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Analysis of cythochrome oxidase sub unit 1 Gene (CO1) of fruit fly (*Droshophila sp.)* from pineapples and aplication in teaching DNA in Senior high school

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Abstract
DNA analysis is still difficult to implement in high schools in Indonesia due to limitations of equipment and materials. Has conducted research that aims to analyze the CO1 gene in fruit flies, from pineapples and its application as a teaching material in DNA study in high school. CO1 gene amplification results pineapple fruit fly has a long 664 bp. The results of the analysis of alignment with the method of BLAST on NCBI website (https://blast.ncbi.nlm.nih.gov/Blast.cgi) obtained pineapple fruit flies CO1 sequence have a level 99% similarity with *Drosophila ananassae* [JQ679117.1]. Reconstruction of phylogenies by Maximum Likelihood models indicate pineapple fruit fly has the closest kinship with *Droshohila pseudoannanasae* [AY757280]. Application as a teaching material in high school subject DNA produce learning outcomes and student learning activities in the very good category. Activities and student learning outcomes are also supported by the response of students and teachers to study media at the very good category.

Keywords: fruit fly, Pineapple, CO1 gene, Education, High School

1. Introduction
Drosophila sp. is a type of fruit fly that can be found on rotten fruit. Fruit flies belong to the class of insects, the Order Diptera who undergo complete metamorphosis. Around 4500 species of fruit flies have been identified (Borrow...). *Drosophila* was one of the first organisms to be studied genetically: its small size, short life cycle (10 - 14 days at 25 ^{o}C), high reproductive rate (an adult female can lay 400-500 eggs in 10 days), and ease of culture and genetic manipulation have made it perhaps the best understood animal genetic system. Many different species, and a large number and wide variety of naturally-occurring and artificiallyinduced genetic variants are available.

The fruit fly, *Drosophila melanogaster*, is the most popular eukaryotic organism used in classrooms to teach Biology (Sinadinos, 2009) [14]. It is used in heredity and biomedical research, where the aims are to understand human genetics and developmental processes. It is also a popular model for teaching Mendelian Genetics. *Drosophila* is a very popular and successful model organism; it has a short life cycle of two weeks, making it possible to study numerous generations in a scholastic year (Kramer, 1986; Sofer and Tompkins, 1994; Flannery, 1997) [9, 15, 4]. It is easy to culture and relatively inexpensive to house large numbers (Jeszenszky, 1997; Poloumpis, 2013, Wen *et al*. 2015) [8, 13, 18]. It is size is suitable to cultivation in school laboratories. In addition, it is large enough that many features can be seen with the naked eye or under low-power magnification [Intra and Pasini, 2016] [7]. In addition, there are many useful tools to facilitate the study of genetics. *Drosophila* is used for many classroom disciplines, such as classical and molecular genetics.

Members of the genus Drosophila, particularly the D. melanogaster, has a lot of mutant type so allowing multiple experiments on the pattern of inheritance, while the wild-type so easily obtained by placing a trap food in the form of fruit that is inserted into the bottle. In Indonesia, North Sulawesi is known as the producer of various types of fruits include papaya, pineapple, Langsat, Durian, Mango, Banana etc. Lobong village, Bolaang Mongondow, known as a superior pineapple production center in North Sulawesi. Fruit flies in this study were isolated directly from the pineapple production center. Cultivation of the fruit fly is then performed at the Laboratory of Molecular Biology bioactivity and Manado State University. The results of DNA analysis of fruit flies, used to study genetics in particular the subject of DNA at the high school.

Mendelian genetics, molecular genetic, genetic engineering and the central nervous system can be perceived as difficult to learn by secondary school students (Cimer, 2011) [3]. Genetics, the central point of developments in the field of biology, is a particularly difficult subject for teachers and students; since it involves relations between the events of different levels of biological organization. Tingkat miskonsepsi pokok bahasan genetika di SMP dan SMA tergolong tinggi baik pada guru maupun siswa SMA di Sulawesi Utara (Sumampouw and Mokosuli, 2005). 76% of students found it difficult to understand the subject of genetics in particular the subject of DNA and 65% of teachers are still relatively low category, the level of understanding on the subject of Heredity and genetics in high school Substance (Wurarah and Mokosuli, 2016).

Genes and DNA are major themes in the study of molecular genetics. Genes and DNA are the object of study of biology that is abstract. Therefore, it is very difficult to understand when not to experiment. Nevertheless experimental DNA analysis can not be done in schools, even in some universities in Indonesia, due to limitations of equipment and materials. It

is known, molecular biology laboratory requires sophisticated equipment and expensive chemicals. This led to experiments on DNA analysis becomes a major problem in schools and several universities in Indonesia and even developing countries. Alternative solutions to the problem is to do a DNA analysis laboratory and use them as audio-visual media to study molecular biology. The object of study is the fruit fly DNA analysis isolates the local fruit is pineapple so that research results can also be utilized in the field of agriculture.

The mitochondrial gene and cytochrome c oxidase subunit I (COI) were often used for animal identification (Hebert *et al*., 2003) [6]. Many previous studies have been used the COI gene for obtaining the divergences among the species (Liu *et al*., 2011; Liao., 2010). The COI gene is used in this study because it appears to be among the most conservative protein-coding genes in the mitochondrial genome of animals (Toda and Murai, 2007) [17]. The COI gene was the slow-evolving gene in the mitochondrial protein coding gene (Funk *et al*., 1995) [5]. The conserved sequence of COI gene allow researchers to use it as a 'universal' primers, and it has been widely used to investigate multiple different taxa and for interspecific analysis. According to Hebert *et al*., (2003) [6], in terms of the degree of variation, it was expected to be low in intraspecific variation such that through a given cluster analysis, the sequences from polymorphic species would cluster together in a genetic distance.

In this study we analyzed the DNA of fruit flies isolate pineapple. Analysis of DNA consists of sample preparation, extraction and purification of DNA of fruit flies, fruit flies CO1 gene amplification, visualization of amplicons CO1 gene of fruit flies and sequencing. Each DNA analysis stage are recorded and packaged in the form of interactive video that later developed as a learning medium molecular genetic material.

2. Materials and Method

A. Analysis of DNA

1. Samples

Samples of fruit flies directly isolated from natural habitat, which is the location of the cultivation of pineapple Village Lobong, Bolaang Mongondow, North Sulawesi. Furthermore, the cultivation of fruit flies in laboratory bioactivity and Molecular Biology Department of Biology, State University of Manado. Population cultivation flies in the laboratory subsequently become a source of tissue for DNA analysis.

2. Extraction and Purification of DNA

Extraction and Purification of DNA is done according to protocol Blood and Tissue DNA Kit Geneiad. Protocol modifications made at the time of immersion of tissue with proteinase-K. DNA extraction, analyzed the purity and concentration on: A260 / A280 nm, using NanoPhotometer, Implan.

3. CO1 Gene Amplification, amplicon Visualization and Sekuensing

CO1 gene amplification was performed using the fruit fly Qiagen Rotor Gene. Amplification was performed using MyTagTM HS Red Mix Bioline (USA). PCR set up is 200 ng DNA templete, 1 mL primer LCO and HCO (Folmer *et al* 1996), myTaq HS Red Mix 2x: 25 mL; ddH2O: up to 50 mL. PCR cyling condition: Initial denaturation 95 ^{0}C, 1 minute; 95 ^{0}C denaturation, 15 seconds; 90 ^{0}C annealing, 15 seconds and Extension 72 ^{0}C, 10 seconds. Amplicon visualization were using automatic electrophoresis qiaexel. Sequencing was performed using sequencing FIRST BASE Services Singapore. Output sequencing is accepted in the form of files seq. Sequence analysis using geneous Program 9.0. Alignment is done using Basic Local Alignment searcing Test (BLAST) on NCBI website (www.ncbi.com). Reconstruction of phylogeny using MEGA 7.0 program. Phylogeny tree model was determined by analysis of the substitution model of 7.0 MEGA program.

B. Application of DNA Analysis Results Fruit Fly, On DNA Learning in High School

The results of the analysis of DNA packaged into teaching materials DNA material. Teaching materials are packaged in the form of interactive video using Camtasia program Power Point 7.0 and MS Office 2010. The application of learning the subject of DNA is done in selected high school in the city of Manado.

1. Student Activities

Student activity level criteria according Sugiyono (2009) [16] as follows:

0 % - 49 %	:	effectiveness is very low
50 % - 59 %	:	effectiveness low
60% - 69 %	:	effectiveness medium
70 % - 84 %	:	effectiveness high
85 % - 100 %	:	effectiveness is very high

2. Student learning outcomes

The data analysis of student learning outcomes aimed to determine students' mastery learning, after the application of learning packages of fruit fly DNA analysis. Data obtained from the learning outcomes of the final test scores, student worksheets scores and scores of students' presentation. Furthermore, the data were analyzed descriptively. The final scores using the formula of Arikunto (2006) [2]. Classical completeness is calculated using the formula of Sugiyono (2009) [16].

3. Response Students and Teachers

The questionnaire used in the form of yes or no answer. The percentage of answers was further confirmed on the criteria according to Sugiyono (2009) [16], namely:

0 % - 49 %	:	ugly
50 % - 59 %	:	not so good
60% - 69 %	:	pretty good
70 % - 84 %	:	good
85 % - 100 %	:	very good

3. Results and Discussion

A. DNA analysis Pineapple Fruit Flies

1. Extraction and Purification of DNA Pineapple Fruit Flies

DNA extraction was performed using the protocol according to the Blood and Tissue Kit Geneiad. Soaking time proteinase-K dimofikasi of the protocol, resulting in purity and concentration of dsDNA better. The concentration of total DNA obtained was 45 pg / ml, with a purity of 1.87. Optimal concentration according to the Blood and Tissue Kit protocol Geneiad is 45-50 ug / ml and purity on the distribution of 1.70

to 2.00 (A260 / 280). When compared with DNA extracted from pineapple fruit flies without modification immersion proteinase K (submersion for 30 minutes) to produce dsDNA concentration of 27.50 ug / ml with 1.24 purity (A260 / 280). DNA extraction abdominal tissue of adult fruit flies were isolated from pineapple with proteinase-K modification soaking for 18 hours resulted in total dsDNA with the best concentration and purity. Further DNA obtained templete used for gene amplification CO1.

Fig 1: The Imago Pineapple fruit flies observed with Stereo Microscope 3D Digital hirox KH 8700 at a magnification of 150 x

2. Amplification and amplicon Visualization CO1 gene Pineapple Fruit Flies

CO1 gene amplification, legible on the length of 664 base pairs (bp). Band formed, showing the quality and quantity of CO1 gene amplicons were successfully amplified well. From elektogram obtained, amplification successful (Figure 2). Thus, universal CO1 gene (Folmer *et al* 1994) successfully used to amplify CO1 gene from fruit flies in pineapple.

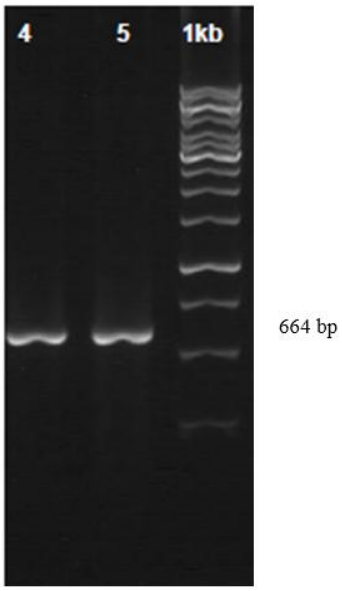

Fig 2: Elektrogram CO1 gene amplicons pineapple fruit flies, shown in wells 4 and 5 wells.

3. Sequencing

Sequencing products in the form of files *seq*, from FIRST BASE Singapura was read on Geneous 9.8.0. CO1 gene sequences pineapple fruit flies after the sequencing results analyzed, has length 685 bp (Figure 3). These results are consistent with the universal CO1 gene length is between 600-700 bp (Folmer *et al*, 1993).

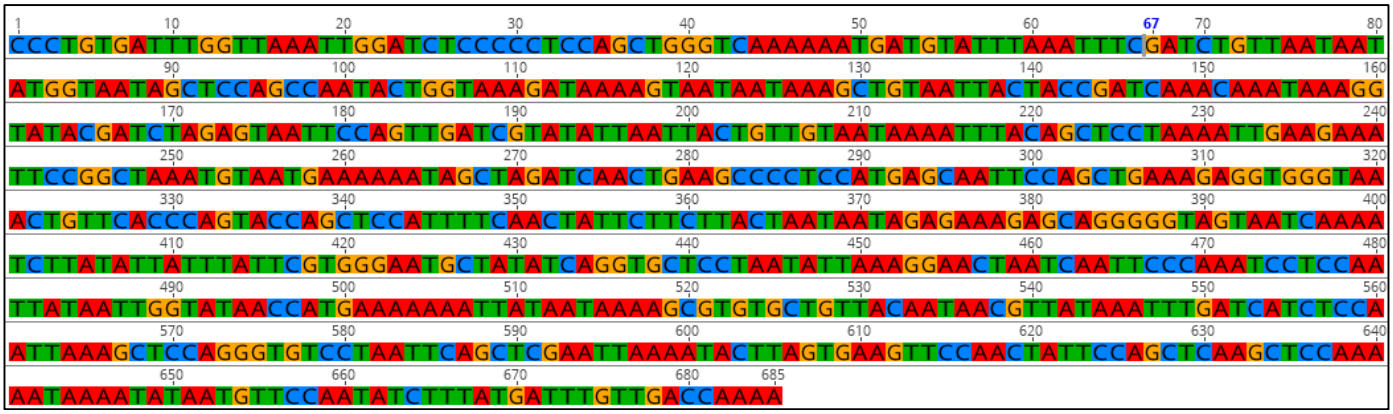

Fig 3: The nucleotide sequence of the fruit fly gene CO1 of Pineapple

4. Analysis of CO1 gene sequences Pineapple Fruit Flies

CO1 gene sequence alignments of pineapple fruit flies conducted using BLAST (Basic loca; alignment searching test) at the NCBI web site (https://blast.ncbi.nlm.nih.gov/Blast.cgi). BLAST results showed the level of sequence similarity with 100 similar sequences which have been recorded in the NCBI gene bank. The red line illustrates the nitrogenous bases of sequence similarity over 200 bp (Fig 3.). Sequences *Droshophila ananassae* [BK006336.1], showed the highest level of similarity with CO1 gene sequences from pineapple fruit flies (Table 1).

Table 1: Twelve sequences identical results NCBI BLAST on site (https://blast.ncbi.nlm.nih.gov/Blast.cgi)

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
TPA: Drosophila ananassae mitochondrion, complete genome	1223	1223	97%	0.0	99%	BK006336.1
Drosophila ananassae cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	1158	1158	93%	0.0	99%	JQ679117.1
Drosophila melanogaster mitochondrial COI gene for ctochrome oxidase I, complete cds, isolate: COI8	1066	1066	87%	0.0	99%	AB907180.1
Drosophila simulans isolate RU259 mitochondrion, complete genome	918	918	97%	0.0	91%	AF200849.1
Drosophila simulans isolate RU07 mitochondrion, complete genome	918	918	97%	0.0	91%	AF200848.1
Drosophila simulans isolate RU00 mitochondrion, complete genome	918	918	97%	0.0	91%	AF200846.1
Drosophila mauritiana isolate BG1 mitochondrion, complete genome	918	918	97%	0.0	91%	AF200831.1
Drosophila mauritiana mitochondrial cytochrome c oxidase subunit I (COI) gene, 5' end, Trp-, Cys-, and Tyr- tRNA genes, NADH dehydrogenase subunit 2 (ND2) g	918	918	97%	0.0	91%	M57912.1
Drosophila pseudoananassae cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	917	917	96%	0.0	92%	AY757280.1
Drosophila parabipectinata cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	913	913	97%	0.0	91%	AY757282.1
Drosophila rufa mitochondrial COI gene for cytochrome oxidase subunit I, partial cds, strain: EH091	911	911	96%	0.0	91%	AB669704.1
Drosophila simulans isolate NC48 mitochondrion, complete genome	907	907	97%	0.0	91%	AF200838.1

5. Reconstruction of Phylogeny Flies Pineapple based gene CO1.

Reconstruction of phylogeny using MEGA 7.0. Phylogeny tree models used were obtained from analysis of the substitution. CO1 gene sequences pineapple fruit flies used for the determination of substitution analysis phylogeny tree model. The results of the analysis model is obtained substitution Minimum Likelihood (Appendix) most suitable to construct a phylogeny tree of pineapple fruit flies. Phylogeny reconstruction produces 2 clade mofofiletik which isolates pineapple fruit flies (LBN) form a monophyletic clade, with 3 BLAST sequence of the fruit fly. LBN form a node with *Droshohila pseudoannanasae* [AY757280] and *Droshophilla parabipectinata* [757 782]. Nevertheless, according to phylogeny tree formed LBH has the closest resemblance to the level *Droshohila pseudoannanasae* [AY757280].

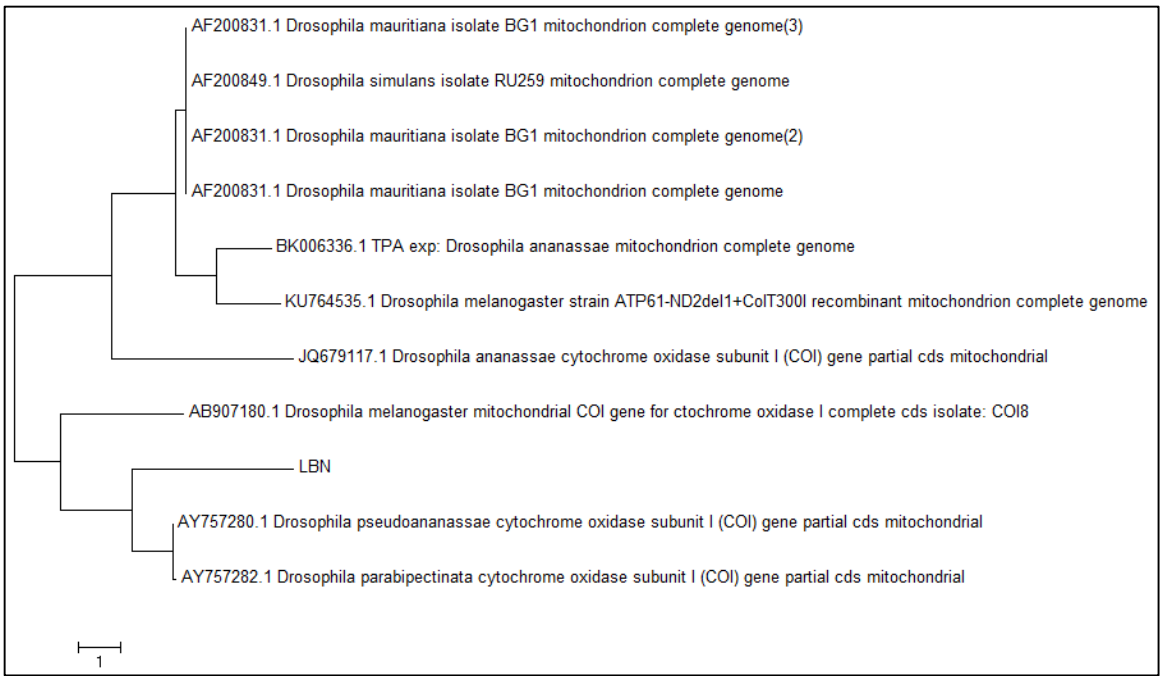

Fig 3: Phylogeny Reconstruction Pineapple Fruit Flies Using gene CO1

B. Application of Audio Visual Media, the results of DNA analysis Pineapple Fruit Flies in Learning in High School

1. Student Activities

Data obtained using the student activity sheet student activity observation conducted by the observer. The results of observation of student activity during the learning process takes place in three classes studied can be seen in the following graph.

The results of observations of the students, 88.89% of students showed high learning activity category. Even 7.78% of students showed learning activity is very high category. Only 5.5% of students who demonstrate learning activity is low and very low (Figure 4).

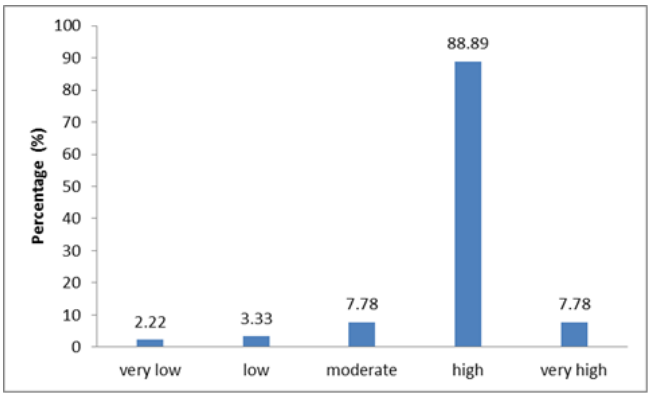

Fig 4: Response to student learning activities

Learning activities of students during the learning process subject of DNA using audio-visual media to show good results. In Table 2, the percentage of students from three classes of activity are investigated in compliance with the prescribed indicators of the effectiveness of learning ie ≥75% of students are in the category of high and very high activeness. According to Table 8 shows that the application of audio-visual media on the subject of DNA has shown pretty good results, in terms of the average value and the percentage of mastery learning.

Table 2: Learning Outcomes

Aspect	The number of Students
The Highest score	92.45
The Lowest Score	72.40
Average Value	83.56
The number of students	95.00
Number of individuals thoroughly studied	92.00
Mastery Learning	96.84

4. Students Response

Students' responses to learning activities through questionnaires with respondents obtained all the students who use the audio-visual media on the subject of DNA. Questionnaire responses of students, given at the end of the lesson. Summary results of the questionnaire responses of students to the learning process can be seen in Table 3.

Table 3: Analysis of the questionnaire responses of students towards learning the DNA material using audio-visual media

S. No	Aspects observed	The number of students	%
1	Students interested in following the lesson	82	91.11
2	Students become easier to learn the material already taught	83	92.22
3	Students better understand the material	80	88.89
4	Students liked the atmosphere of the current class learning	85	94.44
5	Students are motivated to follow the learning	86	95.56
6	Students agree the results of the discussion presented the students a stronger her memory of the material	80	88.89
7	Students were delighted to learn in groups / discussion	78	86.67
8	Percentage of students' response was excellent	85	94.44
9	A good percentage of student responses	85	94.44
10	The percentage of student responses is quite good, less good, bad	5	5.56

Based on Table 3, the students responded very well to the learning process using audio-visual media pineapple fruit fly DNA analysis. Of the three classes are nothing to respond to the criteria ugly, poor and quite well against their lessons. This shows that students are interested in learning to do.

5. Teachers Response

Table 4: Responses to the learning of teachers on the subject of DNA using DNA analysis of audio-visual media pineapple fruit flies

No	Statements	Response
1	Impact on learning	Pretty good for learning has been implemented makes the students active and study results are also good, the students also seems to feel attracted
2	Student motivation during learning	Able to make the students were motivated to learn
3	Activities and student learning outcomes for learning	Students become active and learning outcomes into better
4	Difficulties or obstacles to learning process	It's hard to make students to discuss with fast, because the students have not been accustomed to discussing, a little bit troublesome in preparing media
5	Advantages and disadvantages to learning	Advantages: students feel interested to learn using the media, especially as see video / animation learning, students also was glad to discuss group. Disadvantages: a bit difficult in the division time.
6	Interest to apply learning the material other	Interested to be used on materials biology others, because they were not already never apply learning like this

Table 5: Summary of the results of the study the effectiveness of audio-visual media

Response	Learning Activities	Students Learning Outcomes	Students Response	Teacher Response
Presentation and Criteria	88,23 Very High	90 thoroughly studied	92 Very Good	90 Very Good

6. Discussion

There are still many students, even teachers have a wrong understanding or misconceptions on the subject of genetics. Field of study that is still elusive, among others, cells, genetic material (genes, DNA and chromosomes), genes and heredity, DNA and heredity, gene expression and protein synthesis and mutation (Andrews *et al*, 2012; Nusantari, 2011) [1, 12]. The use of audio-visual media to facilitate students to study the characteristics of genes and DNA abstract becomes concrete. Students' conceptions about the DNA prior to application of instructional media analysis of fruit flies, very different from the concept right. From the interviews conducted, the students thought that "DNA solid form", "different DNA in the cell semue" "DNA is part of the gene", "Chromosomes are in the DNA". Both teachers and students, no idea of how to extract the DNA, the concrete form of the extraction of DNA, how genes are amplified and how the gene expression. After learning media applications fruit fly DNA analysis, there is increasing understanding that a very significant students' understanding of the characteristics of DNA.

Learning activities of students, from 53% to 88.23%, an increase of 25.23%; compared to prior learning applications using audio-visual media analysis of DNA of fruit flies. Student learning outcomes in classical reached 90%, whereas previously on the same subject, only reached 63.50%. Learning the subject of genetic material using audio-visual media, has been able to stimulate students' interest and motivation is characterized by increased activity of student learning. Students are more enthusiastic to learn, discuss, ask questions and solve problems given case. Students can follow the flow or DNA extraction phase, up to the stage of sequencing through audio-visual media. Students can see and understand the concrete form of DNA extraction. Students can understand, that the principle of DNA replication takes place in the process of gene amplification by PCR. Audio-visual media are made attractive, with the actual application of DNA analysis to stimulate the desire to learn more learners. The response of students and teachers to study media made very well belong to the category.

7. Conclusion

Application analysis of DNA, gene CO1 pineapple fruit fly, as a medium of learning material DNA in high school, resulting in learning outcomes and student learning activities are good. Activities and student learning outcomes, also supported by the response of students and teachers to study media at the very good category.

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