Long-term, large scale cryopreservation of insect cells at -80°C

Lucie Vyletova · La'Verne P. Rennalls ·
Kirstin J. L. Wood · Valerie M. Good

Received: 28 April 2014 / Accepted: 10 August 2014 / Published online: 23 August 2014
© Springer Science+Business Media Dordrecht 2014

Abstract Standard tissue culture methods advise freezing cells in small aliquots ($\leq 1 \times 10^7$ cells in 1 mL), and storing in liquid nitrogen. This is inconvenient for laboratories culturing large quantities of insect cells for recombinant baculovirus expression, owing to the length of time taken to produce large scale cultures from small aliquots of cells. Liquid nitrogen storage requires use of specialized cryovials, personal protective equipment and oxygen monitoring systems. This paper describes the long-term, large scale cryopreservation of 8×10^8 insect cells at -80°C , using standard 50 mL conical tubes to contain a 40 mL cell suspension. Sf9, Sf21 and High 5 cells were recovered with a viability $> 90\%$ after

storage for one year under these conditions, which compared favorably with the viability of cells stored in liquid nitrogen for the same length of time. Addition of green fluorescent protein encoding baculovirus demonstrated that cells were “expression ready” immediately post thaw. Our method enables large scale cultures to be recovered rapidly from stocks cryopreserved at -80°C , thus avoiding the inconvenience, hazards and expense associated with liquid nitrogen.

Keywords Cryopreservation · Insect cell culture · Baculovirus expression · Green fluorescent protein

La'Verne P. Rennalls and Kirstin J. L. Wood contributed equally to the work.

Electronic supplementary material The online version of this article (doi:10.1007/s10616-014-9781-5) contains supplementary material, which is available to authorized users.

L. Vyletova · La'Verne P. Rennalls ·
K. J. L. Wood · V. M. Good (✉)
Section of Structural Biology, The Institute of Cancer
Research, 237 Fulham Road, London SW3 6JB, UK
e-mail: vgood@icr.ac.uk

Present Address:

K. J. L. Wood
Laboratory of Protein Crystallography, Centre for
Amyloidosis and Acute Phase, Proteins, UCL Division of
Medicine (Royal Free Campus), Rowland Hill Street,
London NW3 2PF, UK

Introduction

The baculovirus expression vector system (BEVS) is used by many laboratories to produce high levels of recombinant eukaryotic proteins in their native conformation with posttranslational modifications (Drugmand et al. 2011; Hitchman et al. 2009). The eukaryotic nature of the system allows the production of proteins which cannot be expressed in a soluble form with simple prokaryotic recombinant systems (Chambers et al. 2004). It is well suited to convenient suspension culture and avoids the carbon dioxide requirements and safety issues of mammalian expression (Drugmand et al. 2011). The BEVS has become indispensable for researchers interested in producing large amounts of multi-subunit complexes with the introduction of MultiBac vectors designed for this purpose (Barford et al. 2013; Bieniossek

et al. 2012). Elucidation of multi-subunit complex structures with immense biological significance by crystallography and single particle electron microscopy (Chao et al. 2012; Enchev et al. 2010; Schreiber et al. 2011), and production of safer, effective, virus-like particle vaccines (Fernandes et al. 2013) have only been possible by the use of material derived from recombinant baculovirus infected insect cells.

The BEVS is made up of two biological components: an insect cell line host, and a recombinant baculovirus vector. Expression is driven by a baculovirus promoter activated during the infection of insect cells, and depends on healthy host cells for maximum production of foreign protein (Drugmand et al. 2011; Hitchman et al. 2009). Our research was concerned with alleviating the bottleneck associated with insect cell availability by investigating cryopreservation methods to regenerate healthy insect cultures suitable for large scale expression in a timely manner.

Conventionally, animal cells are stored in small aliquots ($\leq 1 \times 10^7$ cells in 1 mL) in liquid nitrogen at -196°C (King and Possee 1992a; Murphy et al. 2004; Summers and Smith 1987). This methodology is inconvenient for laboratories wishing to store large quantities of cells, as the volume of cell suspension is limited by the size of cryovials recommended for use in liquid nitrogen (<5 mL). In addition, liquid nitrogen is a hazardous substance and can only be used after careful risk assessment and health and safety training.

Many mammalian cell lines have been successfully stored at -80°C , including those derived from human (DU145, WM39, HEL, K-562, HL-60), hamster (CHO, TKKL), monkey (COS) and mouse (NIH 3T3) (Ratajczak et al. 1994), while others (human skin epithelium NCTC 2544 and mouse fibroblast ATCC CCL1) have died within a year (Simione 1992). Insect cell cryopreservation protocols recommend overnight storage of insect cells at -80°C , before long term storage in liquid nitrogen (King and Possee 1992a; Murphy et al. 2004; Summers and Smith 1987), and to our knowledge, there is no published data regarding long term survival of insect cells at -80°C . We investigated the recovery of large scale insect cell cultures after long term cryopreservation at -80°C , with the aim of validating a new storage methodology.

We froze the major BEVS insect cell line hosts (Sf9, Sf21 and High 5) in medium containing 7.5 % (v/v) dimethyl sulfoxide (DMSO) as a cryoprotectant. Small aliquots of cells (2×10^7 cells in 1 mL) were stored in

Fig. 1 Schematic diagram illustrating the revival and expression analysis of cryopreserved insect cells. Cells revived from liquid nitrogen (LN2) or freezer (-80°C) storage were transferred into culture medium as follows: 1 mL samples into 20 mL, in 125 mL Erlenmeyer flasks; 20 mL of the 40 mL sample into 400 mL culture medium, in 2 L roller bottles (upright configuration). One roller bottle culture was infected with eGFP virus at a multiplicity of infection = 2. Conditions were standardized by transferring 21 mL cell suspension $\pm$ virus from the roller bottle to 125 mL flask. Cultures were incubated for 2 days at 27°C with shaking at 150 rpm

a mechanical freezer at -80°C and in liquid nitrogen at -196°C , recovered under identical conditions and survival compared. Large aliquots of cells (8×10^8 cells in 40 mL) were stored in a mechanical freezer at -80°C and recovered under two sets of conditions, one of which was identical to that used for recovering the small aliquots (Fig. 1). Cell survival was investigated in relation to storage quantities, times and temperatures.

For the first time, to our knowledge, we demonstrate that storage of Sf9 and High 5 for 14 months (439 days) and storage of Sf21 for 6 months (187 days) in a mechanical freezer at -80°C is as effective in maintaining insect cell viability and growth potential as storage in liquid nitrogen. In addition, the method we describe allows large scale cultures needed for protein production to be recovered rapidly from cryopreserved stocks, avoiding the inconvenience, hazards and expense associated with liquid nitrogen.

Materials and Methods

Cell lines

The following insect cell lines were obtained from Life Technologies (Carlsbad, CA, USA): catalogue #11497-013 Sf21 (IPLB-Sf21AE) from ovaries of the fall

armyworm *Spodoptera frugiperda* (Vaughn et al. 1977), catalogue #11496-015 Sf9 clonal isolate from Sf21 (Summers and Smith 1987) and catalogue #B85502 High 5 (BT1-TN-5B1-4) from embryonic tissue of the cabbage looper, *Trichoplusia ni* (Davis et al. 1992; Wickham et al. 1992).

Culture medium

SF900II (Life Technologies) supplemented with sodium benzylpenicillin at 30 µg/mL and streptomycin sulphate at 50 µg/mL.

Cryopreservation

Freezing medium: 7.5 % (v/v) HYBRI-MAX DMSO (Sigma (St. Louis, MO, USA) D2650), 46.25 % (v/v) culture medium and 46.25 % (v/v) conditioned culture medium (used for growing cells for 2 days). All cells were frozen during mid log growth phase (≥ 98 % viability) and cell pellets were resuspended in freezing medium at a final concentration of 2×10^7 cells/mL. Cell suspension was then aliquoted into Nesco film sealed conical tubes (Falcon (Corning, Tewksbury, MA, USA) 352070; 40 mL each) and into cryoflex sealed internal thread cryovials (Nunc (Roskilde, Denmark) 377224; 1 mL each). The tubes (2 Falcon, 4 Nunc), were placed in an Eprak 5003 box and stored in a -80 °C mechanical freezer (New Brunswick Scientific (Enfield, CT, USA) Premium U410). After 48 h had elapsed, half of the cryotubes were transferred to a liquid nitrogen tank (Taylor-Wharton (Theodore, AL, USA) 3000 RS) and stored at -196 °C. This procedure is based on original insect cryopreservation methodologies (King and Possee 1992a; Murphy et al. 2004; Summers and Smith 1987), modified further by Life Technologies (<http://tools.invitrogen.com/content/sfs/manuals/3910.pdf>).

Thawing of cell lines

At various intervals, cryopreserved cell samples were thawed in a water bath (37 °C) by manual agitation and transferred to culture vessels as shown in Fig. 1.

Baculovirus maintenance

Low passage stocks of eGFP virus were stored at -80 °C. Working stocks of eGFP virus were stored at

4 °C and discarded when the titer dropped below 1×10^8 pfu/mL, as determined by plaque assay (King and Possee 1992b).

Expression analysis

After 48 h incubation, the fluorescence intensities of eGFP infected cells (100 µL) were measured in F96 MicroWell Plates (Nunc 237105) using a BMG POLARstar Omega plate reader (excitation wavelength 485 nm; emission wavelength 520 nm; Gain = 1,460). Prism graphs were plotted after subtraction of medium fluorescence background.

Cell counts

Cells were counted using a Life Technologies Countess automated cell counter. Trypan blue solution [0.2 % (w/v)] was used in order to determine the percentage of viable cells per condition.

Statistical analysis

Cryopreservation results were entered into grouped GraphPad Prism tables (version 6.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com) with cell line/cryopreservation condition/recovery condition as the column factor and length of time frozen as the row factor and analysed using 2-way analysis of variance (ANOVA). The data in each subcolumn was derived from cells frozen on the same day, thawed at various intervals. This arrangement of data permitted repeat measures analysis, i.e. matched samples in sub columns, differentiated by timed analysis according to row. Samples were prepared for all cryopreservation conditions on the same day, hence values were also matched across columns, permitting repeat measures analysis by both factors. Multiple comparisons were made by comparing the column means within each row, and corrected using the recommended Sidak (2 groups) or Tukey (3 groups) post hoc test. Separate Prism tables were created for the inclusion of continuous culture control data. Comparisons were made without repeat measures as the continuous control experiments were not carried out in parallel with the cryopreservations. Multiple comparisons of viability and cell density data were made by comparing the row means within each column to the continuous control row mean on that

Fig. 2 Viability of cryopreserved insect cells. Insect cells were cryopreserved in liquid nitrogen (LN2) in 1 mL volumes and at -80°C in 1 mL and 40 mL volumes. Frozen cells were thawed into medium at an initial density of 9.5×10^5 cells/mL and incubated in roller bottles (RB) and/or Erlenmeyer flasks (EF). After a 2 day recovery period, the trypan blue exclusion test was used to determine the viability of **a** Sf9, **b** Sf21 and **c** High 5 cells. Each 4-bar group represents the percentage viability of cells frozen for 5 (*far left bar*), 187, 439 days or 607 days (*far right bar*) and revived in flasks. Each 5-bar group represents the percentage viability of continuous culture control cells (*far left bar*; no freezing, otherwise same treatment as cryopreserved

cells), or cells frozen for 5, 187, 439 or 607 days (*far right bar*) and revived in roller bottles. Individual bars represent the mean of 7 observations with error bars representing the standard error mean (SEM). Significant differences between the viabilities of cells stored or recovered under different conditions are indicated by * p value <0.05 , ** p value <0.01 or *** p value <0.001 with a line linking the relevant bars. Mean $\pm$ SEM and p values <0.05 are tabulated in online resource 1. Following the initial 2 day post thaw analysis, cells were subcultured and incubated for a further 2 days. Cryopreserved Sf9, Sf21 and High 5 cell viabilities measured 4 days post thaw were found to be similar to their continuous culture controls (data not shown)

column, and corrected using the recommended Dunnett post hoc test. Multiple comparisons of fluorescence data were made by comparing the column means within each row, and corrected using the recommended Sidak (2 groups) or Tukey (3 groups) post hoc test. A p value of <0.05 was considered significant.

Results and discussion

The BEVS has a proven track record in terms of producing soluble eukaryotic protein (Chambers et al. 2004; Drugmand et al. 2011; Hitchman et al. 2009), and is well suited to the co-expression and self-assembly of multi subunit complexes (Barford et al. 2013). Three-dimensional structures of biologically important multi subunit complexes have been

determined after purification from infected insect cells (Chao et al. 2012; Enchev et al. 2010; Schreiber et al. 2011). Large quantities of material are required for crystallographic studies (Barford et al. 2013), hence the need for large scale insect expression cultures. Demand can fluctuate from week to week, however, as researchers divide their time according to the varying expression, purification, crystallization and data analysis needs of their projects. It is helpful, therefore, to re-generate cultures rapidly when required, rather than continuously maintaining expensive large scale cultures. In addition, a catastrophic loss of cultures by contamination can delay research work by several weeks if it is necessary to wait for large scale cultures to be restored from small aliquots of cells stored in liquid nitrogen.

The cryopreservation method presented in this paper represents a useful advance in terms of the

Fig. 3 Growth of cryopreserved insect cells. Insect cells were cryopreserved in liquid nitrogen (LN2) in 1 mL volumes and at -80°C in 1 and 40 mL volumes. Frozen cells were thawed into medium at an initial density of 9.5×10^5 cells/mL and incubated in roller bottles (RB) and/or Erlenmeyer flasks (EF). After a 2 day recovery period, the trypan blue exclusion test was used to determine the viable cell density of **a** Sf9, **b** Sf21 and **c** High 5 cells. Each 4-bar group represents the density of cells frozen for 5 (far left bar), 187, 439 days or 607 days (far right bar) and revived in flasks. Each 5-bar group represents the density of continuous culture control cells (far left bar; no freezing, otherwise same treatment as cryopreserved cells), or the density of cells frozen for 5, 187, 439 or 607 days (far right bar) and revived in roller bottles. Individual bars represent the

mean of 7 observations with error bars representing the standard error mean (SEM). Significant differences between the densities of cells stored or recovered under different conditions are indicated by * p value <0.05 , ** p value <0.01 or *** p value <0.001 with a line linking the relevant bars. Mean $\pm$ SEM and p values <0.05 are tabulated in online resource 2. Following the initial 2 day post thaw analysis, recovered cells were subcultured and incubated for a further 2 days. Cryopreserved Sf9, Sf21 and High 5 cell densities measured 4 days post thaw were found to be similar to their continuous culture controls (data not shown). The revival densities of High 5 cells reached a maximal cell density at approximately 4×10^6 cells/mL, while Sf9 and Sf21 continued growing to approximately 1×10^7 cells/mL (data not shown)

ability to regenerate large scale insect expression cultures in a timely manner. The method was validated with three insect cell lines used frequently for expression, with recovery from -80°C similar to standard liquid nitrogen storage (Figs. 2, 3; online resources 1 and 2).

We carried out cryopreservation studies using Sf9, Sf21 and High 5, as all three insect cell lines have proven utility for baculovirus expression. Titering and amplification of recombinant viruses are carried out in Sf9, as this cell line yields the highest viral titers (Drugmand et al. 2011; Hashimoto et al. 2010). The Sf21 and High 5 cells express higher levels of recombinant protein than Sf9 for the majority of constructs (Drugmand et al. 2011; Hashimoto et al. 2010; Kulakosky and Klinge 2003), but there are

exceptions, and the optimal expressing cell line for any given construct requires empirical determination (Hink et al. 1991). Colleagues at the Institute of Cancer Research have expressed recombinant protein in Sf9 (Dore et al. 2009; He et al. 2013; Oliver et al. 2009), Sf21 (Brennan et al. 2011) and High 5 (Chao et al. 2012; Enchev et al. 2010; Schreiber et al. 2011; Zhang et al. 2013a; b), with the majority of recent work being carried out in High 5.

Historically, the long-term preservation of mammalian cells at -80°C has met with variable success, with some tumor cell lines and cells destined for transplantation surviving (McCullough et al. 2010; Ratajczak et al. 1994; 1997), and other cell lines dying within a year (Simione 1992). We report that insect cells can be preserved at -80°C and extend the

application of this observation by increasing preserved volumes to levels allowing for regeneration of large scale insect cell expression cultures in a timely manner. Large scale working stocks of frozen cells were conveniently stored at -80°C and, following a 2 day post thaw recovery period, regained the viability and growth potential characteristics of continuous culture controls (Figs. 2, 3; online resources 1 and 2). These results illustrate the utility of storing cells at -80°C in the medium term, although it should be noted that there was a small decrease in the recovery viabilities and growth potentials of insect cells stored at -80°C for periods greater than one year (Figs. 2, 3; online resources 1 and 2), indicating that liquid nitrogen remains the optimal condition for indefinite storage.

Trypan blue exclusion is not a reliable marker of viability when carried out immediately post thaw ((Ratajczak et al. 1994) and our unpublished observations), so we chose to measure viability after a two day incubation period. The number of viable cells present at the end point indicates not only the effectiveness of the cryopreservation method but also how quickly the surviving cells are dividing. Initially, cryopreserved cells were resuspended in medium at a concentration of 9.5×10^5 cells/mL, but after the 2 day incubation period, cell densities had increased (Fig. 3 and online resource 2). The cell densities achieved 2 days post thaw increased in ascending order: Sf9 ($1.3\text{--}1.9 \times 10^6$ cells/mL), Sf21 ($1.6\text{--}3.1 \times 10^6$ cells/mL), High 5 ($3.2\text{--}4.2 \times 10^6$ cells/mL) (Fig. 3 and online resource 2). This is consistent with doubling times reported by other workers: Sf9 (40 h) (Drugmand et al. 2011), Sf21 (36–39 h) (Pasumathy and Murhammer 1994) and High 5 (33 h) (Drugmand et al. 2011).

In order to compare cryopreservation in small (2×10^7 cells in 1 mL) and large (8×10^8 cells in 40 mL) aliquots, it was necessary to standardize the recovery procedure. Small aliquots were recovered by adding 2×10^7 cryopreserved cells in 1–20 mL medium in a 125 mL Erlenmeyer flask. Large aliquots were recovered by adding 20 mL (taken from 8×10^8 cryopreserved cells in 40 mL) to 400 mL medium in a roller bottle. The recovery procedure was standardized by the transfer of 21 mL from the roller bottle culture to a 125 mL Erlenmeyer flask, allowing a direct comparison between cells cryopreserved in small and large aliquots (both being recovered under identical conditions). The standard recovery conditions indicated that

storage of Sf9 and High 5 cells for 20 months (607 days) and storage of Sf21 cells for 6 months (187 days) in small or large aliquots at -80°C was equally effective. In contrast, after 20 months (607 days) storage at -80°C , large aliquots of Sf21 cells were recovered with lower viabilities and growth potentials than small aliquot equivalents (Figs. 2, 3; online resources 1 and 2). The reason for this effect is not known, although one might suppose that it relates to the dynamics of freezing and thawing large aliquots, which appear to be more harmful to Sf21 cells than the other cell lines.

The viability and growth potential of Sf9 cells were independent of recovery procedure (Figs. 2, 3; online resources 1 and 2). In contrast, the Sf21 and High 5 recovery densities in 125 mL Erlenmeyer flasks were lower and higher (respectively) than recovery densities in roller bottles (cryopreservation for 5 and 187 days; Fig. 3 and online resource 2). The shape of the Erlenmeyer flasks is optimized for culture aeration, compared with the shape of the roller bottle, and while this is known to have a positive effect on High 5 cell growth (Rhiel et al. 1997), it appears to have a detrimental effect on Sf21. These results illustrate the importance of adequate controls, since if the cells stored in small aliquots recovered in Erlenmeyer flasks had been compared directly with cells stored in large aliquots recovered in roller bottles, there would have been a misinterpretation of results owing to the use of different recovery conditions. Hence, cells from all storage conditions were recovered in Erlenmeyer flasks for the appropriate comparison of cryopreservation conditions.

The practical aim of our cryopreservation studies was to re-establish large scale insect expression cultures rapidly from frozen stocks. We demonstrated that actively growing large (2×0.4 L) scale cultures with good viability could be regenerated within 2 days from a 40 mL frozen aliquot containing 8×10^8 insect cells stored at -80°C . This represents a time saving of approximately 2 weeks compared with regenerating the same amount of culture from a 1 mL aliquot containing 2×10^7 insect cells frozen in liquid nitrogen. We also demonstrated that insect cells stored in a 40 mL aliquot at -80°C were “expression ready” immediately post thaw, although maximum yields required an incubation period prior to infection (Fig. 4; online resource 3). Infecting cells immediately post thaw has the potential to dramatically reduce time

Fig. 4 Expression of baculovirus encoded eGFP. Insect cells (which had been resuspended in freezing medium at a density of 2×10^7 cells/mL with or without freezing) were diluted in culture medium to an initial density of 9.5×10^5 cells/mL in roller bottles, infected with eGFP virus and incubated for 2 days. The eGFP expressing cells were diluted in culture medium and analysed. Each 2-bar group represents the fluorescence of cells not frozen (*left bar*) or frozen for 607 days (*right bar*). Individual bars represent the mean of 7 observations with error bars representing the standard error mean. Significant differences between the eGFP generated fluorescence of control versus frozen cells or between the different cell lines are indicated by **p* value <0.05, ***p* value <0.01 or ****p* value <0.001 with a line linking the relevant bars. Mean $\pm$ SEM and *p* values <0.05 are tabulated in online resource 3. Cryopreserved cells, not infected immediately post thaw, were allowed to recover for 2 days before the addition of eGFP baculovirus. Expression of eGFP in High 5 cells infected after a 2 day recovery period, measured 4 days post thaw, was found to be similar to the expression level of the continuous culture control (data not shown). The medium alone exhibited fluorescence, and this was subtracted from eGFP cell sample results. Blank corrected fluorescence was found to be linear in the range 20,000–150,000 relative fluorescence units (RFU) (data not shown), and all samples were diluted until the fluorescence detected was within this range. Samples of non infected cells and cells infected with a non fluorescent protein encoding virus were also analysed and found to be equivalent to medium (data not shown). No eGFP was detected in trypan blue stained cells when analysed under the fluorescence microscope (data not shown)

scales and offers the flexibility of a prokaryotic type stop/start expression system, so could be the method of choice when absolute yield is not a priority.

Cell line specific expression levels increased in the ascending order: Sf9, Sf21, High 5 (Fig. 4 and online resource 3), which is the same order as the cell line specific growth characteristics and the same pattern of expression as seen with the majority of other constructs (Drugmand et al. 2011; Hashimoto et al. 2010; Kulakosky and Klinge 2003). In contrast, expression

levels for all three cell lines were independent of whether expression took place in a flask or roller bottle (data not shown). This suggests that the mechanisms for eGFP expression in High 5 cells were saturated, and indicates that optimal expression conditions should not necessarily be predicted from optimal growth conditions.

In addition to reporting that insect cells can be stored long term in large scale at -80°C (the main objective of this work), we describe a number of labour and cost saving modifications to the original cryopreservation protocols. Express 5 is recommended by the manufacturer for the cultivation and freezing of High 5 cells, but we grew Sf9, Sf21 and High 5 cells in SF900II, as have other workers (Rhiel et al. 1997). Standardization of media simplifies the large scale maintenance of multiple cell lines. The High 5 cell line was maintained initially as an adherent culture which was only suitable for small scale expression work (Wickham et al. 1992). Adaptation of High 5 to suspension culture was essential for producing large amounts of protein (Rhiel et al. 1997). Our large aliquots of frozen insect cells (8×10^8 cells in 40 mL) were thawed directly into suspension culture, avoiding the need for adherent cultures and associated delays in the return to large scale expression work. Insect cells frozen using the original protocol recovered with <80 % viability (Weiss et al. 1981), whereas the cells frozen using our adapted protocol were recovered with >90 % viability (Fig. 2 and online resource 1). Protocols for the freezing of insect cells recommended filtration of the freezing medium (Summers and Smith 1987), but we omitted this step as all components were sterile. Cryopreservation is reported to be most successful when the temperature is reduced at the rate of 1°C per minute and there are specialized, costly devices designed to achieve optimal cooling rates (<http://www.nalgenelabware.com/techdata/Technical/cryo.pdf>). We froze the insect cell suspensions using inexpensive, polystyrene Eprax 5,003 boxes, as have other workers (Summers and Smith 1987). The polystyrene insulated the insect cell suspension from the surrounding environment of the -80°C freezer, facilitating a slow cooling of the insect cell suspension.

Conclusion

In summary, we made a systematic comparison between the classical liquid nitrogen (-196°C) and the more convenient mechanical freezer (-80°C)

storage of insect cells. This work represents a useful benchmark for those working with the BEVS, enabling expression laboratories to minimize downtime and maximize productivity.

Acknowledgments The baculovirus expressing green fluorescent protein was a gift from Vangelis Christodoulou. The work was funded by Cancer Research UK programme grants to David Barford, Laurence H. Pearl and Dale Wigley. We would also like to thank David Barford and Jane Sandall for assistance with the critical reading of the manuscript.

References

- Barford D, Takagi Y, Schultz P, Berger I (2013) Baculovirus expression: tackling the complexity challenge. *Curr Opin Struct Biol* 23:357–364. doi:[10.1016/j.sbi.2013.03.009](https://doi.org/10.1016/j.sbi.2013.03.009)
- Bieniossek C, Imasaki T, Takagi Y, Berger I (2012) MultiBac: expanding the research toolbox for multiprotein complexes. *Trends Biochem Sci* 37:49–57. doi:[10.1016/j.tibs.2011.10.005](https://doi.org/10.1016/j.tibs.2011.10.005)
- Brennan DF, Dar AC, Hertz NT, Chao WC, Burlingame AL, Shokat KM, Barford D (2011) A Raf-induced allosteric transition of KSR stimulates phosphorylation of MEK. *Nature*. doi:[10.1038/nature09860](https://doi.org/10.1038/nature09860)
- Chambers SP, Austen DA, Fulghum JR, Kim WM (2004) High-throughput screening for soluble recombinant expressed kinases in *Escherichia coli* and insect cells. *Protein Expr Purif* 36:40–47. doi:[10.1016/j.pep.2004.03.003](https://doi.org/10.1016/j.pep.2004.03.003)
- Chao WC, Kulkarni K, Zhang Z, Kong EH, Barford D (2012) Structure of the mitotic checkpoint complex. *Nature* 484:208–213. doi:[10.1038/nature10896](https://doi.org/10.1038/nature10896)
- Davis TR, Trotter KM, Granados RR, Wood HA (1992) Baculovirus expression of alkaline phosphatase as a reporter gene for evaluation of production, glycosylation and secretion. *Biotechnology* 10:1148–1150
- Dore AS, Kilkenny ML, Rzechorzek NJ, Pearl LH (2009) Crystal structure of the rad9-rad1-hus1 DNA damage checkpoint complex—implications for clamp loading and regulation. *Mol Cell* 34:735–745. doi:[10.1016/j.molcel.2009.04.027](https://doi.org/10.1016/j.molcel.2009.04.027)
- Drugmand JC, Schneider YJ, Agathos SN (2011) Insect cells as factories for biomanufacturing. *Biotechnol Adv*. doi:[10.1016/j.biotechadv.2011.09.014](https://doi.org/10.1016/j.biotechadv.2011.09.014)
- Enchev RI, Schreiber A, Beuron F, Morris EP (2010) Structural insights into the COP9 signalosome and its common architecture with the 26S proteasome lid and eIF3. *Structure* 18:518–527. doi:[10.1016/j.str.2010.02.008](https://doi.org/10.1016/j.str.2010.02.008)
- Fernandes F, Teixeira AP, Carinhas N, Carrondo MJ, Alves PM (2013) Insect cells as a production platform of complex virus-like particles. *Expert Rev Vaccines* 12:225–236. doi:[10.1586/erv.12.153](https://doi.org/10.1586/erv.12.153)
- Hashimoto Y, Zhang S, Blissard GW (2010) Ao38, a new cell line from eggs of the black witch moth, *Ascalapha odorata* (Lepidoptera: Noctuidae), is permissive for AcMNPV infection and produces high levels of recombinant proteins. *BMC Biotechnol* 10:50. doi:[10.1186/1472-6750-10-50](https://doi.org/10.1186/1472-6750-10-50)
- He J, Chao WC, Zhang Z, Yang J, Cronin N, Barford D (2013) Insights into degron recognition by APC/C coactivators from the structure of an Acm1-Cdh1 complex. *Mol Cell* 50:649–660. doi:[10.1016/j.molcel.2013.04.024](https://doi.org/10.1016/j.molcel.2013.04.024)
- Hink WF, Thomsen DR, Davidson DJ, Meyer AL, Castellino FJ (1991) Expression of three recombinant proteins using baculovirus vectors in 23 insect cell lines. *Biotechnol Prog* 7:9–14. doi:[10.1021/bp00007a002](https://doi.org/10.1021/bp00007a002)
- Hitchman RB, Possee RD, King LA (2009) Baculovirus expression systems for recombinant protein production in insect cells. *Recent Pat. Biotechnol* 3:46–54
- King LA, Possee RD (1992a) 5.8.1 Freezing and storage of cells in liquid nitrogen. In: *The baculovirus expression system: a laboratory guide*, 1st edn. Chapman & Hall, pp 102–103
- King LA, Possee RD (1992b) 6.3.1 Standard plaque-assay. In: *The baculovirus expression system: a laboratory guide*, 1st edn. Chapman & Hall, London, pp 111–114
- Kulakosky PC, Klinge CM (2003) Maximizing production of estrogen receptor beta with the baculovirus expression system. *Biotechniques* 34(334–338):334–345
- McCullough J, Haley R, Clay M, Hubel A, Lindgren B, Moroff G (2010) Long-term storage of peripheral blood stem cells frozen and stored with a conventional liquid nitrogen technique compared with cells frozen and stored in a mechanical freezer. *Transfusion* 50:808–819. doi:[10.1111/j.1537-2995.2009.02482.x](https://doi.org/10.1111/j.1537-2995.2009.02482.x)
- Murphy CI, Piwnicka-Worms H, Grunwald S, Romanow WG, Francis N, Fan HY (2004) Maintenance of insect cell cultures and generation of recombinant baculoviruses current protocols in molecular biology. Frederick M, Ausubel [et al] (eds) Chapter 16:Unit 16 10 doi:[10.1002/0471142727.mb1610s65](https://doi.org/10.1002/0471142727.mb1610s65)
- Oliver AW, Swift S, Lord CJ, Ashworth A, Pearl LH (2009) Structural basis for recruitment of BRCA2 by PALB2. *EMBO Rep* 10:990–996. doi:[10.1038/embor.2009.126](https://doi.org/10.1038/embor.2009.126)
- Pasumathy MK, Murhammer DW (1994) Clonal variation in the *Spodoptera frugiperda* IPLB-SF21-AE insect cell population. *Biotechnol Prog* 10:314–319. doi:[10.1021/bp00027a012](https://doi.org/10.1021/bp00027a012)
- Ratajczak MZ, Kregenow DA, Kuczynski WI, Ratajczak J, Gewirtz AM (1994) The storage of cells from different tumor lines in a mechanical freezer at –80 °C. Comparison to cryopreservation in liquid nitrogen. *Mater Med Pol* 26:69–72
- Ratajczak MZ, Ratajczak J, Kregenow DA, Marlicz W, Machalinski B, Simon M, Luger S, Gewirtz AM (1997) An optimization study on unprogrammed cryopreservation of human CD34+ bone marrow cells and their subsequent storage in an –80 °C mechanical freezer. *Ann Transplant* 2:5–11
- Rhiel M, Mitchell-Logean CM, Murhammer DW (1997) Comparison of *Trichoplusia ni* BTI-Tn-5B1-4 (high five) and *Spodoptera frugiperda* Sf-9 insect cell line metabolism in suspension cultures. *Biotechnol Bioeng* 55:909–920. doi:[10.1002/\(SICI\)1097-0290\(19970920\)55:6<909::AID-BIT8>3.0.CO;2-K](https://doi.org/10.1002/(SICI)1097-0290(19970920)55:6<909::AID-BIT8>3.0.CO;2-K)
- Schreiber A, Stengel F, Zhang Z, Enchev RI, Kong EH, Morris EP, Robinson CV, da Fonseca PC, Barford D (2011) Structural basis for the subunit assembly of the anaphase-promoting complex. *Nature* 470:227–232. doi:[10.1038/nature09756](https://doi.org/10.1038/nature09756)
- Simione FP Jr (1992) Key issues relating to the genetic stability and preservation of cells and cell banks. *J Parenter Sci Technol* 46:226–232

- Summers MD, Smith GE (1987) A manual of methods for baculovirus vectors and insect cell-culture procedures. Tex Aes Bull pp 1–56
- Vaughn JL, Goodwin RH, Tompkins GJ, McCawley P (1977) The establishment of two cell lines from the insect *Spodoptera frugiperda* (Lepidoptera; Noctuidae). *In Vitro* 13:213–217
- Weiss SA, Smith GC, Kalter SS, Vaughn JL (1981) Improved method for the production of insect cell-cultures in large volume. *In Vitro Cell Dev B* 17:495–502
- Wickham TJ, Davis T, Granados RR, Shuler ML, Wood HA (1992) Screening of insect cell lines for the production of recombinant proteins and infectious virus in the baculovirus expression system. *Biotechnol Prog* 8:391–396. doi:[10.1021/bp00017a003](https://doi.org/10.1021/bp00017a003)
- Zhang Z, Chang L, Yang J, Conin N, Kulkarni K, Barford D (2013a) The four canonical TPR subunits of human APC/C form related homo-dimeric structures and stack in parallel to form a TPR suprahelix. *J Mol Biol* 425:4236–4248. doi:[10.1016/j.jmb.2013.04.004](https://doi.org/10.1016/j.jmb.2013.04.004)
- Zhang Z, Yang J, Kong EH, Chao WC, Morris EP, da Fonseca PC, Barford D (2013b) Recombinant expression, reconstitution and structure of human anaphase-promoting complex (APC/C). *Biochem J* 449:365–371. doi:[10.1042/BJ20121374](https://doi.org/10.1042/BJ20121374)