Project Plan Cell Bank Manufacture
BIOMEVA GmbH

The details listed in chapter 1 and 2 are intended to give an overview of the information required by BIOMEVA before starting the production of a clients GMP microbial cell bank. Chapter 3 describes the different phases of the project and its typical timelines. Chapter 4 informs about the standard manufacturing procedures of a MCB and WCB at BIOMEVA (the proposed procedures can be modified at the request of the client). Contacts for cell banking activities are listed in chapter 5. Please have a look at chapter 6 to get some impressions of our cell banking activities.

1. Basic Information Cell Clone and Expression Construct

The following information on the genetically modified organism is requested from the client according to the German Genetic Engineering Act. BIOMEVA provides the client with appropriate forms that should be completed. Please note that the provided information will be held strictly confidential by BIOMEVA.

Host strain: Source of cells (laboratory or culture collection), citation of relevant references from scientific literature

- species and strain
- genotypic and phenotypic characteristics
- pathogenicity, toxin production

Donor: Description of the origin of the nucleotide sequence coding for the protein (source of the cell)

Vector: Description of the source and function of component parts of the expression construct:

- description of the vector (name, size, structure, source of the plasmid)
- graphic presentation of expression vector with relevant genetic elements and unique restriction sites indicated
- antibiotic resistance genes
- promoter, operator (description and origins)
- detailed component map
- nucleotide sequence of the coding region and associated flanking regions

Risk assessment from client

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2. BASIC INFORMATION CELL BANK MANUFACTURE

The following information is requested in order to assess the scope of manufacture and to perform a feasibility study in advance:

- Type of cell bank to be produced [Master Cell Bank (MCB) and/or Working Cell Bank (WCB)]
- Number of vials to be filled for each cell bank (maximum number: 500 vials)
- Volume of cell suspension to be filled into each vial (preferably: 0,8mL – 1,3mL)
- Final glycerol concentration of cryopreservation medium
- Growth conditions
  - Medium defined by BIOMEVA (complex medium)
    or defined by the client (synthetic or complex medium; in that case the client has to provide information on medium composition such as manufacturer, article number, grade of all components used)
  - Orbit and shaking rate of incubator used for the manufacture of the Research Cell Bank (RCB)
  - Wavelength of optical density measurements
  - Optical density specifications (i.e. for cell harvest)
  - Temperature specifications
  - pH specifications
  - If necessary, antibiotics to be used for manufacture including type and concentration of the antibiotic in mg/litre
- etc.

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3. General Project Schedule

Step	Timeframe	Activity	Prerequisites	Responsibility
1	-	Inquiry for cell bank manufacture to BIOMEVA Inquiry for cell bank testing to GLP laboratory	-	Client Client
2	-	Confirmation of receipt of inquiry, forwarding of information & questionnaire to client	-	BIOMEVA
3	-	Confidentiality agreement Completion of Basic Information Request (see chapter 1 and 2)	-	All involved companies Client
4	-	Issue of Quotation & Contract, approval by client	-	BIOMEVA
5	Week 1 - 2	Shipment of 4 seed stock vials (RCB) to GLP laboratory for pre bank testing: Quarantine Screen Assays (purity & lysogenic prophage / free bacteriophage)	-	Client
6	Week 1 - 2	Start generation of draft manufacturing documentation	Signed contract	BIOMEVA
7	Week 2 - 3	Start review of draft manufacturing documentation Shipment of 4 seed stock vials (RCB) to BIOMEVA	 The seed strain is pure and free of lysogenic prophage / bacteriophage contamination	Client Client
8	Week 3	Implementation of amendments / changes, start generation of pre-final manufacturing documentation	Availability of client's comments on draft manufacturing documentation	BIOMEVA

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STEP	TIMEFRAME	ACTIVITY	PREREQUISITES	RESPONSIBILITY
9	Week 3 - 4	Start cell bank technology transfer run (trial run to determine the growth characteristics of the strain)	Arrival of 4 seed stock vials (RCB)	BIOMEVA
10	Week 4	Start Quality Assurance review, start generation final manufacturing documentation	-	BIOMEVA
11	Week 4 - 5	Approval of final manufacturing documentation	-	BIOMEVA and client
12		Document control and operator's training	Approved final manufacturing documentation	BIOMEVA
13	Week 5	Start cell bank manufacture	-	BIOMEVA
14	Week 6	Shipment of vials to GLP laboratory for post bank testing	-	BIOMEVA, GLP laboratory
15	-	Finalisation review completed manufacturing documentation	-	BIOMEVA

Note: This timeline can be shortened or extended primary due to the progress in providing information on the genetically modified organism (see chapter 1) and due to the progress in providing technology transfer information (see chapter 2).

4. STANDARD MANUFACTURING PROCEDURES (MCB, WCB)

Standard Manufacturing Procedure of a Master Cell Bank (MCB) at BIOMEVA GmbH:
A frozen vial of the RCB is used to inoculate medium agar plates. After incubation on plates and transferring one single colony from the plate into culture medium the culture is cultivated at a specified temperature and shaking rate. Cultivation is continued until optical density of the culture has reached a specified range for harvest. Cells are then harvested by adding cryopreservation media and filling of the cell suspension into a defined number of labelled vials. After cryopreservation of the filled vials using a controlled rate freezer the vials are finally transferred to storage in the vapour phase of liquid nitrogen.

Production step	Description
Weighing procedures	Media ingredients
Preparation of media and solutions	Media solutions NaOH solution Cryopreservation medium
pH adjustment with NaOH	Within a specified pH range
Sterilisation procedures	Solid program Liquid program (each T ≥ 121°C, t ≥30 min)
Labelling of vials	Specified number of vials
Manufacture of agar plates (incl. addition of antibiotic)	6 medium agar plates (antibiotic conc.: to be specified)
Removal and thawing of seed stock 	1 vial of the RCB
Inoculation of agar plates 	Inoculation of 5 plates 1 negative control plate
Incubation on agar plates	Specified temperature and time
Preparation of production flasks (incl. addition of antibiotic) 	1 x “Culture” 1 x “Negative Control” 1 x “Monitoring” 1 x “Dilution” (antibiotic conc.: to be specified)
Inoculation and cultivation of flasks	Specified temperature, time and shaking rate until OD reaches a specified range
Cell harvest (centrifugation step if required)	Addition of freezing medium
Filling of bulk into vials 	Specified number of vials (maximum: 500 vials)
Cryopreservation of filled vials	Cryopreservation program
Storage of vials	Vapour phase of liquid nitrogen

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Standard Manufacturing Procedure of a Working Cell Bank (WCB) at BIOMEVA GmbH:

A frozen vial of the MCB is used to inoculate the culture medium directly. The culture is cultivated at a specified temperature and shaking rate. Cultivation is continued until optical density of the culture has reached a specified range for harvest. Cells are then harvested by adding cryopreservation media and filling of the cell suspension into a defined number of labelled vials. After cryopreservation of the filled vials using a controlled rate freezer the vials are finally transferred to storage in the vapour phase of liquid nitrogen.

Production step	**Description**
Weighing procedures	Media ingredients
Preparation of media and solutions	Media solutions NaOH solution Cryopreservation medium
pH adjustment with NaOH	Within a specified pH range
Sterilisation procedures	Solid program Liquid program (each T ≥ 121°C, t ≥30 min)
Labelling of vials	Specified number of vials
Preparation of production flasks (incl. addition of antibiotic) 	1 x “Culture” 1 x “Negative Control” 1 x “Monitoring” 1 x “Dilution” (antibiotic conc.: to be specified)
Removal and thawing of seed stock 	1 vial of the MCB
Inoculation and cultivation of flasks	Specified temperature, time and shaking rate until OD reaches a specified range
Cell harvest (centrifugation step if required)	Addition of freezing medium
Filling of bulk into vials 	Specified number of vials (maximum: 500 vials)
Cryopreservation of filled vials	Cryopreservation program
Storage of vials	Vapour phase of liquid nitrogen

5. CONTACTS

Please contact the following persons at BIOMEVA if you require additional assistance as for cell banking activities:

Dr. Thomas PULTAR	**Dr. Berengar JAHN**
CEO, Managing Director	Head Fermentation
BIOMEVA GmbH	BIOMEVA GmbH
Czernyring 22	Czernyring 22
D-69115 Heidelberg	D-69115 Heidelberg
Germany	Germany
Fon: +49/6221/9026-0	Fon: +49/6221/9026-61
Fax: +49/6221/9026-90	Fax: +49/6221/9026-90
Email: thomas.pultar@biomeva.com	Email: berengar.jahn@biomeva.com

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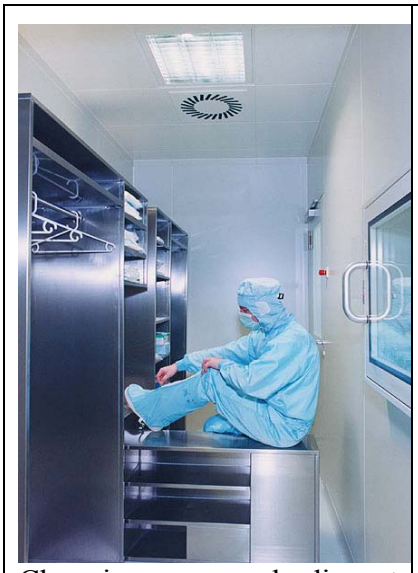
6. IMPRESSIONS

BIOMEVA possesses extensive experience in microbial cell bank manufacture stretching back more than eight years.
Since 2003 cell bank activities are performed in a new "dedicated" cell bank area. More than 50 master and working cell banks have been manufactured in these certificated and state-of-the-art labs:

- Autoclave room (Class 100.000)
- Changing room and air lock (Class 10.000)
- Cell banking lab (Class 100 / 10.000)
- Cell bank storage (Class 100.000)



Changing room and adjacent air lock (both Class 10.000).

Cell banking lab (Class 10.000) with biosafety cabinet (Class 100) and equipment.

Cell bank storage room (Class 100.000). Cells are stored in the gas phase of liquid nitrogen in temperature monitored containers connected to an alarm system.

All rooms are connected by hutches.

Additionally, a room for media preparation (dedicated equipment for cell banking activities) and autoclaving procedures exists within this cell bank area.