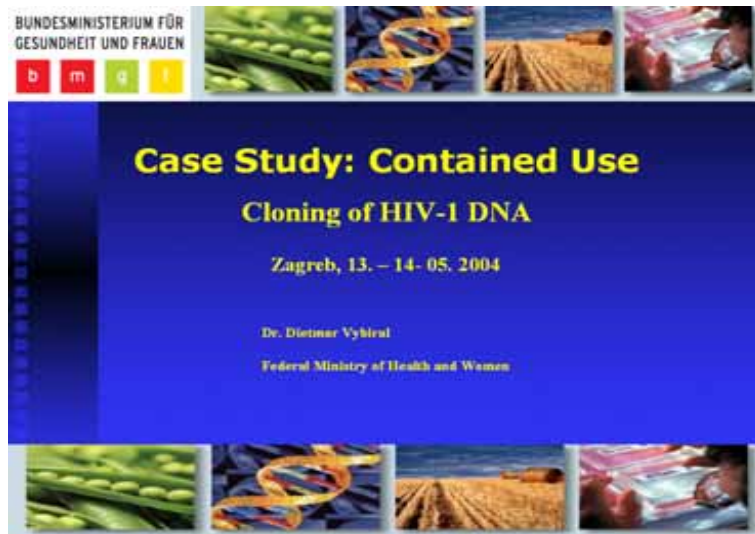


Case Study: Contained Use Cloning of HIV-1 DNA



In this fictional case, one of the major topics of day to day work within a competent authority (hereafter called CA) will be covered. The decision making concerning an application for work with GMM in biosafety level 2. In general it has to be mentioned, that the experience the Austrian CA has with applications shows, that most of the risk assessments provided by the applicants are correct and need only small to none adjustments. In this fictional case, however, some

incorrectness occurred in the risk assessment of the applicant. This case should point out, that it is very important to verify the risk assessment of the applicant carefully and that it is possible to adjust the biosafety level both, up and down. Secondary the Austrian form for applications for first time work with GMO in the contained use in biosafety level 1 or 2 is presented.

The image shows a screenshot of a web-based application form. The form is titled 'NOTIFICATION of work with GMO' and includes checkboxes for 'small' and 'large scale'. It also has sections for 'Intention of the intended work (where applicable)', 'Classification of the intended work (where applicable)', and 'Intention of the BSC method'. To the right of the form, there is a list of bullet points: 'summary of relevant information', 'scale of work', 'biosafety level', 'classification of the GMO', 'culture volume', and 'minutes of the BSC'. The form is set against a blue background with a white text area.

On the front page of the Austrians CA form for applications for first time work with GMO in the contained use in biosafety level 1 or 2, a summary of the basic information of the application is given. This is important, because the given timeframe for the proceeding can be

seen on the first sight. This basic information concerns of statements of the scale of the work, the intended biosafety level, the classification of the GMO, the culture volume and the minutes of the biological safety committee (BSC). In the Austrian law there is a difference between small and large scale work. Small scale work is defined by law as work in biosafety level 1 up to 600 liter culture volume, in biosafety level 2 up to 100 liter culture volume and in biosafety level 3 and 4 up to 10 liter culture volume. Work above this limits has to be declared as large scale work. For large scale work some additional safety precautions has to be made.

In this case, it can be seen that that the given timeframe to make the decision is 30 days because it is an application for a first time work with GMM in small scale in biosafety level 2.

The image shows a screenshot of a computer application window. On the left, there is a form with several sections, each with a title and a list of fields. The sections are:

- 1.1. Carrier:** Fields for Name, Address, Phone, Fax, E-mail, and Qualification.
- 1.2. Biological Safety Officer (BSO):** Fields for Name, Address, Phone, Fax, E-mail, and Qualification. Below the fields, there is a text box containing: "Ph.D. in Chemistry, University of Vienna; 3 years graduate, University of Graz; Postgraduate manager (Mag. Dr.); No responsibilities for the actual work in the laboratory".
- 1.3. Deputy of the BSO:** Fields for Name, Address, Phone, Fax, E-mail, and Qualification. Below the fields, there is a text box containing: "Ph.D. in Biochemistry and Biotechnology, University of Vienna; 2 years graduate, University of Vienna; No responsibilities for the actual work in the laboratory".

On the right side of the window, there is a blue box with the heading "Information on the:" and a bulleted list:

- 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)

As it was mentioned in the talk „Handling of Requests: Contained use“ information about the carrier, the biological safety officer (BSO) and his deputy has to be given. The qualification of the BSO and his deputy has to be judged by the CA. Therefore a short summarize of the qualification and education of the intended persons should be given in the form. For the judgment it is essential that all persons intend to fill a position as BSO, deputy of the BSO, project manager and member of the BSC, send a detailed curriculum vitae attached to the application. It is important to mention, that the BSO has to be a different person than the carrier. This is implicated by the duties of the BSO based by the Austrian law.

Information on the Biological Safety Committee:

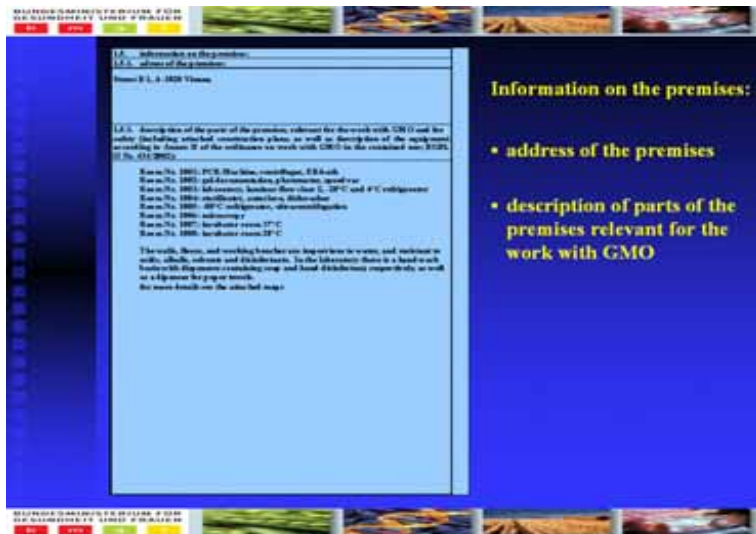
- number of members (total, internal, external)
- qualification of the members of the BSC

The BSC contains the BSO, the deputy of the BSO and at least one additional member (in cases of small scale work), which has to be external. Information about the members of the BSC and their qualification should be given shortly in the form.

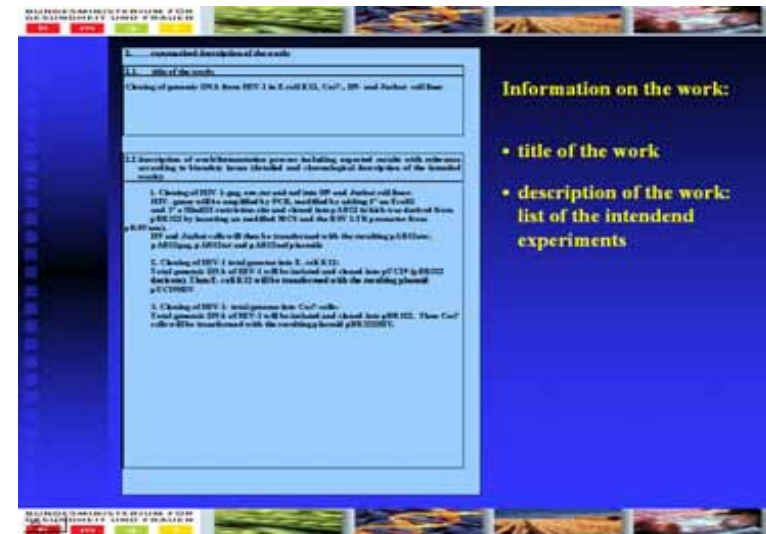
Information on:

- 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)

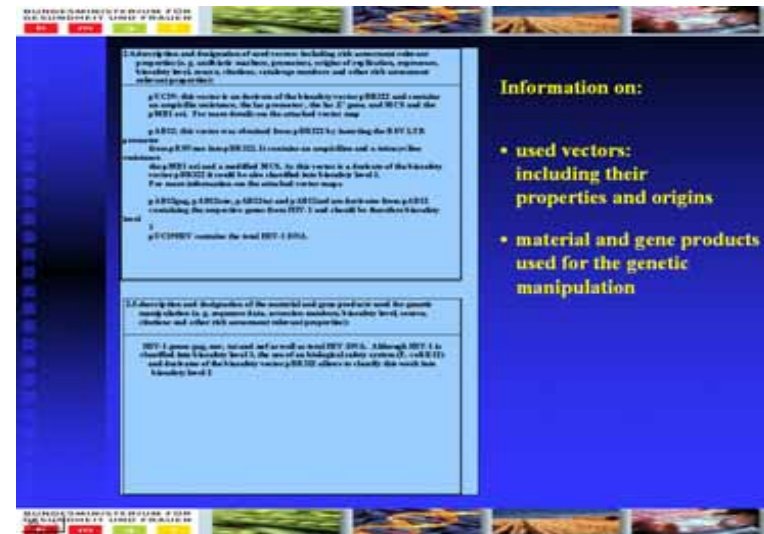
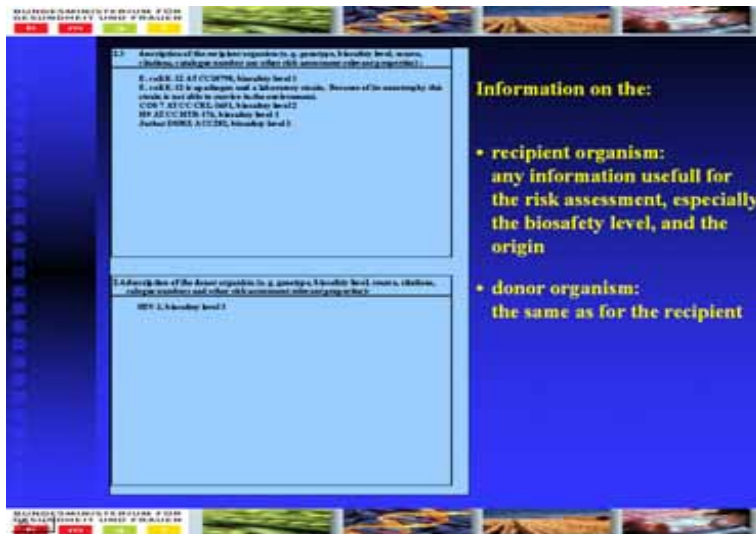
Based on the Austrian law, a project manager is only needed in cases of work with GMO above biosafety level 1. If this is applicable then information about the qualification of the project manager has to be provided in the form.



The information on the premises has to include the address and a detailed description of the parts of the premises, relevant for the work with GMO and for safety. Obligatory attachments are construction drawings of the premises. The detailed description of the equipment of the laboratories makes it possible for the CA to evaluate, if the safety precautions and the equipment is sufficient for the intended work. The Austrian government has released an ordinance for work with GMO in the contained use, where the minimum requirements for each biosafety level is listed.



The second part of the form deals with information of the intended work. First of all a title of the work must be given, since in the Austrian law its laid down, that only works can be notified or permitted and not a premises. One of the most important information the applicant has to give its a detailed description of the intended experiments. In this case, HIV-1 *gag*, *env*, *tat*, and *nef* genes are intended to be cloned into H9 and Jurkat cell lines as well as HIV-1 whole genomic DNA is intended to be cloned into *E. coli* K12 as well as into COS7-cells.

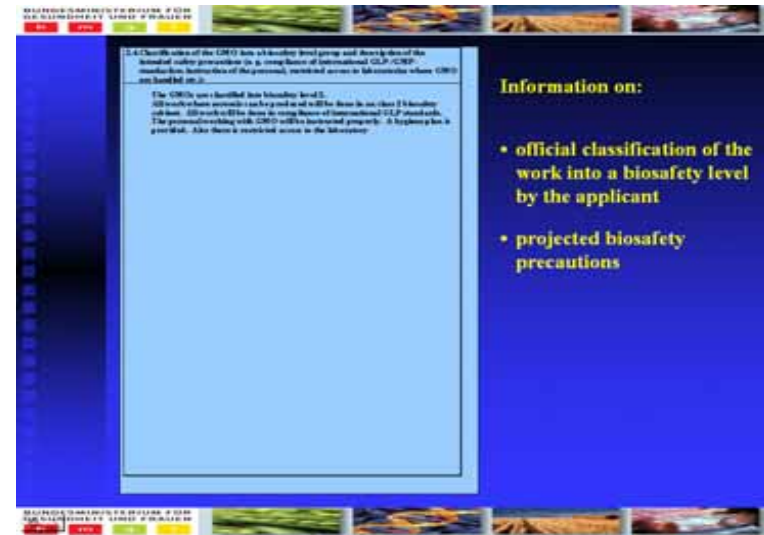
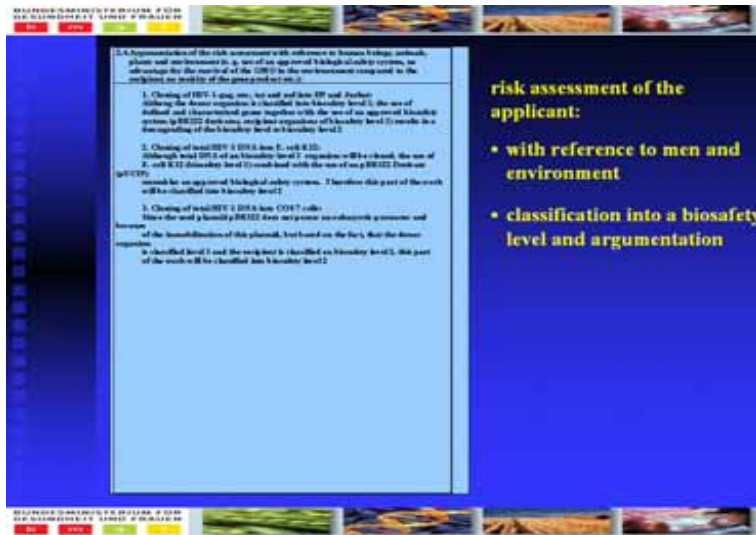


The next important information is about the recipient and the donor organism. Here information about the genotype, the biosafety level based on international lists, the source of the organisms (e.g. commercial cell collections, personal communication etc.) and any risk assessment relevant information should be stated by the applicant.

The information about the used vectors and the intended genetic manipulation is also essential to verify the given risk assessment and the resulting classification of the work into a biosafety level. A lot of vectors are known to be approved biosafety vectors which can together with certain recipient organisms form an approved biological containment and the work can therefore classified into a less stringent biosafety level, where less stringent physical containment has to be applied. Also the genetic manipulation can result in an attenuation of the recipient organism, which is the case in the most applications. However all these manipulations can lead to a higher risk of the GMO compared to the recipient organism, too. Therefore it is essential to

know, what are the used vectors and what is the intended genetically manipulation.

Therefore the risk assessment has not only concern the health of the involved employees of the premises but also possible adverse effects to nature.

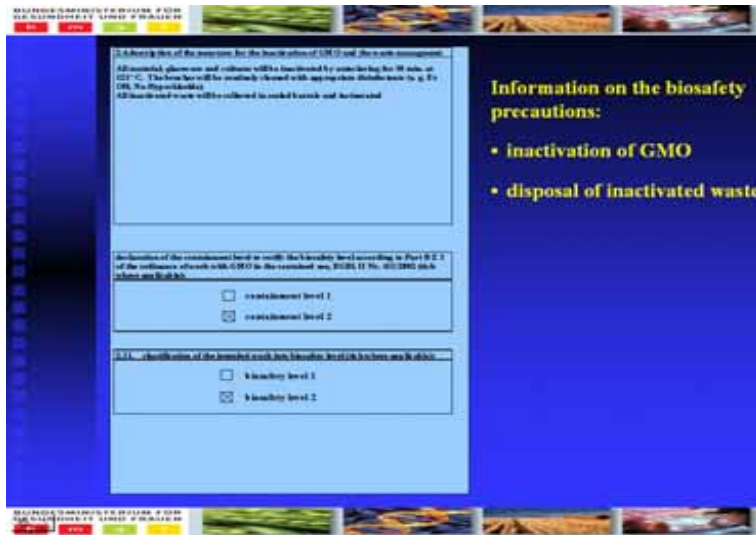


Finally the risk assessment of the applicant is the most important part of the application. Here the applicant should state his argumentation why he classified the intended work into the given biosafety level. This risk assessment should be done with reference to human beings and the environment. In the Austrian Gene Technology Law the principle number one is the precautionary principle. This means, that all human beings, plants, animals, and the environment has to be protected from adverse effects caused by the use on biotechnology.

On this page of the application form, the applicant has to official declare the classification of the GMO into a biosafety level. Additionally he has to mention the projected safety precautions. This is also a very essential point of an application. As an example, at work with organisms, which are classified into biosafety level 2, experiments where aerosols could be produced, should exclusively be

Biosafety Regulation in Croatia – Workshop in Zagreb, 13. – 14- 05- 2004; Case Study: Contained Use – Cloning of HIV-1 DNA; Dr. Dietmar Vybiral, Federal Ministry of Health and Women, Div. IV/B/12, Austria

done in an class 2 biosafety cabinet. Also the applicant should stated how the hygiene plan is look like.



Another point, which is often overseen is the correct inactivation and waste disposal. The needed containment ends only after a proper inactivation of the GMO and a proper disposal of the inactivated GMO waste. This includes of course not only the GMO itself but also all material, glassware etc. which was in contact with the GMO. Therefore a statement of the applicant about the intended inactivation and waste disposal measurements is essential.

According to the above information a containment level is resulting. The level of the containment is based on the regulations laid down in the „Austrian Ordinance for Work with GMO in the Contained Use“. The applicant has to state this containment level. Finally a last statement of the applicant of the classification of the work into a biosafety level has to be done.



If the intended work is above biosafety level 1 the applicant has to mention information about the rules for accident precaution, which has to be established in the premises. Examples are, work in biosafety

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level 2 equipped laboratories, permanently instructions and workshops for the personal, restricted access to the laboratories etc.

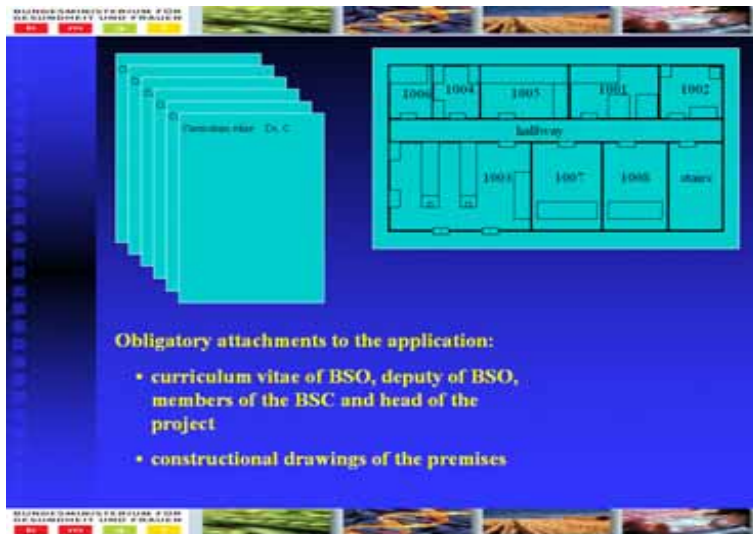
The image shows a screenshot of a computer application window titled "Internal enabling". The window contains a form with several sections. The first section is titled "Internal enabling" and contains a sub-section "1.1.1. Commission of the Biological Safety Committee (BSC)". Below this, there are two rows of checkboxes for "YES" and "NO" under the heading "The classification of the GMO into the biosafety level numbered according to 2.1.1 and 2.1.2 (if not applicable, leave empty)". The second row is for "The minutes of the BSC are attached (if not applicable, leave empty)". Below this, there is a section "1.1.2. Enabling information should be handled confidential according to § 100 and § 104 of the Austrian State Treaty, as well as the BSC members, not become public." followed by a "name:" field. The third section is "1.1.3. Signature of the CARRIER" with fields for "Name: Dr. D." and "Signature: Dr. D.". To the right of the form, there is a blue sidebar with the heading "Enabling:" and a list of three items: "internal enabling of the work by the internal biological safety committee", "signature of the carrier", and "optional: declaration of confidential data".

The Austrian act give the applicant the option to declare some data as confidential. General all information given by the applicant is confidential except for the cases when an public hearing has to be done. This is the case for work in biosafety level 3 in large scale and for work in biosafety level 4. In this case the data declared as confidential are not be made public. The following data can not be declared as confidential:

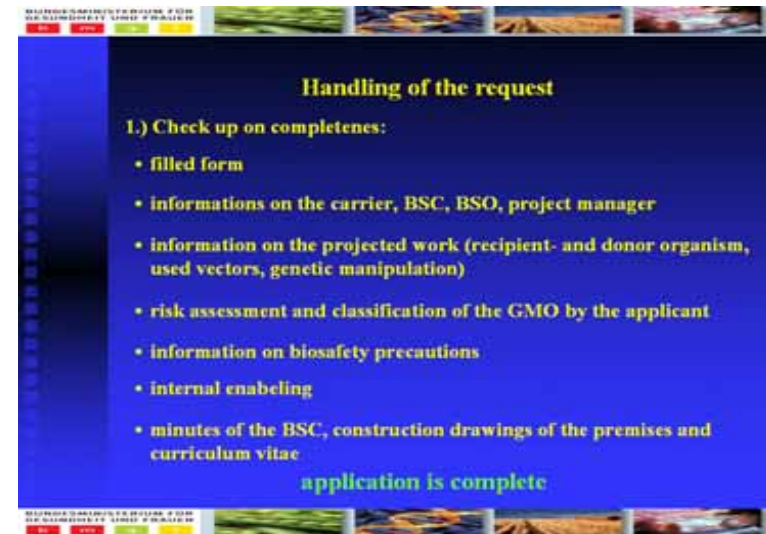
- general description of the GMO, Name and address of the applicant, biosafety level and containment measures, the emergency plan and the risk assessment.

To make the application valid, the carrier has to sign the form. A form signed for example by the BSO is not a valid application.

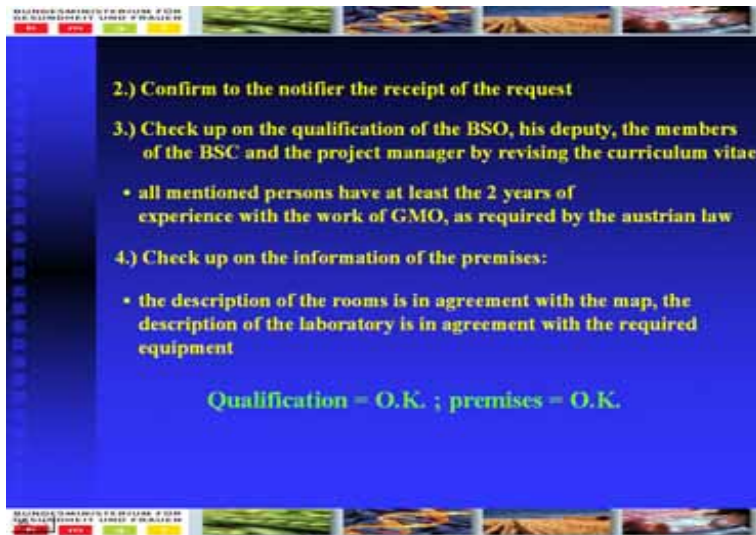
The BSC has to enable the intended work, which means that the internal BSC has to verify the classification into a biosafety level, before the application is send to the CA. For further work in biosafety level 1, where no notification is needed, the BSC has the responsibility that the intended work is correctly classified. This will be controlled by the Austrian CA during their inspections. If the minutes of the BSC are attached, the timeframe for the proceeding will be shortened.



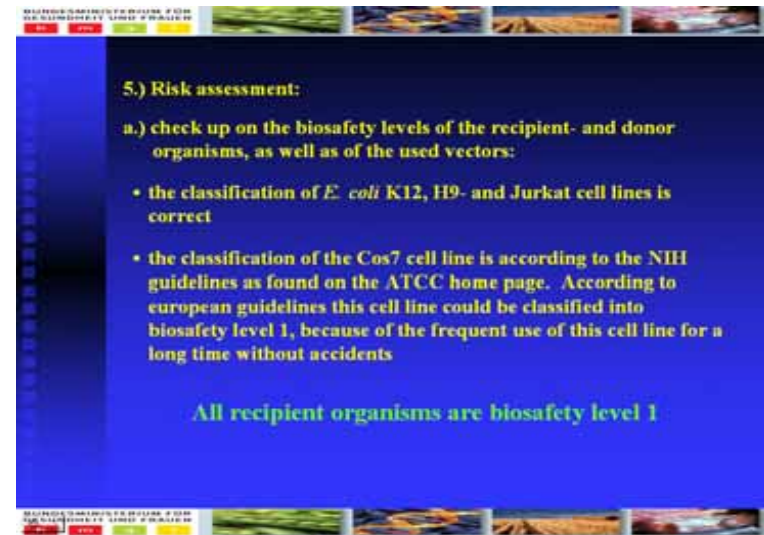
As it was mentioned before a complete application consists of the curriculum vitae of the BSO, the deputy of the BSO, the members of the BSC and the project manager, as well as of constructional drawings of the premises. Based on the curriculum vitae of the intended persons the CA has to evaluate if the required experience is given or not. The constructional drawings are need to verify if the needed containment can be handled at the premises.



As mentioned in the talk „Handling of Requests: Contained Use“, the first thing to do is to check up if the application is complete. In this case, all needed information is given, so the application is complete and the applicant will receive the confirmation of the receipt of the request.



The next step is to check up on the qualification of the BSO, the deputy of the BSO, the project manager and the members of the BSC. Since all named persons have at least 2 years experience in the field of the work with GMO all persons are fulfilling the requirements. The description of the rooms is in agreement with the constructional drawing and the equipment is sufficient to provide the required safety precautions needed for work in biosafety level 2.



The verification of the risk assessment starts with the check up on the stated biosafety levels of the recipient and donor organisms as well as of the used vectors. To look up for the classification of an organism into a risk group, national (when available) or international lists can be used. *E. coli* K12 is an approved biological safety strain of *E. coli*. He possesses some auxotrophy and can therefore not survive outside the lab. So the classification of *E. coli* K-12 as an biosafety level 1 organism is correct. The same is true for the H9- and Jurkat- cell lines.

A different case is the classification of the COS-7 Cells. If looking up at the ATCC classification, this cell line is classified into risk group 2,

because of the presence of a partial SV40-Genome integrated into the genome. This SV-40 DNA is replication defective. The „Zentrale Kommission für die Biologische Sicherheit (ZKBS)“ in Germany has classified these cell line into biosafety level 1, because of the frequent use without accidents for a long time. The deletion in the SV40 origin of replication seems to be stable. So COS-7 cells can be classified into biosafety level 1 in Europe, but will be classified into biosafety level 2 in the USA. This is just an example, that some classifications can be different in different countries.

The classification of the donor organism, HIV-1 into biosafety level 3 is correct. The classification of a vector into a biosafety level is mainly based on his properties. The vector pBR322 is internationally recognized as an approved biosafety vector, because he is not mobilizable. pBR322 is therefore classified into biosafety level 1. Therefore all derivates of pBR322 as for example pUC19, can be classified into biosafety level 1. The fictional vector pAB12, which was derived from pBR322 by inserting an eukaryotic promoter is also classified into biosafety level 1.

• 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 derivate 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:

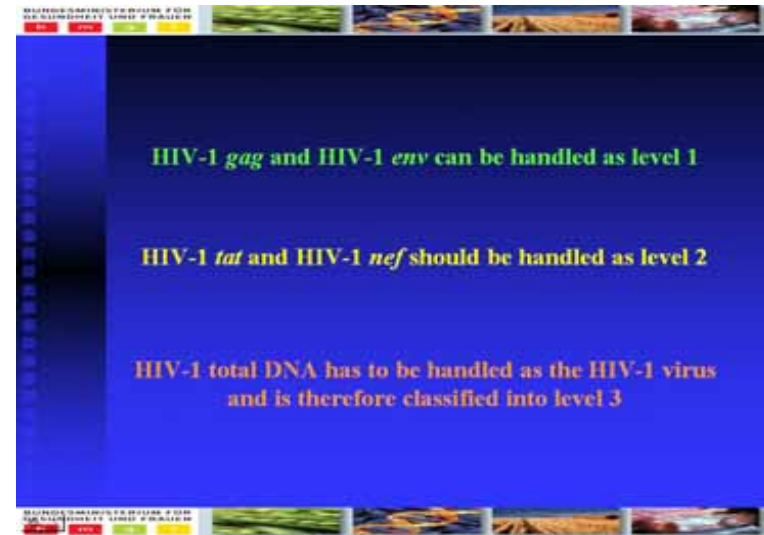
- HIV-1 *gag* and HIV-1 *env* are coding for structural proteins with no known potential hazardous effects
- HIV-1 *tat* is coding for an regulatory protein which is an transcriptional transactivator essential for HIV-1 replication. Possible hazardous effects can not be excluded
- HIV-1 *nef* is coding 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

The next important step in doing an risk assessment is to check up on the material and the resulting gene products, which are intended to use for the genetic manipulation. In this fictional case first four defined genes of the HIV-1 are intended to be cloned into the vector pAB12 followed by transformation of H9 and Jurkat cells. HIV-1 *gag* and HIV-1 *env* are coding for structural proteins. The sequence of this genes is known, and the resulting proteins are well characterized. It is known, that these structural proteins don't possess any hazardous effects. HIV-1 *tat* is coding for an regulatory protein which is an transcriptional transactivator, which is essential for HIV-1 replication. The sequence is known and the protein is well characterized, but possible hazardous effects can not be excluded.

HIV-1 *nef*, codes for an accessory protein which inter alia stimulates the infectivity of HIV-1 virions. The sequence is known and the protein is well characterized, and similar to HIV-1 *tat* possible hazardous effects can not be excluded.

In the second intended experiment whole genomic DNA of HIV-1 is intended to clone into pUC 19 and pBR322, respectively followed up by transformation of *E. coli* K12 and COS-7 cells, respectively. The whole sequence of HIV-1 is known and this virus is well characterized. It is known that the whole genomic HIV-1 DNA

possesses all the known hazardous effects of HIV-1. With this information, it is possible to classify the used DNA into biosafety levels.



The risk assessment of the used genes and their products leads to the following classifications. HIV-1 *gag* and HIV-1 *env* can be handled as biosafety level 1, because the sequence is known, the products are well characterized and it is known, that these proteins do not possess any hazardous effects. HIV-1 *tat* and HIV-1 *nef* should be handled as biosafety level 2, despite the fact that their sequences are known and the proteins are well characterized, because the possibility of

hazardous effects of these proteins can not be completely excluded. It does not mean, that these two proteins have to have hazardous effects, but according to the precautionary principle, these genes should be handled in an higher biosafety level. Last, the whole genomic DNA of HIV-1 is known and HIV-1 is well characterized. Therefore it is known, that HIV-1 whole genomic DNA possesses all the hazardous effects of HIV-1 and therefore the risk potential of the organism has to be fully included into the risk assessment which results in biosafety level 3.

Based on this considerations, the resulting GMO can be checked up. It is essential, to compare the resulting GMO with the donor- and the recipient organism. The classification of the GMO into a biosafety level is the result of the biosafety level of the recipient organism, after the genetic manipulation with material from the donor. This means, the genetic manipulation can lead to an upgrade, to a downgrade or to no change of the biosafety level of the recipient organism. The summary of the H9 and Jurkat cells containing a pBR322 derivate (pAB12) expressing HIV-1 *gag* and HIV-1 *env* leads to following conclusions. The sequences are characterized, there are no potential hazardous effects expected from these two proteins, the recipient organisms are classified into biosafety level 1 and the used vector can be considered as an approved biosafety vector. Therefore no higher risk of the GMO compared with the recipient is expected and these GMO can be classified into biosafety level 1.

c.) check up on the genetically modified organisms:

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

- The sequences of HIV-1 *gag* and HIV-1 *env* are characterised
- There are no potential hazardous effects expected from these two proteins
- 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-derivat (pAB12) expressing HIV-1 *tat* and HIV-1 *nef*

- The sequences of HIV-1 *tat* and HIV-1 *nef* are characterised
- Potential hazardous effects of these two proteins 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.

The summary of the H9 and Jurkat cells containing a pBR322 derivate (pAB12) expressing HIV-1 *tat* and HIV-1 *nef* leads to following conclusions. The sequences are characterized, potential hazardous effects can not be completely excluded from these two proteins, the recipient organisms are classified into biosafety level 1 and the used vector can be considered as an approved biosafety vector. Therefore less risk of the GMO compared with the donor, but higher risk of the GMO compared with the recipient is expected and these GMO should be classified into biosafety level 2.

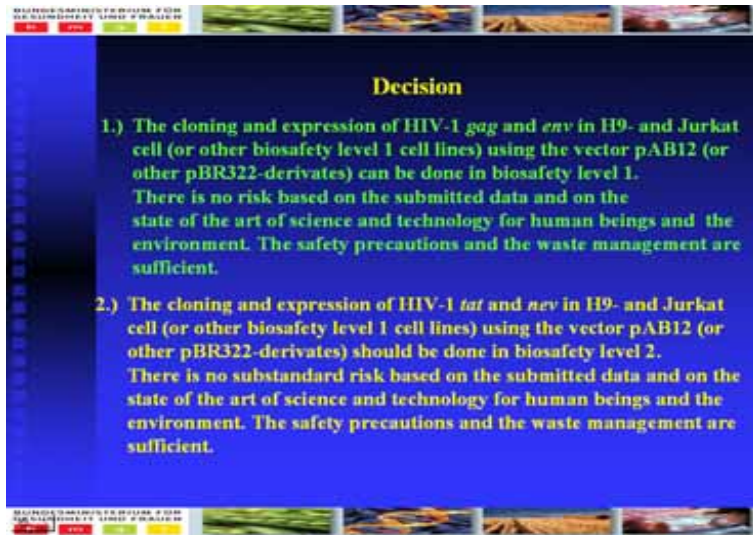
Cos 7 cells and *E. coli* K12 containing a pBR322-derivat (pUC19) containing the total HIV-1 DNA

- 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

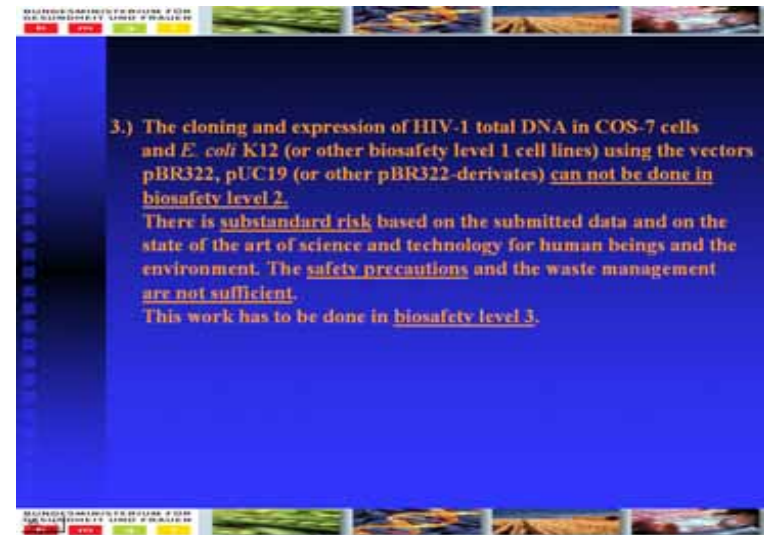
The summary of the COS-7 cells and *E. coli* K12 containing a pBR322 derivate (pUC19) containing the whole genomic HIV-1 DNA leads to following conclusions. The HIV-1 sequence is characterized, the hazardous effects are known, the recipient organisms are classified into biosafety level 1 and the used vector can be considered as an approved biosafety vector. In this case infectious particles of HIV-1 can be produced. Therefore, although an approved biosafety system is used, the risk potential of the donor organism has to be included completely into the risk assessment. There is the same risk of the GMO compared with the donor, and therefore much higher risk of the

GMO compared with the recipient. 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-derivates) 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-derivates) 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.

According to the risk assessment the CA will come to the following 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-derivates) 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-derivates) 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.