

Minutes of the workshop on the Baculovirus reference Material Initiative

Kuopio, 27th of August 2010

Panel: K. Airene, P. Alves, Y. Hashimoto, Y.-C. Hu, **A. Kamen**, O.-W. Merten, M.

Mezzina, M. van Oers, L. Volkmann

Participants: panel and participants of the symposium

- Need for a reference standard or material was generally accepted. This material will be used for biotechnological applications, thus at the base it will consist of budded virus (BV).

- Bacmid/baculovirus – TG and promoter:

- Preference for a recombinant and not a wild-type virus? **(1)**

- Proposition to use GFP as transgene, however, it seems that it is not stable in insect cells **(2)**. Its advantage is the possibility to perform life imaging which is not possible for mSEAP or β -gal. However, luciferase could also be a possibility because it allows life imaging. In favour of GFP: all other reference standards (AdV, AAV2) made use of GFP **(3,4)**.

- Proposition to use combined promoter active in insect and mammalian cells (titration in both systems will be possible):

e.g.: p10/CMV or from arboviruses

- As the true purpose is a reference standard/material for all applications, thus a wt virus might be the better reference (modified as little as necessary) because any modification has to be studied and fully validated **(5)**.

- Baculovirus strain: AcMNPV, strain E2 **(6,7)**.

- Stability issues, titration, controls:

- any baculovirus and the Sf9 cells change over passages. This means that low passage baculovirus should be used. On the cell side, it had been shown for certain Sf9 stocks that they keep their production behaviour for >20 passages **(8)**.

-Titrations should be done with only one cell line/clone and not with different cell lines **(8a)**.

- The baculovirus reference stock has to be partially sequenced, production batch records have to be available, etc.

- **Definition (9):** Reference material = bench mark

Reference standard – much higher quality and QC than reference material

This has to be defined in beforehand

- Manufacturing:

Target titer: 2×10^8 PFU/ml – 10^9 PFU/ml

A higher titer is preferable because at low concentrations, the vector might be lost due to adherence to the vial wall → definition of the vial material is critical.

How many vials to be produced?

What will be the volume per vial?

Cell line: Sf9 – they should be from a cell bank (Invitrogen, ATCC, or else). If cells from a MCB (from a commercial company) are used, their might be licence and IP issues, which could reduce the accessibility of the cells to everybody involved in testing.

Production mode: Batch (the easiest way to produce)

Number of runs: proposition of 2-3 runs for reproducibility reasons (GMP like)
These preparations should be tested for stability over long time.

Purification: if purification, this should be done at the same place as the production (question of logistics for transferring the supernatant to the place of purification)

Final formulation – storage: for stability reasons the baculovirus can be frozen (-80°C) or lyophilized.

Preference for freezing because not much is known on lyophilisation of baculovirus (9a,9b,9c). No protocols are available. Data might be available from the biopesticide field. field (but that is then occlusion bodies not budded virus for which we aim to develop the standard).

On another side, lyophilisation has the advantage that the lyophilized preparation does not have to be stored in a freezer.

Proposition: The vials should be stored at -80°C (freezing leads to titer loss, however, then the titer seems to be stable – only few data are available concerning the stability at -80°C).

Medium (10) is better than PBS for stabilizing baculovirus. The addition of glycerol (11) can be beneficial to avoid aggregation.

Problem: sedimentation of baculovirus during long term storage at 4°C which can lead to particle fusion → storage at -80°C (12).

Characterisation: the material has to be properly characterized else, no value; everybody should be involved in the characterization, the protocols will be distributed, etc.) (13)

Deposit: at a professional depot, such as ATCC.

Next steps:

This initiative will be announced on the web site.

Action: Mauro Mezzina

Proposition for a web-meeting within some months for continuing the discussion and preparing a decision

Action: Amine Kamen

Next possible meeting (follow-up meeting): PEACE meeting, in Portugal in September 2011

Action: Amine Kamen/Paula Alves

The committee should meet earlier: during the ESGCT or Clinigene Meeting

In addition discussed outside the workshop with several members of the committee:

We plan to launch this initiative in a special issue of the Journal of Invertebrate pathology guest edited by Monique van Oers (Amine Kamen principle author).

Annex with the comments from some of the experts of the panel:

YH: Yoshifumi Hashimoto

A: Kari Airene

U: Paula Alves

Andy

Amine

Otto

(the comments from Monique had already been directly integrated into the text)

Comments:

- (1) A: recombinant.
- (2) A: I don't understand this. Why EGFP would be toxic for insect cells ? Any references?
- (3) YH: It is my understanding that GFP needs to be dimerized for excitation and GFP is a relatively large protein. Although GFP has already been used widely, GFP is not stable in insect cells as Loy pointed out. We might need to consider the second reporter, like m-Cherry, which is smaller and monomer reporter protein. This does not relate to the minutes and is just my thought.
- (4) A: KA I also like mCherry, but the use of it requires optimized filters in the equipments. That is why I think EGFP would still be better choice to fit what most labs are familiar to work with already. EGFP as a transgene is also supported by the ad and AAV ref material.
- (5) A: KA Wt virus wouldn't support all applications !
- (6) YH: I think this should be C6, which is originally isolated and sequenced by NERC Oxford group.
- (7) A: The bacmid is based on the E 2 (=L1 ?) variant of AcMNPV ! This is currently the most handy and widely used AcMNPV variant.
- (8) A: KA The bacmid system helps to keep the passage issue in better control, because new low passage virus lots can be easily generated from the characterized and purified clonal "master Bacmid DNA".
 - (8a) U: PA Should the cells for titration be also provided with the reference material? If we need to be sure that a cell bank is prepared and fully characterized (screened for viruses, etc) or use ATCC or ECACC
 - (9) YH: Do we provide the information of the reference materials or do we actually distribute the reference materials based on request ? I still do not fully understand our goal of this initiative....
 - (9a) U: PA Decision on storage temperature will affect the formulation and this also affects the yes or no regarding purification/concentration steps requirements for exchange of medium, buffers etc. If no purification or concentration step is performed we have to evaluate the impact of freezing in culture medium, even if glycerol or other cryo-stabilizer e.g. sucrose or trehalose, is added. Impact of cryo-temperature on pH during long term storage. Stability studies required.
 - (9b) Andy: I have only one remark at this time: the lyophilization might not work. I have reviewed a manuscript recently, which address the issue of baculovirus lyophilization. The authors tried to mimic the lyophilization condition by drying the baculovirus on culture plates, and then add medium back to the plate to reconstitute the virus solution. They found that baculovirus infectivity is greatly lost. So it seems that the baculovirus infectivity might be lost after dring.
 - (9c) Amine/Otto: drying os not lyophilisation
- (10) YH: With light protection

- (11) A: KA or Sucrose.
- (12) A: KA Better formulation needed to guarantee the virus quality in all cases.
Glycerol/sucrose helps to avoid aggregation?
- (13) U: PA Intra lab reproducibility may also add useful information. (inter-lab is already considered).