

## APPENDIX F-IV

### SPECIFIC APPROVALS OF TOXINS

Confidential proprietary information has been deleted

1. Permission has been granted to clone the Exotoxin A gene of *Pseudomonas aeruginosa* under BL1 conditions in *Pseudomonas aeruginosa* and in *Pseudomonas putida*.
2. The pyrogenic exotoxin type A (Tox A) gene of *Staphylococcus aureus* may be cloned in an HV2 *Bacillus subtilis* host-vector system under BL3 containment conditions.
3. Restriction fragments of Corynephage  $\beta$  carrying the structural gene for diphtheria toxin may be safely cloned in *E. coli* K-12 in a high containment BL4 facility. Laboratory practices and containment equipment are to be specified by the IBC. If the investigators wish to proceed with the experiments, a prior review will be conducted to advise the institution and the investigators whether the proposal has sufficient scientific merit to justify use of the BL4 facility.
4. The genes coding for the *Staphylococcus aureus* determinants, A, B, and F, which may be implicated in toxic shock syndrome may be cloned in *E. coli* K-12 under BL2 + EK1 conditions. The *Staphylococcus aureus* strain used as the donor is to be alpha toxin minus. It is suggested that, if possible the donor *Staphylococcus aureus* strain should lack other toxins with LD<sub>50</sub> in the range of one microgram per kilogram body weight such as the exfoliative toxin.
5. Fragments F-1, F-2, and F-3 of the diphtheria toxin (tox) gene may be cloned in *E. coli* K-12 under BL1 + EK1 containment conditions and may be cloned in *Bacillus subtilis* host-vector systems under BL1 containment conditions. Fragment F-1 and fragment F-2 both contain: (i) some or all of the transcriptional control elements of tox; (ii) the signal peptide; and (iii) fragment A (the center responsible for ADP-ribosylation of elongation factor 2). Fragment F-3 codes for most of the non-toxic fragment B of the toxin and contains no sequences coding for any portion of the enzymatically active fragment A moiety.
6. The gene(s) coding for a toxin, designated labile enterotoxin (LT), isolated from *E. coli* which is similar to the *E. coli* heat LT with respect to its activities and mode of action but is not neutralized by antibodies against cholera enterotoxin or against LT from human or porcine *E. coli* strains, and sequences homologous to the *E. coli* LT-like toxin gene may be cloned under BL1 + EK1 conditions.
7. Genes from *Vibrio fluvialis*, *Vibrio mimicus*, and non 0-1 *Vibrio cholerae*, specifying virulence factors for animals, may be cloned under BL1 + EK1 conditions. The virulence factors to be cloned will be selected by testing fluid induction in suckling mice and Y-1 mouse adrenal cells.
8. The intact structural gene(s) of the Shiga-like toxin from bacterial species classified in the families Enterobacteriaceae or Vibrionaceae including Campylobacter species may be cloned in *E. coli* K-12 under BL3 + EK1 containment conditions.

*E. coli* host-vector systems expressing the Shiga-like toxin gene product may be moved from BL3 + EK1 to BL2 + EK1 containment conditions provided that: (i) the amount of toxin produced by the modified host-vector systems is no greater than that produced by the positive control strain *Shigella dysenteriae* 60R, grown and measured under optimal conditions; and (ii) the cloning vehicle is an EK1 vector preferably belonging to the class of poorly mobilizable plasmids such as pBR322, pBR328, and pBR325.

Nontoxinogenic fragments of the Shiga-like toxin structural gene(s) may be moved from BL3 + EK1 to BL2 + EK1 containment conditions or such nontoxic fragments may be directly cloned in *E. coli* K-12 under BL2 + EK1 conditions provided that the *E. coli* host-vector systems containing

the fragments do not contain overlapping fragments which together would encompass the Shiga-like toxin structural gene(s).

**9.** A hybrid gene in which the gene coding for the melanocyte stimulating hormone (MSH) is joined to a segment of the gene encoding diphtheria toxin may be safely propagated in *E. coli* K-12 under BL4 containment. If the investigators wish to proceed with the experiment, a prior review will be conducted to advise the institution and the investigators whether the proposal has sufficient scientific merit to justify the use of the BL4 facility. Before any of the strains may be removed from the BL4 facility, data on their safety shall be evaluated by the Working Group on Toxins and the recommendation shall be acted upon by the institution.

**10.** The gene segment encoding the A subunit of cholera toxin of *Vibrio cholerae* may be joined to the transposons Tn5 and Tn5-131 and the A-subunit Tn5-131 hybrid gene cloned in *E. coli* K-12 and *V. cholerae* under BL1 containment conditions.

**11.** A hybrid gene in which the gene coding for interleukin-2 (IL-2) is joined to a specific segment of the gene encoding diphtheria toxin may be propagated in *E. coli* K-12 host-vector systems under BL2 containment plus BL3 practices, with the use of poorly mobilizable plasmid vectors such as EK2 certified plasmids.

**12.** The modified diphtheria toxin gene of *Corynebacterium diphtheriae* reassembled from a DNA segment encoding the A chain and a modified fragment encoding the B chain lacking the C-terminal 17 amino acid, may be propagated in *E. coli* K-12 under BL1 + EK1 containment. The gene encoding the A chain may be cloned in *Corynebacterium glutamicum* under BL1 condition. July 27, 1984.

**13.** A hybrid gene in which the gene coding for the melanocyte stimulating hormone (MSH) is joined to a segment of the gene encoding diphtheria toxin, may be propagated in *E. coli* K-12 and the physical containment level may be reduced from BL4 to BL2 plus BL3 practices. The truncated gene for diphtheria toxin including fragment A and fragment B up to but not beyond the Sph1 site may also be propagated under BL2 plus BL3 practices. September 30, 1985.

**14.** The gene segment encoding diphtheria toxin A chain may be propagated in *E. coli* K-12 under BL1 + EK1 containment. The original cloning of the tox A fragment from tox-176 beta phage should be under BL3 or BL4 until demonstration that only the tox A fragment was cloned. November 1, 1985.

**15.** The genes encoding *Bacillus anthracis* toxin components (LF, PA and EF) may be cloned in *Bacillus subtilis* under BL2 containment conditions. The LF and PA genes cannot be transferred into the host together. February 21, 1986.

**16.** The gene encoding Bordetella pertussis toxin may be cloned in *B. pertussis* to construct hyper-toxigenic strains under BL2 containment plus BL3 practices. The BL2 containment conditions may be used for the non-virulent strain of *B. pertussis* (BP348). September 23, 1986.

**17.** The gene encoding ricin toxin may be cloned in sterile strains of yeast under BL3 containment conditions. November 12, 1986.

**18.** The gene encoding the streptococcal pyrogenic exotoxin A (speA) may be cloned in *Staphylococcus aureus* under BL2 containment plus BL3 practices. November 26, 1986.

**19.** The genes for type D enterotoxin (entD) and type E enterotoxin (entE) of *Staphylococcus aureus* may be cloned in *E. coli* K-12 under BL2 containment conditions. March 4, 1987.

- 20.** The gene for pertussis toxin may be transferred from *Bordetella pertussis* into *Bordetella parapertussis* under BL2 containment plus BL3 practices, and Animal Biosafety Level 2 for experiments involving animals. March 10, 1987.
- 21.** The gene for Staphylococcal enterotoxin A (entA) cloned in *E. coli* may be transferred back to *Staphylococcus aureus* under BL2 containment conditions. April 13, 1987.
- 22.** The gene for pertussis toxin (ptx) may be transferred from *Bordetella pertussis* into *Bordetella bronchiseptica* under BL2 containment plus BL3 practices. May 15, 1987.
- 23.** The gene for diphtheria toxin fragment A (DT-A) may be cloned in retroviral vectors and expressed in mammalian cells under BL2 containment conditions. September 3, 1987.
- 24.** The gene for protective antigen (PA) of anthrax toxin may be cloned into *Bacillus anthracis* under BL3 containment conditions. The chloramphenicol acetyl transferase (CAT) gene of *E. coli* may also be expressed with the PA gene promoter in *B. anthracis* under BL3 containment conditions. March 29, 1988.
- 25.** The genes for snail conotoxin, alpha scorpion toxin, cobra snake toxin and rattlesnake toxin may be cloned in *Saccharomyces cerevisiae* under BL3 containment conditions. These genes may also be expressed in Chinese hamster ovary (CHO) cells by eukaryotic expression vectors under BL3 containment conditions. July 25, 1988.
- 26.** The gene for *Listeria monocytogenes* hemolysin, listeriolysin, may be cloned in an asporogenic strain of *B. subtilis* or back into *L. monocytogenes* under BL2 containment conditions. May 5, 1989.
- 27.** The gene encoding a truncated form of pseudomonas exotoxin A (PEA) may be transfected into and expressed in mammalian cells under BL2 containment conditions. Experiments to construct and to express genes encoding fusion proteins of PEA and nerve growth factor specific for cholinergic neurons may be performed under BL3 containment conditions. March 26, 1990.
- 28.** The cloned and mutagenized genes for enterotoxins of *Staphylococcus aureus*, entA, entB and entE, may be expressed in *Bacillus subtilis* under BL2 containment plus BL3 practices. October 18, 1990.
- 29.** The gene for ricin A chain may be genetically linked to the gene for diphtheria toxin B fragment to construct a chimeric toxin under BL2 + EK1 containment conditions. April 2, 1991.
- 30.** The listeriolysin gene of *Listeria monocytogenes* may be cloned and expressed in Bacille Calmette-Guerin (BCG) under BL3 containment conditions. April 3, 1991.
- 31.** The gene for proricin may be cloned, mutagenized at the linker between A and B subunits, and expressed in *E. coli* under BL2 + EK1 containment conditions. October 2, 1991.
- 32.** The gene encoding a potential toxin, zonula occludens toxin (ZOT), from *Vibrio cholerae* 01 may be cloned back to *V. cholerae* under BL2 containment conditions. Genes for additional virulence factors may also be cloned in *V. cholerae* under BL2 containment conditions. November 8, 1991.
- 33.** The physical containment level may be reduced from BL3 to BL1 for cloning genes encoding diphtheria toxin-IL-2 fusion proteins, DAB<sub>486</sub>-IL-2 and DAB<sub>389</sub>-IL-2. The genes encoding the fusion proteins DAB<sub>389</sub>-mIL-4, DAB<sub>389</sub>-C3d (a construct containing 28 amino acids of complement 3d) and DAB<sub>389</sub>-EBV (a construct containing 14 amino acids of Epstein-Barr virus gp350/220) may be cloned in *E. coli* K-12 under BL2 containment plus BL3 practices. April 7, 1992.

- 34.** The gene for castor bean ricin cloned into a vector may be used to transform *Agrobacterium tumefaciens* under BL2 containment conditions. The transformed bacteria may be used to construct a transgenic tobacco plant under BL-3P containment and the transgenic plant in the pre-seeding stage may be grown in BL-2P containment. May 22, 1992.
- 35.** The gene for *Clostridium botulinum* neurotoxin A may be linked to DNA encoding the nontoxic A2 peptide of cholera toxin, and the resulting fusion protein isolated from *E. coli* K-12 may be combined in vitro with the B subunit of cholera toxin to form a recombinant holotoxin under BL2 containment conditions. April 30, 1993.
- 36.** The physical containment level may be reduced from BL3 to BL2 for cloning the gene for protective antigen (PA) of anthrax toxin into *Bacillus anthracis* (Appendix F-IV-24). September 23, 1994.
- 37.** The gene for diphtheria toxin A chain (DT-A) may be cloned in replication-incompetent adenoviral vectors and expressed in mammalian cells and in rodents under BL2 containment plus BL3 practices. June 10, 1998
- 38.** The gene for diphtheria toxin A chain (DT-A) may be cloned and expressed in replication-incompetent retroviral vectors that are infectious to human cells under BL2 containment plus BL3 practices. August 26, 1998.
- 39.** The genes for chimeric toxins containing portions of the gene encoding the botulinum neurotoxin A or B heavy chain (the neuronal cell binding and translocation domains) and a portion of the gene encoding the ricin toxin A chain, may be cloned and expressed in *E. coli* BL21 (DE3) under BL2 containment plus BL3 practices. February 25, 2000
- 40.** The gene for diphtheria toxin A chain (DT-A) may be cloned in a helper-dependent (“gutless”) adenoviral vector under BL2 containment plus BL3 practices. Initial infection of the severe combined immunodeficiency (SCID) mice may be performed under BL3 containment until the vector is no longer detected in the blood and is no longer shed. April 24, 2000
- 41.** The genes for Toxin A and Toxin B of *Clostridium difficile* may be cloned and over-expressed in *E. coli* BL21 (DE3) under BL2 containment plus BL3 practices. June 12, 2000
- 42.** The experiment involving transfer of Shiga-like toxin encoding phage from natural isolate into an *E. coli* O157:H7 strain that does not harbor these genes may be conducted under BL2 containment. July 28, 2000
- 43.** The gene for diphtheria toxin A chain (DT-A) may be cloned into a plasmid that will be shuttled into *Yersina enterocolitica* under BL2 containment. November 13, 2000
- 44.** The gene for botulinum C3 exoenzyme may be cloned and expressed in *Drosophila* under BL2 containment. January 3, 2001
- 45.** The genes for either the light or heavy chain fragments of botulinum neurotoxins that are not known to be non-toxic, may be cloned in *E. coli* under BL2 containment. February 2, 2001
- 46.** The gene for diphtheria toxin A chain (DT-A) may be cloned under BL2 containment plus BL3 practices in a replication-incompetent lentiviral vector under transcriptional control of a prostate-specific antigen (PSA) promoter. April 11, 2001
- 47.** A transgenic mouse model has been constructed using diphtheria toxin A subunit (DT-A) gene for ablation of retinal pigment epithelial cell lineages. BL2 containment may be used for initial transfection of the embryonic stem cells. Lower containment levels may be used for later

steps of the transgenic experiments provided that DT-A is not expressed, secreted and circulated at high levels in transgenic animals, and DT-A is not then excreted. October 15, 2001

**48.** A transgenic mouse model has been constructed using diphtheria toxin A subunit (DT-A) gene for studying modulation of feeding and metabolism by opioid peptides. BL2 containment may be used for generation of the transgenic mice. April 11, 2003.

**49.** Diphtheria toxin A subunit gene has been used as a negative selection marker to study gene regulation in chicken bursal lymphoma (DT40) cells *in vitro*. BL2 containment may be used for the tissue culture experiments. April 27, 2004.

**50.** A plasmid expressing diphtheria toxin gene has been used as a negative selection marker in mouse embryonic stem cells *in vitro*. BL2 containment may be used for the tissue culture experiments. August 5, 2005.

**51.** Transgenic mice and zebrafish have been constructed to express either diphtheria toxin Tox 176, an attenuated form of the subunit A, or tetanus toxin light chain, as a negative selection marker for ablation of retinal cells. BL2 containment may be used for initial transfection experiments involving embryonic stem cells, and BL1 containment for subsequent stages (e.g., housing of transgenic animals) of the research. May 4, 2006.

**52.** Cloning of the Diphtheria toxin A subunit (ToxA) driven by ubiquitous promoters in a 3<sup>rd</sup> generation lentivirus vector has been approved at BL2+ containment for *in vitro* (tissue culture) work. Subsequent inoculation into mice is approved at BL2+ for initial administration, until animals and bedding have been decontaminated (per RAC recommendations in the Lentiviral Biosafety Guidance document). Subsequent reduction of containment to BL2 or BL1 is at the discretion of the IBC pending local review and risk assessment. May 21, 2007.

**53.** The production of the lentivirus vector expressing diphtheria toxin A subunit (DT-A) and experiments using this vector in tissue culture setting should be performed under Biosafety Level 2 (BL2) physical containment using BL3 practices. For experiments in mice, BL2 with BL3 practices may be used for all procedures involving administration of the vector construct with particular caution when using sharps. Once the vector is administered, BL2 containment may be used since mice cannot support replication of infectious HIV-1, the parental virus of the vector. February 13, 2008.

February 13, 2008.