

**§ 725.420 Recipient microorganisms.**

The following recipient microorganisms are eligible for either exemption under this subpart:

- (a) *Acetobacter aceti*.
- (b) *Aspergillus niger*.
- (c) *Aspergillus oryzae*.
- (d) *Bacillus licheniformis*.
- (e) *Bacillus subtilis*.
- (f) *Clostridium acetobutylicum*.
- (g) *Escherichia coli* K-12.
- (h) *Penicillium roqueforti*.
- (i) *Saccharomyces cerevisiae*.
- (j) *Saccharomyces uvarum*.

**§ 725.421 Introduced genetic material.**

For a new microorganism to qualify for either exemption under this subpart, introduced genetic material must meet all of the criteria listed in this section.

(a) *Limited in size*. The introduced genetic material must consist only of the following:

- (1) The structural gene(s) of interest.
- (2) The regulatory sequences permitting the expression of solely the gene(s) of interest.
- (3) Associated nucleotide sequences needed to move genetic material, including linkers, homopolymers, adaptors, transposons, insertion sequences, and restriction enzyme sites.
- (4) The nucleotide sequences needed for vector transfer.
- (5) The nucleotide sequences needed for vector maintenance.

(b) *Well-characterized*. For introduced genetic material, well-characterized means that the following have been determined:

- (1) The function of all of the products expressed from the structural gene(s).
- (2) The function of sequences that participate in the regulation of expression of the structural gene(s).
- (3) The presence or absence of associated nucleotide sequences and their associated functions, where associated nucleotide sequences are those sequences needed to move genetic material including linkers, homopolymers, adaptors, transposons, insertion sequences, and restriction enzyme sites.

(c) *Poorly mobilizable*. The ability of the introduced genetic material to be transferred and mobilized is inactivated, with a resulting frequency of

transfer of less than  $10^{-8}$  transfer events per recipient.

(d) *Free of certain sequences*. (1) The introduced genetic material must not contain a functional portion of any of the toxin-encoding sequences described in this paragraph (d).

(i) For the purposes of this section, a functional portion of a toxin-encoding sequence means any sequence which codes for a polypeptide that has one of the following effects:

(A) It directly or indirectly contributes to toxic effects in humans. Directly contributes to toxic effects in humans means those sequences encoding polypeptides that have direct toxicity to target cells. An example of a sequence which directly contributes to toxic effects in humans is one which encodes the portion of diphtheria toxin, listed in paragraph (d)(2) of this section, capable of interacting with elongation factor 2, leading to inhibition of protein synthesis in target respiratory, heart, kidney, and nerve tissues. Indirectly contributes to toxic effects in humans means a sequence whose encoded polypeptide is not directly toxic to target cells, yet still adversely affects humans. An example of a sequence which indirectly contributes to toxic effects is the sequence which encodes the portion of the botulinum toxin, listed in paragraph (d)(3) of this section, capable of blocking the release of acetylcholine from gangliosides. Botulinum toxin affects neuromuscular junctions by its blockage of acetylcholine release, leading to irreversible relaxation of muscles and respiratory arrest.

(B) It binds a toxin or toxin precursor to target human cells.

(C) It facilitates intracellular transport of a toxin in target human cells.

(ii) While these toxins are listed (with synonyms in parentheses) in paragraphs (d)(2) through (d)(7) of this section according to the source organism, it is use of the nucleotide sequences that encode the toxins that is being restricted and not the use of the source organisms. The source organisms are listed to provide specificity in identification of sequences whose use is restricted. Although similar or identical sequences may be isolated from organisms other than those listed