ZKBS
Zentrale Kommission für die Biologische Sicherheit

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Position statement of the ZKBS on the risk assessment of do-it-yourself (DIY) kits for genetic engineering experiments which are freely available from retailers

Numerous online commercial traders currently offer science kits for simple microbiological experiments, so-called ‘do-it-yourself‘ (DIY) kits. As a result, interested parties addressed inquiries on the legal regulation and risk assessment of these kits to the highest state authority of the German federal states. Against this backdrop, the German Federal Ministry of Food and Agriculture asked the ZKBS for a statement on the risk assessment of the DIY kits under concern. In particular, the following kits have been evaluated on the basis of the information the respective manufacturers have published on the internet:

"Engineer-it" kit (Amino Labs), Canada

The "Engineer-it" science kit offered for sale by Amino Labs contains all necessary components (consumables, reagents, a recombinant plasmid as well as cells of the bacterial strain *Escherichia coli* K12) to carry out a transformation experiment which produces genetically modified *E. coli* bacteria. According to the instruction manual enclosed, the bacteria are first propagated and rendered chemically competent. Afterwards, the bacteria are transformed by a plasmid, which contains an antibiotic resistance gene and a gene for a freely selectable colored or fluorescent protein. The transformed bacteria are plated and cultured.

According to Art. 3 Paragraph 2 of the German Genetic Engineering Act (GenTG), the experiment described is a genetic engineering operation. The recipient organism *E. coli* K12 is assigned to **risk group 1** and recognized as being a part of a biological safety measure pursuant to Art. 6 Paragraph 4 of the German Genetic Engineering Safety Regulation (GenTSV). The employed plasmid contains an antibiotic resistance gene and a gene encoding a colored or fluorescent protein. Whether the vector fulfills the conditions for recognition as being part of a biological safety measure pursuant to Art. 6 Paragraph 5 of the GenTSV cannot be derived from the description available. The nucleic acid segments transferred by using the vector do not increase the hazard potential of *E. coli* K12. The genetically modified

organisms are therefore – under the condition that neither the vector nor the insert contain nucleic acid segments with a hazard potential of their own – are assignable to **risk group 1** pursuant to Art. 5 Paragraph 1 Sentence 2 of the German Genetic Engineering Safety Regulation (GenTSV).

"Engineer GFP Yeast" kit (The ODIN), USA

The "Engineer GFP Yeast" science kit is offered for sale by The ODIN. It contains all necessary components (consumables, reagents, a recombinant plasmid as well as cells of the *Saccharomyces cerevisiae* strain BY4742) to carry out a transformation experiment which produces genetically modified yeast cells. The experimental procedure is described in the instructions enclosed. First, the yeast cells are propagated and rendered chemically competent. Afterwards, the yeast cells are transformed by a plasmid which contains the gene for the green fluorescent protein as well as the *ura3* gene of yeast. The *ura3* gene codes for the enzyme orotidine-5‘-phosphate decarboxylase which enables the growth of the Δ*ura3* strain BY4742 on a selection medium without uracil. The transformed yeast cells are plated and cultured.

According to Art. 3 Paragraph 2 of the German Genetic Engineering Act (GenTG), the experiment described is a genetic engineering operation. The recipient organism *S. cerevisiae* BY4742 is assigned to **risk group 1** and recognized as being a part of a biological safety measure pursuant to Art. 6 Paragraph 4 of the German Genetic Engineering Safety Regulation (GenTSV). The employed plasmid contains a selection marker gene and a gene coding for a fluorescent dye. Whether the vector fulfills the conditions for recognition as being part of a biological safety measure pursuant to Art. 6 Paragraph 5 of the GenTSV cannot be derived from the description available. The nucleic acid segments transferred using the vector do not increase the hazard potential of *S. cerevisiae* BY4742. The genetically modified organisms are therefore – under the condition that neither the vector nor the insert contain nucleic acid segments with a hazard potential of their own – assignable to **risk group 1** pursuant to Art. 5 Paragraph 1 Sentence 2 of the German Genetic Engineering Safety Regulation (GenTSV).

"Bacterial Gene Engineering CRISPR" kit (The ODIN), USA

The "Bacterial Gene Engineering CRISPR“ science kit is offered for sale by The ODIN. It contains all necessary components (consumables, reagents, two recombinant plasmids, a template DNA as well as cells of the bacterial strain *Escherichia coli* HME63) to carry out a transformation experiment to produce genetically modified, Streptomycin-resistant *E. coli*-

bacteria. According to the instruction manual enclosed, the bacteria are first propagated and rendered chemically competent. Afterwards, the bacteria are transformed by (*i*) one plasmid which contains the gene for Cas9, the tracrRNA and perhaps an antibiotic resistance gene, (*ii*) a second plasmid which bears the crRNA and perhaps an antibiotic resistance gene, and (*iii*) a specific template DNA with the desired K43T mutation of the *rpsL*-gene of *E. coli*. The transformed bacteria are plated and cultured.

According to Art. 3 Paragraph 2 of the German Genetic Engineering Act (GenTG), the experiment described is a genetic engineering operation. The recipient organism *E. coli* HME63 is a derivative of the *E. coli* strain K12. It is thus assigned to **risk group 1** and is also recognized as being a part of a biological safety measure pursuant to Art. 6 Paragraph 4 of the German Genetic Engineering Safety Regulation (GenTSV). The two plasmids employed probably contain an antibiotic resistance gene as well as the components of the CRISPR/Cas9 system [1] from *Streptococcus pyogenes* (**risk group 2**). Whether the vectors fulfill the conditions for recognition as being part of a biological safety measure pursuant to Art. 6 Paragraph 5 of the GenTSV cannot be derived from the description available. The CRISPR/Cas9 system introduces a double-strand break into the *rpsL* gene. Besides, a specific template DNA, which serves as a template for the bacterial DNA repair machinery, is introduced into the cells. The template DNA comprises a segment of the *rpsL* gene for the ribosomal S12 protein of *E. coli* and also contains a mutation (K43T) which confers resistance to the antibiotic streptomycin [2]. The transferred nucleic acid segments are without a hazard potential of their own and do not increase the hazard potential of *E. coli* HME63. The genetically modified organisms are therefore – under the condition that neither the vector nor the insert contain nucleic acid segments with a hazard potential of their own – assignable to **risk group 1** pursuant to Art. 5 Paragraph 1 Sentence 2 of the German Genetic Engineering Safety Regulation (GenTSV).

Notice

Pursuant to Art. 8 Paragraph 1 Sentence 1 of the German Genetic Engineering Act (GenTG), genetic engineering operations may only be carried out in genetic engineering installations. Conducting genetic engineering operations outside genetic engineering installations is an offence and/or punishable [3]. The genetic engineering operations of **biosafety level 1** here described must be carried out in genetic engineering installations observing the legal requirements of biosafety level 1.