively. After immunization of rabbits with GFP and mCherry proteins optimal dilution for polyclonal antibodies was determined, that is sufficient for Western-blotting. High specificity and sensitivity of produced antibodies were experimentally confirmed on bacterial, eukaryotic cell lysates and also on the yeast cultures secreting GFP.

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ТҮЙІН

Жұмыстың мақсаты GFP және mCherry рекомбинантты флуоресцентті ақуыздарды алу болып табылады. pET-28c/gfp және pET-28c/mcherry экспрессиялаушы векторлары алынып, *Escherichia coli* жасушаларын трансформациялау арқылы флуоресцентті ақуыздардың бактериалды штамм-продуценттері алынды. 0,5 мМ ИПТГ көмегімен 16 сағаттық индукциядан кейін 400 мл Rosetta(DE3)/pET-28c/gfp бактериалды дақылынан металлафинді хроматография әдісімен 67,7 мг тазартылған гетерологиялық GFP ақуызы бөлініп алынды, ал Rosetta(DE3)/pET-28c/mcherry дақылынан 25,0 мг Cherry тазартылған ақуызы алынды. Бөлініп алынған ақуыздардың 98% электрофоретикалық тазалық деңгейге ие болды, сонымен қатар ультракүлгін сәулесінің әсерінен фракцияларда ақуыздардың жоғары деңгейде шоғырлануы ақуыздардың қарқынды сәулеленуін қамтамасыз етті. Рекомбинантты GFP және mCherry ақуыздарымен ор қояндардың

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иммунизациясы өткізілді, сарысуларынан 35% аммоний сульфатымен сәйкес иммуноглобулиндер бөлініп алынды. Тазартудан кейін антиденелер шоғырлануы 4,4 мкг/мкл болды. anti-GFP және anti-mCherry антиденелері үшін оңтайлы сұйылту 1:3000 болып табылады. Антиденелер арнайылылығы бактериалды, ашытқы текті жиынтықты ақуыздар экстрактыларында және HEK293 жасушаларында расталды.

Кілтті сөздер: GFP, mCherry, рекомбинантты ақуыздар, поликлоналды антиденелер, вестерн блот.