Certification of *Kluyveromyces lactis* as a new Host-Vector 1 (HV1) system

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Request for Certification

- **OBA has received a request from the IBC at New England BioLabs (NEB) to certify two strains of *K. lactis* CBS2359 (ATCC 8585) and GG799 (a.k.a. YCT391) and their associated plasmids as a HV1 system and then to exempt certain research with *K. lactis* from the requirements of the *NIH Guidelines* (research under Section III-F-6).**
- **Most of the plasmid vectors are derived from pGBN1, a shuttle plasmid that is designed to integrate into the chromosome at the *K. lactis LAC4* promoter locus.**

Biological Containment of Host-Vector Systems

- Combination of vector and host so as to provide biological containment and therefore minimize the following:
  - (i) survival of the vector in its host outside the laboratory, and
  - (ii) transmission of the vector from the host to other non-laboratory hosts.

Appendix I , *NIH Guidelines*

Information to be considered for Certification of HV1 Systems

(App. I-II-B)

- The strain's natural habitat and growth requirements.
- Physiological properties, including those related to its reproduction, survival, and the mechanisms by which it exchanges genetic information.
- The range of organisms with which the organism normally exchanges genetic information and the type of information exchanged.
- Relevant information about its pathogenicity or toxicity.

Information to be considered for Certification of HV1 Systems

(App. I-II-B-2)

- A description of the history of the particular strains and vectors to be used, including data on any mutations which render this organism less able to survive or transmit genetic information.

- A general description of the range of experiments contemplated with emphasis on the need for developing such an HV1 system.

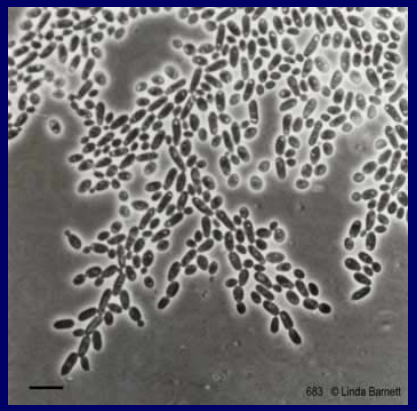
Natural Habitat and Growth

- *K. lactis* is a natural and indispensable component of cultured dairy processes (yogurt, cheese, buttermilk). It possesses a highly expressed lactase (β-galactosidase) that degrades lactose to galactose and glucose monosaccharides.

- Fermentation results in the production of acetic acid and ethanol – growth under aerobic conditions produces no ethanol.

- Optimum growth at 30 °C, and generally replicates poorly above 34 °C thereby limiting survival within humans and most animals.

Physiology and Reproduction



- Lower eukaryote, member of the budding yeast, Phylum Ascomycota (spore forming) capable of both respiration and fermentation.
- Originally classified as *Saccharomyces lactis;* closely related to *S. cerevisiae*, currently certified as a HV system.
- Can have haploid or diploid genomes.
- Genetic transfer occurs primarily by reproduction between haploid mating types.

Genetic Exchange and Survival

- **Though closely related, *K. lactis* will not mate with *Saccharomyces* spp.; *g*enetic exchange will thus be limited to few *Kluyveromyces* species.**
- ***Kluyveromyces* spp. rarely cause any disease in humans, aside from rare reports of superficial skin disease reported for *K. marxianus,* a closely related yeast species.**

Pathogenicity and Toxicity

- *K. lactis* is naturally isolated from dairy products.
- *K. lactis* has been extensively used in food industry to express heterologous proteins (*e.g.* recombinant bovine chymosin, a rennet enzyme and lactase both granted “GRAS” status by the FDA (GRAS: Generally Recognized As Safe).

Pathogenicity and Toxicity (2)

- An oral form of purified native lactase isolated from *K. lactis* is used to treat lactose intolerance.
- Dried, inactivated *K. lactis* powder has been used as a natural human dietary supplement.
- No known acute pathogenicity or toxicity has been described for this organism.

Host-Vector System Proposed for Certification
(Proposed Action-part 1)

- Proposed strains include:
  - Lab adapted wild-type strains, and
  - Lab adapted protease-deficient strains
    - designed to enhance production and secretion of functional heterologous proteins.
- Protease deficient strains may have selective disadvantage over wild-type strains.
- Wild-type strains adapted for growth under laboratory conditions are also less likely to compete effectively in the wild due to the selection of strains adapted to an optimal nutrient environment.

Host-Vector System Proposed for Certification
(Proposed Action-part 1)

- **Plasmids used for experimental purposes are low copy number:**
  - **Cannot be efficiently transferred between *K. lactis* strains.**
  - **Not transferrable to other yeast species by virtue of a restricted mating capacity with other yeast.**

- **Plasmids are mainly designed for protein expression.**

Current Status Under the *NIH Guidelines*

- Research with these strains of *K. lactis* and associated plasmids could be conducted at BL1 and be initiated upon registration with the IBC.
- Large scale growth (>10L) would be conducted under BL1-Large Scale (BL1-LS) (see App. K *NIH Guidelines*).

Research Advantages for all Certified Host Vector Systems

- For research involving < 10 liters, BL1 containment is likely to be used but no IBC review is required and work could be conducted at institutions without a registered IBC.

- For research involving > 10 liters, IBC review and Biosafety Officer oversight is required, but minimum containment is in accordance with Good Large Scale Practice (GLSP), (Appendix K-II) not BL1-LS (Appendix K-III).

Selection of Physical Containment Levels-Large Scale

- Good Large Scale Practice (GLSP):
  - recommended for large-scale research or production involving viable, non-pathogenic, and non-toxigenic recombinant strains derived from host organisms that have an extended history of safe large-scale use.
  - recommended for organisms such as those included in Appendix C, *Exemptions under Section III-F-6*, which have built-in environmental limitations that permit optimum growth in the large-scale setting but limited survival without adverse consequences in the environment.
- BL1-Large Scale (BL1-LS):
  - recommended for large-scale research or production of viable organisms containing recombinant DNA molecules that require BL1 containment at the laboratory scale and that do not qualify for Good Large Scale Practice.

Additional Requirements for BL1-LS not Mandated for GLSP

- Reporting to lab director any spills, accidents and overt exposures with medical evaluation as necessary.
- Organism containing recombinant DNA shall be handled in a closed system, including transfers, and sampling must minimize aerosols or contamination of surfaces.
- Culture fluids shall only be removed from closed systems if the viable organisms containing the rDNA have been inactivated using a validated system.
- Must use HEPA filtration of exhausts or other method (e.g. incineration) to minimize possible environmental release.
- Emergency plans in place for handling large losses of culture.

Question for Consideration by the RAC

Do all non-recombinant <u>laboratory-adapted</u> strains of *K. lactis* and associated plasmids satisfy the criteria for HV1 certification?

Characteristics of *K. lactis*: Eligible for Certification?

- **Limited natural habitat and growth requirements, mainly restricted to lactose rich environments, with extremely poor, if any, survival in humans and animals**
  - **Lab-adapted strains grown on defined media should be less competitive than the original wild type strain.**

Characteristics of *K. lactis*: Eligible for Certification?

- **Exchange of genetic information limited to mating of haploid strains with other *Kluyveromyces* spp. only; does not exchange with closely related yeast such as *Saccharomyces* spp.**
- **Not pathogenic and used for over 50 years in food industry.**

Question for Consideration by the RAC

If eligible for certification, should certain research using this newly certified HV1 system be exempt from the requirements of the *NIH Guidelines*?

Exemption Criteria under the NIH Guidelines

- **Research that does not pose a significant risk to health or the environment as determined by the NIH Director, with advice of the RAC, after public comment, can be classified as exempt from the *NIH Guidelines* (see Appendix C).**

Section III-F-6

Host-Vector System Proposed for Certification

(Proposed Action-part 2)

All research with this newly certified HV-1 system would be exempt from *NIH Guidelines* unless, the system:

- **Is capable of producing a functional vertebrate toxin**
- **Contains DNA from a Risk Group 3 or 4 organism**
- **Is for large scale production (> 10L)**

Proposed Appendix C Language

Experiments involving *Kluyveromyces lactis* host-vector systems, with the exception of experiments listed in Appendix C-(*new*)-A, are exempt from the *NIH Guidelines* provided laboratory-adapted strains are used. For these exempt experiments, BL1 physical containment is recommended.

For large-scale fermentation experiments, the appropriate physical containment conditions need be no greater than those for the host organism unmodified by recombinant DNA techniques; the Institutional Biosafety Committee may specify higher containment if deemed necessary.