THE SINGAPORE BIOSAFETY GUIDELINES FOR RESEARCH ON GENETICALLY MODIFIED ORGANISMS (GMOs)

May 2021

TABLE OF CONTENTS

Section 6: IMPORT, EXPORT AND TRANSPORT FOR GMOs AND/OR GMO-DERIVED MATERIALS

APPENDICES

SECTION 1: INTRODUCTION

1.1 BACKGROUND

The Genetic Modification Advisory Committee was established under the purview of the Ministry of Trade and Industry to oversee and provide scientifically-sound advice on the research and development, production, release, use and handling of genetically modified organisms (GMOs) in Singapore

The objective of GMAC is to ensure public and environmental safety while maintaining an environment that is conducive for commercial exploitation of GMOs and GMO-derived products.
The responsibilities of GMAC are:

- Provide advice and recommendations concerning research, development, production, use and handling of GMOs;
- Review, monitor and provide advice on matters related to the release of GMOs into the environment;
- Inform the public, where deemed necessary, on planned releases of GMOs;
- Establish mechanisms for exchange of information with overseas agencies and facilitate the harmonization of guidelines with regional and international authorities;
- Facilitate public education and create awareness on GMO issues.

These guidelines were drawn up after a review of relevant guidelines, regulations and publications including those from Australia, the United States of America (USA), European Union (EU), World Health Organization (WHO) and United Nations Environment Programme (UNEP). A list of all relevant documents and references are listed in Appendix 1.

All communications with GMAC should be addressed to the Secretariat.

Secretariat, Genetic Modification Advisory Committee (GMAC)

Contact details: Please refer to https://www.gmac.sg/
Email: info@gmac.sg

1.2 OBJECTIVES OF GUIDELINES

These guidelines are established to ensure the safe containment, handling, transport and disposal of genetically modified organisms used in research and to provide a framework for the review and assessment of research.

1.3 SCOPE OF GUIDELINES

The scope of the Singapore Biosafety Guidelines for Research on GMOs covers experiments that involve the manipulation of genomic composition of all biological entities (cells, organisms, microorganisms, prions, viroids or viruses).

1.4 DEFINITIONS OF ABBREVIATIONS

For purposes of these Guidelines, the following abbreviations shall be defined as follows.

- “GMOs” refers to Genetically Modified Organisms
- “GMMs” refers to Genetically Modified Microorganisms
- “GMAC” refers to the Genetic Modification Advisory Committee of Singapore.
- “NParks” refers to the National Parks Board, Singapore
- “MOH” refers to the Ministry of Health, Singapore
- "NEA” refers to the National Environment Agency, Singapore
- "MOM” refers to the Ministry of Manpower, Singapore
- “BATA” refers to the Biological Agents and Toxins Act (Chapter 24A), regulated by the Ministry of Health
- “IBC” refers to the Institutional Biosafety Committee
- “NACLAR” refers to the National Advisory Committee for Laboratory Animal Research
- “BAC” refers to the Bioethics Advisory Committee

A detailed glossary of terms can be found in Appendix 12.

SECTION 2: PURVIEW - EXTENT AND EXEMPTIONS

2.1 EXTENT OF GUIDELINES

2.1.1 **These guidelines cover experiments that involve the experimental manipulation of genomic composition of all biological entities (cells, organisms, microorganisms, prions, viroids or viruses). These guidelines do not cover work involving human subjects (e.g. clinical trials).**

The categories of experiments which fall under these guidelines are described in Section 3. A list of other relevant documents is included in Appendix 1.

2.1.2 Commercial and non-research applications that involve the controlled intentional release of genetically manipulated organisms are not covered under these Guidelines but should adhere to the “Singapore Guidelines on the Release of Agriculture-Related Genetically Modified Organisms”. Kindly refer to the GMAC Website (http://www.gmac.sg/Index_Singapore_Guidelines_on_the_Release_of_Agriculture_Related_GMOs.html) for more information.

2.1.3 If an investigator is unsure whether their research proposal falls within these guidelines, a description of their proposed research should be submitted, in writing, to their Institutional Biosafety Committee(s) (IBC) for clarification, before the commencement of their research work.

The responsibilities of GMAC, IBCs and principal investigators (laboratory leaders) are defined in Section 5.

2.2 EXEMPTIONS

2.2.1 The following classes of experiments ((i) - (vii)) are exempt from the guidelines unless they fall within Categories A (regulated experiments with significant risks) or B (notifiable experiments with low risks) in Section 4. Such experiments exempt from the guidelines are classified as Category C (experiments with no significant risks, refer also to section 4.3).

(i) Experiments involving the fusion of mammalian cells which generate a non-viable organism, for example, the construction of hybridomas to make monoclonal antibodies.

(ii) Fusion of protoplasts between non-pathogenic microorganisms.

(iii) Protoplast fusion, embryo-rescue, *in vitro* fertilisation or zygote implantation in plant cells.

(iv) Use of Model Organisms[+], in which:

(a) the genetic modification involves knock-out, deletion and inactivation of genes; and/or

(b) the genetic modification using knock-in, activation, gene substitution and activation of genes in which the outcome does not confer any survival advantage to the animal and cause subsequent detrimental effects in human health and environment through the expression of toxins and/or human pathogens. These may include selectable reporter/marker genes such as fluorescent proteins.

[+]Please refer to the GMAC website link (https://www.gmac.sg/pdf/Research/List%20of%20GMAC-approved%20model%20organisms%202020.pdf) for the updated list of approved model organisms.

If further manipulations (e.g. breeding with genetic crossing) are performed on any of the above described genetically modified organisms, they may not be automatically exempted from the guidelines and could fall within Category B(i) (Risk Group 2) or Category A as assessed separately.

(v) Research involving the introduction of naked nucleic acids into plants or animals (other than humans), unless the nucleic acid is both recombinant and able to give rise to infectious agents.

(vi) Work involving the introduction of genetically manipulated somatic cells into animals, unless they are able to give rise to infectious agents.

(vii) Experiments involving approved host/vector systems (refer to Appendix 2 and List of Approved Host/Vector systems on the GMAC website) provided that the donor DNA:

- is not derived from plant or animal pathogens and that the DNA to be introduced is characterised fully and will not increase host or vector virulence;

- is derived from mammalian sources and is used to construct shot-gun libraries in an approved host/vector system mentioned in Appendix 2 and List of Approved Host/Vector systems on the GMAC website;

- does not code for a vertebrate toxin having a LD50 of less than 100 µg/kg;

- does not represent or comprise more than two-thirds of a viral genome, and is not being used in any experiment in which missing segments of the viral genome that are essential for infection are available in the host cell or will become available by further breeding processes.

2.2.2 All experiments, whether exempt or not, should be carried out under conditions of standard microbiological laboratory practice. When pathogenic organisms are used, appropriate containment levels should be used and the personnel should be properly trained and have had the recommended vaccinations as stipulated in the guidelines issued or recommended by regulatory authorities such as MOH and NParks e.g. Laboratory Biosafety Manual, 3rd Ed, World Health Organization.

2.2.3 Exemptions under Sections 2.2.1 (i) – 2.2.1 (vii) **do not apply** should the experiments involve intentional releases of genetically manipulated organisms, which include contained field trials. Such experiments must adhere to the Singapore Guidelines on the Release of Agriculture-related Genetically Modified Organisms.

2.2.4 Exemption from these guidelines does not equal exemption from statutory provisions applying to any aspect of a project involving genetic manipulation (e.g. importation, quarantine legislation).

2.3 WORK SUBJECTED TO OTHER AND/OR ADDITIONAL REGULATORY REQUIREMENTS

2.3.1 Work with GMOs derived from biological agents known to be hazardous to human health are regulated, under the BATA according to the classification of the organism. Large scale production of GMOs derived from biological agents known to be hazardous to human health may be regulated under the BATA. Large scale production refers to the production of 10 or more litres of biological agent at any one time.

2.3.2 Work in the field of human health such as gene therapy, or other genetic manipulations on humans involving stem cells, whole organs or individuals will be assessed by its designated agency(ies). These investigations include the introduction of nucleic acids (genetically manipulated or chemically synthesised and their derivatives), or genetically manipulated microorganisms, or cells into human subjects for the purposes of gene therapy, cell marking, or for stimulating an immune response against a subject's own cells, as used for the treatment of some cancers.

2.3.3 Research proposals where the introduction into human subjects of nucleic acids (genetically manipulated or chemically synthesised), or genetically

manipulated microorganisms, or cells, is designed to stimulate an immune response to antigenic determinants of infectious agents, as in the case of classical vaccine, should be submitted to the appropriate Bioethics committees. If necessary, advice from GMAC could also be obtained.

2.3.4 Vaccines which have been approved for use in Singapore, as well as the transfer of non-genetically manipulated autologous host cells, organ and tissue transplants are subjected to other relevant authority requirements. If necessary, advice from GMAC could also be obtained.

2.4 REGULATORY AGENCIES

The national agencies responsible for legislation which include relevant GM technology and GMOs in Singapore are:

- Regulation of laboratories dealing with GMO research, involving:
    a) Animal pathogens and plant pests – NParks
    b) GM microorganisms - MOH

- Importation of organisms including GMOs – NParks, MOH and NEA (Please also see Section 6)

- Certification or Inspection of Laboratories handling biological agents or toxins regulated under the BATA – MOH

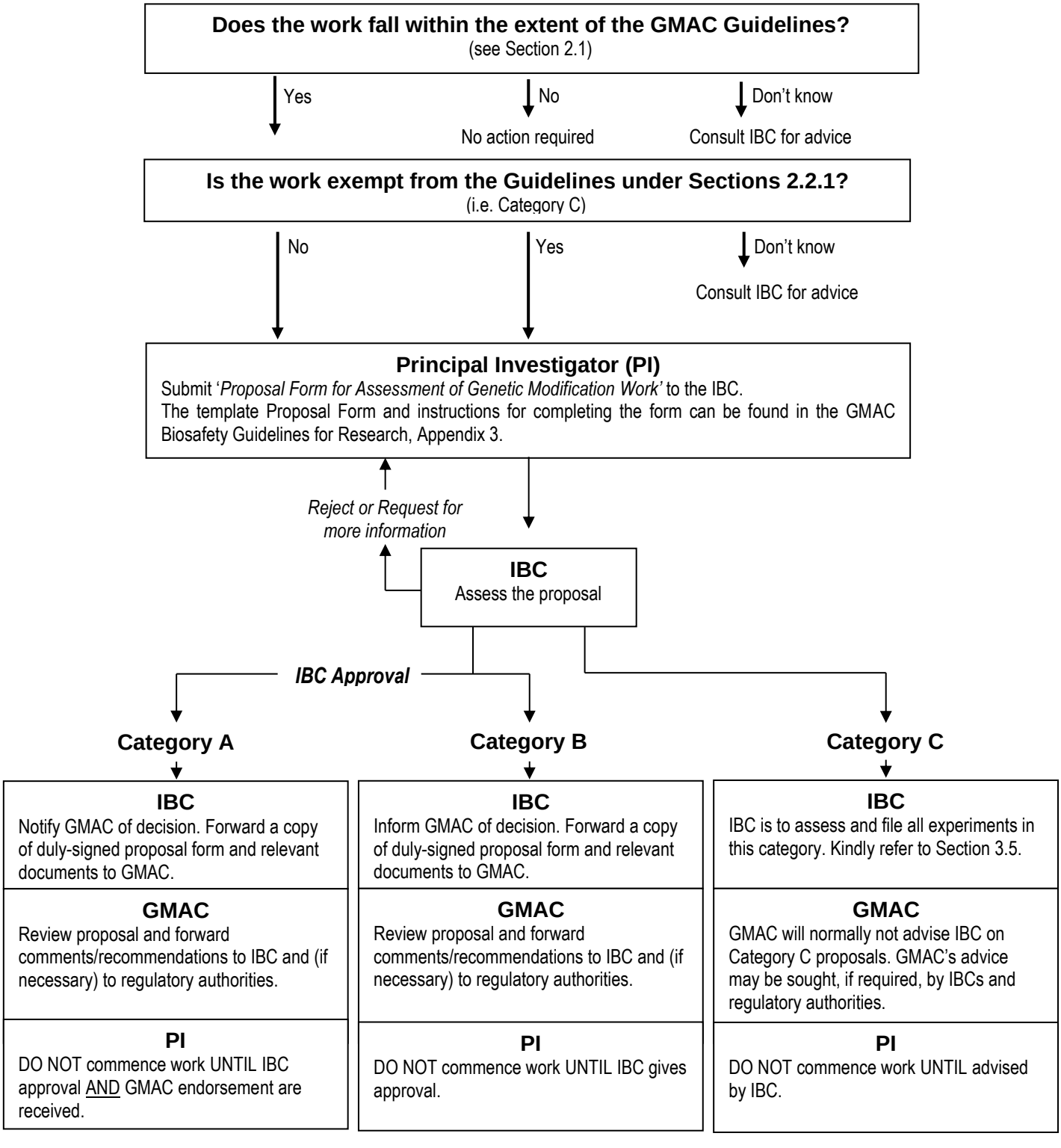
- Regulation of Workplace Safety and Health - MOM

The requirements of the regulatory bodies should be met; see Appendix 1 for the relevant local Acts.

SECTION 3: SUMMARY OF PROCEDURES

3.1 DECISION FLOW CHART FOR ASSESSMENT AND NOTIFICATION OF RESEARCH WORK

The relevant procedures for assessment and notification of research work involving genetic manipulation are detailed in Sections 3.3 – 3.5. A simplified Decision Chart is shown below for easy referral.



3.2 IMPORTATION OF GMOs FOR RESEARCH

Note that importation or possession of GMOs, not relating to human health, is regulated under Section 9 of the Animals and Birds Act (Cap 7). Additionally, importation or procurement to import specific biological agents and toxins, which is capable of causing death, disease or malfunction in a human, as those specified under First, Second, Fourth and Fifth Schedules, is regulated under the BATA.

Principal investigators intending to import GMOs for research should approach the IBC for assessment and the relevant regulatory agency for import approval. If necessary, the IBC and/or regulatory agency may seek GMAC's advice.

3.3 CATEGORY A – EXPERIMENTS REQUIRING IBC APPROVAL AND GMAC NOTIFICATION (REGULATED EXPERIMENTS WITH SIGNIFICANT RISKS)

Please refer to Section 4.1 for a description of experiments falling into Category A.

Experiments in this category **require both IBC approval and GMAC endorsement before starting**. Principal investigators **should not** start work on proposals assessed as Category A until advised by their IBC, after obtaining GMAC endorsement.

Principal Investigators intending to conduct experiments classified as Category A should submit a 'Proposal Form for Assessment of Genetic Manipulation Work' to their respective IBCs for assessment. The IBC shall assess the proposal and determine the appropriate working and containment measures and facilities necessary.

A proposal in this category should be submitted by the Principal Investigator to the IBC for assessment on a GMAC 'Proposal Form for Assessment of Genetic Manipulation Work'. The IBC should assess the proposal and determine the appropriate working and containment conditions. Upon approval, the IBC should forward the proposal to the GMAC secretariat, together with a summary of the IBC's recommendations or comments, for notification.

Instructions for filling out the forms are included in Appendix 3. When completing the forms and assessing the experiments, the IBC and the investigator should identify potential hazards and their types, and decide upon any special procedures necessary for the proposed experiments.

The GMAC Secretariat will return a copy of the GMAC 'Proposal Form for Assessment of Genetic Manipulation Work', carrying a GMAC case reference number, to the IBC within 10 working days to acknowledge receipt of the proposal. The case reference number should be quoted in all future correspondences relating to the proposal. The acknowledgment of receipt does not imply GMAC's endorsement of IBC's decision. Work must not commence until GMAC has released its endorsement to the IBC.

3.4 CATEGORY B – EXPERIMENTS REQUIRING IBC APPROVAL (NOTIFIABLE EXPERIMENTS WITH LOW RISKS)

Please refer to Section 4.2 for a description of experiments falling into Category B.

Experiments in this category **require assessment by the IBC** before starting. Principal investigators **should not** start Category B experiments until advised by the IBC.

Principal Investigators intending to conduct experiments classified as Category B should submit a 'Proposal Form for Assessment of Genetic Manipulation Work' to their respective IBCs for assessment. The IBC shall assess the proposal and determine the appropriate working and containment measures and facilities necessary.

The IBC shall forward a copy of the approved proposal form, together with a summary of the IBC's recommendations, to the GMAC Secretariat. GMAC will review and forward comments/recommendations to IBC and if necessary, to relevant regulatory authorities.

The GMAC Secretariat will return a copy of the GMAC 'Proposal Form for Assessment of Genetic Manipulation Work', carrying a GMAC case reference number, to the IBC within 10 working days to acknowledge receipt of the proposal. The case reference number should be quoted in all future correspondences relating to the proposal. The acknowledgment of receipt does not imply GMAC's endorsement of IBC's decision. Principal Investigators can commence work without waiting for GMAC's endorsement.

Should IBC be notified by GMAC Secretariat that a Category B proposal may be reclassified as Category A, the IBC should request PI to stop work whilst awaiting confirmation and endorsement from the GMAC Subcommittee. The GMAC Secretariat will copy the regulatory authorities in the correspondences with IBC. This will allow regulatory authorities to provide advice on any additional measures needed. Applicants may appeal to continue work subject to evaluation by GMAC on a case-by-case basis.

3.5 CATEGORY C – EXPERIMENTS EXEMPT FROM THE GUIDELINES (EXPERIMENTS WITH NO SIGNIFICANT RISKS)

Please refer to Section 4.3 for a description of experiments falling into Category C.

Experiments in this category are exempt from the guidelines and therefore, the 'Proposal Form for Assessment of Genetic Manipulation Work' does not need to be submitted to GMAC. However, experiments in this category still **require assessment by the IBC** before commencement. Principal Investigators are to inform IBC of their projects by forwarding the duly-signed proposal form and relevant documents.

Principal investigators who are unsure if their work falls under the exemptions in Sections 2.2.1 (i) – 2.2.1 (vii) should submit a proposal form to their respective IBCs for assessment. The IBC shall assess the proposal and determine the appropriate categorisation. Principal investigators **should not** start Category C experiments until advised by the IBC.

SECTION 4: EXPERIMENTS COVERED BY THE GUIDELINES

4.1 CATEGORY A – EXPERIMENTS REQUIRING IBC APPROVAL AND GMAC NOTIFICATION (REGULATED EXPERIMENTS WITH SIGNIFICANT RISKS)

This category includes experiments which may pose high risks to laboratory workers, the community or the environment. This category also includes experiments for which the type or level of hazard is unclear. The level of containment required will vary depending on the kind of experiments and their assessed hazard.

In general, experiments involving biological agents or toxins that are defined as First, Second and Fifth Schedules of the BATA and/or classified as Risk Group 3 and 4 of the WHO Laboratory Biosafety Manual will fall within Category A.

The facility shall meet the requirements stipulated for handling First, Second Schedule biological agent and/or Fifth Schedule toxin under the BATA, where applicable.

This category of work **requires IBC assessment and approval, followed by GMAC endorsement before work begins**. Principal investigators **shall not start work** on proposals assessed as Category A until advised by the IBC, following IBC's receipt of GMAC endorsement. Please refer to Section 3 for procedures for submitting proposal forms and obtaining GMAC advice.

The following classes of experiments fall within Category A:

A(i) Experiments with toxin producers:

- Experiments using DNA which encodes a vertebrate toxin having an LD50 of less than 100 µg/kg. Appendix 4 lists some toxins falling under this sub-category.

- Experiments in which toxin genes are expressed at a high-level, even if the LD50 is greater than 100 µg/kg. Experiments using uncharacterised DNA from toxin-producing organisms and, which therefore could contain toxin sequences also fall under this sub-category. However, experiments using DNA which has been fully characterised and shown not to code for a toxin, from a toxin-producing organism as donor, is not included in this sub-category.

A(ii) Experiments using viral vectors whose host range includes human cells, and where the viral vectors contain one or more inserted DNA sequences coding for a product known to be an oncogene or to be toxic to human cells. (Special conditions for working with viral vectors encoding oncogenes are given in Appendices 5 and 6.)

A(iii) Experiments involving introduction of DNA into microorganisms which can cause plant or animal (including human) diseases when used as host or vector, except:
(a) microorganisms listed as approved hosts or vectors (see Appendix 2); or

(b) If the DNA will not increase the virulence of the host or vector and the DNA is fully characterised, in which case it is classified as B(iii).

This sub-category does not include experiments using replication-defective viruses as host or vector. However, experiments using replication-defective vector/helper virus combinations which have the potential to regenerate replication competent recombinant virus are included in this sub-category.

A(iv) Introduction of pathogenicity genes into non-approved hosts. This sub-category includes those genes whose products are suspected of or have a risk of initiating autoimmune diseases.

A(v) Cloning or transfer of entire viral genomes, viroids, or fragments of a genome capable of giving rise to infectious particles with the capacity to infect human, animal or plant cells. Experiments involving cloning of less than two-thirds of an entire viral genome do not fall within this sub-category. Cloning of a viral genome which lacks a vital component of its replication or packaging activity that is not supplied by the experimental system, also does not fall within this sub-category.

A(vi) Experiments involving recombination between entire viral genomes, viroids and/or complementary fragments of these genomes, where one or more fragments encode virulence or pathogenic determinants. This sub-category includes experiments that could alter the host range of pathogens or increase pathogen virulence or infectivity.

A(vii) Experiments where a fragment of or the entire genome of a virus is injected into an embryo to produce a transgenic animal which secretes or produces infectious viral particles (see Appendix 8)

A(viii) Experiments not falling within the A sub-categories listed above or into Category B, but which fall within the extent of the guidelines (see Section 2).

A(ix) Experiments involving gene drives (systems that ensure biased inheritance by enhancing the likelihood a sequence of DNA passes between generations through sexual reproduction and potentially throughout an entire population)

4.2 CATEGORY B – EXPERIMENTS REQUIRING IBC APPROVAL (NOTIFIABLE EXPERIMENTS WITH LOW RISKS)

This category includes experiments which may pose low-level risks to laboratory workers, the community or the environment. These experiments require at least Biosafety Level 2 physical containment (laboratory, plant house, animal house, insectary, bird house or aquarium), as determined by the IBC and with reference to the Biosafety level detailed by the ATCC and CDC. Some experiments may require additional precautions or higher containment because the donor DNA or its components are hazardous or infectious, for example special containment features are needed for the housing of transgenic animals. Recommendations for procedures for GM-BSL2 and other containment levels are in the appendices (Appendix 7 *et seq*).

IBC assessment is required before work begins on this category of experiments. Principal investigators **shall not commence work** on proposals assessed as Category B until specifically advised by the IBC. Procedures for submitting proposal forms and obtaining IBC advice are in Section 2.

If the proposed experiments should fall within both Categories A and B, Category A classification shall take precedence i.e. Category A conditions shall be applied.

The following classes of experiments fall within Category B:

B(i) Experiments with whole animals (including non-vertebrates) which involve genetic manipulation of oocytes, zygotes or early embryos to produce a novel organism. For transgenic animal work, prior approval from the institution's bioethics committee is needed. (See Appendix 1 for other relevant documents, Appendix 8 on the relevant administrative procedures required by these Guidelines and transgenic animal facility requirements.)

B(ii) Genetic manipulation experiments involving the production of modified whole plants.

B(iii) Work with non-approved host/vector systems (i.e. other than those in the List of GMAC-approved host, vector systems) where the host or vector either:
(a) does not usually cause disease in plants, humans or animals; or
(b) is able to cause disease in plants, humans or animals but the introduced DNA is completely characterised and will not cause an increase in the virulence of the host or vector.

B(iv) Experiments with approved host/vector systems, in which the gene inserted is: (a) a pathogenic determinant; or (b) DNA that is not fully characterised from microorganisms which are able to cause disease in humans, animals or plants; or (c) an oncogene. (See Appendices 5 and 6 for work with oncogenes.)

Shot-gun cloning of mammalian DNA in approved host/vector systems does not fall into this category. Approved host/vector systems can be found at the GMAC website. Investigators may request to have new host/vector systems added to the list by making a detailed submission to GMAC through their IBC.

Note that experiments not falling within B sub-categories or in Category A, but falling under the Extent in Section 2, require GMAC advice and IBC approval (see sub-category A(viii)).

4.3 CATEGORY C – EXPERIMENTS EXEMPT FROM THE GUIDELINES (EXPERIMENTS WITH EXTREMELY LOW RISKS)

This category includes experiments which do not pose significant risks to laboratory workers, the community or the environment.

Experiments falling into this category include those classes of experiments as outlined in Section 2.2.1 (i) – 2.2.1 (vii).

Experiments in this category are exempt from the guidelines and therefore, the 'Proposal Form for Assessment of Genetic Manipulation Work' does not need to be submitted to GMAC. However, experiments in this category still **require assessment by the IBC** before commencement. Principal Investigators are to inform IBC of their projects by forwarding a duly-signed proposal form and relevant documents.

Principal Investigators who are unsure of the categorisation of their experiments are required to seek advice from their respective IBCs, by submitting a 'Proposal Form for Assessment of Genetic Manipulation Work'. The IBC shall assess the proposal and determine the appropriate categorisation status. Principal investigators **should not** start Category C experiments until advised by the IBC.

SECTION 5: ROLES AND RESPONSIBILITIES

5.1 INSTITUTIONS

Any institution, company or organisation that carries out genetic manipulation, imports organisms arising from such work, produces such organisms, or plans to sell or release such organisms into the environment, should abide by all existing legislation and relevant guidelines, especially current GMAC Guidelines.

Such institutions, companies or organisations are required to establish an IBC and provide the resources and facilities which are necessary for safe work in laboratories. The IBC should carry out its duties adequately, and ensure, by recruitment, procedures and other measures that adequate supervision of work occurs. Institutions may consider making compliance with the GMAC Guidelines a term of their employment contracts, if appropriate. Those Institutions conducting large scale or industrial scale work should have a Biological Safety Officer.

GMAC appreciates the difficulties that small institutions and companies may have in establishing an IBC. These institutions and companies can choose to be supervised by another IBC. Such arrangements should be formalised between the institutions concerned. A representative of the smaller institution should closely liaise with, or be a member of, the IBC.

The responsibilities of IBCs are described in Section 5.2. Institutions should recognise the essential roles of their IBC and give it the authority and support it needs to undertake its duties.

5.1.1 Recruitment and staff training

The institution should ensure that laboratory staff is informed of hazards and have adequate training to make sure that their work is carried out within these guidelines. The IBC Chair or Biological Safety Officer should be readily accessible to give advice.

5.1.2 Certification of Biosafety Level 3 (BSL3)

BSL3 facilities (laboratories, animal houses, plant houses, insectaries, bird houses, aquaria) are to be approved by the regulatory agency(ies). Researchers shall indicate the level of containment in the research proposal submissions to GMAC for experiments conducted in BSL3 facilities. The necessary advice regarding the structural requirements on BSL3 facilities are included in Appendix 9. Guidance may also be found in the Laboratory Biosafety Manual, 3rd Ed, WHO.

Appropriate door signs and universal Biohazard signs and labels are available from laboratory and reagent suppliers.

5.2 INSTITUTIONAL BIOSAFETY COMMITTEES

IBCs are vital for executing these guidelines and thus the monitoring and surveillance of genetic manipulation work. The calibre and experience of IBC members should be such that it can competently undertake its duties. The Chair of the Committee should be of sufficient standing in the institution for decisions and advice by the IBC to be effectively carried out. Appropriate deputising arrangements should be made when the Chair is on leave.

5.2.1 Where applicable, duties and obligations in relation to biological agents and toxins, as stipulated in the Part V of the BATA legislation must be adhered to.

5.2.2 Composition

The IBC should comprise:

- individuals with requisite knowledge and expertise to evaluate and oversee work being conducted in the institution;

- the Biological Safety Officer, if appropriate;

One microbiologist, and one molecular biologist and/or a geneticist, should be included as well as a scientist with expertise relevant to the organisms being studied in the institution. Different disciplines need only be represented when work falling within that area is performed in the institution. For example, an institution working only on plants need not have an animal geneticist.

Responsibilities may be combined in the same person if appropriate.

5.2.3 Biological Safety Officer

Institutions should either appoint a Biological Safety Officer or assign such duties to the IBC. The officer should ideally have experience in working with containment conditions and should be sufficiently trained and competent to offer advice on, or participate in staff training. Suitable deputising arrangements should be made when the officer is on leave.

The Biological Safety Officer or the IBC Chair should act as adviser to the head of the institution or company in all biosafety matters. Regular safety audits and the supervision of a regular evaluation programme for relevant pieces of equipment should be carried out by the Biological Safety Officer or the IBC. The Biological Safety Officer should also consult the WHO Laboratory Biosafety Manual for additional requirements that need to be fulfilled.

5.2.4 Conflicts of Interests

To avoid any potential conflicts of interest, IBC members should not assess their own proposals that they have submitted. The IBC should have sufficient scientifically qualified members to ensure that proposals can be adequately evaluated.

IBC members who may have commercial interests on an item of the agenda being assessed should declare their interests and be excluded from the meeting.

5.2.5 Monitoring of Work

The IBC should ensure that GMAC's and its own advice on proposals are received by principal investigator(s) and, if necessary, are acted upon. The IBC should visit laboratories and facilities occasionally to monitor biosafety aspects and implementation.

In order to effect the intent of these guidelines, an IBC may draft whatever rules it considers necessary to supplement these guidelines. Furthermore, IBCs should have appropriate powers to ensure that all aspects of these guidelines are observed.

5.2.6 Duties

The main functions of the IBC are to:

- assess all research proposals it receives, (including changes to Category C projects), so as to identify potential hazards to the researchers, the public and the environment. It should also advise the investigator(s) about these hazards and their management;

- ascertain the containment level and procedures for experiments falling within GMAC Categories A and B (see Section 4), and determine the storage and transportation requirements for genetically manipulated organisms falling within these Guidelines;

- IBCs are recommended to send a copy of the GMAC 'Proposal Form for Assessment of Genetic Manipulation Work', together with the IBC's assessment, to the GMAC for assessment of experiments falling under Category A and B, and make sure that GMAC advice is followed. For proposals falling under Category C, a copy of the proposal form should be sent to the IBC for assessment. Research proposals falling under Category C need not be submitted to GMAC. See Appendix 3 for instructions on completing proposal forms.

- inspect plant houses, animal houses, bird houses, insectaries and aquaria before they are used for genetic manipulation work. The IBC should also conduct inspections and monitor procedures in **all** the institution's containment facilities. At least annual inspections of these facilities should be carried out to make sure that they continue to meet the relevant containment standards. The detailed requirements for these facilities are in Appendices 7 *et se*q;

- monitor ongoing work within the institution from time to time and make recommendations to investigators, if appropriate;

- assess the qualifications and experience of personnel involved in research proposals, to make sure that they are adequate for good microbiological practice and the supervision of junior personnel;

- maintain a register of approved projects with their assessment as well as projects exempted under Sections 2.2.1(i) to 2.2.1 (vii) of these guidelines;

- maintain a list of the personnel who work in containment facilities, and ensure that new workers are familiar with the appropriate containment procedures and the correct use of laboratory equipment.

- take responsibility for drafting rules and making decisions about specific procedural safety matters. GMAC does not need to be consulted about these, as long as they are consistent with these guidelines.

5.2.7 Medical Surveillance of Workers

For personnel using GM-BSL2 physical containment facilities, GMAC advises that no special arrangements are necessary outside the normal institutional practices for laboratory workers.

Institutions doing microbiological research may take baseline serum samples from personnel. Such samples are stored for diagnostic tests on workers exposed to accidents or who develop unexplained illness.

For experiments requiring a physical containment level of GM-BSL3 or higher, laboratory workers should have an initial medical examination, and other requirements as stipulated under the WHO Laboratory Biosafety Manual.

5.2.8 Accidents and Incidents

The IBC or the Biological Safety Officer should record both the accident or incident and the follow-up action. MOH shall be notified as soon as possible of any adverse incident involving First, Second Schedule biological agent and/or Fifth Schedule toxin. If the IBC Chair is satisfied that the accident or incident was directly attributable to genetic manipulation work, and was significant, they should make a report to the relevant regulatory agency(ies). IBCs are encouraged to keep GMAC informed during the process. An example of such an incident could be the intentional failure to comply with these guidelines, or an incident which might have risked human health or the environment. Under the WSHA, it may be required for such incidents to be reported to the MOM and/or relevant regulatory agencies.

5.3 PRINCIPAL INVESTIGATORS

The Principal Investigator should be thoroughly familiar with the requirements of these guidelines and should ensure that, the guidelines are adhered to, for any project he/she is responsible and for which, involves the use of genetic manipulation. Specifically, he/she should:

- assess the proposal to decide if it falls within the guidelines. If unsure, the investigator should consult the IBC;

- provide all information on the proposal that the IBC may need for assessment or monitoring of the proposal;

- follow through on GMAC's and IBC's advice and recommendations;

- fill out a typed original GMAC ‘Proposal Form for Assessment of Genetic Manipulation Work’ and hand a copy (keeping a second copy) to their IBC, before starting work on any project which falls under these Guidelines, and ensure that work does not commence until approval is granted by the IBC;

- submit a new proposal form to the IBC before any major change is made to the experimental system of a proposal, which may result in a change of category or which may affect the exempt status of Category C projects.

- conduct experiments under the conditions of physical containment approved by regulatory agency(ies) and IBCs in the case of Category A proposals (see Section 4);

- ensure that students, maintenance workers, subordinates, and other co-workers are aware of the nature of potential hazards of the work and have been given relevant training. They should also arrange for training, if needed;

- inform the IBC of any changes to the project team;

- promptly report accidents, unexplained illnesses and absences to the IBC;

- advise the IBC when intending to import biological material(s) falling within these guidelines (see also Section 6.7).

SECTION 6: IMPORT, EXPORT AND TRANSPORT FOR GMOs AND/OR GMO-DERIVED MATERIALS

6.1 GENERAL CONSIDERATIONS FOR TRANSPORT AND PACKAGING OF MATERIAL

6.1.1 Basic Requirements

The basic requirement for transport of viable genetically modified organisms is that there should be a minimal risk to humans, animal, plants and the environment.

Risk is minimized through a) proper packaging that will withstand rough handling and contain liquid material within the package without leakage to the outside; b) labelling of the package with the biohazard symbol and other labels to alert the workers in the transportation chain to the hazardous contents of the package; c) documentation of the hazardous contents of the package should such information be necessary in an emergency situation; and d) training of workers in the transportation chain to be able to respond appropriately to emergency situations.

The samples should be transported in packaging that corresponds to their respective GMAC risk categories. The recipients should have facilities to contain the organisms at the required level.

6.1.2 Transport Requirements

For transport within an institution, standard operating procedures (SOP) implemented by the facility operator should include risk assessment and packaging according to risk category. Generally, GMOs of categories A and B that are transferred out of a containment laboratory must be triple packaged in sealed unbreakable containers or bags.

For transport outside an institution, procedures must have been set up for the safe transport of biological materials by air, rail and road. Packaging and transport arrangement should correspond to its risk level. Risk levels are categorised according to GMAC risk classification- Category A, B and C (See Section 4).

We do not recommend shipping animals deliberately infected with Category A and B agents between institutions. Though the risk of escape is low, the outcome could be severe if there was damage to a crate and animals escaped.

It is the responsibility of the sender and the recipient to make sure that all packaging and transport regulations are followed.

The transport of biological materials is controlled by the following regulations. The latest version should be consulted:

- The IATA *Dangerous Good Regulations* are comprehensive and incorporate the many provisions of the other regulations.

The IATA *Live Animals Transport Regulations* is the worldwide standard for transporting live animals and arthropods; they aim to ensure that both safety and animal welfare are addressed during transportation.

The International Air Transportation Association (IATA), *Dangerous Goods Regulation*s and *Live Animals Transport Regulations*; (IATA online store – http://www.iata.org/)

- The Biological Agents and Toxins (Transportation) Regulations

  In Singapore, the BATA regulates transportation of specified biological agents and toxins covered under the legislation (MOH Biosafety Branch- https://www.moh.gov.sg/docs/librariesprovider7/about-bata-documents/bata_transportation_regulation.pdf).

6.1.3 Risk Classification

For the purpose of transport, biological materials are classified into different risk categories as follows:

Table 1: Risk Classification for transport of GM biological agents

Risk Classification	**Characteristics of Biological Materials**	**Packaging Specifications**	**Regulatory Bodies**
Category A	GMOs and GMMs that 1) are infectious and pathogenic to humans and/or animals and plants; 2) pose high risks to operators, the community or the environment; and/or 3) are biological agents or toxins defined as First, Second and Fifth Schedule of the BATA and/or are defined as Risk Group 3 and 4 (WHO Laboratory Biosafety Manual).	Infectious materials should be transported in packaging that complies with the IATA Dangerous Goods Regulations. For live GM animals/arthropods, they may only be transported in packaging that complies with Container Requirement 62 of the IATA Live Animals Regulations. Transportation of all schedules of biological agents and toxins of the BATA shall comply with the packaging and transportation requirements stated in the Biological Agents and Toxins Act and BATA Transportation Regulations.	National Parks Board (NParks), Plant Science and Health – For Plants; Animal & Veterinary Service (AVS) – Industry & Biosecurity Management Division; Ministry of Health (MOH), Biosafety Branch; National Environment Agency (NEA), Environmental Health Department.

Category B	GMOs and GMMs that 1) are non-infectious and non-pathogenic to humans and/or animals and plants; 2) pose low-level risks to operators, the community or the environment; and/or 3) are defined as Risk Group 1 and 2 (WHO Laboratory Biosafety Manual).	GMMs and GMOs should be transported in packaging that complies with the IATA Dangerous Goods Regulations. For live GM animals/arthropods, they may only be transported in packaging that complies with Container Requirement 62 of the IATA Live Animals Regulations. Transportation of all schedules of biological agents and toxins of the BATA shall comply with the packaging and transportation requirements stated in the Biological Agents and Toxins Act and BATA Transportation Regulations.	National Parks Board (NParks), Plant Science and Health – For Plants; Animal & Veterinary Service (AVS) – Industry & Biosecurity Management Division; Ministry of Health (MOH), Biosafety Branch; National Environment Agency (NEA), Environmental Health Department.
Category C	GMOs and GMMs that 1) are non-infectious and non-pathogenic to humans and/or animals and plants; 2) do not pose significant risk to operators, the community or environment; and/or 3) are defined as Risk Group 1 and 2 (WHO Laboratory Biosafety Manual).	May be subjected to the provision of the transport regulations.	National Parks Board (NParks), Plant Science and Health – For Plants; Animal & Veterinary Service (AVS) – Industry & Biosecurity Management Division; Ministry of Health (MOH), Biosafety Branch.

Packaging, labelling and transportation requirements should comply with the BATA and BATA (Transportation) Regulations, which are available on the MOH Biosafety Website.

Packaging of imports transported via air shall adhere to the packaging instructions of the International Air Transport Association (IATA). The above regulations detail: the certification requirements; the maximum quantities that can be transported by cargo or passenger aircraft; the external labelling requirements (including the identifying UN number); and the details to be included in the Shipper's Declaration for Dangerous Goods. Please refer to the International Air Transportation Association (IATA) for packaging specifications.

6.1.4 Documentation

When infectious material is being transported, a Shipper's Declaration for Dangerous Goods must be completed indicating information including origin, contents and date of dispatch, and should be placed in a separate leak-proof bag so as to protect the declaration form from potential contamination by the contents of the package. Recipients should be informed, before delivery, of all known hazards associated with the material.

6.1.5 Labelling

The package must be labelled (according to IATA standards) to clearly show the name, address and contact details of the persons responsible for the materials, so that the person can be contacted should the package be lost, damaged or misdirected.

6.1.6 General

- Only trained personnel may undertake the packaging for transport which should be done according to the above regulations.

- Facilities should be provided for after-hours delivery of samples, and all staff including night staff should be warned of any hazards.

- Procedures and precautions for unpacking should be appropriate to the type of package being unpacked.

- When infectious waste is removed from a laboratory, waste should be disposed of by waste disposal contractors licensed by NEA and in accordance to NEA guidelines on disposal of such waste.

6.2 TRANSPORT OF GENETICALLY MODIFIED MICROORGANISMS

6.2.1 General considerations

- All genetically modified microorganisms should undergo risk assessment (See Table 1) to determine its risk category.

- The transport and packaging of any First, Second, Third, Fourth Schedule biological agents and Fifth Schedule toxins regulated in the Biological

Agents and Toxins Act (BATA) in Singapore shall comply with the BATA and the Biological Agents and Toxins (Transportation) Regulations. No person shall transport or procure the transportation of any biological agent or toxin under the BATA within Singapore by mail or public transport.

- Transport of biological agents via air shall adhere to the specified packaging requirements under the IATA Dangerous Good Regulations.

- Both sender and addressee need to ensure that no sample vials/canisters containing GMMs are missing in the delivery process. It is the responsibility of both sender and addressee to exercise diligence **respectively** in checking the number of sample vials/canisters containing the GMMs tally at the point of delivery to that received.

6.2.2 Packaging and Transport Requirements

- Transgenic or genetically modified microorganisms (GMMs) to be transported must be wholly contained inside a watertight, leak-proof, sealed, unbreakable primary container packed in a secondary container and finally packed into a rigid outer container.

- Sufficient quantity of absorbent materials should be wrapped around the primary container to absorb all fluid (if any) in the event of a breakage of or leakage from the primary container.

- The packaging should be sufficiently strong to withstand any impact which the package would normally be subject to during the transportation, loading and unloading.

6.2.3 Labelling

- The outermost container must be labelled to clearly show the species/origin of the GMM, the name, address and contact details of the person responsible for the dealings, so that the person can be contacted should the package be lost, damaged or misdirected.

- A biohazard label must be attached to at least the outermost container holding any GMOs which fit into the classification of Cat A and B, and C if it contained biological agent regulated under the BATA.

6.2.4 Treatment of containers after transport

Please refer to Appendix 10 for disposal and decontamination requirements.

Any materials transported with the GMMs must be either retained with the organisms under containment or decontaminated after transport has occurred.

6.3 TRANSPORT OF TRANSGENIC ANIMALS

6.3.1 General considerations

In making transport arrangement for transgenic animals, two principles are paramount:

- the need to prevent the animals from escaping, to ensure that transgenic animals will not interbreed with feral populations; all reasonable scenarios such as accidents should be considered*;*

- the need to ensure that the animals are properly identified, that they arrive at the intended destination, and that a competent laboratory animal professional with experience in handling transgenic animals takes delivery of them. Accounting procedures should be instigated to make sure that all animals sent are delivered - whether dead or alive.

We do not recommend international shipping of animals deliberately administered with infectious agents, as crates are unattended during flights and may be damaged during in-flight turbulence.

6.3.2 Packaging and Transport requirements

- The IBC should formulate general rules to meet these conditions.

- Animal boxes should comply with IATA standards. Modifications can be made to the boxes especially for pathogen-free animals. The boxes must be escape-proof and allow easy observation during an import inspection without opening the box.

- Transport of all animals should also adhere to the NACLAR Guidelines on the Care and Use of Animals for Scientific Purposes.

6.4 TRANSPORT OF TRANSGENIC ARTHROPOD AND THEIR PATHOGENS

Genetically manipulated arthropods (including live arthropods and arthropod cell cultures infected with genetically manipulated pathogens and microorganisms)

6.4.1 General Considerations

- Transport of live transgenic arthropods requires packaging that prevents the escape of the arthropods, maintains their viability and protects personnel in the transportation chain from exposure to the contents.

- The selection of transport packaging begins by establishing the phenotypic change in the arthropod and/or microorganism due to genetic modification, and potential impact of escaped transgenic arthropods.

- Transport packaging is directly correlated with its risk of infection, pathogenicity and transfer of transgenes when handled by operators in the transport chain and in the event of accidental release.

- Accounting procedures should be instigated to make sure that the same number of organisms and containers that are sent are delivered.

6.4.2 Packaging and Transport Requirements

- The IBC should formulate general rules to meet these conditions.

- Transport packaging should prevent leakage/escape of the contents; unbreakable, able to tolerate pressure changes and other conditions incident to ordinary handling in transportation.

- It is recommended that this packaging consist of three layers of containment; a sealed primary receptacle surrounded by padding enclosed in a solid, transparent, unbreakable secondary container with the lid tightly secured and lastly in an appropriate transport container.

- If aquatic stages are transported, the container should also contain sufficient absorptive material to absorb and contain all of the water.

- IATA Live Animals Regulation 26th Edition (LARs) describes containers that are appropriate for the shipment of arthropods (See Container requirements 62 of LARs).

- Upon arrival, the arthropods should immediately be transferred from the holding container to a new container.

6.4.3 Labelling

- The outermost container should bear a ‘biohazard label’ and must be labelled to clearly show the species, strain/origin of the transgenic arthropods.

- The label must include the name, address and contact details of the person responsible for the transgenic arthropods, in order that the person can be contacted should the package be lost, damaged or misdirected.

6.4.4 Treatment of containment after transportation

Please refer to Appendix 10 for disposal and decontamination requirements.

- After transfer of the transported arthropods into new containers, all of the transport material should be decontaminated by autoclaving or incinerated.

6.5 TRANSPORT OF TRANSGENIC PLANTS

6.5.1 Packaging and Transport requirements

- Vegetative transgenic plant material should be transported within and between institutions in a primary container (for example, a plastic bag for cuttings, an envelope for seeds), which is packed in another unbreakable container.

- Whole transgenic plants should be netted and deflowered and all seed or fruit removed before transport. Plants may be transported in pots, contained in boxes or crates.

6.5.2 Labelling

- The outer container should be labelled to indicate that it contains transgenic plant material, and the label should include the telephone number of a person to contact should the package be lost or damaged. Labels on seed packets should include the number of seeds being transported.

6.5.3 Documentation

- Accounting procedures should be instigated to make sure that the same number of plants and containers that are sent are delivered.

6.6 SUPPLY OF GENETICALLY MANIPULATED MATERIAL TO OTHER RESEARCH WORKERS

6.6.1 Investigators supplying people in Singapore with genetically manipulated material shall make sure that recipients who are unfamiliar with the genetic manipulation field are made aware of the existence of these guidelines and of the need to observe them. If the genetically manipulated materials fall under the First, Second and/or Fifth Schedule of the BATA, MOH shall be notified of the transfer of the materials.

6.6.2 When genetically manipulated animals are supplied to investigators overseas, the requirements of the export legislations of Singapore must be met. After obtaining an NParks/AVS export licence, the exporter should apply for an export permit (strategic goods export permit if the export is subject to Strategic Goods (Control) Act) through TradeNet® (Link can be found on URL: https://www.customs.gov.sg) prior to exportation.

For strategic goods export procedures:

Singapore Customs

Contact details: https://www.customs.gov.sg/
Email: customs_stgc@customs.gov.sg

For export of laboratory animals:

National Parks Board
Animal & Veterinary Service
Industry & Biosecurity Management Division

Contact details: Please refer to https://www.nparks.gov.sg/avs
Email: animal_feedback@nparks.gov.sg

To ensure Singapore's responsibility as an exporting country, individuals or organisations exporting genetically manipulated organisms must include the following information to authorities in the recipient country.

- details of the risk assessments that have been carried out in Singapore, and the conditions under which the organism has been approved for use in Singapore (e.g. contained use in a BSL2 laboratory, or small scale field trial under specified conditions);

- any information known to the sender about possible adverse effects of the organism in the recipient country. (In many cases, a disclaimer that no assessment has been made of potential effects in the recipient country may be suitable.)

6.7 IMPORT OF GENETICALLY MANIPULATED ORGANISMS OR MATERIAL

6.7.1 Importation or possession of GMOs and other animal-related pathogens, not relating to human health, is regulated under Sections 8 and 9 of the Animals and Birds Act (Cap 7), Part IV of the Control of Vectors and Pesticides Act (Cap 59) and Second Schedule of Control of Plants (Plant Importation) Rules.

6.7.2 Importation or procurement to import specific biological agents and toxins, which are capable of causing death, disease of malfunction in a human, such as those specified under the First, Second, Fourth and/or Fifth Schedules, is regulated under the BATA.

6.7.3 Work with imported genetically manipulated viral genomes, microorganisms, arthropod, plants, animals or animal-related pathogens fall under the extent of these Guidelines. Investigators should seek the approval of the relevant regulatory agency for intended import and submit a proposal for assessment if appropriate. Regulated organisms are stated in the NParks Bio-security Assurance Arrangement Guidelines and the list of regulated animal-related pathogens[a] of NParks/AVS. The import of any arthropod vectors capable of transmitting infectious diseases is regulated in the Infectious Diseases Act (Cap 137) of NEA.

6.7.4 Permission should be obtained from the relevant agency to import all biological materials, including transgenic microorganisms, plants, animals and arthropods. After obtaining the permission from the relevant agencies, the importer should apply for an import permit through TradeNet® (Link can be found on URL: https://www.customs.gov.sg/). Permission can be obtained by

[a] Available at: https://www.nparks.gov.sg/avs/pets/bringing-animals-into-singapore-and-exporting/veterinary-biologics/import-licence-requirements-for-pathogens

submitting an 'Application for Import Permit' to the appropriate quarantine officer given on the form. The form is available from:

For plants or plants-related pathogens and pests:

National Parks Board
Plants Science and Health – For Plants

Contact details: Please refer to
https://www.nparks.gov.sg/services/plant-health-services
Email: https://www.nparks.gov.sg/feedback

For animals or animal-related pathogens and pests:

National Parks Board
Animal & Veterinary Service
Industry & Biosecurity Management Division

Contact details: Please refer to https://www.nparks.gov.sg/avs
Email: animal_feedback@nparks.gov.sg

For Human related pathogens:

Ministry of Health
Biosafety Branch
Public Health Group

Contact details: Please refer to https://www.moh.gov.sg/biosafety
Email: MOH_BioSafety@moh.gov.sg

For vectors[b] (arthropod or rodent) carrying or causing any disease to human beings:

National Environment Agency (NEA)
Central Licensing Unit

Contact details: Please refer to http://www.nea.gov.sg
Email: contact_NEA@nea.gov.sg

6.7.5 The information provided should contain any relevant information about the genetically manipulated material which falls within these Guidelines. In particular, the following information should be included if appropriate:

- details of the donor organism, which is the DNA source and characteristics of the genes transferred;
- the method or vector used to transfer the DNA to the host organism;
- host organism;
- complete details should be provided if a plant or animal is infected with a genetically manipulated microorganism.
- Risk assessment on import, handling and transport of the genetically manipulated material.

[b] "Vector" means any arthropod, including its egg, larva and pupa, and any rodent, including its young, carrying or causing, or capable of carrying or causing any disease to human beings.

6.7.6 If appropriate, the relevant agency may make the information provided on this form available to the GMAC, or to other assessors, and may request specific information about any aspect of the genetically manipulated material. GMAC's assessment of the Application to Import will be accelerated if a small-scale proposal for the work has already been received.

APPENDICES

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APPENDIX 1 : OTHER RELEVANT DOCUMENTS

Documents relevant to and mentioned in the guidelines are listed below.

1. Relevant Local Legislation

The requirements of the regulatory bodies should be met. All local legislation is available on the Singapore Statutes Online [https://sso.agc.gov.sg/]

1.1. Animal and Birds Act (Cap 7)
1.2. Biological Agents and Toxins Act (Chapter 24A)
1.3. Control of Plants Act (Cap 57A)
1.4. Workplace Safety and Health Act 2006 (Act 7 of 2006)

2. Relevant Local Guidelines

2.1. Singapore Guidelines on the Release of Agriculture-Related GMOs, GMAC

The publication is available from:

- *GMAC Secretariat or*
- *As a downloadable PDF from the GMAC website at https://www.gmac.sg*

2.2. Guidelines on the Care and Use of Animals for Scientific Purposes, NACLAR

The publication is available from:

- *The National Advisory Committee for Laboratory Animal Research NACLAR Secretariat*
  As a downloadable PDF from https://www.nparks.gov.sg/-/media/avs/migrated-content/animals-and-pets/animal-health-and-veterinarians/naclar-guidelines.pdf?la=en&hash=8CA0D838280D125EE463D32588F54F96B924734B

3. Worldwide guidelines specific to genetic manipulation

3.1. Australia

i. Guidelines for Certification of Facilities/Physical Containment Requirements
ii. Guidelines for the Transport, Storage and Disposal of GMOs

3.2. European Commission

i. Safety Considerations for Biotechnology 1992, OECD, Paris, 1992

Publication (i) above is available from:

- *OECD Publications Service*
  2 rue André-Pascal
  75775 Paris, Cedex 16
  France

3.3. United Kingdom

i. The Scientific Advisory Committee on Genetic Modification (SACGM) Compendium of guidance, The Health and Safety Executive, UK

3.4. United States of America

i. Guidelines for Research Involving Recombinant DNA Molecules, National Institutes of Health, Department of Health and Human Services, USA

3.5. World Health Organization

i. Laboratory Biosafety Manual, 3rd edition, World Health Organization, 2004, (ISBN 92-4-154650-6); relevant to the Biological Agents and Toxins Act

Publications of the WHO can be obtained from:
Marketing and Dissemination, World Health Organization
20 Avenue Appia, 1211 Geneva 27, Switzerland
Email:bookorders@who.int

4. Other relevant guidelines, regulations and publications

i. PICS Guide for Good Manufacturing Practice for Medicinal Products 2009

ii. Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, NHMRC, CSIRO, Australian Research Council, 2013, ISBN Online: 1864965975

iii. National Statement on Ethical Conduct in Human Research (2007)

iv. Infection Control in the Health Care Setting - Guidelines for the Prevention of the Transmission of Infectious Diseases, April 1996

v.	AS 2243: 2005	Safety in laboratories
vi.	AS/NZS 2243.1: 2005:	Safety in laboratories: Planning and operational aspects
vii.	AS/NZS 2243.3: 2010:	Safety in laboratories: Microbiological safety and containment
viii.	AS 2252:	Biological safety cabinets

ix.	AS 2252.1: 2002:	Biological safety cabinets (Class 1) for personnel and environment protection
x.	AS 2252.2: 2009:	Controlled environments – Biological safety cabinets classes I and II – Installation and Use
xi.	AS 2647:	Biological safety cabinets - installation and use, 1994
xii.	AS 2982: 2010	Laboratory design and construction
xiii.	AS/NZS 2982.1: 1997:	General requirements
xiv.	AS 1324:	Air Filters for use in air conditioning and general ventilation
xv.	AS 1324.1: 1996:	Construction
xvi.	AS 1324.2: 1996:	Tests
xvii.	AS 1807.6: 2000	Cleanroom, workstations, safety cabinets and pharmaceutical isolators – Methods of test: Determination of integrity of terminally mounted HEPA filter installations, 2000

xviii. The requirements manual for agricultural chemicals

xix. The requirements manual for veterinary chemicals

xx. Vaccination of Laboratory Workers Handling Vaccinia and Related Poxviruses Infectious for Humans, 1990, Advisory Committee on Dangerous Pathogens and Advisory Committee on Genetic Modification, ISBN 0-11-885450-X

xxi. HIV - the Causative Agent of AIDS and Related Conditions, January 1990 Advisory Committee on Dangerous Pathogens

xxii. Biosafety in Microbiological and Biomedical Laboratories, 5th edition, Washington DC, 2009, US Department of Health and Human Services

xxiii. Collins C H, Laboratory Acquired Infections: History, Incidence, Causes and Preventions, 4th edition, 2000, ISBN 0-750640235

xxiv. *Convention on Biological Diversity,* June 1992, United Nations Environment Programme

xxv. Arthropod Containment Guidelines (Version 3.1)

APPENDIX 2: LIST OF GMAC-APPROVED HOST/VECTOR SYSTEMS

BIOLOGICAL CONTAINMENT

The objective of biological containment is to minimise both the survival of the host and vector outside the laboratory, and the transmission of the vector from the propagation host to a non-laboratory host. Please kindly refer to the GMAC website link (https://www.gmac.sg/pdf/Research/List%20of%20GMAC-approved%20host,%20vector%20systems%202021.pdf) for the updated list of host/vector systems.
This Appendix lists the host/vector systems currently approved by GMAC as providing a level of biological containment.

Note:
The approved host/vector systems may also be used in experiments where DNA is inserted into the host cell without the use of a biological vector (for example, by mechanical, electrical or other means), provided that the DNA:

- is not derived from microorganisms able to cause disease in humans, animals or plants, unless the DNA to be introduced is fully characterised and will not increase the virulence of the host or vector;

- does not code for a toxin for vertebrates with an LD50 of less than 100 µg/kg, and is not an oncogene;

- does not comprise or represent more than two-thirds of the genome of a virus and is not being used in an experiment in which the genetic material missing from the viral genome and essential for producing infection is available in the cell into which the incomplete genome is introduced, or made available by subsequent breeding processes.

- Any commercially available Host-Vector Systems.

Such a system with an approved host and the DNA meeting these conditions would constitute an approved host/vector system for the purposes of these Guidelines, and hence would fall under exemption section 2.2.1.

APPENDIX 3: INSTRUCTIONS FOR COMPLETING GMAC PROPOSAL FORMS

The Institutional Biosafety Committee will use the information provided in the GMAC 'Proposal Form for Assessment of Genetic Manipulation Work' to determine the category into which the project fits and the containment level. GMAC will use the information in the form to assess proposals falling into Category A and Category B. In order to enable GMAC and the IBC to carry out those functions, a clear statement of what the applicant proposes to do is needed, and if this cannot easily be fitted into the space provided, a separate description shall be attached.

GMAC's endorsement will be valid from the date of receipt of the endorsement for the total number of years corresponding to project duration as indicated in question 9 of the GMAC proposal form. For example, if the PI indicates that the duration of experiment is 3 years, the expiration would occur in 3 years' time from the date on which IBC receives the endorsement from GMAC.

If there are no changes made to the purpose of the project, protocol, vectors and transgenes used in a previously endorsed proposal that has expired, the PI shall submit a declaration to the IBC, indicating "no changes in purpose, genes of interest, vectors, and protocols to the project". The IBC Chair shall endorse the PI's declaration and forward the correspondence to GMAC. The correspondence will be used to extend the endorsement administratively; without risk assessment by GMAC.

The following suggestions are intended to ensure that GMAC has sufficient relevant information to make a prompt decision on the application.

Title of Project and Aims

When describing the aim of the proposal, include a brief description of the main steps involved. If both immediate and long term broader aims are included, make clear which component of the work needs IBC approval (or GMAC advice) now.

If the project is complex and likely to take several years to complete, it will help if the work to be undertaken first is described separately. If recombinant DNA is ultimately to be inserted into whole animals or plants, or into bacteria not listed in Appendix 2 of these Guidelines, it may well be that approval can be given in the first instance for cloning and characterisation of specific genes or other DNA, with approval for later stages being possible only after such characterisation. If the stages are made clear to the Committee, approval or advice for the first stage may be given to enable the work to start.

If the intention is to import biological material which falls under the Guidelines, the title may read 'Intention to import...'.

For projects using the same Project Titles, IBCs should introduce reference numbers to distinguish the proposal forms.

Source of DNA

If the DNA has already been cloned, please give details of the construct: e.g. who made it, how it was made, and what is known of its properties.

If several genes or species etc. are to be used, list all of them, because one proposal may cover them all. For example, if appropriate, request advice for chickens, ducks and other avian species. This will alleviate the need for repeated applications.

Details of the transgene such as the gene name, origin of the gene, targeted site of delivery of gene etc. should be provided for assessment to understand if there may be potential changes in host range, virulence, resistance etc. Whole transcripts of the gene are not required.

Host Organism

If more than one host is to be used, particularly if different containment levels apply, clarify when and how each is to be used. Comments made above on dividing a project into stages may be relevant. Please also complete the supplementary information form for experiments involving whole plants.

Vectors

Make the description for prokaryotic vectors as broad as is necessary to cover the intended work. For example, specify 'non-conjugative plasmids such as pBR322 and pUC9' if the project is likely to require a range of specific vectors as the project progresses. If only pBR322 and pUC9 are requested, the approval will be limited to the two vectors and will not cover the many closely related vectors which may turn out to be more useful.

The description of vectors should comprise more than a series of letters, symbols and numbers. Some description of their properties is also required.

In the case of retroviral vectors, be specific and indicate what is known about their properties, and provide details of the construct, if appropriate, on a separate sheet of paper, including a genetic map and/or a description of its construction.

Assessment on DURC

Researchers will find in the GMAC proposal form a question on DURC for experiments involving microorganism/virus/toxins. The purpose of the question is to screen for possible dual use research of concern for the research project.

According to the WHO[3], dual use research of concern (DURC) is life sciences research that is intended for benefit, but which might easily be misapplied to do harm. The possibility that dual use research might result in misuse, either intentionally or accidentally, is a long-standing concern of science. The issues are broad and encompass not only research and public health, but also security, scientific publishing and public communications, biotechnology and ethics and wider societal issues.

As GMAC has an established review process, it is appropriate to include a screen for such concerns within its proposal form. As such, this question serves as a checklist for DURC that is relevant to research work involving GMOs.

Please also find a relevant document for more information on DURC, "United States Government Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern":
https://www.phe.gov/s3/dualuse/Documents/oversight-durc.pdf

[3] Definition of DURC available on WHO website: https://www.who.int/csr/durc/en/

Assessment of Potential Hazards

The increasing range of hosts and vectors being used makes it imperative for every applicant to assess assiduously any potential hazards associated with the proposed work and to discuss such hazards and their containment in the proposal. Failure to do so may delay assessment until after additional information has been sought and obtained from the applicant.

Details of Personnel

For the ‘full details’ requested, please include the extent and nature of relevant experience on a separate sheet of paper. These details need not be sent to GMAC but should be checked by the IBC.

Appendix I

Please complete the table to assess if the HIV-based lentiviral vector system used suffices the revised criteria of HIV-based lentiviral vector system under the BATA Fourth Schedule. Information required includes the HIV-based lentiviral vector names, features of the vector system according to the BATA Fourth Schedule and supporting documents, if any. Upon completion of the table, please indicate if the HIV-based lentiviral vector system fits to be regulated under BATA Fourth Schedule.

Proposal Form

A copy of the proposal form is attached to this Appendix. The proposal form is self-duplicating and allows for sufficient copies to be made for GMAC, the IBC and the principal investigator’s records.

PROPOSAL FORM FOR ASSESSMENT OF GENETIC MANIPULATION WORK

GMAC Ref No.: _____
(For official use only)

Name of Principal Investigator: _____

Name of Institution : _____

Experiment Risk Group (please check the appropriate box):

☐ Category A ☐ Category B ☐ Category C

A. Experimental detail (attach separate sheet if necessary)

1.	Project title (Please provide reference numbers for projects with the same title.)
2.	Research unit involved
3.	Experimental objective
4.	Rationale for the experiment

5. Scope of experiment – involvement of

- ☐ Microorganisms and/or viruses
- ☐ Toxins
- ☐ Animals
- ☐ Cells
- ☐ Others, please specify: _____

Note: For experiments that involve animals, cells and/or others, please proceed to 6 and the rest, but skip 7 & 8. For experiments that involve microorganisms, viruses and/or toxins, please skip 6 and proceed to the rest of the form. For experiments that involve multiple experimental organisms (i.e. animals/cells together with microorganisms/viruses/toxins), please proceed to fill up all relevant questions. For experiments that involve HIV-based lentiviral vectors, please also fill up the table in Appendix I.

6. Project with experiment involving animals/cells/others

a. Can the modification result in a predictable change of the following:

i) Increased oncogenicity:

☐Yes

☐No

ii) Potential to change natural microbiome/ecology of the organism:

☐Yes

☐No

Details, if "yes":

b. Description of gene(s) involved, gene construct(s) and intended experimental host system.

c. Method of gene delivery (bacteriophage, vectors, breeding, injection, biological delivery vehicle/carrier etc.)

7. Project with experiment involving microorganisms/viruses/toxins

a. Name of microorganism/virus/toxin: _____

b. Is the microorganism/virus/toxin listed under the BATA List of Biological Agents and Toxins, and/or a potential human pathogen?

i) If Yes, provide the BATA Schedule: _____
ii) If No, provide the risk grouping (for biological agent): _____

c. Brief description of gene modification on the microorganism/virus:

i) Gene(s) involved and gene construct(s) and intended experimental host system *(if chimeric microorganism is created, please specify the backbone and the inserted genes)*: __

ii) Natural host of microorganism/virus: _____

iii) Method of gene delivery (transformation, conjugation, vectors, breeding, injection, biological delivery vehicle/carrier etc. For retroviral vectors, please specify the (viral) origin, and also note all safety features included in the constructs. For HIV-based lentiviral vectors, please specify the BATA Schedule of HIV lentiviral vectors):

_____

8. For dual use research of concern (DURC)

(This section is to screen for DURC relating to research work involving GMOs-microorganisms/viruses/toxins. Kindly fill up the following questions to the best of your abilities.)

Is there a reasonable possibility that the modification might result in a change of the following?

(If unsure, choose "Yes" and explain under Details).

i) Increase in host range:
☐Yes
☐No
ii) Increased virulence:
☐Yes
☐No
iii) Immunogenicity:
☐Yes
☐No
iv) Increased toxicity:
☐Yes
☐No
v) Increased transmissibility/ability to disseminate:
☐Yes
☐No
vi) Increased drug resistance:
☐Yes
☐No
vii) Enhanced susceptibility of host population:
☐Yes
☐No
viii) Increased stability:
☐Yes
☐No
ix) Potential to change natural microbiome/ecology of the organism:
☐Yes
☐No

Details, if "yes" to any of the above:

(e.g. if the resultant product has increased drug resistance, please provide info on the extent of the resistance, and if there is still effective drug or treatment for infected individuals)

9. Duration of the experiment

☐1 year

☐2 years

☐3 years

10. The proposed work will be performed in the following biocontainment level:

☐BSL1/ABSL1

☐BSL2/ABSL2

☐BSL2+/ABSL2+

☐BSL3/ABSL3

11. Measures to ensure containment, safe handling, storage and disposal

12. For organism/microorganism

a. Experimental GMO Material to be obtained from (please note requirements for import permit for importation of biological agents and/or toxins regulated under the BATA):

b. Anticipated date of transfer or receipt:

13. PI's declaration:

☐ I declare that the above information is accurate and complete based on risk assessment, and to the best of my knowledge.

☐ I recognise that the actual risk may defer from the assessed risk. I will continue to monitor the risk of the project and the genetically modified organism. Should the risk assessment change with respect to 8 and/or 10, I will stop work immediately and notify the IBC, which will then notify MOH and GMAC.

Submitted by PI:

_____ _____ _____

PI's Name and Signature **Appointment / Laboratory** **Date**

Contact Details

Address : _____

_____

Business Tel Number : _____ Fax Number : _____

Business Email : _____

Reviewed by IBC:

_____ _____

IBC Chairman's Name and Signature **Date**

.....

The following section is applicable for Category A experiments only:

Please indicate if approval has been sought from relevant regulatory authority (MOH/NEA/NParks) for *use/possession/import/transport of the GMO.

If yes, please provide copy of document or reference.

If no, please explain why.

**(highlight where applicable)*

For a list of regulatory contact points, please refer to Section 6.6 and 6.7 (page 26-28).

Appendix I

Please complete the table below to assess if the HIV-based lentiviral vector system (HIV LVS) used suffices the revised criteria of HIV LVS under the BATA Fourth Schedule.

A HIV LVS that possesses at least 2 of the following features is classified under the Fourth Schedule of the BATA:

I. the U3 region of the 3'LTR in the transfer vector is absent or altered, which results in a stable self-inactivating (SIN) configuration;
II. the HIV genes for packaging function are split to a minimum of 2 packaging plasmids (excluding the env plasmid);
III. the vpr, vpu, vif and nef genes are either absent or altered to be non-functional; and
IV. the vector system requires minimally 4-recombination to achieve Replication Competent Lentivirus (RCL)

S/N	HIV-based Lentiviral Vector System (include vector names)	Features incorporated in vector system	Supporting Documents	Suffice HIV LVS criteria regulated under BATA Fourth Schedule
1	Example- XXX Vector System Transfer vector – ABC plasmid Packaging vector 1 – 123 plasmid Envelope vector – XYZ plasmid	Feature I Feature III	Attachments	Y
2	Example- BBB Vector System Transfer vector – XYZ plasmid Packaging vector 1 – 456 plasmid Envelope vector – RST plasmid	Feature I only	NA	N

SUPPLEMENTARY INFORMATION FORM
FOR EXPERIMENTS INVOLVING WHOLE PLANTS
(Attach separate sheet if necessary)

1. Are the experimental plant noxious weeds or closely related to species which are noxious weeds?

   If 'yes', please elaborate:

2. Are the microorganisms/fungi etc. involved in this work known to be harmful to humans, animals or plants?

   If 'yes':

   a) Give further information about the harmful agent:

   b) Detail the known and likely transmission modes (including carrier insects) for this agent:

3. Are the genetically manipulated plants to be grown?

   If 'yes':

   a) What developmental stage will they reach?

b) Describe the techniques to be employed to contain plant materials (including pollen, seeds, spores, vegetative materials) during and at the completion of the experiments.

c) What is the proposed method of disposal of plant materials at the conclusion of the experiment?

4. a) Is soil or soil substitute to be used? (Specify.)

b) How will it be sterilised?

5. Describe the facility to be used for cultivation of the plants. Include information such as location, proximity to containment laboratory etc.:

6. Give any additional information which may be relevant to the assessment of this work:

APPENDIX 4: TOXINS

For work involving toxins that fall under Fifth Schedule of the BATA, prior approval for its use must be sought from the Ministry of Health.

Fifth Schedule Agents (as of 6th October 2011)

1. Botulinum toxins (Types A, B, C, D, E, F and G)
2. *Clostridium perfringens* toxins
3. Staphylococcal Enterotoxin
4. Shigatoxins
5. T-2 Toxin
6. HT-2 Toxin
7. Tetanus Toxin
8. Verotoxins

Section 4.1, Category A(i), of these Guidelines requires that, for work involving the cloning of genes for toxins (or uncharacterised DNA from organisms that synthesise toxins for vertebrates) with an LD50 of less than 100 µg/kg, the IBC seek a recommendation from GMAC before giving approval for work to commence.

Below is a list of a number of toxins with an LD50 of less than 100 µg/kg. The list is not exhaustive. If it is not known whether a substance should be regarded as toxic, GMAC advice shall be sought.

SOME TOXINS WITH AN LD50 OF LESS THAN 100 µg/kg[4]

Abrin
Bacillus anthracis lethal factor
Bordetella pertussis toxin

Cholera - see *Vibrio cholerae*
Clostridium botulinum toxins
Clostridium perfringens epsilon toxin
Clostridium tetani toxin
Corynebacterium diphtheriae toxins

Escherichia coli heat labile (LT) enterotoxin and LT -like toxin
Oxygen-labile haemolysins such as streptolysin O
Yersinia pestis murine toxins
Pseudomonas aeruginosa exotoxin A

Ricin
Shigella dysenteriae toxin
Staphylococcus aureus determinants A, B and F, alpha and beta toxin, exfoliative toxin
Vibrio cholerae toxin and toxins neutralised by antiserum monospecific for cholera toxin (e.g. heat labile toxins of *E. coli*, *Klebsiella* and other related enterotoxins)
Yersinia enterocolitica heat stable toxin

[4] Information derived from the NIH Federal Register, Vol 51, No 88, May 1986 (Appendix F) and information provided by the NIH Office of Recombinant DNA Activities.

APPENDIX 5: GUIDANCE FOR WORK INVOLVING GM VIRUSES FOR GENE TRANSFER INTO ANIMAL & HUMAN CELLS IN A LABORATORY SETTING

1. General Considerations

Genetic modification of viruses is a common practice in medical research laboratories to study the biology of the viruses. Viruses are also used as efficient vehicles for gene transfer into animal and human cells.

The hazards associated with the use of genetically-modified viruses depend on the following:

(a) The virus' host range;
(b) Its ability for repeated rounds of infection;
(c) Its competence for replication inside the cell;
(d) The possibility of generating replication-competent virus from replication-defective vectors by recombination (e.g. in the case of retroviruses);
(e) The ability of the genetic material of the virus to be integrated into the chromosome of the infected cell;
(f) The stability of the virus inside the cell and exposed in the environment;
(g) The means of transmission of the virus (e.g. through aerosol or skin abrasions and other physical contact);
(h) The nature of the introduced DNA sequences or its encoded protein.

2. Approval and Consultation

Principal Investigators should seek the clearance from IBC prior to the start of any experimentation with genetically-modified viruses.

Exhaustive references for the Biosafety Level and Guidelines with regards to the use of specific animal and human-infectious viruses shall be obtained by the Principal Investigator prior to the start of the project. The following organisations provide good references for specific animal and human viruses:

(a) American Biological Safety Association (ABSA)
www.absa.org
(b) Center for Disease Control (CDC)
www.cdc.gov
(c) American Tissue Culture Collection (ATCC)
www.atcc.org

3. Procedures for handling rodent and other non-human viruses

Viruses that are capable of infecting **ONLY animal (non-human and non-primate)** species are not considered to be associated with any hazard to the manipulator or other laboratory personnel. Practices associated with good tissue culture technique will be adequate in containing and handling these viruses. While the risks associated with the use of these viruses are considered negligible, solutions and contaminated cells shall be decontaminated/autoclaved before disposal to prevent accidental infection of other animal cell lines.

4. Procedures for handling viruses that can potentially infect human cells ('human infectious' viruses)

The primary hazard associated with the use of live recombinant viruses that have the capacity or that could potentially infect human cells lies on the type of the viruses and the nature of the introduced genetic sequences. Primate-infectious viruses shall be considered "potentially human-infectious". For all human-infectious viruses handled in a class II biological safety cabinet, the primary hazard to the scientist is the possibility of infection by viruses through broken skin brought about by needles and other commonly used sharps. The potential danger to other laboratory personnel depends on the stability and infectivity of the virus in the extracellular environment and the nature of the introduced genetic material.

For all human-infectious or potentially human-infectious viruses, the major requirement is for good virological and tissue culture practice on the part of the scientist with regard to the following precautions:

(a) A facility of containment of level BSL2 or higher (as determined by the IBC and with reference to the Biosafety level detailed by the ATCC and CDC) will be required. All manipulations shall be conducted in a class II biological safety cabinet or equivalent. Only one individual shall use the cabinet at any one time.

(b) Laboratory gowns, gloves and face mask shall be worn, as appropriate PPE during manipulations with recombinant human-infectious viruses.

(c) Dishes and plates of cells containing human-infectious viruses shall be placed on a larger tray or a secondary container to provide traps for accidental spills.

(d) All pipettes, glassware and plasticware shall be decontaminated with an efficacious chemical disinfectant or autoclaved before disposal, taking into considerations sharps disposal requirements.

(e) For viruses which are able to persist in the environment (e.g. adenovirus, vaccinia virus, hepatitis virus, papillomavirus), decontamination and bagging of waste should be done within the biosafety cabinet prior to removal and autoclaving. Care should be taken to ensure that the amount of material held in the biosafety cabinet is minimised, in order to avoid interference with the air flow in the cabinet.

(f) Mouth pipetting is strictly prohibited.

(g) Open flames that could interrupt the air-flow in the biosafety cabinet should be avoided.

(h) The use of sharp instruments (sharps) such as syringe needles, glass pipettes, razors, scissors and surgical knives, wherever possible, should be avoided, since skin abrasions represent the most likely portal of entry to the body.
Where the use of sharps is unavoidable, these instruments shall be placed in separate biological disposal receptacles and sterilized before disposal.

(i) Tissue cultures infected with human-infectious or potentially human-infectious viruses shall be kept in specially dedicated incubators.

(j) Likewise, frozen stocks of human-infectious or potentially human-infectious viruses should be kept in specially designated and clearly marked liquid nitrogen tanks and freezers. Laboratory personnel who leave the laboratory for new employment shall

ensure that these materials are either discarded or entrusted to another worker. A central register shall be maintained which includes a record of stored cell lines and human-infectious viruses. Principal Investigators are responsible for providing information for the register and maintaining a record of the viruses and infected cell lines used in their laboratories. The maintenance of a central register for the institution/ company/organisation is the responsibility of the IBC.

(k) Great care shall be taken to decontaminate spills immediately. The correct disinfectant to use in any given situation depends on the organism being handled and is the responsibility of the Principal Investigator in charge of the work to select an appropriate disinfectant. After each session, work surfaces shall be wiped down with an appropriate disinfectant. Where a biosafety cabinet/laminar flow hood has been used for handling amphotropic retroviruses, subsequent use with non-amphotropic viruses can be undertaken following appropriate decontamination.

(l) Only trained individuals shall be permitted to handle human-infectious recombinant viruses. It is the responsibility of the Principal Investigator to ensure the proper training of personnel in consultation with the IBC.

(m) Under no circumstances should investigators be infecting cultures of their own cells, or of their immediate relatives, or those of other members of the laboratory.

(n) Before beginning work with human-infectious (genetically manipulated) viruses where vaccination with the corresponding virus is regarded as an effective means of preventing subsequent infection (e.g. vaccinia, hepatitis), investigators shall be vaccinated.

5. Infection of animals with recombinant viruses

(a) Viruses unable to infect human cells

Viruses of this group are not considered hazardous and accordingly good animal handling practices are appropriate. Infected animals shall be kept in separate cages and be held in the biohazard room separate from non-infected animals. Infected animals should be clearly marked. If possible, the use of micro-isolators should be encouraged to prevent cross-infection. Precautions should be taken to avoid animals escaping and coming into contact with other animals. All waste generated from animals of this group shall be autoclaved before disposal.

(b) Viruses with the capacity to infect human cells

Animals infected with human-infectious viruses shall be kept in a separate cage which is clearly labelled as containing the particular virus in question. They should be kept in a separate biohazard room from non-infected animals. The main risk is to the handler who shall take great care to avoid being scratched, bitten or exposed to aerosols. Gloves, face mask and protective clothing must be worn to avoid direct contact with tissue and body fluids. Work area should be lined with absorbent protective material which shall be changed regularly. Only trained staff shall handle these infected animals under the supervision of the Principal Investigator. Precautions should be taken to prevent the infected animals from escaping and coming into contact with other animals. All waste should be autoclaved prior to disposal.

6. Gene therapy

In projects where viruses are being used to deliver genes to human subjects (gene therapy), the approval from the relevant institutional ethics committees and the Medical Clinical Research Committee (Ministry of Health) should be obtained, unless the therapy has already been established by the MOH as an approved treatment. A separate guideline governs the control of virus production and safety testing procedures in gene therapy experiments.

APPENDIX 6: PROCEDURES FOR WORK WITH HAZARDOUS FRAGMENTS OF DNA

Note for Category B (iv), Section 4.2

When working with isolated DNA molecules or amplifying DNA molecules using techniques such as the polymerase chain reaction (PCR), there are some cases where caution is warranted. Some degree of risk may exist and the extent of this is uncertain.

Such cases include:

1. DNA which encodes an active oncogene product or tumour suppressor gene product, particularly when this is associated with a gene promoter with high activity in human cells. DNA containing more than one active oncogene is associated with increased risk.

2. DNA encoding growth factors, their receptors or other substances that might directly or indirectly alter the growth patterns of human cells.

3. DNA or RNA representing complete viral genomes or fragments with the potential to regenerate live virus. Complete genomes for HIV or papilloma viruses, for example, warrant careful handling.

There is some risk that such molecules could enter the cells of the operator, the principal routes of entry being through breaks in the skin. It is therefore recommended that work of this type be carried out using gloves in order to avoid skin contact. Special care shall be taken when using needles or other sharp instruments.

Precautions for handling genetically manipulated viruses with the capacity to infect human cells are described in Appendix 5.

APPENDIX 7: REQUIREMENTS FOR BIOCONTAINMENT AT BIOSAFETY LEVELS 1, 2 and 3

Please refer to the requirements for Biosafety Levels in

(a) the Laboratory Biosafety Manual (3rd Edition, WHO) at https://www.who.int/csr/resources/publications/biosafety/en/Biosafety7.pdf;

(b) the Biosafety in Microbiological and Biomedical Laboratories (5th Edition, CDC) at https://www.cdc.gov/biosafety/publications/bmbl5/; and

(c) the WHO Biorisk management: Laboratory Biosecurity Guidance at https://www.who.int/ihr/publications/WHO_CDS_EPR_2006_6/en/

The following appendices provide additional guidance not covered by the requirements in the WHO Manual referenced above. This is for work with microorganisms, animals, fish and other aquatic organisms and plants.

Guidance should be sought prior to commencement of research work that does not fall under these categories.

Principal investigators and research personnel shall be trained to assess the risks (both safety and security) involved for the experiments and organisms and apply the appropriate biosafety and biosecurity measures at the correct biocontainment level. For guidance on biosafety requirements for genetic manipulation of microbes, plants and animals, please refer to the biosafety levels which are appropriate. IBCs shall provide guidance on the appropriate facility for the experimental work conducted. The sub-sections define the additional requirements for facilities that deal with such organisms. Please note that some research facilities for genetic manipulation of microbes, plants and animals require regulatory approval from MOH, NParks (from April 2019) and NEA in Singapore.

APPENDIX 8: BIOSAFETY REQUIREMENTS FOR BIOSAFETY LEVEL 2

For facilities handling Genetically Modified Microorganisms/Viruses:

1. All technical procedures shall be performed in a way that minimises the creation of aerosols. Sonication shall be done in an enclosure to minimise the release of aerosols.
2. Materials shall be spun down or allowed to settle after vortexing to reduce aerosols. Equipment used for handling cultures or contaminated materials which is not readily steam sterilised shall be chemically disinfected after use.
3. Culture spills shall be dealt with immediately, and the spill area decontaminated properly.
4. Culture incubators and culture shakers shall be regularly maintained, cleaned and chemically decontaminated. Sticky mats, if used in culture incubators/shakers, shall be replaced regularly to prevent spills. Non-breakable containers are to be used for culturing of infectious agents to prevent breakage or spillage.

For facilities handling Genetically Modified Animals:

Genetic Manipulation work which involve the use of animals include:

- Introduction of DNA into the fertilized oocyte or zygote or early embryo or which may be carried out in or involve whole animals
- Introduction of a fragment of the whole genome or a virus into an embryo to produce a transgenic animal secreting infectious viral particles.
- Use of genetically modified microorganisms to inoculate animals

All investigators and IBCs responsible for genetic manipulation work which involve transgenic animals shall be familiar with the requirements of the NACLAR Guidelines on the Care and Use of Animals for Scientific Purposes and laboratory biosafety.

Personnel must receive training in the handling of animals to be used and an appropriate standard of work supervision must be maintained.

Researchers are responsible for laboratory animal care and maintaining basic welfare standards (References: Guidelines for the Care and Use of Animals for Scientific Purposes, NACLAR 2004; Guide to the Care and Use of Experimental Animals, Canadian Council on Animal Care). Researchers should also be aware that they are subject to Singapore Laws that legislate against cruelty to animals. This is contained in Part IV of the Animals and Birds Act (Cap. 7).

Infrastructure

1. A double door vestibule is recommended for the prevention of escape of animals.
2. Windows are discouraged in the construction of the facility, but if present, shall be sealed and resistant to breakage.

Operating Procedures

1. Personnel entering the facility shall be trained in the handling of the type of animals housed within the facility. An immunisation programme shall be in place for all staff working in the facility.
2. IACUC and IBC approval shall be obtained prior to conducting work in the facility.

3. Cages shall be disinfected chemically and/or autoclaved if used with infectious material.
4. Cages are to be non-breakable to prevent animal escape.
5. Autoclaving is recommended for all animal materials prior to disposal.
6. Breeding and experimental facilities are to be kept separate to avoid cross contamination.
7. A clear inventory of the animals housed in the facility shall be kept and maintained by authorised personnel in charge of animal care.
8. Accidental releases should be reported immediately and remedial action taken should be documented and maintained.
9. Equipment transferred into and out of the facility shall be disinfected prior to transfer.
10. A rodent control programme shall be put in place to prevent entry and escape of pests at the facility.

For facilities handling Genetically Modified Arthropods[5]:

Genetic Manipulation work which involve the use of arthropods include:

- Work with genetically engineered insects or with insects that contain genetically engineered pathogens. These work involve all life stages of the arthropods; embryo lines, eggs and larvae are included.

Infrastructure

1. The facility shall be separated from areas that are open to unrestricted personnel traffic.
2. A double door vestibule is recommended for the prevention of arthropod escape.
3. Windows are discouraged in the construction of the facility, but if present, shall be sealed and resistant to breakage.
4. Interior surfaces should be light coloured for easy location of escaped arthropod.
5. Floor drains should be sealed or modified to prevent accidental release of arthropods and prevent arthropods from breeding.
6. Facilities shall have an effective arthropod trapping system to monitor and prevent the escape of arthropods. This may encompass the use of oviposition traps, flea traps, light traps etc.

Operating Procedures

1. Personnel entering the facility shall be trained in the handling of arthropods and adequately immunised for work with arthropods.
2. IBC approval shall be obtained prior to conducting work in the facility.
3. Containers shall be disinfected chemically and/or autoclaved if used with infectious material.
4. Containers are to be non-breakable and sealed with a screen mesh to prevent arthropod escape. Openings designed to prevent escape such as the use of valves are highly recommended.
5. Autoclaving is recommended for arthropod materials prior to disposal.
6. Containers shall be clearly marked for use with infectious versus non-infectious arthropods.
7. No infected arthropod material is to be disposed down the sewer.

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[5] Please refer to American Committee of Medical Entomology; American Society of Tropical Medicine and Hygiene. Arthropod containment guidelines. Vector-Borne Zoonotic Diseases, 2003; 3(2): 63-67. https://www.liebertpub.com/doi/pdf/10.1089/153036603322163457

8. Harbourage and breeding areas should be eliminated; if not possible, these shall be monitored. Equipment in which water may accumulate shall be screened regularly and treated to prevent arthropod survival.
9. Use of sterile, pathogen-free blood sources are highly recommended.
10. The disease status of the animal/human must be ascertained prior to feeding sessions.
11. Arthropod specific personal protective equipment may be worn when appropriate, such as respirators, particle masks etc.
12. Loose arthropods should be killed and disposed of. Care shall be taken when dealing with loose infectious arthropods.
13. An inventory should be kept for arthropods in the facility.
14. Accidents of releases should be reported immediately and remedial action taken should be documented and maintained.
15. Equipment transferred into and out of the facility shall be disinfected and cleared of viable arthropod material.

For facilities handling Genetically Modified Fish and Other Aquatic Organisms:

Genetic manipulation work involving the use of fish and other aquatic organisms include:

- Production or use of transgenic aquatic organisms;
- Use of genetically modified organisms to infect aquatic organisms (e.g. microorganisms, sea lice etc.)

All investigators and IBCs responsible for genetic manipulation work which involve transgenic animals shall be familiar with the requirements of the NACLAR Guidelines on the Care and Use of Animals for Scientific Purposes and laboratory biosafety.

Personnel must receive training in the handling of animals to be used and an appropriate standard of work supervision must be maintained.

Adherence to the biosafety guidelines does not exempt researchers from practising responsible laboratory animal care and maintaining basic welfare standards (References: Guidelines for the Care and Use of Animals for Scientific Purposes, NACLAR 2004; Guide to the Care and Use of Experimental Animals, Canadian Council on Animal Care). Researchers should also be aware that they are subject to Singapore Laws that legislate against cruelty to animals. This is contained in Part IV of the Animals and Birds Act (Cap. 7).

Infrastructure

1. The rearing area shall be confined in a secured building and be restricted to authorised personnel only.
2. All water shall leave the facility through a common drain.
3. All effluent water shall be passed through at least two screens. The size of the screens shall be set to retain the smallest life history stage of the organism in use. The screens shall be cleared regularly to prevent blockage and overflow.
4. Effluent water shall not discharge into a major system containing related fish species.
5. The building shall be structurally sound and of sufficient elevation to preclude flooding or unintentional escape.
6. To prevent escape of adult aquatic organisms into the sewers, a grill or mesh, with an appropriate grid size to prevent passage of adults of the species being use, shall be fitted to outlets used for disposal of tank water.
7. Water from tanks containing only adult transgenic aquatic organisms, and not involving the use of recombinant infectious agents, may be discarded untreated

down the sink, provided that a filtering mechanism to retain adult aquatic organisms is in place.
8. To prevent escape of adult aquatic organisms into the sewerage system, a grill or mesh, with an appropriate grid size to prevent passage of adults of the species being used, shall be fitted to outlets used for disposal of tank water.

Operating Procedures

1. General practices shall be as required for GM-BSL2 or GM-BSL3 laboratory work depending on the microorganism.
2. Tank water that has any potential to contain embryos, sperm, eggs or larvae of transgenic aquatic organisms shall be treated to ensure inactivation of viable transgenic material before disposal. The proposed treatment procedures and evidence for their efficacy shall be provided to NParks/AVS for case-by-case assessment.
3. Tank water used for work with infectious recombinant microorganisms, or tank water that has any potential to contain embryos, sperm, eggs or larvae of infected aquatic organisms, shall be treated to ensure inactivation of viable material before disposal. The proposed treatment procedures shall be provided to NParks/AVS for case-by-case assessment.

For facilities handling Genetically Modified Plants:

Genetic manipulation work involving the use of plants include:
- Production or use of transgenic plants;
- Use of genetically modified microorganisms to infect plants

Infrastructure

1. The plant house shall have a floor of concrete, or some other substance approved by IBC. Any openings in the walls or roof (e.g. windows, vents, and air supply and exhaust inlets and outlets) shall be screened with fine screens (thirty–gauge 30/32 mesh wire gauze). The drainage exits shall be designed to avoid entry of invertebrates, rodents and insects. Transparent sections of the plant house shall be made of impact-resistant material selected to maintain the integrity of the structure during all foreseeable impact events, including windstorm, and impacts from golf balls, stones picked up by grass mowers, hailstones and the like. If ordinary glass is used, a protective screen shall be fitted.
2. Entrances to the plant house shall be posted with a sign identifying the type of plant house and listing the procedures applicable, including emergency and maintenance procedures.
3. If the plant house is free-standing, it shall have an anteroom for entry and exit. The anteroom shall be fitted with a sticky pest strip or automatic insecticide aerosol device designed to kill arthropods which gain entry. An anteroom is not necessary if the plant house connects directly with a certified small or large scale containment facility.
4. A wash-basin shall be provided within the plant house adjacent to the entry door. Effluent should be disinfected. Where a laboratory is directly connected to the plant house, the basin may be in the laboratory.
5. The plant house shall be inspected regularly to ensure that its containment features are intact. Screens, filters and the like shall be cleaned regularly (in accord with manufacturer's specifications when provided).
6. All doors to the plant house shall be locked for the duration of the work except for those periods when personnel are actually working inside it.

Operating Procedures

1. Hands shall be washed with soap and water before leaving the plant house.
2. Only persons authorised by the facility operator are to enter the plant house. All such persons shall be trained to follow normal plant house routines as well as these operating procedures.
3. All plants in the plant house shall be treated as containing genetically manipulated DNA. Work in the plant house other than that involving genetic manipulation shall be discouraged.
4. Operations which may generate aerosols shall be done in a biological safety cabinet as specified for BSL2 laboratory containment.
5. Plants and tissues taken into or out of the plant house shall be carried in closed containers. Waste plants, tissues, soil, soil substitutes and the containers shall be sterilised.
6. Living plants or tissues shall not be taken from the plant house except to a containment laboratory or, with the approval of the IBC, when they are being transferred to another organisation.
7. If the work permits, plants shall be sprayed regularly with a systemic insecticide. The plant house shall be sprayed or fumigated to kill other arthropods (especially mites) at regular intervals, and at the end of each series of experiments. The organisation shall have an effective insect and rodent control programme.
8. The experimental materials shall be inspected regularly for signs of arthropod infestation. The inspection regimen shall pay particular attention to mites as they would not normally be excluded by the window and vent screens.

APPENDIX 9: BIOSAFETY REQUIREMENTS FOR BIOSAFETY LEVEL 3

BSL-3 facilities handling BATA First and Second Schedule biological agents are required to undergo annual certification by MOH-Approved Facility Certifier using MOH Containment Facility Certification Checklist which is available at the MOH Biosafety website (http://www.moh.gov.sg)

Access/ Personnel

Authorised Personnel include facility staff and maintenance staff. External staff (e.g. maintenance) must be accompanied by lab/facility staff. The equipment/area to be maintained/serviced/repaired must by decontaminated prior to maintenance/service/repair. Access to the zone must be restricted to authorised personnel only. Visitors to the laboratories have to be escorted by authorised laboratory staff.

The Laboratory shall not be located adjacent to, nor open onto, corridors used by the general public. Doors shall be self-closing, and interlocking whenever relevant. A break-glass procedure shall be in place for emergencies.

The laboratory shall be labelled with an internationally recognised biohazard warning symbol and sign. The laboratory shall be labelled with a sign indicating the personal protective equipment required for work at this level of containment.

Regular medical surveillance shall be provided for all personnel working within the facility. A medical record card is recommended for all personnel; the card is to include details of the containment facility and is to be carried by the holder of the card.

Containment

All laboratories shall be equipped with hands-free sinks for handwashing, preferably foot-operated, elbow-operated or sensor operated. Eyewash station must be provided, and shower shall be available if indicated by risk assessment.

Respiratory protective equipment shall be provided when necessary for certain laboratory procedures and/or when working with animals infected with certain pathogens. Manipulation of all infectious materials must be conducted within a biological safety cabinet or primary containment device.

The facility must be sealable for decontamination. HVAC systems in place must provide for gaseous decontamination of the facility. There shall be inward directional airflow into the facility shall be inward; animal rooms shall maintain inward directional airflow compared to the adjoining corridors. The system shall also be supported to ensure that operational malfunctions allow for alerts to be sent to the facilities' support personnel. Backflow-precautions shall be put in place for the water supply. Waste water shall be treated and free of infectious pathogens prior to release.

Biological safety cabinets shall be located away from doors and areas with high human traffic, air supply diffusers or exhaust vents as these could affect the performance of the cabinet. An autoclave shall be available within the containment facility for decontamination of waste. All decontamination procedures in the facility must be validated prior to their use.

Procedures/Documentation

1. All aerosol-producing equipment such as that for sonication and vortexing shall be kept and used in the biosafety cabinet or a gas-tight aerosol generation chamber.
2. All spills shall be decontaminated immediately with appropriate disinfectant. Records of spills and/or any other incidents (including accidents and near miss) must be documented, detailing the date, time, personnel involved and the measures taken for clean-up, as well as the corrective actions. Such records must be available for inspection at all times.
3. Protective clothing shall not be worn outside the laboratory; they shall be decontaminated/autoclaved prior to disposal or reuse.
4. An on-site autoclave must be available for BSL3 facilities. Laboratory wastes shall be bagged and placed in unbreakable containers or in biohazard bags with secured lids or is tied up, prior to autoclaving. All wastes must be effectively autoclaved or chemically decontaminated before they are allowed to be transferred out of the BSL3 facilities.
5. Provision shall be made to decontaminate the biological safety cabinet(s) and the room via gaseous decontamination with an appropriate fumigant, e.g. chlorine dioxide gas, vaporised hydrogen peroxide (VHP), and for the fumigant to be neutralised and/or purged safely upon completion of the procedure.
6. Decontamination of the safety cabinets(s) shall be performed in accordance with the requirements of established International Standards (see Appendix 1) and/or manufacturer's specifications.
7. A pest control programme against insects, rodents, birds, etc. shall be instituted.
8. Biosafety cabinets and all other laboratory equipment shall be regularly inspected and maintained to ensure optimum performance.
9. Decontamination of the entire laboratory is required prior to renovations and maintenance.
10. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis and other major chemicals.
11. Should the facility be segregated for work with different infectious agents, PPE shall be disposed of within the room before movement to another area of the facility. Ideally, a single equipment shall not be utilised by two different infectious agents. If this situation cannot be avoided, the equipment shall be fully decontaminated with the appropriate disinfectants prior to use.
12. BSL3 facility design, operating parameters and procedures must be verified and documented at all times. Certification of the BSL3 facility has to be conducted yearly.
13. Emergency standard operating procedures have to be developed and implemented. Personnel shall be trained and competent with the procedures.
14. Individuals must be accompanied by at least one other staff when carrying out work in the facility after normal working hours.

Personal Protective Equipment

Personnel shall don the required personal protective equipment as advised by the facility based on risk assessment prior to entering the facility.

For facilities handling Genetically Modified Microorganisms/Viruses:

1. Criteria for facilities handling Genetically Modified Microorganism/Virus under BSL2 conditions apply.
2. All experimental work involving the manipulation of infectious material must be conducted within at least a Class II Biological Safety Cabinet or other physical containment devices.
3. Avoid sharps used. If unavoidable, policies for safe handling and disposal of sharps must be developed and implemented
4. All procedures must be carried out in such a way to prevent spills and the creation of aerosols.
5. Work surfaces must be decontaminated prior to commencement of and after completion of work. Appropriate disinfectants shall be used in accordance to their effectiveness against the type of microorganisms/viruses.
6. Appropriate disinfectants shall be used to decontaminate all cultures, stocks and other infectious material before disposal via incineration.
7. Personnel shall wear appropriate PPE as indicated by risk assessment, including gloves. Gloves shall be worn and removed aseptically.
8. Equipment that may produce aerosols must be contained in primary barrier devices. Exhaust air must be filtered and treated prior to release from the laboratory.

For facilities handling Genetically Modified Animals:

1. Criteria for facilities handling Genetically Modified Animals under BSL2 conditions apply.
2. Personal protective equipment shall be used to reduce risk of exposure to infectious agents, animals and contaminated equipment
3. Access to the facility shall be limited to the fewest number of individuals at any one time, with a minimum number of 2 individuals.
4. The use of sharps (such as needles, cutters etc.) is strongly discouraged. Extra precautions shall be taken when handling and disposing of sharps.
5. Personnel shall wear gloves when handling animals. Gloves shall be worn and removed aseptically.
6. Work surfaces must be decontaminated prior to commencement of and after completion of work. The appropriate disinfectant shall be used for the type of work conducted.
7. Actively ventilated caging systems shall be designed to prevent the escape of microorganisms.
8. Animal bedding, feed, waste, tissues, carcasses etc. must be decontaminated prior to disposal. Animal bedding shall not be shared and must be exclusive to the animal hosted to prevent cross-contamination.
9. Cages shall be washed in a mechanical cage washer. Cages shall be autoclaved prior to washing.

Note: Considerations for animals with driving transgenes. Should a project be conducting research on genetically manipulated animals to skew the inheritance of a particular gene (Kindly refer to A(ix)), containment of research material may be escalated to a BSL3 – Animal Laboratory so as to minimise risk of animals escaping the containment facility. All escaped animals are to be killed and destroyed by incineration.

For facilities handling Genetically Modified Arthropods[6]:

1. Criteria for facilities handling Genetically Modified Arthropods under BSL2 conditions apply.
2. Only personnel trained in handling of arthropods and equipped to work with biological agents requiring BSL3 containment should clean up spills and eliminate escaped arthropods.
3. Arthropods isolated and housed in a BSL3 facility should not be mixed with arthropods from a BSL2 facility. Should arthropods from a BSL2 facility be housed in a BSL3 facility, the arthropods are not to be re-housed back to the BSL2 facility. The arthropods are to be autoclaved and incinerated before exiting the BSL3 facility.
4. The use of sharps in the facility should be limited.
5. Appropriate training should be provided to personnel prior to allowing them to work in an arthropod BSL3 facility.
6. Access to the facility shall be strictly restricted to staff only. Visitors are to be briefed before entering the facility and must always be accompanied by staff. The facility shall be cleaned prior to visitation.
7. All work with arthropods is to be done within a primary containment equipment.
8. Rearing of transovarial infected arthropods shall be performed in a designated and isolated room from the main arthropod facility.
9. A clear inventory shall be maintained for all arthropods kept in the facility.
10. Personnel shall wear appropriate PPE, including gloves when handling arthropods and host animals. Gloves shall be worn and removed aseptically.
11. White Laboratory coats shall be worn at all times within the facility. Wrap-around or solid-front gowns shall be worn over the laboratory coats. All personal protective equipment should be left within the facility. All apparel should be decontaminated prior to removal and disposal.
12. Protective footwear shall be used. Shoe covers are highly recommended over protective footwear.
13. Emergency pesticides should be housed in clear and easy to reach areas within the facility.
14. Floor drains are not recommended within the facility. If present, floor drains shall be fully sealed and treated frequently under the pest programme to prevent the release and entry of arthropods.
15. An autoclave should be available within the facility.
16. Regular reviews by the IBC shall be conducted, ensuring proper functionality of the facility.

Note: Considerations for arthropods with driving transgenes. Should a project be conducting research on genetically manipulating arthropods to skew the inheritance of a particular gene

[6] Please refer to Vector-Borne and Zoonotic Diseases, Introduction for the Definition and the Intent of Use of Arthropods, Pages 63-67. Website: https://www.liebertpub.com/doi/pdf/10.1089/153036603322163457

(Kindly refer to A(ix)), containment of research material may be escalated to a BSL3 – Arthropod Laboratory so as to minimise risk of arthropods escaping the containment facility. All escaped arthropods are to be killed and destroyed through incineration.

Please refer to additional guidance from Benedict et al. (2018) Recommendations for Laboratory Containment and Management of Gene Drive Systems in Arthropod and the NIH Guidelines for Guidance on the facility containment, handling and disposal of such research material.

For facilities handling Genetically Modified Fish and Other Aquatic Organisms:

1. Criteria for facilities handling Genetically Modified Fish and Other Aquatic Organisms under BSL2 conditions apply.
2. Effluent water shall not discharge into a major system containing related fish species. Effluent water must be filtered, treated and purified prior to discharge.
3. The use of sharps (such as needles, cutters etc.) is strongly discouraged. Extra precautions shall be taken when handling and disposing of sharps.
4. Tanks must be washed mechanically preferably with cage-wash machines. Tanks and items used in the tank (such as gravel, nets etc.) are to be decontaminated and preferably autoclaved prior to use and removal.
5. Work surfaces must be decontaminated prior to commencement of and after completion of work. The appropriate disinfectant shall be used for the type of work conducted.
6. Personnel shall wear gloves when handling animals. Gloves shall be worn and removed aseptically.
7. Air pumps shall be uni-directional and regularly detached for routine cleaning and disinfection.
8. Lighting for tanks shall be decontaminated prior to disposal.

Note: Considerations for fish and other aquatic organisms with driving transgenes. Should a project be conducting research on genetically manipulated fish and other aquatic organisms to skew the inheritance of a particular gene (Kindly refer to A(ix)), containment of research material may remain a BSL2 – Fish and Aquatic Organisms Laboratory on a case-by-basis. IBCs are to advise on the containment facility required for such animals. Additional fixtures to contain these animals must be in place to prevent the release of viable material.

For facilities handling Genetically Modified Plants:

1. Criteria for facilities handling Genetically Modified Plants under BSL2 conditions apply.
2. All structural joints in the plant house, including transparent sections, shall be fully sealed using an elastomeric sealant.
3. If the plant house is free-standing it shall have an anteroom for entry and exit. The innermost door shall have a door -closing device fitted. The anteroom shall be fitted with a sticky pest strip or automatic insecticide aerosol device designed to kill arthropods which gain entry. An anteroom is not necessary if the plant house connects directly with a certified small or large scale containment facility.
4. Personnel shall decontaminate their hands by washing with soap and warm water in the wash-basin provided on entering and leaving the plant house. When entering, personnel shall put on overshoes, covering clothes (e.g. gown/boiler suit) and a hat in the anteroom. These garments shall be removed on leaving the plant house and kept in the anteroom (or laboratory) between uses. They shall be laundered regularly.
5. Materials and equipment taken into or out of the plant house shall be treated by a technique demonstrated to be effective in destroying or removing all stages of the life-cycle of arthropods. This requirement applies to soil substitutes and where feasible to soil. Soil substitutes which can be readily decontaminated shall be used whenever possible. Use of soil is discouraged.

Note: Considerations for plants with driving transgenes. Should a project be conducting research on genetically manipulated plants to skew the inheritance of a particular gene (Kindly refer to A(ix)), containment of research material may remain a BSL2 – Plant Laboratory on a case-by-case basis. IBCs are to advise on the containment facility required for such plants. Additional fixtures to contain plants and their spores must be put in place to prevent release of viable plant material. A vestibule is highly recommended for this purpose.

APPENDIX 10: DISPOSAL

I. Inactivation

Waste contaminated with GMMs should be inactivated prior to discharge or disposal. Inactivation is defined (as applied to treatment of waste prior to disposal) as '*the complete or partial destruction of GMMs so as to ensure that any contact between the GMMs and humans or the environment is limited to an extent commensurate with the risks identified in the risk assessment and to provide a high level of protection for humans and the environment*'. This means that the degree of 'kill' achieved by the inactivation must be related to the risk posed by the GMM. For Category A hazardous GMMs, sterilisation will be required. For instance, physical methods and alkaline hydrolysis can be validated to effectively achieve a complete kill. For Category B and C hazardous GMMs, a lower standard might be acceptable, provided that the risks are sufficiently reduced to a 'safe' level. For instance, chemical disinfection typically gives a 5-log reduction in viability of GMMs.

For Category B and C activities, inactivation may not need to be a separate, discrete step in the procedure. Inactivation may be a consequence of other processing steps or experimental methods. For example, extraction methods frequently involve disruption or lysis steps that will achieve sufficient inactivation of the GMM. Alternatively, collection of waste generated from these low risk activities by licensed waste collectors and sending for incineration without autoclaving are acceptable. Care should be taken, however, to ensure that such methods are consistent and provide a level of inactivation that is adequate from a risk management perspective.

A. Physical inactivation methods

1. Physical methods of inactivation are arguably the most reliable way to achieve a high kill rate. For large-scale operations, inactivation will usually involve heat inactivation of cultures. For other operations, the use of an incinerator (for example to dispose of infected animal carcasses) or rendering procedure might be appropriate or sufficient. However, the use of a validated autoclave cycle remains the most effective means of inactivating GMMs.

2. A range of autoclave cycle parameters are suitable for inactivating microorganisms and a typical cycle would be 121 °C, maintained for 15 minutes. Holding-time may need to be increased for work with particular organisms (for example spore-forming bacteria), large amounts of contaminated material or where steam penetration is inefficient. Users should consult the technical specifications for their particular autoclaves. A higher heat setting is recommended for work with transmissible spongiform encephalopathy agents (TSEs) (134-138 °C). Further information on the handling of TSEs can be found in ACDP/SEAC guidance document *Transmissible spongiform encephalopathy agents: Safe working and the prevention of infection*.

3. An autoclave should be available for all laboratory-scale activities with GMMs and those that involve animals. Therefore, the use of an autoclave should always be an option for these activities and it is recommended that one be used for waste inactivation, either by itself or in combination with chemical methods. Care should be taken, however, to ensure that chemical disinfectants do not damage the autoclave. The autoclave should be serviced regularly and its performance tested frequently.

4. Any inactivation method used should be validated or verified under working conditions.

B. Chemical inactivation methods

1. Chemical inactivation methods are commonly used in laboratory-scale operations to avoid the need to autoclave bulk waste (eg spent media and liquid cultures). Chemical disinfection is an inherently less reliable method of inactivation as there are many factors that can come to bear on the effectiveness of the chosen disinfectant. For example, the presence of organic matter can impede the performance of certain disinfectants. Similarly, the disinfection regime may be compromised if cultures are buffered or proteinaceous. Furthermore, the requirement to validate or verify the effectiveness of the procedure stands, and it can be technically difficult or impractical to adequately remove the disinfectant and screen for viable GMMs. For these reasons it is recommended that, for high-risk activities, chemical methods are not relied upon as a sole means of inactivation but are used in combination with physical methods. However, the sole use of chemicals does represent an acceptable means of inactivation for lower-risk activities.

2. When selecting a disinfectant, its toxicity to humans and the environment should be considered. Appropriate safety precautions should be adopted. Different disinfectants must not be mixed together or used in combination unless the possibility of hazardous reactions or the formation of toxic products has been properly assessed. Appropriate procedures should be used to ensure suitable disinfectants at the correct dilution are available at the point of need. Personnel should be trained in the correct use of disinfectants and in the emergency spillage protocols associated with them.

3. There are advantages in limiting the number of different disinfectants available in the workplace to the minimum necessary, in order to avoid confusion and to reduce costs. Once a disinfectant has been selected, in-use tests should be carried out to monitor both the performance of an individual chemical but also the way in which it is used (for example tests to detect incorrect dilution, old solutions and mixtures of incompatible reagents).

4. Disinfectants and chemical inactivation methods in common use are discussed below, including considerations relating to the characteristics, advantages and disadvantages of each.

II. Disposal of Animals

1. When an animal containing recombinant DNA or a recombinant DNA-derived organism is euthanized or dies, the carcass shall be disposed of to avoid its use as food for human beings or animals unless food use is specifically authorised by an appropriate agency.

2. Following project closure, a 3-year record (NACLAR requirement) shall be maintained of the experimental use and disposal of each animal or group of animals.

III. Disposal of Plants

1. There is a regulatory requirement that all GMM contaminated materials and waste must be inactivated by a validated means prior to disposal. In plant growth facilities, this may include growing media, pots and tools, as well as plant material and other incidentally contaminated items. Autoclaving will generally provide the best assurance of inactivation, but it may not be appropriate for all contaminated materials. When autoclaving, the equipment should be operated so as to comply with the manufacturers' instructions. For example, small amounts of plant material may be inactivated using 121°C for 15 minutes but appropriate times and temperatures may vary. Larger volumes of waste may necessitate a longer holding time or higher temperature. The key requirement is that the system is validated to ensure sufficient steam penetration to the centre of the load for the

required time period is achieved. Incineration is an appropriate alternative, although the risk assessment should detail the risk management procedures in operation. Where the incinerator is located off site, there is a regulatory requirement that the incinerator premises be registered as a GM centre. For Class 1 GMMs, waste material should be double bagged and placed in a suitable container for transfer to waste management facilities.

2. Since Class 2 GMMs have been identified as being able to infect plants in the environment, the containers used for transporting to the incinerator should be sufficiently robust (see M18). Where small amounts are involved validated containment vessels (e.g. incinerator bins) may be sufficient. One-way burn bins may also be appropriate, but for larger volumes burn bags contained within wheelie bins are acceptable. Local rules should be used to clearly outline the expected fate of all material within the facility and GM and non-GM material may have to be subject to the same waste inactivation measures unless fully justified in the risk assessment.

3. Inactivation of GMMs in effluents from washbasins and showers might be required in Containment Level 3 facilities, where the risk assessment shows that this is necessary. Where this is required, effluents should be collected in a sump and inactivated, or pass through a 'kill tank'. Given the hazardous nature of the material, the containers used for transporting to the incinerator should be sufficiently robust (see M18). One-way burn bins should be sufficient. Local rules should be used to clearly outline the expected fate of all material within the facility and GM and non-GM material may have to be subject to the same waste inactivation measures unless fully justified in the risk assessment.

IV. Disposal of GMM contaminated waste
(Applicable for both contained use and deliberate release activities)

1. The inactivation of waste is another area that causes concern among GM users, as the requirement to inactivate GMMs in contaminated waste under the Contained Use Regulations is interpreted as being more onerous than the steps taken when dealing with normal clinical waste. This is not necessarily the case and GM clinical waste should be dealt with in a pragmatic way.

2. Both the form of contaminated material generated and the procedure for dealing with it should be described in the risk assessment. As there is no formal definition of 'waste' in Contained Use Regulations, the risk assessment should identify all types of material that could be regarded as GMM contaminated.

3. The intention of the Regulations is to limit contact with the environment and people, as opposed to preventing all contact. In practice, there may be a need for specific inactivation procedures to limit contact, although the approach taken should be commensurate with the risk. For example, the needle and syringe used to with draw blood samples following patient treatment can be disposed of as any other contaminated sharps. Used vials containing the inoculated material could be treated with a chemical disinfectant or autoclaved prior to disposal.

4. Dirty laboratory coats should be cleaned using routine procedures. This would normally involve a high temperature wash (eg. 65 °C), and as the procedures are considered sufficient to deal with blood-borne viruses or enteric pathogens, they should be sufficient for GMMs used in hospitals. Similarly, surgical equipment should be cleaned in the standard way, which has been proven to be effective at preventing cross-contamination.

5. Unused or excess clinical materials containing viable GMMs may be returned to the trial sponsor, or inactivated on site through disinfection or autoclaving, prior to disposal in the clinical waste stream.

V. Disposal of Genetically Modified Arthropods

Transgenic insects should be destroyed by proven appropriate methods such as autoclaving or boiling prior to disposal. Waste materials generated from the ACL2 or ACL3 that could contain any life stages must be treated to render them non-viable before disposal.

APPENDIX 11: PRECLINICAL RESEARCH INVOLVING HIV-BASED LENTIVIRAL VECTORS

Introduction:

- The use of lentiviral vectors for gene delivery has been increasing in preclinical research because these systems have attractive features.
- However, the use of lentiviral vectors may also raise biosafety issues.
- The major risks to be considered for research with HIV-based lentiviral vectors are:

  (i) Potential for generation of replication-competent lentiviral (RCL) particles
  (ii) Potential for oncogenesis and/or perturbation of cell cycle, as a result of overexpression of the inserted gene or cDNA carried within the lentiviral vector.

- These risks can be mitigated by the nature of the vector system (and its safety features) or exacerbated by the nature of the transgene insert encoded by the vector.

Procedures:

Investigators planning work which involves HIV-based lentiviral vectors should:

1. Consider a range of parameters including:

   (i) the nature of the vector system and the potential for regeneration of replication competent virus from the vector components,
   (ii) the nature of the transgene insert (e.g., known oncogenes or genes with high oncogenic potential may merit special care)
   (iii) the vector titer and the total amount of vector,
   (iv) the inherent biological containment of the animal host, if relevant

2. Carry out complete, case-by-case, risk assessments of the planned experiments based on the parameters listed above and choose the lentiviral vector system that is most suitable in terms of safety and project requirement.

3. Unless the vector system is covered in the List of GMAC-approved host, vector systems, submit a GMAC 'Proposal Form for Assessment of Genetic Manipulation Work'. In addition, submit other appropriate institutional forms and additional relevant information to the respective local Institutional Biosafety Committee (IBC), which will advise if additional regulatory application is required.

4. Complete the table in Appendix I of the GMAC Proposal Form to determine if the vector system falls under the BATA Fourth Schedule. In addition, submit relevant documents of the vector system, if any.

5. Determine the measures and procedures to be instituted to ensure proper containment, handling, storage, and disposal of infectious materials. Some recommendations are provided in Appendix 5. In addition, the risk assessments and proposed measures and procedures may be subjected to review and approval by the IBC or other regulatory body.

6. With regard to the GMAC evaluation criteria, experiments involving the use of lentiviral vectors (excluding lentiviral vectors that fall under the BATA Fourth Schedule[7]) whose host range includes human cells, and where the lentiviral vectors contain inserted DNA sequence(s) coding for a product known to be oncogenic, or to play a role in the regulation of cell growth, or to be toxic to human cells, could typically be classified under Category A (i.e. Regulated Experiments with Significant Risks). In this case, the experiments using lentiviral vectors should be carried out under Biosafety Level 2 containment with enhanced safe practices and procedures.

7. Conversely, where the vectors used do not fall under the BATA Fourth Schedule, and where they do not contain inserted DNA sequence(s) coding for a product known to be oncogenic, or to play a role in the regulation of cell growth, or to be toxic to human cells, the experiments could typically be classified under Category B (i.e. Notifiable Experiments with Low Risks). In this case, the experiments using lentiviral vectors should be carried out under Biosafety Level 2 containment provided that the amount of virus-containing culture produced is less than 1 liter.

8. Satisfy the requirements of the NACLAR Guidelines on the Care and Use of Animals for Scientific Purposes (if animal work is involved) and ensure that the appropriate animal facilities are available (refer to Appendices 8 and 9).

[7] To find out if the HIV-based lentiviral vector system falls under the Fourth Schedule, please refer to the MOH Biosafety FAQs under Category 3. Possession "How do I know if a HIV lentiviral vector system falls under the BATA Fourth Schedule?" – https://www.moh.gov.sg/biosafety/faqs

APPENDIX 12: GLOSSARY

Note: Words are defined in this Glossary according to the use they have in these Guidelines

ACL	Arthropod Containment Level
Agrobacterium tumefaciens	A bacterium which infects plants and contains a plasmid (*q.v.*) that can be used to introduce foreign DNA into plant cells.
amphotropic retrovirus	A retrovirus (*q.v.*) that will grow in the cells from which it was isolated and also in cells from a wide range of other species.
amplify	To increase the number of copies of a gene or DNA sequence
autoclave	A device in which materials are sterilised using steam under high pressure.
bacterium	A single-celled prokaryotic (*q.v.*) organism.
bacteriophage	A virus that infects bacteria; also called **phage**.
baculovirus	A group of viruses that infect insects and can be used as vectors (*q.v.*) to produce foreign proteins in insect cells.
biological safety cabinet/biosafety cabinet	Specially constructed cabinets which are designed to protect workers and the environment from dangerous agents, especially bacteria and viruses.
BATA	Biological Agents and Toxins Act
cell	The smallest structural unit of living organisms that is able to grow and reproduce independently.
characterised DNA	DNA which has been sequenced and for which there is an understanding of the gene products.
chromosome	A structure in the cell, consisting of DNA and proteins, that carries the organism's genes.
clone	As a noun: a group of genes, cells or organism derived from a common ancestor and genetically identical. As a verb: to generate replicas of DNA sequences or whole cells using genetic manipulation techniques.
conjugative plasmid	A plasmid (*q.v.*) which codes for its own transfer between bacterial cells by the process of conjugation ('mating').
construct	As a noun: genetically manipulated DNA.

containment	Prevention of the spread of genetically manipulated organisms outside the laboratory. Physical containment is accomplished by the use of special procedures and facilities. Biological containment is accomplished by the use of particular strains of the organism which have a reduced ability to survive or reproduce in the open environment.
containment level	The degree of physical containment provided by a laboratory or facility, which depends on the design of the facility, the equipment installed, and the procedures used. GMAC physical containment levels are numbered from 2 to 4, 4 being the highest level.
defective virus	A virus that is unable to reproduce in its host without the presence of another ('helper') virus.
deliberate release	Intentional release of a genetically modified organism into the open environment.
DNA	Deoxyribonucleic acid, the molecule which carries the genetic information for most organisms; consists of four bases and a sugar-phosphate backbone.
donor	The organism or cell from which DNA is derived for insertion into another organism (the host).
Drosophila	A genus of flies whose genetics has been extensively studied.
embryo-rescue	The process in plant breeding whereby tissue from young embryo plants is excised and propagated *in vitro* for subsequent growth as differentiated plants.
***Escherichia coli* (*E. coli*)**	A bacterium that inhabits the intestinal tract of humans (and other animals).
***Escherichia coli* K12**	A strain of *E. coli* that has been maintained in culture in laboratories for many years. It has lost the ability to colonise the intestinal tract of humans and animals, is well-characterised genetically, and is often used for molecular cloning work.
***Escherichia coli* B**	Another well-characterised laboratory strain of *E. coli.*
eukaryotic	Belonging to the group of organisms whose cells contain a true nucleus. Eukaryotic organisms include animals, plants and fungi.
expression	Manifestation of a characteristic that is specified by a gene; often used to mean the production of a protein by a gene that has been inserted into a host organism.
fungi	Non-photosynthetic eukaryotic organisms, including moulds, that feed on organic matter.

fusion	Joining of the cell membranes of two cells to create a daughter cell that contains the genetic material from both parent cells.
gamete	A reproductive (egg or sperm) cell.
gene	A hereditary unit of nucleic acid which specifies the structure of a protein or RNA molecule.
gene drives	Gene drives are systems that enable biased inheritance of a genetic element so that offspring within a population have a >50% change of inheritance of a given trait.
gene therapy	The replacement of a defective gene in a person or other animal suffering from a genetic disease.
genetic engineering	See **genetic manipulation**.
genetic manipulation	A technology used to alter the genetic material of living cells or organisms in order to make them capable of producing new substances or performing new functions.
genome	The total genetic complement of a given organism.
genotype	The genetic make-up of an organism, as distinguished from its physical appearance (the phenotype).
germline cells	Gametes and the cells from which they are derived. The genetic material of germline cells, unlike that of somatic cells (*q.v.*), can be passed to succeeding generations.
GA-BSL	Biosafety Level for genetic modification of animal
GI-BSL	Biosafety Level for genetic modification of insects
GM-BSL	Biosafety Level for general genetic modification work
GP-BSL	Biosafety Level for genetic modification of plants
GMAC	Genetic Modification Advisory Committee.
GMO	Genetically Modified Organism.
GMM	Genetically Modified Microorganism.
growth factor	A protein that stimulates cell division when it binds to its specific cell-surface receptor.
helper virus	A virus which, when used to infect cells already infected by a defective virus (*q.v.*), enables the latter to multiply by supplying something the defective virus lacks.
HEPA filter	High efficiency particulate air filter.
HIV	Human immunodeficiency virus (a retrovirus).

host	A cell or organism into which foreign DNA is introduced to enable production of proteins or further quantities of the DNA.
host range	For a virus, the range of species that can be infected by that virus.
host-vector system	Combination of host (*q.v.*) and the vector (*q.v.*) used for introducing foreign DNA into the host.
hybridoma	A hybrid cell, used in production of monoclonal antibodies (*q.v.*), which is produced by fusing an antibody-producing cell (a B lymphocyte) with a tumour cell.
IBC	Institutional Biosafety Committee.
in vitro	Literally ‘in glass’; performed in a test tube or other laboratory apparatus.
in vivo	In a living organism.
knockout mouse	A mouse that has been genetically modified by deletion or inactivation of a specific gene.
LD50	The dose of a toxin or infectious agent which will kill half of a population of organisms.
microorganism	An organism that can be seen only with the aid of a microscope.
MOH	Ministry of Health, Singapore
MOM	Ministry of Manpower, Singapore
monoclonal antibody	An antibody that is derived from a single clone (*q.v.*) of hybridoma (*q.v.*) cells and recognises only one antigen.
NEA	National Environment Agency, Singapore
NParks	National Parks Board, Singapore
oncogene	An activated (modified) cellular gene which causes normal cells to become cancerous.
oocyte	A cell that divides to form the female reproductive cell.
packaging	In the process of virus replication, the assembly of the components of the virus to form the complete virus particle.
pathogen	An organism that causes disease.
PCR	See **polymerase chain reaction**.

phage	See **bacteriophage**.
plasmid	A small, self-replicating molecule of DNA which contains a specific origin of replication. Plasmids are often used as cloning vectors (*q.v.*).
polymerase chain reaction	A technique for generating, *in vitro*, an increased quantity of a target segment of DNA.
prion	An infectious agent of unknown etiology which causes spongiform encephalopathies of humans and animals.
prokaryotic	Belonging to the group of microorganisms whose DNA is not enclosed within a nuclear membrane.
promoter	A DNA sequence, located in front of a gene, that controls expression of the gene. It is the sequence to which RNA polymerase binds to initiate transcription.
protein	A molecule composed of amino acids.
protoplast	A plant or bacterial cell which has had the outer cell wall removed.
receptor	Cell-surface protein to which molecules such as hormones and growth factors bind to exert their effects on the cell, or to which viruses bind to gain entry to the cell.
recombinant	Organisms, cells, viruses etc. which contain recombinant DNA (*q.v.*).
recombinant DNA	DNA formed by joining, *in vitro*, segments of DNA from different organisms.
recombination	The occurrence or production of progeny with combinations of genes other than those that occurred in the parents.
replication	Reproduction.
retroviral vector	A retrovirus (*q.v.*) which is used to introduce foreign DNA into animal cells, usually by replacing part of the viral genome with the foreign DNA of interest.
retrovirus	A virus that uses the enzyme reverse transcriptase to copy its RNA genome into DNA, which then integrates into the host cell genome.
RNA	Ribonucleic acid, a molecule similar to DNA, whose functions include decoding the instructions for protein synthesis that are carried by the genes; comprises the genetic material of some viruses.
sharps	Sharp laboratory items such as syringe needles, scalpel and razor blades, and broken glass.

shot-gun cloning	The production of a large random collection of cloned fragments of the DNA of an organism, from which genes of interest can later be selected.
somatic cell	Any cell of a multicellular organism other than germline cells (*q.v.*).
Ti plasmid	A large plasmid of the bacterium *Agrobacterium tumefaciens* (*q.v.*) which carries genes for tumour induction in some plants. A disarmed form of the plasmid which lacks the tumour-inducing genes is often used as a vector to introduce foreign DNA into plant cells.
tissue culture	*In vitro* growth of tissue cells in nutrient medium.
toxin	A poisonous substance, produced mainly by microorganisms, but also by some fungi, plants and animals.
transgenic (organism)	An organism whose cells, including the germline cells, contain foreign DNA; transgenic animals are produced by the insertion of the foreign DNA into the newly fertilised egg or embryo.
tumour suppressor gene (or anti-oncogene)	A type of gene in which inactivating mutations contribute to tumour development.
vector	A self-replicating agent (e.g. plasmid or virus) used to transfer foreign DNA into a host cell.
viroid	A disease-causing agent of plants, which is smaller than a virus and consists of a naked RNA molecule.
virulence	Ability of an organism to cause disease.
virus	A submicroscopic infectious particle, containing genetic material (DNA or RNA) and protein, which can replicate only within the cell of an organism (plant, animal or bacteria).
zygote	The cell produced by the union of the male and female gametes.