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The development of molecular genetics concept test for senior high school students using Rasch analysis

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ABSTRACT

Developing a high-quality test item requires substantial time and effort. A well-developed item bank is conducted using rigorous development and validation procedures. This study aimed to describe the development process of molecular genetics concept test (MGCT) for senior high school students using Rasch analysis under Berkeley evaluation and assessment research (BEAR) assessment system framework. The test consists of 50 multiple-choice items to assess conceptual understanding of molecular genetics concepts. The MGCT was developed based on curriculum analysis from the Indonesian ministry of education and culture and content-validated by three content experts comprising an expert in biology, an expert in bioinformatics, and an experienced Indonesian biology teacher in a senior high school. The MGCT was then piloted to 114 students who had taught the molecular genetics unit from a senior high school to conduct the empirical validation. The results from Rasch analysis showed that the MGCT is acceptable because all items have outfit and infit mean-square values in the acceptable range of 0.7 to 1.3 and the reliability is 0.43. So, the MGCT can be used to assess the understanding of the molecular genetics concept. However, several items were too difficult to discriminate the student ability. So, future studies need to develop more this MGCT to get a more appropriate instrument.

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1. INTRODUCTION

One of the main challenges in education is developing test items to determine the extent of students' understanding after carrying out the learning process [1]. Test items are important for teachers to assess the understanding level of students in a particular topic, assess their lessons' effectiveness, and test the effectiveness of new instructional tools and learning methods [2]. This challenge is particularly acute in biology education, in that the rapid scientific advances, the plentifully of information, and the complexity of phenomena [1].

The genetics concept assessment was developed using a game to examine students' conceptual understanding of the genetics concept [3]. Additionally, the understanding of central dogma was measured by 23 multiple-choice questions targeting undergraduate biology levels [2]. However, the previous instruments

focus on genetics concepts it needs to be extended to molecular genetics [1]. According to the Biology curriculum framework in Indonesia for senior high school, genetics comprises some basic and advanced concepts, including cell division, inheritance, the relationship between structural and the functional of genes, deoxyribonucleic acid (DNA), chromosomes, mutation, gene regulation, the principles of biotechnology and its applications as an effort to improve human welfare (also known as DNA technology), and theories, principles, and also mechanism of evolution and speciation [4]. So, we need to develop an assessment tool to measure advanced understanding, especially understanding the molecular genetics concepts as difficult concepts to be understood by high school students [1], [3].

Rasch model is a statistical model used to develop test items. It provides relevant information regard to students' learning progression [5]. Additionally, Wright Map is a graphical representation in Rasch analysis that provides a comprehensive outlook of the person ability and item difficulty in the form of a map in which person abilities and item difficulties use the same logit ruler [5]. Rasch model can estimate the parameters including item difficulty, person-ability, reliability index, infit-outfit indices, and a Wright Map. There are numbers of statistical software that can run Rasch analysis, such as Winstep, RUMM, and R [6]. This current study used R to conduct Rasch analysis. R is one of the programming languages for statistical analysis that provides commands like handling and storing data, performing calculations, and includes a core collection of packages for data analysis [7]. Various third-party programs provide R studio and R commander, consisting of package management, file importation, and more features [8]. This study described the development of the molecular genetics concept test (MGCT), including evidence of its validity from experts and Rasch modeling to give information on reliability, infit and outfit index, and a Wright Map.

2. RESEARCH METHOD

Berkeley evaluation and assessment research (BEAR) assessment system were used as a guideline to develop a new instrument in this study. BEAR assessment system provides meaningful interpretations of student work relative to a curriculum's cognitive and developmental goals [9]. This assessment system is based on four principles: assessment should be based on a developmental perspective of student learning; what is taught and what is assessed must be clearly aligned; teachers are the managers and users of assessment data; and classroom assessment must uphold sound standards of validity and reliability. BEAR consists of a step-wise process including construct maps, items design, outcome space, and measurement model [9], [10].

2.1. Construct map

A construct map is an initial step usually accomplished through domain analysis using extant literature and the particular goals of related curricula [11]. Our main purpose is to develop an instrument to examine students' understanding of molecular genetics concepts. According to the Indonesian ministry of education and culture [4] the molecular genetics concepts consist of six basic competencies, 12 targets of molecular genetic concepts, and 28 indicators of the target concept, as shown in Table 1. Basic competency is a general description of what students can do and a more detailed breakdown of what is expected from students, which is described in the learning outcome indicators. In Indonesia, basic competencies are references for developing subject matter, learning activities, and assessment standards according to students' characteristics, initial abilities, and subject characteristics [12]. The target concepts of molecular genetics are the concepts used in developing MGCT to achieve students' understanding.

We built a hypothetical construct map of molecular genetics concepts based on basic competencies in Table 1 from the Indonesian biology curriculum for a high school level. We used structure of observed learning outcomes (SOLO) taxonomy to sequence the sub-concept of molecular genetics concepts from the easiest to the hardest. The SOLO taxonomy is a systematic way of describing how students' performance improves in understanding materials, assignments, and instructions from the lower end (pre-structural) to the higher end (extended abstract) [13]. Svensäter and Rohlin [14] described five SOLO, consisting of SOLO 1 (the pre-structural level), that the student does not understand but uses irrelevant information or misses the point altogether. The students may have obtained bits and pieces of information, but they are disorganized and unstructured. SOLO 2 (uni-structural level) that students can tackle a single aspect and make explicit connections. So, the possibility of students having the ability to memorize or remember, find, say names, paraphrase, count, and perform simple instructions. SOLO 3 (multi-structural level), where students can handle several aspects but are still independent or unrelated. Thus, a student can have the competence to categorize, describe, apply methods, carry out procedures, and combine. SOLO 4 (relational level), where students understand the relationship between several aspects and relate them to a suitable whole. Thus, a student may have the competence to connect, compare, analyze, apply theory, and explain in terms of cause and effect. SOLO 5 (the extended abstract level), where students can generalize the given structure and

understand the structure from various perspectives. Based on the SOLO average from basic competencies of molecular genetics concept for a high school level, we constructed a hypothetical construct map for molecular genetics concepts in Figure 1.

Table 1. Basic competencies and SOLO taxonomy of molecular genetics concept

Basic competencies (Code)	Target of molecular genetic concepts (Code)	Indicators of the target concept (SOLO taxonomy)	SOLO average
Analyzing the process of cleavage cell as a basis for inheritance from parent to offspring (COMP1)	The cell cycle (MG1)	- Identify (SOLO 2) the role of phases of cell division - Explain (SOLO 4) the purpose of the cell cycle is to produce new cells where each cell carries genetic information in DNA	(2+4)/2=3
Analyzing the relationship between structural and the functional of genes, DNA, chromosomes (COMP2)	The structure and replication of DNA (MG2)	- Describe (SOLO 3) the structure of DNA - Describe (SOLO 3) the structure of nucleic acids in order of size, from the largest to the smallest - Comparing (SOLO 4) the structure and components between DNA and RNA - Explain (SOLO 4) the function of components involved in the process of DNA replication (e.g DNA polymerase, restrictions, ligases)	(3+3+4+4)/4=3.5
	Central dogma (MG3)	- Explain (SOLO 4) how an organism's DNA genotype produce its phenotype - Explain (SOLO 4) the phase of transcription process from initiation, elongation, to termination - Explain (SOLO 4) the function of the components involved in the translation process (e.g ribosomes, codons, mRNAs) - Translating (SOLO 4) the mRNA molecule from the simple nucleotide sequence into the corresponding amino acid sequence	(4+4+4+4)/4=4
Analyzing mutation in living things (COMP3)	Mutation (MG4)	- Explain (SOLO 4) the two types of mutations (substitution and deletion) and their effects - Explain (SOLO 4) the causes of mutations and their prevention	(4+4)/2=4
Analyzing gene regulation in living things (COMP4)	Gene regulation (MG5)	- Explain (SOLO 4) the role of gene regulation in cellular differentiation - Explain (SOLO 4) the cell signal that can be the effect to transcription of particular genes	(4+4)/2=4
	The DNA microarrays (MG6)	- Read the results (SOLO 5) of DNA microarrays between normal DNA samples and cancer DNA	(5+4)/2=4.5
	The genetic basis of cancer (MG7)	- Explain (SOLO 4) three types of causes of proto-oncogenes to become oncogenes (mutation, multiple copies, and gene moved to new DNA locus under new controls)	4
Analyzing principles of Biotechnology and its application as an effort to improve human welfare (DNA Technology) (COMP5)	Recombinant DNA technology (MG8)	- Explain (SOLO 4) the recombinant DNA techniques - Explain (SOLO 4) the function of the components involved in recombinant DNA technology - Explain (SOLO 4) the genomic library ways for bring a gene of interest - Explain (SOLO 4) the principal DNA amplification by PCR - Explain (SOLO 4) the RFLP method to compare DNA samples - Read visualization (SOLO 5) of restriction fragment patterns resulting from DNA fragment electrophoresis gel	(4+4+4+4+4+5)/6=4.2
	DNA fingerprinting and forensic science and human gene therapy (MG9)	- Explain (SOLO 4) the steps to compare DNA fingerprints in a murder case - Explain (SOLO 4) bone marrow stem cell ideally suited as targets for gene therapy	(4+4)/2=4
	Genomics (MG10)	- Explain (SOLO 4) DNA sequencing as one of the techniques for genome mapping	4
Explaining the theories, principles and mechanism of evolution also the latest views of experts related to speciation (COMP6)	Evidence of evolution (MG11)	- Explain (SOLO 4) how molecular biology can confirm the fossil record and other evidence that supports Darwin's view of kinship among all life	4
	Classification and Phylogeny (MG12)	- Making (SOLO 4) phylogenetic trees using comparison DNA and amino acid sequences	4

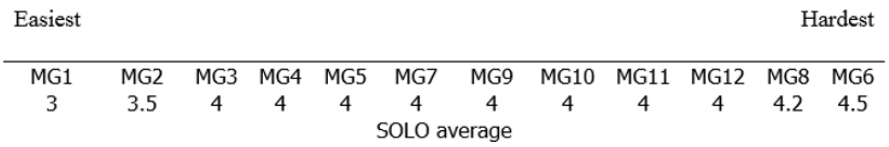

Figure 1. A hypothetical construct map for molecular genetics concepts

2.2. Items design

After the construct map was defined and visualized, the next step is items design to develop 50 multiple-choice test items aligned with the hypothetical construct map. Jolin and Wilson [9] stated that items go through iterative development and quality control processes are carried out to ensure good quality and adequate coverage of the item construct map design. We used three experts to validate each item. They are experts in molecular genetics, biology education, and experienced biology teacher in senior high school. Before conducting a field test of the instrument, we revised according to experts' suggestions. Furthermore, the 50 multiple-choice items were translated into Indonesian and confirmed by the English-Indonesian language specialist.

2.3. Outcome space

Essentially, outcome space is a value on student's work [11]. Due to the test format is a multiple-choice test. The outcome space is dichotomous, meaning each item scored as 1 if it was correct or 0 if it was incorrect.

2.4. Measurement model

In this step, the researchers field-tested the instrument on students who had learned the molecular genetics concept. There were 114 Indonesian senior high school students voluntarily took the test in November 2020. The students were given 90 minutes to complete the MGCT. The researchers used Google form as a platform to administer the MGCT and collect their responses.

After outcome space, the measurement model is the next step to describe how inferences about students' understanding were drawn from the field test through numerous models such as item response models, factor analysis, or latent class models [11]. We validate the proposed model with empirical data using Rasch analysis with the R program's test analysis module (TAM) package. TAM can estimate student and item measures. The probability that a student will respond to an item correctly was determined by the difference between the student's achievement level and the item's difficulty [15].

According to the Rasch model, some of the criteria stated are when student fit, and difficulty are on the same interval scale, independent of each other, and the size is in log odds or logits, which can vary from -∞ to +∞. In our study, the average item difficulty was set to zero. Placing the item positively far above zero is the most difficult, while the item difficulty negatively far below zero is the easiest [16]. Herrmann-Abell and DeBoer [17] stated that if a measure of student achievement is at the same level on the map as a measure of item difficulty, students have a 50% chance of answering the item correctly.

3. RESULTS AND DISCUSSION

MGCT undergone revisions based on feedbacks from three experts, including the accuracy and relevancy of each item [2]. We collected feedback on whether each question was valid, clear, and scientifically accurate, aligned with the stated learning objective (competencies), and was appropriate for senior high school level. There were 31 items MGCT undergone revisions, and the experts agreed with other nineteen items. For example, MG3.15 need revision because of two experts gave the comments for the answer and the question parts as shown in Figure 2.

A total of 114 students took MGCT test using Google Form in 90 minutes. They were proctored by their teachers. They took this test in their schools. Table 2 provides the fit analysis, including standard errors, infit-outfit mean-square, point-biserial correlation, and reliability to determine the acceptance, validity, and reliability of items of MGCT [16].

The final fit analysis in Table 2 shows that the standard errors for MGCT items within ranged from 0.3 to 0.39. All items have outfit and infit mean-square values in the acceptable range of 0.7 to 1.3. Infit-outfit mean-square values of more than 1.3 shows that the seen items had 30% more variety than was predicted by Rasch and if the outfit mean-square values less than 0.7 shows that the seen items had 22% less variety than was predicted by Rasch model [16]. Outfit (outlier-sensitive fit) is the criteria that more sensitive

to responds the items with difficulty far from a person, and vice-versa [18]. Analysis of point-biserial correlation indicates that the construct validity of the items where the point-biserial decreases to zero regardless of the quality of the item [19]. The point-biserial correlation for the items ranged from 0.02 to 0.44. Alagumalai, Curtis, and Hungi [20] classified the point measure correlation value into five categories, namely very good (>0.40), good (0.30–0.39), sufficient (0.20-0.29), unable discriminatory (0.00-0.19), and requires examination of items (<0.00). Based on that category, there are 4 items with a very good category, 7 items have a good category, 12 items have sufficient category, and 27 items have unable discriminatory category. So, 27 items need further investigation to revise. The instrument reliability analysis obtained from 50 items is 0.43. Reliability is the consistency of a measure that the values for reliability coefficients range is from 0 to 1.0. A coefficient of 0 means no reliability and 1.0 means perfect reliability [18]. Landis and Koch [21] classified the reliability from kappa value into six categories, namely poor (<0.00), slight (0.00-0.20), fair (0.21-0.40), moderate (0.41-0.60), substantial (0.61-0.80), and almost perfect (0.81-1.00). Based on those criteria, MGCT has a moderate quality that has consistency in measuring the student's understanding of the molecular genetics concept in high school level.

<table>
<tr><td>Item</td><td>See this codon strand
Mationin, sistein, valin, histidin, alanin histidine, valin, leusin (AUG-UGC-GUG-CAU-GCA-CAU-GUG-UAA)
How many amino acids will be produced?
a. 5
b. 6
c. 7
d. 8
The answer is A</td></tr>
<tr><td>Expert 1
Expert 2
Expert 3</td><td>“Even though is the same AA, still the number will be 8. What you mean is type not number”
“Please, add the table of amino acid”
“Affordable for high school students”</td></tr>
<tr><td>Revision item</td><td>See the table of amino acid
<table>
<tr><td></td><td></td><td colspan="4">Second Letter</td><td></td></tr>
<tr><td></td><td></td><td>U</td><td>C</td><td>A</td><td>G</td><td></td></tr>
<tr><td rowspan="4">1st letter</td><td>U</td><td>UUU Phe
UUC
UUA Leu
UUG</td><td>UCU
UCC Ser
UCA
UCG</td><td>UAU Tyr
UAC
UAA
UAG</td><td>UGU Cys
UGC
UGA
UGG Trp</td><td>U
C
A
G</td></tr>
<tr><td>C</td><td>CUU
CUC Leu
CUA
CUG</td><td>CCU
CCC Pro
CCA
CCG</td><td>CAU His
CAC
CAA Gln
CAG</td><td>CGU
CGC Arg
CGA
CGG</td><td>U
C
A
G</td></tr>
<tr><td>A</td><td>AUU
AUC Ile
AUA
AUG Met</td><td>ACU
ACC Thr
ACA
ACG</td><td>AAU Asn
AAC
AAA Lys
AAG</td><td>AGU Ser
AGC
AGA Arg
AGG</td><td>U
C
A
G</td></tr>
<tr><td>G</td><td>GUU
GUC Val
GUA
GUG</td><td>GCU
GCC Ala
GCA
GCG</td><td>GAU Asp
GAC
GAA Glu
GAG</td><td>GGU
GGC Gly
GGA
GGG</td><td>U
C
A
G</td></tr>
</table>
3rd letter
See this codon strand
AUG-UGC-GUG-CAU-GCA-CAU-GUG-UAA
How many amino acid will be produce:
a. 5
b. 6
c. 7
d. 8
The answer is D</td></tr>
</table>

Figure 2. The example of feedbacks from three experts

Table 2. Summary of Rasch fit statistics

	Min	Max	Median
Standard error	0.3	0.39	0.31
Outfit mean-square	0.86	1.26	0.99
Infit mean-square	0.89	1.12	0.99
Point biserial correlation	0.02	0.44	0.18
Reliability		0.43	

Table 3 summarizes the result of Rasch difficulty. This result shows the difficulties of molecular genetics concept items. We can see that the easiest molecular genetics concept is the genetic basis of cancer (MG7) and the hardest of molecular genetics concept is genomics (MG10). The description of the target concept for MG7 are 'explain why cancer is a genetic disease' and 'technology that can assist the researcher in getting the diagnosis of cancer.' High schools have successfully provided cancer concept and students can understand it. That is because of cancer concept is not only about conceptual understanding for students, but also about experiences learning of problems encountered in everyday life [22], [23]. The description of the target concept for MG10 is 'explain DNA sequencing as one of the techniques for genome mapping.' The genomics concept was first introduced at the senior high school level in Indonesia that very briefly introduced DNA sequencing and the technique for genome mapping. Whitley *et al.* [24] found that high school level is one of the earliest academic institutions where students were introduced to genomics. It is especially important to start early studying genomics, give the background for future health professionals, and familiarize all citizens.

Table 3. Difficulty of MGCT

Molecular genetics concept	No of items	Rasch difficulty (logits)			
		Min	Max	Mean	
The genetic basis of cancer (MG7)	2	-1.986	-0.804	-1.395	The easiest
Central dogma (MG3)	9	-2.288	0.281	-0.641	|
The structure and replication of DNA (MG2)	9	-1.636	0.754	-0.508	|
DNA fingerprinting and forensic science and human gene therapy (MG9)	2	-0.153	-0.002	-0.078	|
Mutation (MG4)	4	-0.040	0.701	0.165	|
Classification and phylogeny (MG12)	4	-0.301	0.552	0.258	|
The DNA microarrays (MG6)	4	-0.078	0.198	0.285	|
The cell cycle (MG1)	2	-0.913	1.676	0.380	|
Evidence of evolution (MG11)	2	0.077	0.982	0.529	|
Recombinant DNA technology (MG8)	8	-0.040	1.044	0.559	|
Gene regulation (MG5)	2	0.077	1.488	0.783	↓
Genomics (MG10)	2	0.922	1.676	1.299	The hardest

The Wright Map in Figure 3 shows that the distribution of students' abilities on the left side and item difficulties on the right side. Positive numbers indicate higher achievement or difficulty. Rasch analysis shows that the item difficulty is 0.136 and means of person ability is -0.601. It means the items on average, relatively difficult for the students. Based on Figure 3, it shows that some items are too easy, so they cannot discriminate student's ability, including item number 32 and 16. These items come from the genetic basis of cancer concept (MG7) and the central dogma concept (MG3). In addition, some items are too difficult to discriminate the student's ability, including items number 1, 43, 25, 42, 39, 46, 41, 7, 38, 40, 47, 27, 21, and 35. The majority of these items come from recombinant DNA technology (MG8) and genomics (MG10).

Figure 3 shows several contradiction items with the hypothetical construct map, especially MG1, MG5, and MG7. MG1 (the cell cycle) has two items, but only one item far from the prediction in a hypothetical construct map. The item asks the phase of the cell cycle when chromosomes multiply. The item should be the easiest, but some students misunderstood the cycle concept, especially about describing phases in the cell cycle and connecting it with chromosomes. Suwono *et al.* [25] found that some students were confused about the cell division phase and the events that occurred in that phase. MG5 (the gene regulation) has two items, but only one item far from the prediction in a hypothetical construct map. The indicator for that item is explain the role of gene regulation in cellular differentiation.' The item should be the easy item based on a hypothetical construct map, but it is the difficult item. Biology teachers have to give a clear explanation and good examples about gene regulation. Stefano and Kream [26] stated that the gene regulation concept was one of the genetic concepts for medical professionals. One of the items for MG7 was about explaining why cancer is a genetic disease. The item should be the difficult item in the hypothetical construct map, but it is the easy item. It shows that the MG7.32 in Figure 3 is too easy. So, we need to revise the question to more difficult accordance with SOLO 4 in SOLO taxonomy for further research.

The instrument was developed with the intent of biology teachers to measure students' understanding of molecular genetics concepts at the senior high school level. In Indonesia, molecular genetics concepts at the senior high school level taught in grade 12 are more complex than those at the middle level [27]. So it is essential to measure students' understanding of the concept of molecular genetics regularly [27]. Based on Figure 3, it shows that 34 items of MGCT indicate a reasonable way to measure students' molecular genetics concepts before and after an introductory molecular genetics concept in the classroom. Multiple choice in MGCT has plausible options, only examines the important facts, and the

distractors in options are on the syllabus. So, MGCT has potential to identify high school students' misconceptions on the concept of molecular genetics. Multiple choice can inform teachers about students' misconceptions [28], [29]. Vlckova, Kubiatko, and Usak [30] analyzed Czech high school students' misconceptions about basic genetic concepts using multiple choice with four possibilities and one correct answer. Their study shows that Czech high school students had several difficulties in learning genetics, such as students having difficulty in the size relationships among genetic concepts and solving the function of genetics concept problems. The developed instrument implies that MGCT provides a place for discussion to see and overcome students' misconceptions or difficult concepts in subsequent learning. Student scores can be looked back at competence by viewing a hypothetical construct map used for individual improvement concerning a given variable and student learning over time. This study used the BEAR assessment to develop an MGCT that allows describing student learning progress [31]. Learning to plan educator preparations, progress procedures, and classroom exercises centered on learning steps around information [32]. In addition, the BEAR Assessment System is a comprehensive and integrated system to assess the level of student understanding, interpret it, and monitor student performance [10].

Measure	Students	Map	Items
		|	
2		#	
		|	MG1.1 | MG10.43
		|	MG5.25
		|	
		|	
1		#	MG10.42 | MG8.39 | MG11.46 | MG8.41
		|	MG2.7 | MG8.38
		|	MG8.40 | MG12.47 | MG6.27 | MG4.21 | MG8.35
	I	|	MG3.13 | MG3.18 | MG8.36 | MG12.49 | MG6.30 | MG12.48
	III	|	MG6.28 | MG8.33
0	IIIII IIII	#	MG4.22 | MG4.23 | MG8.34 | MG9.37 | MG4.24 | MG5.26 | MG11.45
	IIIII IIIII IIIII III	|	MG9.44 | MG3.15 | MG2.6 | MG2.11 | MG6.29
	IIIII IIIII IIIII IIII	|	MG12.50 | MG3.17 | MG3.19
	IIIII IIIII IIIII III	|	MG2.9 | MG2.8 | MG2.5
	IIIII IIIII IIIII IIII	|	MG7.31 | MG2.10 | MG3.20
-1	IIIII IIIII IIII	#	MG1.2
	IIIII I	|	MG2.3
	III	|	MG3.14 | MG3.12
	III	|	MG2.4
	I	|	
-2		#	MG7.32
		|	MG3.16
		|	

Figure 3. Item–person map (a Wright Map) of MGCT

4. CONCLUSION

Based on the Rasch analysis result, the researchers can conclude that the MGCT is acceptable and reliable. So, biology teachers can use it to measure understanding of molecular genetics concepts. However, items number MG1.1, MG10.43, MG5.25, MG7.32, and MG3.16 need further revision and validation by experts because the items are too difficult and too easy for students.

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