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Molecular biology techniques in real life — case study introducing medical students to QF-PCR technique used in medical diagnostics

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Abstract: QF-PCR is a widely used molecular biology method. To name just a few of its uses, it is considered to be useful in paternity tests, identification tests or prenatal diagnostics. Therefore, there is a good chance that medical faculty students would come into contact with this technology — directly or indirectly — during their professional work. The following article proposes a teaching classes scenario conducted in the problem-based learning manner, which aims to familiarize students with the QF-PCR technique. In addition, other modern methods of molecular genetics are among topics that students can learn during the problem-based learning modules. The classes are divided into three parts. In the first part, students learn about the possible usage of QF-PCR in paternity tests. The second part focuses on learning about the advantages and limitations of QF-PCR in prenatal diagnosis. Learning activities in the last part are designed to show the limitations of the diagnostic properties of the method — students analyze the case study, in which QF-PCR must be replaced by other modern methods of molecular genetics. By analyzing three independent stories, students learn about usage, advantages and limitations of QF-PCR, and additionally gain knowledge in basic, pre-clinical and clinical sciences. This course is designated as an elective course for final year medical students who have completed either: a basic genetics course, a molecular genetics course, a biochemistry course or a molecular biology course. The focus of the classes is to draw students' attention to the possible application and rapid development of molecular biology techniques, which is the base for modern therapeutic and diagnostic strategies.

Keywords: Problem Based Learning, medical education, QF-PCR, prenatal diagnostics, paternity tests, Next Generation Sequencing.

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Introduction

The following paper presents a teaching classes scenario that focuses on Quantitative Fluorescence Polymerase Chain Reaction (QF-PCR) method. The main learning outcome of this Problem Base Learning (PBL) curriculum is to familiarize students with the mentioned technique. PBL was selected as a main teaching method in accordance with the study performed at Jagiellonian University Medical College where 95.5% of students, who took part in the survey, positively assessed the way of classes conducted using this method and majority of students found this way of teaching useful in both: acquiring the knowledge and integration of acquired knowledge with pre-clinical subjects [1].

Nevertheless, although the formula of the modules is based mainly on PBL method, due to the difficulty and specificity of the subject matter, an initial selection of materials is proposed. Designated and carefully selected publications, book chapters and films regarding the subject should be delivered so that the students' learning is based on sources allowing them to adapt the required content. At the same time control over the amount of content will allow them to assimilate the required knowledge in an accessible, but not overwhelming way. The classes are divided into three stages, in each part students are given a description of the situation that can theoretically happen in everyday life. For each of the parts there are detailed learning outcomes defined, which the students realize during the discussion about individual stories. An important aspect of each section is the ability to explain an advanced molecular biology technique to a person outside of medical sciences. The course is designed to be taught in groups of 6 to 8 students. Each part will take two hours (2×45 minutes). Students' work will be evaluated according to a pre-established criterion in the form of Rubrics tables. The workshop is planned as a module in the optional course "Molecular biology techniques in medicine". Criteria for participation in this course will be completion of one of the following: a basic genetics, molecular genetics, biochemistry or molecular biology course.

The first class module — materials for students

The script scenario along with Figure 1 should be provided for students.

— Have you heard that Amanda found her father? They did a DNA test and it confirmed they are relatives.

— After all these years...

— It's amazing, these DNA-based techniques fascinate me, I'd like to understand how it actually works.

— I think I know who can explain it to us! Wasn't it Paul who bragged the other day that he passed the genetics exam?

— But, you know, he's more into theory than actual research.
— Even better! He will have a chance to put the theory into practice!

DNA research laboratory

Ordering person: [redacted]

samples/code analysed	date of sample receipt	type of sample
A/1234567	10.06.2020	swab
B/2211334	10.06.2020	swab

	Sample A		Sample B	
Marker	Profile A		Profile B	
Amelogenin	X		X	Y
D3S1358	16	18	16	18
D1S1656	11	14	12	14
D2S441	14	15	10	14
D10S1248	14	14	14	15
D13S317	7	11	10	11
Penta E	8	12	7	12
D16S539	12	12	12	12
D18S51	15	18	15	17
D2S1338	23	23	17	23
CSF1PO	10	11	10	13
Penta D	11	12	12	13
TH01	7	9	7	9
v WA	15	18	15	16
D21S11	29	30	29	33
D7S820	12	12	10	12
D5S818	11	11	11`	11
TPOX	8	8	8	11
DYS391	9	-	-	-
D8S1179	12	15	11	15
D12S39I	20	21	21	21
D19S433	13	15	13	15.2
FGA	20	21	21	24
D22S1045	11	15	15	16

paternity rate:	probability of paternity: 99.9999998%
830,244,467	

[redacted]
podpis osoby potwierdzającej

[redacted]
podpis osoby

Fig. 1. The paternity test results. The report's chart displays the genetic profiles of the child (sample A) and the alleged father (sample B). As presented in the table the DNA profiles of the child and the alleged father were matched, so the test is considered a non-excluded paternity case. In addition, the amelogenin (sex) gene analysis is presented for each participant as together with STR analysis the gender determination is commonly performed using PCR products generated from the amelogenin gene that occurs on both the X- and Y-chromosome. A commonly used PCR primer set targets a 6 bp deletion that occurs on the X-chromosome, which makes it possible to distinguish amplicons generated from the X- and Y-chromosome, respectively.

The first class module — materials for teachers

After the group has worked with the first part of the classes, students should be able to explain how QF-PCR works in the identification tests [2]. They should also be able to define concepts such as: Short Tandem Repeat (STR), polymorphism as well as STR areas polymorphism. From the provided data they should be able to find the answers to the following issues: how is the paternity index (PI) and likelihood of paternity determined, as well as the answer to the following question: "how gender is determined in identification tests?". The discussion may also raise questions about the stability of STR areas [3]. The leader may redirect the discussion to this topic if they notice that the group is doing well with the basic issues. After the first part, students should practice a scene in which one student acts as a doctor, explaining the steps of the identification tests, while the second asks questions from a patient's perspective. After the exercise the roles can be reversed [4].

Intended learning outcomes

Knowledge

1. The student knows the following terms: STR, polymorphism, STR areas polymorphism.
2. The student knows the use of QF-PCR in identification tests in terms of paternity probability determination.
3. The student knows how gender is determined in identification tests.

Skills

The student is able to interpret the result of an identification test performed using QF-PCR.

Competencies

The student is able to transfer the knowledge of the principle of identification tests to potential person without medical/biological background.

The second class module — materials for students

The script scenario along with Figure 2 should be provided for students.

A phone call between two friends:
— Hey Anna, I have the results of the amniocentesis.
— Yeah? So fast? — in just two days — so what's the news?
— It's bad. The results confirmed trisomy, Patau syndrome.

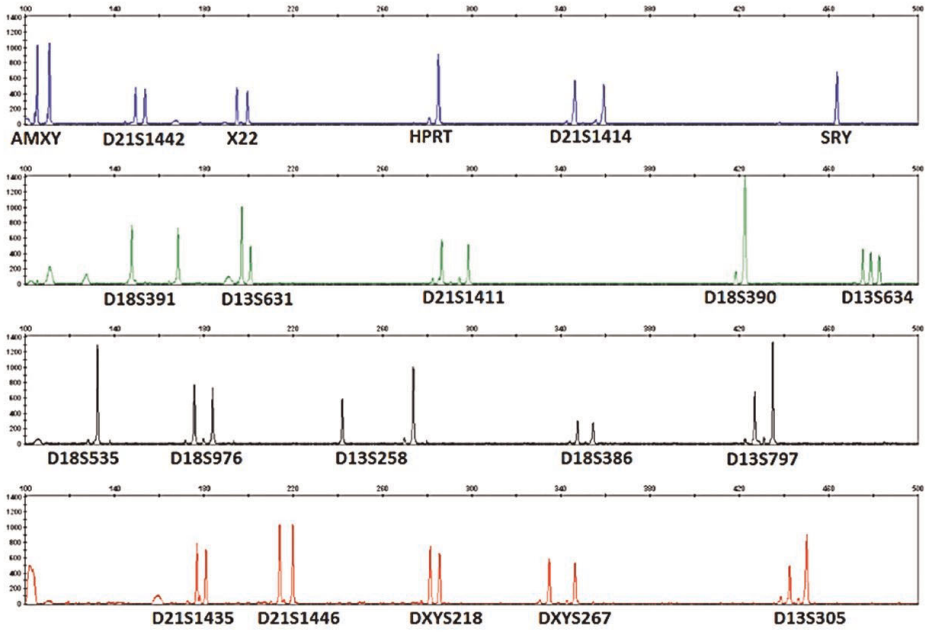

Fig. 2. Detection of trisomy 13. In the presented example trisomy 13 was confirmed by the trisomic diallelic pattern for D13S631, D13S258, D13S305 (two peaks with ratio 2:1) and trisomic triallelic (three peaks with ratio 1:1:1) for D13S634. The genetic profile markers for chromosome 21 (D21S1442, D21S1414, D21S1411, D21S1435, D21S1446) and genetic profile markers for chromosome 18 (D18S391, D18S390, D18S535, D18S976, D18S386) confirmed normal chromosome copy number (peaks ratio 1:1). In addition, the amelogenin gene (indicated as AMXY on the scheme), SRY gene (nonpolymorphic Y-specific marker) and HPRT loci (present only on X chromosome) were used to allow for the assessment of fetal sex (male gender was confirmed). DXY267, DXYS218 markers, present on both X and Y chromosomes confirm the lack of gender chromosome abnormalities. For the purpose of case study part 2, the figure without description should be provided for the students. The figure was adapted based on Aneufast TM user manual of Multiplex QF-PCR for rapid detection of trisomy 13, 18, 21 and sex chromosomes aneuploidies.

— Listen, for now, don't panic, stay calm and wait for the results of the karyotype. Only the karyotype gives the diagnosis.

— Anna, I'm 43, the doctor said the result is sure, no need to order a karyotype.

— You must change your doctor, the karyotype may not confirm it. I even read about this case on the website of an American prestigious clinic, I will find it and send you a link.

The second class module — materials for teachers

In the second part students get acquainted with screening tests and diagnostic tests used in prenatal diagnosis. QF-PCR appears here as one of the methods of prenatal diagnosis. Students are supposed to learn about the possibilities and limitations of the method. In this part, students should discuss which tests are appropriate for prenatal diagnosis to identify chromosome aberrations and after the discussion be able to distinguish between screening prenatal tests that estimate the risk of chromosome aberrations and prenatal diagnosis, where fetal cells are analyzed. Students list the markers analyzed in maternal blood (PAPP-A and beta-hCG test) in the first trimester of pregnancy and the fetal anatomical evaluation in the first trimester ultrasound (with particular attention paid to the measurement of nuchal translucency). At this point of discussion, the additional aspect of the topic concerning the assessment of chromosomal aberrations occurrence probability without the analysis of fetal cells could be investigated. The solution to solve this particular problem may be an investigation of exemplary epidemiological data showing the statistical relationship between abnormalities for the parameters studied in prenatal screening (PAPP-a, beta-hCG, nuchal translucency) and chromosome aberrations of fetuses [5]. It is noteworthy that even the extreme deviation of marker levels does not determine the presence of chromosome aberrations [6]. Following the previous discussion students should explain what the significance of calculated high risk from screening-based tests is and what are their further steps to be considered regarding the diagnosis. Positive results of screening tests are an indication for further prenatal diagnostics thus the following topics will include the comparison of pros and cons of chorion biopsy and amniocentesis (as it is included in the obligatory intended learning outcomes). In accordance with the Polish Gynecological Society recommendations, "the gold standard" is to perform karyotype analysis (that is characterized by its main disadvantage — long awaiting time). In some other countries QF-PCR dedicated to the diagnosis of trisomy 13, 18 and 21 is recommended to be performed independently and no further confirmation of a positive result is required. Therefore, it is important for students to analyze the data showing the sensitivity and specificity of QF-PCR compared to the classical karyotype analysis for trisomy 13, 18 and 21 [7, 8]. In the second part students should also consider the situation mentioned by

Anna in the dialogue. Hypothetically, the initial DNA analysis isolated from cytotrophoblast cells (chorionic biopsy) confirms trisomy. The karyotype analysis carried out on cultured cells of the extra-embryonic mesoderm (chorionic biopsy, culture method) indicated a normal karyotype [9]. The description of such a case is given to students in the materials and they should find the reason for inconsistency of results. In this case we are dealing with mosaicism (relatively often observed in case of chorionic biopsy). In such a situation QF-PCR may give a positive result, due to the DNA source for QF-PCR analysis coming from cytotrophoblast cells. Moreover, if karyotype is preceded by in vitro culture, performed on the cells of non-germ mesoderm, in case of mosaicism it may be normal. In such situations the analysis of amniocytes is necessary to confirm the fetal karyotype. However, the dialogue situation analyzed by students states clearly that the patient has had an amniocentesis performed. Therefore, for this particular circumstance the karyotype cannot be expected to rule out the presence of trisomy which was confirmed by QF-PCR test. The limitation of mosaicism detection (minimum percentage of abnormal cells) in QF-PCR [9, 10] for trisomy 13, 18, 21 can be considered for further discussion if the previous learning outcomes are completed. In this part students are already familiar with the principle of QF-PCR, so there should not be a problem with analyzing the obtained results. However, it is of great importance for their future practice to consider the cause of trisomy and the molecular basis of the so-called age-related maternal effect [11]. The new aspect of this part is a bi-allelic trisomy, which will be visible in the form of a higher and lower peak on the electropherogram (Fig. 2). The data from the diagram may initiate a discussion about the stages of cell division in which abnormal chromosome propagation may occur.

Intended learning outcomes

Knowledge

1. The student knows the differences between prenatal screening and prenatal diagnostics.
2. The student knows the algorithm of the National Gynecological Society's conduct in relation to screening tests.
3. The student knows biochemical markers and ultrasound markers used in the first trimester screening according to the National Gynecological Society guidelines.
4. The student knows the pros and cons of chorion biopsy and amniocentesis.

Skills

1. The student is able to explain the reason for the divergent results of QF-PCR and classical karyotype in the analysis of cells from the chorionic biopsy.
2. The student is aware of the limitations of QF-PCR in case of mosaicism (low percentage of abnormal cells).
3. The student is able to interpret the QF-PCR result for the diagnosis of trisomy 13, 18 and 21.

Competencies

The student is able to transfer the knowledge related to the interpretation of the prenatal diagnostics tests to a person without medical/biological background (potential patients).

The third class module — materials for students

The script scenario should be provided for students.

Investigation Jail — Visiting Room:

— I know from a trusted person that they've found biological traces. You have no chance of getting out.

— They won't prove anything.

— I repeat, they have biological traces, DNA, you know, even one hair is enough to confirm that you're the one.

— They ain't gonna be a threat to me if my twin brother lives in Krakow, they may have DNA, but how will they confirm that it was me and not him who robbed that bank?

The third class module — materials for teachers

The aim of the third part of this PBL class is to show students other modern methods of molecular genetics that provide solutions to questions that cannot be answered by using QF-PCR. In this part, students already know and understand how QF-PCR works in analyzing the length of STRs polymorphism. Thus, they will know that the QF-PCR used in identification tests will not distinguish the DNA of monozygotic twins. The role of the trainer is to start a brainstorming exercise to answer the question of what actually differentiates the monozygotic twin's DNA. Possibly, the first answer will be the epigenetic changes. Part of the discussion may be focused on the talk about epigenetics, the epigenetic changes described in the literature and their influence on genetic information. It is also important to mention that the epigenetic pattern (DNA

methylation pattern) may indicate the tissue from which the DNA comes, as well as the age of the person whose genetic material has been analyzed. The basic epigenetic modification that is the easiest to analyze is DNA methylation, and students should be aware of that fact. However, the question will arise how to investigate it, where to look for differences and whether the epigenetic pattern should be compared at the level of the whole genome. The materials the students will receive include various articles on the use of epigenetic analysis in forensic medicine. The basic limitation here will be the amount of DNA that has to be analyzed. Therefore, we propose to focus on the method of high-resolution melting curve analysis, where a DNA sample is subjected to sodium bisulfite dependent modification. Only specific areas of so-called markers are amplified by PCR in this approach. In the case of different methylation profiles, the sequence of amplified fragments will be different and thus the differences in the melting curves between the monozygotic twins occur. This is a fast and relatively inexpensive method, which, according to the literature, distinguishes monozygotic twins based on differences in epigenetic profiles [12]. Epigenetic changes should not be the only difference in the genome that the students will talk about. During brainstorming, they should also talk about mutations that appear during embryonic development and are present later in most cells of an adult organism. In this case the teacher may pose the questions directly to the students: "Where do the mutations come from?", "What DNA damage leads to the mutation?" and "What is the connection between DNA damage and specific changes in the genome sequence?". The crucial question for discussion and solving the problems from this part is: "Where should we look for these mutations?", with the provided answer — in the whole genome. Therefore, another issue that needs to be addressed is the sequencing of new generation. Students do not need to fully understand how exactly this technology works. It is important they are aware that in the next generation of sequencing (NGS), millions of templates are sequenced simultaneously, which significantly increases throughput and reduces the time needed to assemble a full genome sequence. At this point of the class, students should also become aware of the exceptionally rapid development of new DNA sequencing technologies in recent years. The materials include a description of the process that took place in Boston in 2014, where classical DNA analysis could not indicate which of the two twin brothers raped the victim https://www.eurofins.com/scientific-impact/scientific-innovation/examples-of-our-scientific-innovations/busting-the-identical-twin-myth/. The new generation sequencing carried out by Eurofins revealed differences in the genome between the twins at the level of single nucleotides that were acquired in embryonic development, which allowed to find one of the brothers guilty [13]. The new generation sequencing technology can also be used in paternity tests if there is any doubt which of the twin brothers is the father of the child. Just as at the end of the first part teacher may propose a role-playing scene where the question "How to differentiate between the identical twins using molecular genetic techniques?" will be included.

Some students act as individuals without a medical background (as for example potential twin brothers, who want to answer the question who is the father of the child) while others play a role of the expert answering the questions.

Intended learning outcomes

Knowledge

1. The student knows the definition of epigenetics.
2. The student knows basic epigenetic modifications and their influence on gene expression.
3. The student knows the general principles of DNA methylation testing.
4. The student knows the causes of the most common spontaneous DNA damages (tautomeric mutations, depurination, deamination) and the induced ones (by UV light, ionizing radiation, alkylating agents, reactive oxygen species (ROS), induced deamination).

Skills

1. The student is able to explain the relationship between DNA damage and genome mutations.
2. The student is able explain the basic differences between Sanger sequencing and the new generation sequencing.
3. The student is able to discuss methods of distinguishing the DNA of monozygotic twins based on epigenetic changes.

Competencies

1. The student is ready to describe the principle of new DNA technologies to a person without a medical or biological background.
2. The student is ready to expand their knowledge in rapidly developing fields such as molecular genetics.
3. The student is aware that being up to date with new therapeutic and diagnostic possibilities requires constant monitoring of the latest literature.

Discussion with final conclusions

After completing the PBL classes, students should understand the principles of QF-PCR method as well as the limits of its application in specific diagnostic situations. Given the rapid development of molecular biology techniques, it is crucial for them to

gain knowledge about the methodology of new diagnostic tests as well as the ability to interpret its results. We reason that demonstrating the application of QF-PCR in everyday life will make the subject much easier for the participants of the workshop. According to our concept, all the materials from which students gain knowledge and interpret the presented results, should be selected by the teacher. This is due to our opinion that selection will significantly increase the effectiveness of self-study and will also allow for efficient usage of time during classes. Students focusing on the provided specific materials will avoid deviations from the subject matter. At the same time the given formula of PBL workshop, self-regulation of the work and time considered for learning and investigating each part of the classes (and the prepared materials) will give the students a sense of self and group work responsibility. Moreover, it will enable an equal chance for a positive final result, while simultaneously achieving the same learning outcomes. The formula in which students widen their molecular genetics knowledge through authentic examples seems to be a right direction towards interesting and modern education. The real-life context and practical aspects create a better background for students to gain knowledge and at the same time motivates them to acquire it. It is sufficient to integrate basic knowledge of biochemistry and basic genetics with molecular biology to solve three hypothetical situations presented in this scenario. However, the most important aspect of the presented scenario is enabling students to see that the basic knowledge allows them to understand the application of complicated molecular biology methods to solve issues that everyone can encounter in everyday life. A key aspect we want to address here is the opportunity to discuss the possibilities and limitations of molecular biology methods, as it is presented in Module 2 and 3. After completing Module 2, students should see that the correct interpretation of the QF-PCR result for chorionic biopsy is possible only if embryology understanding is included. Similarly, in Module 3, students will see the spontaneous mutations in genomic DNA, or epigenetic modifications (from the basic course curriculum), become the basis for distinguishing between theoretically "identical" DNA of monozygotic twins. In all three modules, the analysis and its results are translated into the solution of a concrete real-life life situation. This is particularly important as often the relation between molecular biology and clinical science is not obvious for students. So far, we have used the elements of described in different medical-related courses. Each time we have observed a high motivation of students to understand the methodology and to solve a specific case. Therefore, we think that combining these three cases and using such a scenario in optional classes for final year medical students could be beneficial for them. What is more, we have observed that despite having completed basic courses in genetics and biochemistry, students in their final years of medicine often cannot explain neither how identification tests work nor how to distinguish DNA of monozygotic twins. In our opinion, the problem arises due to a great deal of theoretical knowledge acquired through basic science classes and not

enough time dedicated to understanding the application of the acquired topics into clinical aspects. Therefore, the course outline is designed in such a way as to enable students to talk about the methods and their limitations, which is important for their further medical practice. They will potentially meet patients who will question the results of the analysis based on fake news or pseudo-scientific knowledge with an easy access to the internet or media or simply they will meet patients who will want to understand (to some possible extend) how a result that can change their whole life was obtained — as it was presented in the analyzed cases. According to the analysis performed by Zawiślak *et al.* the trend of integrating the knowledge of basic and clinical sciences is observed in medical education at various medical schools across the world [14]. In fact, this approach in medical teaching can help to break the barrier between basic science and clinical science as reported by Brauer *et al.* [15]. Nevertheless, it should be emphasized that the integration of basic science with clinical science requires the creation of a new curriculum for which the class scenario described in this manuscript would be definitely suitable. In conclusion, the scheme of the course is not designed to considerably widen the theoretical knowledge of genetics or molecular biology, but its primary goal is to focus on integrating the acquired knowledge needed to understand the principle of molecular biology in clinical practice. Medicine students to whom this workshop is addressed, directly or indirectly, will certainly encounter all the discussed scenario problems during their medical practice.

Knowledge and competencies

The authors of the article have acquired didactic knowledge and competences participating in the courses and in the project:

The Didactic Courses

"Basics of academic didactics — Ars Docendi workshops" (KKP, ABI, PD) and "Basics of problem-based learning (PBL) (KKP, ABI), a course implemented as part of the Ars Docendi project" — development of didactic competences of the Jagiellonian University staff co-financed by the European Union under the European Social Fund.

The Didactic Project

"Masters of Didactics" (KKP, ABI) under Measure 4.3. Transnational cooperation of the Operational Program Knowledge Education Development (POWR.04.03.00-00-0074/17).

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Conflict of interest

None declared.

Abbreviations

beta-hCG — beta-human Chorionic Gonadotropin
DNA — Deoxyribonucleic Acid
NGS — Next Generation Sequencing
PAPP-A — Pregnancy Associated Plasma Protein-A
PBL — Problem Based Learning
PI — Paternity Index
QF-PCR — Quantitative Fluorescence-Polymerase Chain Reaction
ROS — Reactive Oxygen Species
STR — Short Tandem Repeat

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