

QUOTATION

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| Date: | Name: | Organisation |
| Prepared by: | | |

| Service Notes | Service Detail | Cost | Time-scale | Deliverables | Customer Requirement |
|---------------|--|--|------------|--|----------------------|
| 1.1 | Gene synthesis or PCR cloning into transfer plasmid | from £1050 <small>Large/complex genes may incur an extra charge</small> | 3-4 weeks | Agreed transfer plasmid with gene ready to make virus and full report | |
| 1.2 | Sub-cloning into transfer plasmid | from £700 <small>Complex strategies may incur an extra charge</small> | 2-3 weeks | Agreed transfer plasmid with gene ready to make virus and full report | |
| 2.1 | Production of recombinant virus (50 ml) and test expression in 2 cell lines | £1250 flashBAC £1350 flashBAC GOLD £1450 flashBAC ULTRA | 2-3 weeks | 50 ml P1 virus stock, titrated by QPCR Western blot test expression report | |
| 2.2 | Optimisation of expression (moi, time to harvest, pellets/culture medium) by Western blot | £650 per cell line | 2 weeks | Western blot and optimisation report with recommended culture conditions to optimise expression | |
| 2.3 | Optimisation as 2.2 plus 1-2 ml samples sent to customer for analysis | £700 per cell line | 2 weeks | As 2.2 plus 1-2 ml samples to test in own laboratory | |
| 3.1 | Protein production (amplification of P2 virus stock and infection of cells to produce recombinant protein) | £500 per 1 L £2000 per 5 L (pro rata other volumes) | 2 weeks | Amplification and titration of required amount of P2 virus (100-200ml/L protein) Production of cell pellet and/or culture medium containing recombinant protein | |

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| | | | | to ship to customer or for use in 3.3 | |
| 3.2 | Protein production in monolayer T flasks | £300 per 500 ml virus £375 per 40 T175 flasks | 2 weeks | Amplification and titration of required amount of P2 virus (500 ml) Production of cell pellet and/or culture medium containing recombinant protein to ship to customer or for use in 3.3 | |
| 3.3 | Protein purification using His tag (1 column) from step 3.1 | £1250 (up to 5 L) | 1 week | Purified protein and report including gel analysis | |
| 3.4 | Protein purification using His-tag (1 column) plus cleavage | £1550 (up to 5 L) | 1 week | Purified, cleaved protein including gel analysis | |
| 3.5 | Additional column steps | £300 per column | 1 week | Purified protein and report including gel analysis | |
| 3.6 | Alternative purification e.g. strep/HA tag | Dependent upon protocol – please enquire | 1 week | Purified protein and report including gel analysis | |
| 3.7 | Characterisation of purified protein by Mass Spec | Depends on analysis – please enquire | 2 weeks | Report on findings of Mass Spectrometric analysis | |
| 4.1 | All-in-one basic gene expression service: gene synthesis/PCR cloning, making virus, test expression, 1 L protein production (1.1, 2.1, 3.1) | £2,800 | 6-8 weeks | Transfer plasmid as agreed with gene 50 ml P1 virus stock (FB GOLD) Test expression western blot 1 L protein (as pellet/culture medium) and residual P2 virus stock (if any) | |
| 4.2 | All-in-one basic gene expression service: sub-cloning then as 4.1 (1.2, 2.1,3.1) | £2,500 | 5-7 weeks | As 4.1 | |
| 4.3 | All-in-one basic gene expression and protein purification service. As 4.1 but including one-step His-tag protein purification (1.1, 2.1, 3.1, 3.2) | £3,800 | 7-9 weeks | Transfer plasmid as agreed with gene 50 ml P1 virus stock Test expression western blot Purified protein and residual P2 virus stock (if any) | |
| 4.4 | All-in-one basic gene | £3,500 | 6-8 weeks | As 4.3. | |

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| | expression and protein purification service. As 4.1 but sub-cloning gene and one-step His tag protein purification (1.2, 2.1, 3.1, 3.2) | | | | |
| 4.5 | All-in-one basic gene expression service using clients own transfer plasmid to 1 L protein production (2.1, 3.1) | £1800 | 4-5 weeks | 50 ml P1 virus (titrated by QPCR) Test expression Western blot 1 L protein as pellets/culture medium and residual P2 virus (if any) | |
| 4.6 | All-in-one basic gene expression service using clients own transfer plasmid to 1 L protein purification (2.1, 3.1, 3.2) | £2950 | 5-6 weeks | 50 ml P1 virus (titrated by QPCR) Test expression Western blot Purified protein and residual P2 virus (if any) | |
| 5.1 | Virus amplification in Sf9 cells and titration by QPCR | ≤500 ml: £300 ≥500ml: £200 per 500 ml + £80 | 1 week | Virus of known titre (by QPCR) | |
| 5.2 | Virus amplification in Sf9 cells and titration by plaque-assay | ≤500 ml: £400 ≥500ml: £200 per 500 ml + £180 | 2 weeks | Virus of known titre (by plaque-assay) | |
| 5.3 | Virus titration by plaque-assay to determine accurate titre | £180 per virus | 1 week | Accurate titre of virus (essential for titration of older virus stocks) | |
| 5.4 | Virus titration by QPCR | ≤5: £80 per virus ≥5: £60 per virus | 1 day | Accurate titre of fresh virus stocks | |
| 5.5 | Recovery of old virus: 2-3 rounds of virus amplification as required, initial titration by plaque-assay and interim/final by QPCR. Test expression optional. | £850 per virus with test £700 per virus w/o test of expression | tbc | 50 ml high titre virus stock Western blot of test expression (if required) | |
| 5.6 | Virus safe deposit: Preparation of 40 1 ml vials | £950 per virus £210 annual OET charge | tbc | 50 ml high titre virus 40 x 1 ml vials – test recovery and | |

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| | virus (20 stored at OET; 20 at ECACC. Test recovery of virus and gene expression. | £700 annual test recovery and expression ECACC storage charges are extra | | expression 20 vials stored at OET 20 vials stored ECACC Annual recovery and test expression (as required) | |
| 5.7 | Use of own cell line in expression work. Initial recovery of cells and weekly passaging. Use of cells in all experimental work | £355 initial charge £210 annual storage (up to 20 vials) £120 weekly maintenance charge | | Full data sheets for initial recovery and weekly passaging using agreed medium | |
| 5.8 | Shipping charges | As charged to us | n/a | Added to all invoices for shipping virus, cell pellets, culture medium, purified protein – as agreed and at cost. We also ship using customers own carrier if preferred or local customers can collect by agreement. | |
| 6.1 | Other – customer specific | | | | |
| 6.2 | Other – customer specific | | | | |

Notes

2.1 A recombinant baculovirus will be produced using:

1. *flashBAC*TM with chitinase deletion, or;
2. *flashBACGOLD*TM has both chitinase and cathepsin gene deletions – further increasing yield, especially for difficult to express proteins. The absence of cathepsin (a protease) also greatly reduces protein degradation by proteolysis and improves protein stability, or;
3. *flashBACULTRA*TM offers the deletions present in *flashBACGOLD* with the additional deletions of the viral p10, p26 and p74 genes. These genes are not essential in cell culture and their deletion removes an unnecessary genetic burden and provides an additional boost to recombinant protein production

*flashBAC*TM is a patented baculovirus system that is much faster to use than traditional methods as no plaque assay is required to separate parental from recombinant viruses. It can also be used in automated systems, allowing many viruses to be produced simultaneously. More information on *flashBAC*TM is available on our website www.oetltd.com.

Insect Sf9 cells will be transfected with *flashBAC*[™] and the transfer vector. The culture medium containing 100% recombinant virus will be harvested. We will then amplify the recombinant virus to a P1 stock in Sf9 cells (50 ml). The P1 stock will be titrated by QPCR. We will confirm expression of the foreign gene by simple Western blot using anti-His antibody in Sf9 and Tni cells (**Client provides primary antibody if a tag has not been added or provides OET with details to purchase commercial source of primary antibody at extra cost**).

Note 1: Clients will understand that we cannot guarantee expression of any particular gene, nor can we guarantee expression levels of proteins or virus titres. Virus titres of the P2 stock are typically in the range 4×10^7 to 1×10^8 pfu/ml. If the titre is exceptionally low we will re-amplify and titrate at no extra cost.

Note 2: For regulatory purposes, we recommend that the recombinant virus is plaque-purified so that the recombinant virus produced is clonal in origin. Insect Sf21 cells will be transfected with the *flashBAC*[™] DNA and the transfer vector. The culture medium containing 100% recombinant virus will be harvested. In order to obtain a clonal version of the recombinant virus, we will plaque-purify the recombinant virus and select 3 plaques for small-scale amplification (3 x 2 ml for each virus type). We will then amplify the recombinant viruses to a P2 stock in Sf21 cells (3 x 50 ml for each *flashBAC* virus type). The P2 stock will be titrated by QPCR to confirm an exact titre. At this stage a clonal virus will be selected for further study. Full report for QC and regulatory purposes will be provided. **Cost may vary depending on requirements and can be provided on request.**

2.2/2.3 OET recommends that expression levels are optimised before proceeding to large-scale production of recombinant protein. For example, various cell lines can be assessed for expression levels, the optimum moi (in pfu/cell) can be determined (this may well save litres in virus inoculum at a later stage) and the best time to harvest after infection can be determined. It will also indicate whether proteolysis is likely to be a problem or not.

OET will set up small-scale cultures of cells (20-25 ml) from one, two or three cell lines and infect with virus at a range of multiplicities of infection (e.g., 2, 5, 10 pfu/cell). Samples will be harvested at 0, 24, 48, 72 and 96 hpi (culture medium or cell pellets as needed) and will be analysed by SDS-PAGE and Western blot (or other method if preferred). OET can undertake the Western blotting and/or samples (1-2 ml) can be sent to the client for analysis.

3.1 OET will need to produce batches of high titre inoculum for use in protein production. The viruses will be amplified in Sf9 cells in serum-free medium and titrated by QPCR. This produces a P2 (or P3) virus stock depending on the virus inoculum used. For protein production, after optimal expression conditions have been agreed upon, the appropriate cells, in shake culture in serum-free medium, will be infected with virus at appropriate moi and cell pellets and supernatant will be harvested at the agreed time after infection. We can ship culture medium and/or cell pellets to clients or we can proceed to protein purification. Where we are undertaking a pilot protein purification we will normally produce 1 L of infected cells.

Note 1: to give an idea of the amounts of virus inoculum required for protein production, if a virus amplifies to 1×10^8 pfu/ml, we require 100 ml to infect 1000 ml Sf9 cells at an moi of 5 pfu/cell. If the titre is only 5×10^7 pfu/ml, then 200 ml of virus will be required for the Sf9 cells.

Note 2: we will advise on expected yields during the course of the project so that culture volumes can be optimised to generate required yields of protein.

3.2 Protein production in T-flasks. T-Flasks (175cm T-flasks) will be seeded at 2.5×10^7 cells per flask and infected at required MOI. The cells will be harvested at 48 hours post-infection (or as required) by tapping flasks sharply to dislodge cells followed by low speed centrifugation. Cell pellets and/or culture medium can be provided as required.

3.3-3.6 Protein purification will be carried out by affinity chromatography in agreement with the customer. We can perform a pilot experiment normally using 1 L infected cell culture to optimise conditions, if required. Our normal service quote allows for one column chromatography step using a his-tagged protein with analysis by SDS PAGE. Further chromatography steps can be used to increase purity as required and agreed or we can quote for using different purification tags. We can also attempt to purify native protein without tags if sufficient detail is available to plan a suitable strategy. We are pleased to discuss this prior to providing a quote.

We can provide more detailed analysis of the purified protein by Mass Spectrometry to verify identity and profile post translational modifications etc

5.1/5.4 We can titre fresh (< 3 months old) stocks of virus using BaculoQuant (QPCR).

5.2/5.3 We can titre virus by plaque-assay; older virus stocks must be titrated by this method to be accurate.

5.5 We can take your old virus stock and attempt to recover a high titre stock of virus by amplifying virus first in monolayer cultures and then in shake cultures. We will do a plaque-assay first to assess whether there is any virus remaining to make a feasible attempt. If there is not, we will only charge for the plaque-assay and can offer to remake the virus for you (see relevant prices depending on whether you still have the transfer vector or not); in which case we will waive the plaque-assay fee.

5.6 We offer a virus storage service in collaboration with ECACC(European Cell Culture Collection). We will amplify a high titre batch of virus (50ml) and dispense into vials for storage at OET (1 ml x 20 vials at -80°C with 2% FCS) and ECACC (20 x 1 ml vials). We check that stored vials are viable and that they still express the required protein by Western blot. We can perform an annual assessment if required and at cost. Our costs as stated are **exclusive of ECACC costs** but we can facilitate the transfer of vials to ECACC on your behalf. Please ask for more details if you require this service.

5.7 Some projects may require the use of the client's own insect cell line. We can set up and maintain a stock of cells at OET for exclusive use in the client's project. We can provide all passaging history and can obtain specific cell culture medium as required. At the end of the project the cell line will be destroyed or vials can be stored in liquid nitrogen for up to one year in case further projects are required.

Terms and Conditions

Oxford Expression Technologies Ltd Standard Terms and Conditions apply. Copies available on request. In addition, OET cannot guarantee virus titres or protein expression levels/yields. OET undertake to keep all work confidential and will sign a confidentiality agreement if this is required. Clients are also reminded that if any recombinant virus or expressed protein is used for commercial activities then the appropriate licences must be sought and that this is the Client's responsibility.

The OET safe deposit service is intended as a second site repository to serve as a precaution against the possibility of damage to the Depositor's primary stock. OET recommends that its safe depository should not be the primary, and/or only storage site for any Deposited Material unless by special agreement with OET. We strongly recommend that safe

storage at OET is combined with that offered by ECACC (<http://www.hpacultures.org.uk>) and OET offers a service to facilitate such storage arrangements. ECACC terms and conditions and costs will apply to such arrangements.

However, it shall be the responsibility of the Depositor to verify that viable Deposited Material can be resuscitated from the OET depository, either by requesting the return of a sample of the Deposited Material or by requesting that OET conducts test resuscitations on selected parts of the Deposited Material. The current scale of OET charges shall apply.

OET accepts no responsibility for Deposited Material that is found to be damaged or nonviable after storage at its Safe Depository and OET shall never be liable for any direct or indirect losses connected herewith other than:

(i) Replacement of the affected Deposited Material, if necessary using fresh seed stock obtained from the Depositor, at no cost to the Depositor if it can be reasonably demonstrated that the loss is attributable to negligence on the part of OET.

(ii) Reimbursement of any advance rental fees covering that part of a calendar year following the date when control of the storage conditions was lost.

Start date

Once a quotation has been accepted we are normally able to commence work with one to two weeks' notice, however, in exceptionally busy periods or for large projects we may need to agree a start date within one month to allow for appropriate scheduling of other projects. Before starting we require an official purchase order number (or a formal contract can be agreed if this is a requirement). We also require as much detail as possible about the virus to be sent and a statement of safety category. We can only work with viruses/genes that fall into UK ACGM and ACDP Class I pathogens/recombinant viruses

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