József Szeberényi

EXPERIMENTS IN MOLECULAR CELL BIOLOGY

A Problems Book With Multiple-choice Question-based Tests

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Schenk Verlag

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Bibliografische Information. Der Deutsche Bibliothek
Die Deutsche Bibliothek verzeichnet diese Publikation in der Deutschen Nationalbibliografie; detaillierte bibliografische Daten sind im Internet über http.//dnb.ddb.de abrufbar.

ISBN 3-939337-13-7
ISBN 978-3-939337-13-3

Printed in Hungary

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Preface

"Medical practice is mainly problem-solving, so why not teach medicine through problem-solving?" This slogan originates from E. Reerink of Maastricht Medical School, Limburg University (The Netherlands), one of the leading universities in experimenting in medical education. This philosophy of medical education can be extended: it is true not only for clinical instruction, but for the teaching of basic medical sciences as well. Molecular cell biology is one of the most rapidly developing fields of natural sciences: its revolution has had a very strong impact on clinical disciplines leading to the establishment of a new science, ***molecular medicine****. The spectacular advances in cell biology are mainly caused by the development of new, more and more sophisticated techniques. Without understanding this methodology, it is impossible to digest and understand the new findings, processes and principles of modern cell biology. Teaching and learning molecular medicine thus requires a novel, problem-oriented approach.*

Application tests *have been used for teaching and knowledge assessment of medical students for more than two decades in the Molecular Cell Biology course at the University Medical School of Pécs, Hungary. This type of test was designed to enhance the problem-solving capabilities of medical students and to objectively assess (by using multiple-choice questions) the higher domains of their intellectual skills in cell biology. Applicaton tests are usually based on original scientific papers: they raise an experimental problem, briefly describe the methods used in the study and present the results in figures or tables. The student is expected to evaluate the data and to draw conclusions by answering a set of multiple-choice questions. To solve these questions, a thorough factual knowledge of the topic is required, but is not sufficient: the student has to apply his knowledge creatively. According to the principles of pedagogy, such a knowledge is more valuable and durable than knowledge acquired by sheer memorization of facts.*

I hope that solving these tests will not only help the reader to have an insight into how science works, to acquire useful knowledge in cell biology, but maybe even to have intellectual excitement and satisfaction.

January, 2006

József Szeberényi
MD, PhD, DSc

Types of multiple-choice questions

Five-choice completion

This type of question consists of a question or incomplete statement followed by five suggested answers or completions. Select the *one* best answer.

Five-choice association

This type of question consists of a list of lettered headings followed by a list of numbered words or phrases. For each numbered word or phrase, select the *one* heading which is most closely related to it.

Four-choice association

In this type of question a set of lettered headings is followed by a list of numbered words or phrases. Select

A if the word or phrase is associated with A only;
B if the word or phrase is associated with B only;
C if the word or phrase is associated with Aand B;
D if the word or phrase is associated with neither A nor B.

Relationship analysis

This type of question consists of a sentence with two main parts: an assertion and a reason for that assertion. Select

A if both assertion and reason are true statements and the reason is a correct explanation of the assertion;
B if both assertion and reason are true statements but the reason is not a correct explanation of the assertion;
C if the assertion is true but the reason is a false statement;
D if the assertion is false but the reason is a true statement;
E if both assertion and reason are false statements.

Quantitative comparison

In this type of question paired statements describe two entities that are to be compared in a quantitative sense. Select

A if A is greater than B;
B if B is greater than A;
C if the two are equal or very nearly equal.

Variation relationship

This type of question consists of pairs of phrases which describe conditions or quantities which may or may not vary in relation to each other. Select

A if increase in the first is accompanied by increase in the second or if decrease in the first is accompanied by decrease in the second;
B if increase in the first is accompanied by decrease in the second or if decrease in the first is accompanied by increase in the second;
C if changes in the second are independent of changes in the first.

Multiple completion

A question or incomplete statement is followed by four numbered completions, one or more of which are correct. Select

A if 1, 2 and 3 are correct;
B if 1 and 3 are correct;
C if 2 and 4 are correct;
D if only 4 is correct;
E if all four are correct.

Text / Figure / Table analysis

The following statements are related to the information presented in the description of the experiment. Based on the information given, select

A if the statement is supported by the information given;
B if the statement is contradicted by the information given;
C if the statement is neither supported nor contradicted by the information given.

1. RESTRICTION MAPPING OF A PLASMID DNA

Terms to be familiar with before you start to solve the test

*restriction endonucleases * plasmid * agarose gel electrophoresis * ethidium bromide staining * circular DNA (see G.M. Cooper: The Cell. A Molecular Approach. ASM Press/Sinauer Associates, Inc., 1997, pages 102-105, 108-109.)*

The experiment

Restriction mapping of small DNA molecules is an important technique of modern biology. An example of the mapping of a circular plasmid (**pBR322**) DNA is described in this test. Purified samples of plasmid DNA were digested with the restriction enzymes *Acc*I, *Ava*I, *Bam*HI, *Eco*RI or *Pvu*II, or with various combinations of these enzymes, and the reaction mixtures were fractionated by **agarose gel electrophoresis**. Bands of DNA fragments were visualized by staining with **ethidium bromide**. The image of the gel is displayed in Figure 1.1., and the cleavage sites on the circular DNA are shown in Figure 1.2.

Using the information in Figure 1.1. determine the cleavage sites of the enzymes. (An *Eco*RI site is already shown in Figure 1.2.)

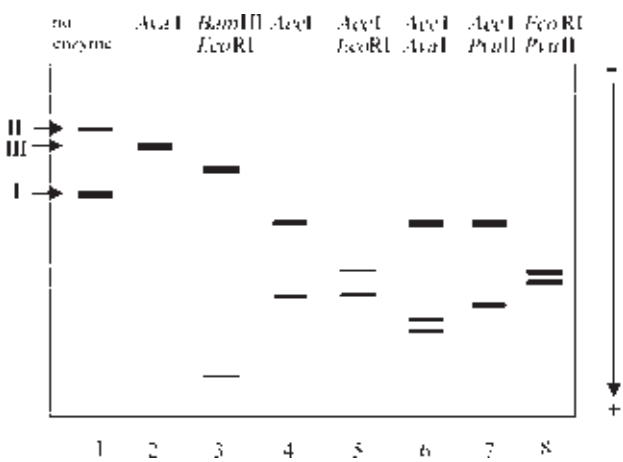

Figure 1.1. Fractionation of the products of restriction endonuclease digestions by agarose gel electrophoresis. (Undigested circular DNAs are usually present in the sample in three forms: closed, superhelical circular DNA /I/, relaxed circular DNA /II/ or linear DNA /III/; see sample 1. The arrow on the right side indicates the direction of electrophoresis.)

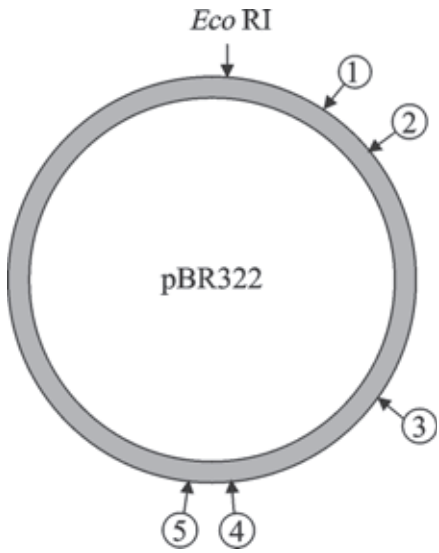

Figure 1.2. The map of plasmid pBR322.
(The numbers indicate restriction sites to be identified on the basis of Figure 1.1.)

Five-choice association

A: *Acc*I
B: *Ava*I
C: *Bam*HI
D: *Pvu*II
E: *Eco*RI

1.1._____ This enzyme cuts at site 1.
1.2._____ This enzyme cuts at site 2.
1.3._____ This enzyme cuts at site 3.
1.4._____ This enzyme cuts at site 4.
1.5._____ This enzyme cuts at site 5.

Correct answers

1.1. C
1.2. A
1.3. B
1.4. D
1.5. A

Explanations

Agarose gel electrophoresis fractionates DNA fragments according to their size: the smaller the molecule, the faster it runs. Restriction mapping can be done by comparing fragment sizes.

Digesting pBR322 with *Acc*I generates two fragments (sample 4 in Figure 1.1.), therefore the plasmid must contain two cleavage sites. All the other enzymes have a single recognition site in pBR322: alone they produce a single DNA fragment (e.g. *Ava* I in sample 2) or in pairs they generate two fragments (samples 3 and 8). *Eco*RI and *Pvu*II give fragments of similar size (sample 8): the *Pvu*II site is therefore either site 4 or site 5. *Acc*I gives two medium-sized fragments (sample 4): sites 1 or 2 and sites 4 or 5 are the candidates for *Acc*I cleavage. *Pvu*II cuts into the small *Acc*I fragment (compare samples 4 and 7): site 4 is thus for *Pvu*II (**MCQ 1.4. D**) and site 5 is for *Acc*I (**MCQ 1.5. A**). *Bam*HI generates a large and a very small fragment with *Eco*RI (sample 3) indicating that the two enzymes cleave near to each other (**MCQ 1.1. C**). Site 2 is thus left for *Acc*I (**MCQ 1.2. A**) and site 3 for *Ava*I (**MCQ 1.3. B**).

FURTHER READING

Bloch, K.D.: Mapping by multiple endonuclease digestions. In "Current Protocols in Molecular Biology" (eds. Ausubel, F.M. et al.), John Wiley & Sons, Inc., 1994, Chapter 3.2.

Brooks, J.E.: Properties and uses of restriction endonucleases. In "Guide to Molecular Cloning Techniques" (eds. S.L. Berger, A.R. Kimmel). Academic Press, Inc., 1987, pages 113-129.

2. RESTRICTION MAPPING OF A LINEAR DNA FRAGMENT

Key terms to be familiar with before you start to solve the test

*restriction endonucleases * DNA polymerase I * in vitro DNA synthesis * chloroform extraction * polyacrylamide gel electrophoresis * autoradiography * 5′ → 3′ exonuclease * template * primer * DNA structure (see G.M. Cooper: The Cell. A Molecular Approach. ASM Press/Sinauer Associates, Inc., 1997, pages 46-47, 92-93, 102-105, 175-184.)*

The experiment

A DNA fragment generated by *Eco*RI (cleavage site: 5'-G↓AATTC-3') and *Bam*HI (cleavage site: 5'-G↓GATCC-3') digestion was analyzed in this experiment. (One strand of the recognition site of the enzymes is shown only; the cleavage sites are indicated by the arrows in the sequences.) The purified DNA fragment was incubated *in vitro* with a **DNA polymerase I** lacking 5' → 3' exonuclease activity, dATP, dGTP, dTTP, [α-^{32}P]dCTP, ions and pH required for enzyme activity. After incubation the DNA fragment was purified by chloroform extraction, divided into five aliquots and treated with *Alu*I restriction enzyme for various periods of time (0, 2, 5, 10 and 120 minutes). The products of the reaction were fractionated by **polyacrylamide gel electrophoresis** and then analyzed by **autoradiography**. Figure 2.1. shows the autoradiogram.

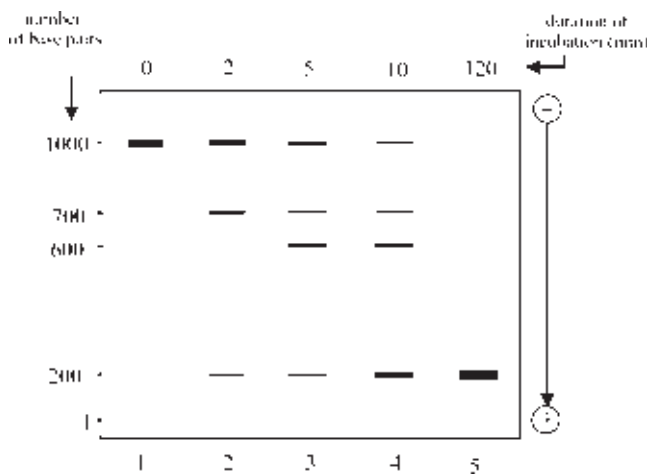

Figure 2.1. Autoradiogram of AluI digests fractionated by gel electrophoresis.

Figure analysis

2.1._____ The DNA fragment was labelled by repair synthesis.

2.2._____ All *Bam*HI-*Eco*RI fragments contain one labelled phosphate group.

2.3._____ The radioactive phosphate is present in the 3' end nucleotide of labelled strands.

2.4._____ The *Bam*HI-*Eco*RI fragment is 1000 base pairs (bp) long.

2.5._____ There are 100 bp fragments among the products of *Alu*I digestion.

2.6._____ The *Alu*I enzyme used in the experiment is contaminated by a 3' → 5' exonuclease.

2.7._____ The sample digested for 2 minutes contains labelled DNA molecules only.

Five-choice completion

2.8._____ Which map of Figure 2.2. is compatible with the experimental results?

A: Map A
B: Map B
C: Map C
D: Map D
E: None of them

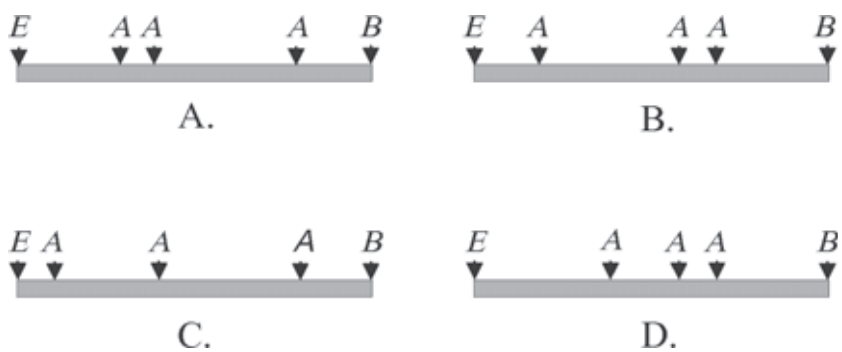

Figure 2.2. Possible maps of the EcoRI-BamHI fragment. (E, EcoRI; A, AluI; B, BamHI)