Work instruction for determining the purity and identity of genetically modified organisms and the vectors and insertions used

Introduction

The aim of this instruction is to determine when and how the purity and identity of genetically modified organisms (GMOs), including *E. coli* K12 strains, are determined. It also specifies how the DNA constructs for the creation of genetically modified organisms are identified. The results are documented in the researcher's lab journal.

These activities are required based on Section 2, Article 9 (3d) of the Regulation on Genetically Modified Organisms, Environmental Management 2013. The permit/notification from the Ministry of IenW, Bureau GGO, including categorization of the applicable physical containment level, is based on the specified host, vector, insert, and origin insert.

Procedure

1. 1. Identity of E. coli K12 strains

1.1. Commercially obtained bacteria

- Check the certificate supplied with the cells
- Record the batch number in the lab journal when storing the cells.
- Freeze the cells, in portions if necessary (with repeat thawing, the cells lose their competence; with long-term culturing, there is a greater risk of mutation).
- Record the batch number in the lab journal when the cells are used.

1.2. Self-isolated bacteria or bacteria obtained from colleagues

- Before freezing in small portions, verify the accuracy and purity of the cells in accordance with the methods below, using a new portion on each occasion.
- *E. coli* strains may be distinguished from other gram-negative bacteria and rod-shaped bacteria by means of a PCR test. Consult the Medische Microbiologie Infectieziekten en Infectiepreventie, MUMC+ for further information.
- A multiplex PCR test may be used to distinguish *E. coli* K12 strains and wildtype *E. coli* strains (that could be pathogenic for people and must therefore be handled under ML-II conditions). Consult the Medische Microbiologie Infectieziekten en Infectiepreventie, MUMC+ in case of doubt.
- In case of doubt about the purity of the cells, order a new batch and repeat the above.

2. Identity of cells

- Assess the growth characteristics of the cells under the microscope at the start of the experiments and each cultivation.
- Eukaryote cells can be further distinguished by their morphology and colouring by means of antibodies, FACS analyses, and PCR testing.

3. Identity of genetically modified rodents

- Determine the accuracy of the modification by means of genotyping

4. Identity of vectors and insertions

- Determine the identity and integrity of the vector and the insert by means of sequencing, restriction analysis, gel electrophoresis, and PCR testing. The best method is sequencing.

Abbreviations

BSO	Biosafety Officer
GMO	Genetically Modified Organism
IenW	Ministry of Infrastructure and Water Management; executive body: Bureau GGO
ML-II	Microbiological Laboratory Class II
PCR	Polymerase Chain Reaction

References

- KNVM: Veilig werken met micro-organismen (Working safely with micro-organisms), ISBN: 978-90-825105-0-8
- AP Bauer et al. Rapid identification of E. coli safety and laboratory strain lineages based on Multiplex-PCR. FEMS Microbiol Lett 269, 2007, 36-40.
- The Medische Microbiologie Infectieziekten en Infectiepreventie, MUMC+ can be consulted at any time if you have received a sample whose identity you are unsure about.
- Decree and Regulation on Genetically Modified Organisms, Environmental Management 2013, IenW April 2014.
- Website HSBM Maastricht

Further information

For further information, please contact the BSO.