



Federal Ministry of Health and  
Women - Division IV/B/12  
Radetzkystraße 2  
A - 1030 Wien

November 2003

**NOTIFICATION  
of work with GMO**  
 small  large scale  
(tick where applicable)

**Classification of the intended work** (tick where applicable)

- Biosafety level 1  
 Biosafety level 2

**Classification of GMO** (tick where applicable)

- |   |  |
|---|--|
| <input checked="" type="checkbox"/> bacteria          | <input checked="" type="checkbox"/> other eucaryotic cells |
| <input type="checkbox"/> virus                        | <input type="checkbox"/> agricultural crops                |
| <input type="checkbox"/> fungi                        | <input type="checkbox"/> other plants                      |
| <input type="checkbox"/> other microorganisms         | <input type="checkbox"/> farm animals                      |
| <input checked="" type="checkbox"/> human cell tissue | <input type="checkbox"/> other animals                     |

**Work with GMM:**

culture volume: 500 ml - 1 l

- more than 600 liter ( l)  
(declare volume)

**minutes of the BSC attached:**

- YES  NO

**1. Informations on the carrier, BSC, BSO, project manager and premises:**

<b>1.1. carrier:</b>	
<b>name:</b>	<b>Company A</b>
<b>address:</b>	<b>Street B 1 A-1020 Vienna</b>
<b>phone:</b>	<b>123456</b>
<b>fax:</b>	<b>1234567</b>
<b>e-mail:</b>	<b>office@companyA.at</b>

<b>1.2. biological safety officer (BSO):</b>	
<b>name:</b>	<b>Dr. B</b>
<b>phone:</b>	<b>123456</b>
<b>fax:</b>	<b>1234567</b>
<b>e-mail:</b>	<b>Dr.B@companyA.at</b>
<b>qualification and education:</b>	
<p style="text-align: center;"><b>Ph.D. in Genetics, University of Vienna 3 years postdoc, University of Graz 5 years project manager Comp. D for more details see the attached curriculum vitae</b></p>	
<b>1.2.1. deputy of the BSO:</b>	
<b>name:</b>	<b>Dr. C</b>
<b>phone:</b>	<b>123456</b>
<b>fax:</b>	<b>1234567</b>
<b>e-mail:</b>	<b>Dr.C@companyA.at</b>
<b>qualification and education:</b>	
<p style="text-align: center;"><b>Ph. D. in Biochemistry and Biotechnology; University of Rome 2 years postdoc, University of Vienna for more details see the attached curriculum vitae</b></p>	

<b>1.3. members of the biological safety committee (BSC):</b>
<b>1.3.1. number: overall: 4 internal: 3 external: 1</b>
<b>1.3.2. internal members:</b>
<b>name: Dr. B</b>
<b>qualification and education:</b>  see 1.2
<b>name: Dr. C</b>
<b>qualification and education:</b>  see 1.2.1
<b>name: Dr. D</b>
<b>qualification and education:</b>  Ph. D. in Microbiology, University of Texas 10 years Biosafety Officer of Comp. G for more details see the attached curriculum vitae
<b>name:</b>
<b>qualification and education:</b>
<b>name:</b>
<b>qualification and education:</b>

<b>1.3.3. external members:</b>	
<b>name:</b>	<b>Prof. Dr. E</b>
<b>address:</b>	<b>Inst. of Microbiology, University of Vienna, A-1030</b>
<b>phone:</b>	<b>112233</b>
<b>fax:</b>	<b>1122334</b>
<b>e-mail:</b>	<b>Dr.E@mibi.univie.ac.at</b>
<b>qualification, education and present occupation:</b>	
<b>Ph.D. in Biotechnology, University of London  since 10 years , a. o. Prof. University of Vienna  for more details see the attached curriculum vitae</b>	
<b>name:</b>	
<b>address:</b>	
<b>phone:</b>	
<b>fax:</b>	
<b>e-mail:</b>	
<b>qualification, education and present occupation:</b>	

<b>1.4. project manager (not applicable for work with GMO in biosafety level 1):</b>	
<b>name:</b>	<b>Dr. F</b>
<b>phone:</b>	<b>123456</b>
<b>fax:</b>	<b>1234567</b>
<b>e-mail:</b>	<b>Dr.F@companyA.at</b>
<b>qualification and education:</b>	
<b>Ph D. in Genetics, University of Vienna  3 years postdoc, University of Vienna  for more details see the attached curriculum vitae</b>	

**1.5. information on the premises:**

**1.5.1. adress of the premises:**

Street B 1, A-1020 Vienna

**1.5.3. description of the parts of the premises, relevant for the work with GMO and for safety (including attached construction plans, as well as description of the equipment according to Annex II of the ordinance on work with GMO in the contained use; BGBl. II Nr. 431/2002):**

**Room Nr. 1001: PCR-Machine, centrifuges, EB-bath**

**Room Nr. 1002: gel-documentation, photometer, speed vac**

**Room Nr. 1003: laboratory, laminar-flow class 2, -20°C and 4°C refrigerator**

**Room Nr. 1004: sterilisator, autoclave, dishwasher**

**Room Nr. 1005: -80°C refrigerator, ultracentrifugation**

**Room Nr. 1006: microscopy**

**Room Nr. 1007: incubator room 37°C**

**Room Nr. 1008: incubator room 28°C**

The walls, floors, and working benches are impervious to water, and resistant to acids, alkalis, solvents and disinfectants. In the laboratory there is a hand wash basin with dispensers containing soap and hand disinfectant, respectively, as well as a dipenser for paper towels.

for more details see the attached maps

**2. summarized description of the work:**

**2.1. title of the work:**

**Cloning of genomic DNA from HIV-1 in E.coli K12, Cos7-, H9- and Jurkat- cell lines**

**2.2 description of work/fermentation process including expected results with relevance according to biosafety issues (detailed and chronological description of the intended work):**

**1. Cloning of HIV-1-gag, env,tat and nef into H9 and Jurkat cell lines:  
HIV- genes will be amplified by PCR, modified by adding 5' an EcoRI and 3' a HindIII restriction site and cloned into pAB12 (which was derived from pBR322 by inserting an modified MCS and the RSV LTR promotor from pRSVneo).**

**H9 and Jurkat cells will then be transformed with the resulting pAB12env, pAB12gag, pAB12tat and pAB12nef plasmids**

**2. Cloning of HIV-1 total genome into E. coli K12:**

**Total genomic DNA of HIV-1 will be isolated and cloned into pUC19 (pBR322 derivate). Then E. coli K12 will be transformed with the resulting plasmid pUC19HIV**

**3. Cloning of HIV-1- total genome into Cos7-cells:**

**Total genomic DNA of HIV-1 will be isolated and cloned into pBR322. Then Cos7 cells will be transformed with the resulting plasmid pBR322HIV.**

**2.3 description of the recipient organism (e. g. genotype, biosafety level, source, citations, catalogue number and other risk assessment relevant properties) :**

**E. coli K-12 ATCC10798, biosafety level 1**

**E. coli K-12 is a pathogen and a laboratory strain. Because of its auxotrophy this strain is not able to survive in the environment.**

**COS 7 ATCC CRL-1651, biosafety level 2**

**H9 ATCC HTB-176, biosafety level 1**

**Jurkat DSMZ ACC282, biosafety level 1**

**2.4. description of the donor organism (e. g. genotype, biosafety level, source, citations, catalogue numbers and other risk assessment relevant properties):**

**HIV-1, biosafety level 3**

**2.5. description and designation of used vectors including risk assessment relevant properties (e. g. antibiotic markers, promoters, origins of replication, repressors, biosafety level, source, citations, catalogue numbers and other risk assessment relevant properties):**

**pUC19:** this vector is a derivative of the biosafety vector pBR322 and contains an ampicillin resistance, the lac promoter, the lac Z' gene, and MCS and the pMB1 ori. For more details see the attached vector map

**pAB12:** this vector was obtained from pBR322 by inserting the RSV LTR promoter

from pRSVneo into pBR322. It contains an ampicillin and a tetracycline resistance

the pMB1 ori and a modified MCS. As this vector is a derivative of the biosafety vector pBR322 it could be also classified into biosafety level 1.

For more information see the attached vector maps.

**pAB12gag, pAB12env, pAB12tat and pAB12nef** are derivatives from pAB12 containing the respective genes from HIV-1 and should be therefore biosafety

level

1

**pUC19HIV** contains the total HIV-1 DNA.

**2.6. description and designation of the material and gene products used for genetic manipulation (e. g. sequence data, accession numbers, biosafety level, source, citations and other risk assessment relevant properties):**

**HIV-1 genes gag, env, tat and nef as well as total HIV-DNA.** Although HIV-1 is classified into biosafety level 3, the use of a biological safety system (*E. coli* K12) and derivatives of the biosafety vector pBR322 allows to classify this work into biosafety level 2

**2.7. Argumentation of the risk assessment with reference to human beings, animals, plants and environment (e. g. use of an approved biological safety system, no advantage for the survival of the GMO in the environment compared to the recipient, no toxicity of the gene product etc.):**

**1. Cloning of HIV-1-gag, env, tat and nef into H9 and Jurkat:**

**Although the donor organism is classified into biosafety level 3, the use of defined and characterised genes together with the use of an approved biosafety system (pBR322 derivatives, recipient organisms of biosafety level 1) results in a downgrading of the biosafety level to biosafety level 2**

**2. Cloning of total HIV-1 DNA into E. coli K12:**

**Although total DNA of an biosafety level 3 organism will be cloned, the use of E. coli K12 (biosafety level 1) combined with the use of an pBR322 Derivate (pUC19) resembles an approved biological safety system. Therefore this part of the work will be classified into biosafety level 2**

**3. Cloning of total HIV-1 DNA into COS 7 cells:**

**Since the used plasmid pBR322 does not possess an eukaryotic promoter and because of the immobilization of this plasmid, but based on the fact, that the donor organism is classified level 3 and the recipient is classified on biosafety level 2, this part of the work will be classified into biosafety level 2**

**2.8. Classification of the GMO into a biosafety level group and description of the intended safety precautions (e. g. compliance of international GLP-/GMP-standards, instruction of the personal, restricted access to laboratories where GMO are handled etc.):**

**The GMOs are classified into biosafety level 2.  
All work where aerosols can be produced will be done in an class 2 biosafety cabinet. All work will be done in compliance of international GLP standards. The personal working with GMO will be instructed properly. A hygiene plan is provided. Also there is restricted access to the laboratory**

**2.9. description of the measures for the inactivation of GMO and the waste management:**

All material, glassware and cultures will be inactivated by autoclaving for 30 min. at 121° C. The benches will be routinely cleaned with appropriate disinfectants (e. g. Et-OH, Na-Hypochloride).

All inactivated waste will be collected in sealed barrels and incinerated

**declaration of the containment level to verify the biosafety level according to Part B Z 3 of the ordinance of work with GMO in the contained use, BGBl. II Nr. 431/2002 (tick where applicable):**

**Einschließungsstufe 1**

**Einschließungsstufe 2**

**2.11. classification of the intended work into biosafety level (tick where applicable):**

**biosafety level 1**

**biosafety level 2**

**3. safety precautions:**

**3.1. information on the rules for accident precaution (only for work in biosafety level 2):**

**All work will be done in biosafety level 2 equipped laboratories. Process steps where hazardous quantities of aerosols are formed will be done in class two biosafety cabinets. The personal will be instructed properly. There will be restricted access to the laboratories**

**4. internal enabling:**

<b>4.1. Assessment of the biological safety committee (BSC):</b>
the classification of the GMO into the biosafety level mentioned at point 2.11 was affirmed (tick where applicable):
<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
minutes of the BSC are attached (tick where applicable)
<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO

<b>4.2. following information should be handled confidential according to § 105 and § 106 of the Austrian Gene Tecnology act and should therefore not become public:</b>
none

<b>4.3. siganture of the CARRIER:</b>
name: Dr. Z
date: 05.05.2004
signature: Dr. Z