



DEALING WITH GMO'S IN EUROPE

Case Study: Contained Use Cloning of HIV-1 DNA

Zagreb, 22. 10. 2004

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Division IV, Department B/12
Biotechnology and Genetic Engineering





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

1.2 biological safety officer (BSO):	
name:	Dr. B
phone:	123456
fax:	1234567
e-mail:	Dr.B@companyA.at
qualification and education:	
<p>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</p>	

1.2.1 deputy of the BSO:	
name:	Dr. C
phone:	123456
fax:	1234567
e-mail:	Dr.C@companyA.at
qualification and education:	
<p>Ph. D. in Biochemistry and Biotechnology; University of Rome 2 years postdoc, University of Vienna for more details see the attached curriculum vitae</p>	

Information on the:

- carrier (name, address, (phone, fax, e-mail))
- biological safety officer (name, phone, fax, e-mail qualification)
- deputy of the BSO (name, (phone, fax, e-mail, qualification))





1.3. members of the biological safety committee (BSC):

1.3.1. number: overall: 4 internal: 3 external: 1

1.3.2. internal members:

name: Dr. B

qualification and education:

see 1.2

name: Dr. C

qualification and education:

see 1.2.1

name: Dr. D

qualification and education:

Ph. D. in Microbiology, University of Texas
10 years Biosafety Officer of Comp. G
for more details see the attached curriculum vitae

name:

qualification and education:

name:

qualification and education:

Information on the Biological Safety Committee:

- number of members (total, internal, external)
- qualification of the members of the BSC





1.3.2. external members:

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

1.4. project manager (not applicable for work with GMO in biosafety level 1):

name:	Dr. F
phone:	123456
fax:	1234567
e-mail:	Dr.F@companyA.at
qualification and education:	Ph D. in Genetics, University of Vienna 3 years postdoc, University of Vienna for more details see the attached curriculum vitae

Information on:

- external members of the BSC (name, address, phone, fax, e-mail, qualification and present occupation)
- project manager (only above level 1; name, phone, fax, e-mail, qualification)





1.5. information on the premises:

1.5.1. address 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

Information on the premises:

- address of the premises
- description of parts of the premises relevant for the work with GMO





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 promoter 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.

Information on the work:

- title of the work
- description of the work:
list of the intendend experiments





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 apathogen und 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

Information on the:

- **recipient organism:**
any information useful for the risk assessment, especially the biosafety level, and the origin
- **donor organism:**
the same as for the recipient





2.4.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 an derivate of the biosafety vector pBR322 and contains an ampicilin resistance, the lac promotor , 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 ampiciline and a tetracycline resistance the pMB1 ori and a modified MCS. As this vector is a derivate 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 derivates from pAB12 containing the respective genes from HIV-1 and should be therefore biosafety level 1
pUC19HIV contains the total HIV-1 DNA.

2.5.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 an biological safety system (E. coli K12) and derivates of the biosafety vector pBR322 allows to classify this work into biosafety level 2

Information on:

- used vectors: including their properties and origins
- material and gene products used for the genetic manipulation





2.4. 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 a biosafety level 3 organism will be cloned, the use of E. coli K12 (biosafety level 1) combined with the use of a pBR322 Derivative (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

risk assessment of the applicant:

- with reference to men and environment
- classification into a biosafety level and argumentation





2.4. 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 personnel, 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 personnel working with GMO will be instructed properly. A hygiene plan is provided. Also there is restricted access to the laboratory

Information on:

- official classification of the work into a biosafety level by the applicant
- projected biosafety precautions





2.4.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):

containment level 1

containment level 2

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

biosafety level 1

biosafety level 2

Information on the biosafety precautions:

- inactivation of GMO
- disposal of inactivated waste





2. 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

Information on:

- accident prevention





4. internal enabling:

4.1. Assessment of the biological safety committee (BSC):

the classification of the GMO into the biosafety level mentioned at point 2.11 was affirmed (tick where applicable):

YES

NO

minutes of the BSC are attached (tick where applicable)

YES

NO

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:

none

4.3. siganture of the CARRIER:

name: Dr. Z

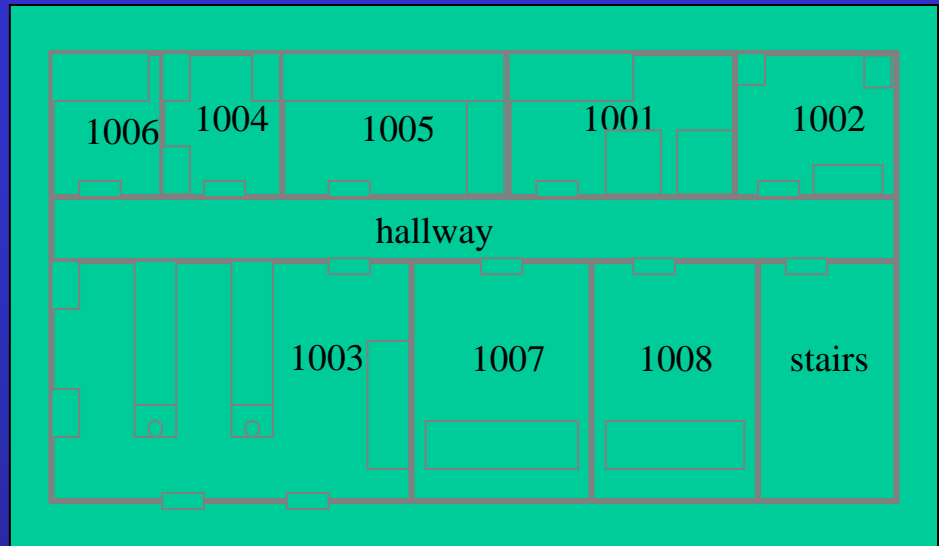
date: 05.05.2004

signature: Dr. Z

Enabling:

- internal enabling of the work by the internal biological safety committee
- signature of the carrier
- optional: declaration of confidential data





Obligatory attachments to the application:

- curriculum vitae of BSO, deputy of BSO, members of the BSC and head of the project
- constructional drawings of the premises





Handling of the request

1.) Check up on completeness:

- ✓ filled form
- ✓ informations on the carrier, BSC, BSO, project manager
- ✓ information on the projected work (recipient- and donor organism, used vectors, genetic manipulation)
- ✓ risk assessment and classification of the GMO by the applicant
- ✓ information on biosafety precautions
- ✓ internal enabeling
- ✓ minutes of the BSC, construction drawings of the premises and curriculum vitae

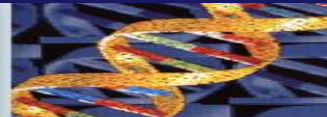
application is complete





- 2.) Confirm to the notifier the receipt of the request
- 3.) Check up on the qualification of the BSO, his deputy, the members of the BSC and the project manager by revising the curriculum vitae
 - ✓ all mentioned persons have at least the 2 years of experience with the work of GMO, as required by the Austrian law
- 4.) Check up on the information of the premises:
 - ✓ the description of the rooms is in agreement with the map, the description of the laboratory is in agreement with the required equipment

Qualification = O.K. ; premises = O.K.





5.) Verify the applicants risk assessment:

a.) check up on the biosafety levels of the recipient- and donor organisms, as well as of the used vectors:

- ✓ the classification of *E. coli* K12, H9- and Jurkat cell lines is correct
- ✓ the classification of the Cos7 cell line is according to the NIH guidelines as found on the ATCC home page. According to european guidelines this cell line could be classified into biosafety level 1, because of the frequent use of this cell line for a long time without accidents

All recipient organisms are biosafety level 1





a.) check up on the biosafety levels of the recipient- and donor organisms, as well as of the used vectors (continued):

- ✓ **the classification of the donor organism HIV-1 is correct**
- ✓ **the plasmid pBR322 is commonly used and is an approved biosafety vector, pUC19 is a derivative of pBR322 and therefore also an approved biosafety plasmid. The newly constructed plasmid pAB12 is derived from pBR322 and can therefore also be considered as a biosafety vector**

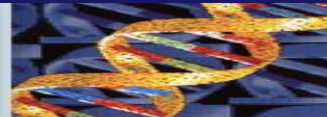
The used donor organism has to be classified into biosafety level 3 and the used vectors can be classified into biosafety level 1





b.) check up on the material and gene products used for genetic manipulation:

- ✓ **HIV-1 *gag* and HIV-1 *env* are coding for structural proteins with no known potential hazardous effects**
- ✓ **HIV-1 *tat* is coding for an regulatory protein which is an transcriptional transactivator essential for HIV-1 replication. Possible hazardous effects can not be excluded**
- ✓ **HIV-1 *nef* is coding for an accessory protein which inter alia stimulates the infectivity of HIV-1 virions. Possible hazardous effects can not be excluded**
- ✓ **total HIV-1 DNA possesses all the know hazardous effects of HIV-1**





HIV-1 *gag* and HIV-1 *env* can be handled as level 1

HIV-1 *tat* and HIV-1 *nef* should be handled as level 2

**HIV-1 total DNA has to be handled as the HIV-1 virus
and is therefore classified into level 3**





c.) check up on the genetically modified organisms:

H9- and Jurkat cells containing a pBR322-derivative (pAB12) expressing HIV-1 *gag* and HIV-1 *env*

- ✓ The sequences of HIV-1 *gag* and HIV-1 *env* are characterised
- ✓ No potential hazardous effects are expected
- ✓ The recipient organisms are classified into biosafety level 1
- ✓ The used vector is an approved biosafety vector
- ✓ No higher risk of the GMO compared with the recipient

These GMO can be classified into biosafety level 1





H9- and Jurkat cells containing a pBR322-derivative (pAB12) expressing HIV-1 *tat* and HIV-1 *nef*

- ✓ The sequences of HIV-1 *tat* and HIV-1 *nef* are characterised
- ✓ Potential hazardous effects can not be completely excluded
- ✓ The recipient organisms are classified into biosafety level 1
- ✓ The used vector is an approved biosafety vector
- ✓ No infectious virus particles are produced
- ✓ There is less risk of the GMO compared to the donor organism, but higher higher risk of the GMO compared to the recipient organisms

These GMO should be classified into biosafety level 2





Cos 7 cells and *E. coli* K12 containing a pBR322-derivative (pUC19) containing the total HIV-1 DNA

- ✓ The whole sequence of HIV-1 is characterised
- ✓ The hazardous effects of HIV-1 are known
- ✓ The recipient organisms are classified into biosafety level 1
- ✓ The used vector is an approved biosafety vector
- ✓ Infectious virus particles can be produced
- ✓ Although an approved biosafety system is used, the risk potential of the donor organism, has to be included completely into the risk assessment

These GMO have to be classified into biosafety level 3





Decision

- 1.) **The cloning and expression of HIV-1 *gag* and *env* in H9- and Jurkat cell (or other biosafety level 1 cell lines) using the vector pAB12 (or other pBR322-derivatives) can be done in biosafety level 1. There is no risk based on the submitted data and on the state of the art of science and technology for human beings and the environment. The safety precautions and the waste management are sufficient.**
- 2.) **The cloning and expression of HIV-1 *tat* and *nev* in H9- and Jurkat cell (or other biosafety level 1 cell lines) using the vector pAB12 (or other pBR322-derivates) should be done in biosafety level 2. There is no substandard risk based on the submitted data and on the state of the art of science and technology for human beings and the environment. The safety precautions and the waste management are sufficient.**





3.) The cloning and expression of HIV-1 total DNA in COS-7 cells and *E. coli* K12 (or other biosafety level 1 cell lines) using the vectors pBR322, pUC19 (or other pBR322-derivatives) can not be done in biosafety level 2.

There is substandard risk based on the submitted data and on the state of the art of science and technology for human beings and the environment. The safety precautions and the waste management are not sufficient.

This work has to be done in biosafety level 3.

