**HOW TO PLACE A REQUEST:**

* Complete this form in full and submit it by email to vvcf-enquires@ndm.ox.ac.uk

If additional services are required, please detail these in the email.

* Upon receipt, your order will be reviewed by VVCF staff and a quote raised for the services required.
* If the quotation is suitable, the form will be sent to our local BSO for GMO / risk assessment and approval.
* Once an order has been checked VVCF will get back to your to arrange a time for plasmid samples to be brought to the unit.

Please note; available shuttle vectors are listed at the end of this form.

The bulk of our production work is to produce vectors for pre-clinical evaluation. This material is not suitable for use as a seed stock for clinical manufacture. If you require this service please get in touch to discuss.

**SECTION 1: GENERAL ORDER INFORMATION:** to be completed by all customers

|  |  |
| --- | --- |
| **Requestors name** |  |
| **Institution** |  |
| **e-mail address** |  |
| **Oxford PI**  Please state Oxford PI’s group (grant holder) that this is being funded by |  |
| **Name of virus to be prepared**  (this will be on all paperwork and labels) |  |
| **Full name of antigen(s) being expressed:**  Please include the host species from which this is derived and / or accession number |  |
| **Is there any additional information you require to be included on the production record?** (please type in info in box provided) |  |
| **Quantity required:**  Standard prep is 5x108 to 1x109 PFU in total |  |
| **Aliquot sizes required:**  Typically we prepare 1 x 500µl plus any spare. |  |
| **Do you require any other services with regards to this order** (e.g. end point sequencing) | Please give full details and get in touch for additional costs associated. |
| **Funding**  If an Oxford customer, how is this order funded (please delete and leave that which is appropriate) | Full Economic Cost (FEC), WT funded, NIH funded, EU funded |

**SECTION 2: PLASMID INFORMATION:** to be completed by all customers

|  |  |  |
| --- | --- | --- |
| **Entry plasmid information:**  Please delete accordingly leaving only the correct information visible. | | |
| Locus | TK B8 F11 | |
| Promoter | p7.5 mH5 ssp F11 | |
| Marker | GFP mcherry BFP none | |
| Please provide other information here | | |
| **Name of plasmid being supplied**  (please include any plasmid number as well as the name given to the plasmid) | |  |
| **Location of plasmid map**  (If external to PSI or Jenner please submit your plasmid map via email when submitting the request. If PSI or Jenner please indicate where the plasmid map is saved. | |  |
| **Confirmation that plasmid has been sequenced** (all plasmids submitted should be sequenced) | | Has sequencing been performed across the antigen site to determine this is correct?  YES / NO |

**Plasmids are only to be submitted to VVCF once the order has been placed and the risk assessments been performed. When submitting plasmids please note we require:**

For MVA with a marker please prepared plasmid as VVCF-PRO-500. This is to be supplied along with 50-100µl control plasmid at 10ng/µl for our internal QC testing. Both of these must fully labelled including the DNA concentrations.

For markerless MVA please supply uncut plasmid (6-20µg is required in a volume of approx. 10-20µl) plus 50-100µl control plasmid at 10ng/µl for our internal QC testing. Both of these must be supplied in fully labelled tubes with the DNA concentrations on them.

**SECTION 3: RISK ASSESSMENT INFORMATION AND DETAILS OF USE:** to be completed by all customers.

|  |  |  |  |
| --- | --- | --- | --- |
| **What containment level will be required for the recombinant viral vector** | | | |
|  | | | |
| **EXTERNAL CUSTOMERS ONLY**: please complete the External customer GMO RA appendix 2 form below and return to VVCF | | GMO RA appendix form attached YES/NO | |
| VVCF USE ONLY: Approval given from GMO committee YES/NO | |
|  | | | |
| **UNIVERSITY OF OXFORD CUSTOMERS ONLY**: please complete the UoOx customer GMO RA appendix 1 form below and return a fully signed copy to VVCF. (Note: It is your responsibility to get the required approval. VVCF can not start work without a signed copy.) | | GMO RA appendix form attached YES/NO | |
| VVCF USE ONLY: Approval given from GMO committee YES/NO | |
| **Please give a brief description of the research area and proposed experiments**  (not more than half a page)  N.B. It is the customer’s responsibility to ensure the correct documentation is in place for use and disposal of the vector. | | | |
|  | | | |
| **How will the expression of the insert from the recombinant viral vector be demonstrated?**  N.B. VVCF do not check expression of antigens as part of QC process and recommend western blot or equivalent is performed on batches received. | | | |
|  | | | |
|  | | **APPENDIX 1: GMO APPROVAL FORM university of oxford customers only** | |  |
|  | |  | |  |
| GMO (*delete as appropriate for proposed VVCF work*): | | Bacteria  Adenovirus  MVA | |
| Antigen/Transgene (species the transgene is from and any other sequences present eg. TPA, invariant chain) | | Origin of transgene i.e. species:  Any other sequences present e.g. TPA, invariant chain: | |
| **UNIVERSITY OF OXFORD CUSTOMERS**: Please paste the GMO RA statement or attach full GMO RA below exactly as documented and approved by your GMO safety committee.  (Note: Jenner personnel this will be the statement as it appears in R004 appendix document to R001, R002 and R003).  **It is the responsibility of all University of Oxford staff to ensure they have the correct approvals in place for the generation and use of their GMOs. GMO approval must be sought through your departmental BSO and GMO committee prior to the submission of this viral request form.**  Once a signed form is returned the information and approval status will be transferred to VVCF GMO RA documents. | | | |
|  | | | |
| TO BE SIGNED BY REQUESTERS LOCAL BIOLOGICAL SAFETY OFFICER (BSO) | | | |
| I declare that the above information (vebatim) or attached GMO RA has been submitted to the University H&S office and local GMO committee and approval given for the generation and use of this GMO in the parent department.  BSO PRINT NAME:……………………………………BSO Signature………………………………………………… | | | |
|  | | **APPENDIX 2: GMO APPROVAL FORM**  **external customers only** | |  |
|  | |  | |  |
| GMO (*delete as appropriate for proposed VVCF work*): | | Bacteria  Adenovirus  MVA | |
| Antigen/Transgene (species the transgene is from and any other sequences present e.g. TPA, invariant chain) | | Origin of transgene i.e. species with accession number if available:  Any other sequences present e.g. TPA, invariant chain: | |
| **EXTERNAL CUSTOMERS:** Please provide below a GMO risk assessment statement.  Please note this is a statement of risk to the environment and human health during laboratory handling and vector generation, not a general description of the transgene.  **It is the responsibility of all Customers to ensure they have the correct approvals in place for the generation and use of their GMOs.**  1. Please state what the transgenes are and what they do in their natural host.  Assess the risks associated with the inserted transgene in all GMO hosts required for VVCF to complete the work proposed i.e. bacteria, adenovirus, MVA. Consider the following: what is the worst case scenario in case of accidental exposure, will the GMO host range be changed by the inserted DNA, does the inserted DNA complement any replication deficient deletions in the host, is there any chance of recombination with natural host and what would be the outcome if released into the environment.  2. BLAST search any artificial junctions of sequence e.g. in fusion peptides to ensure there are no human self-antigen sequences generated (provide details of BLAST search). Upon submission VVCF will seek approval from their local GMO committee before work can commence. | | | |
|  | | | |
| TO BE SIGNED BY VVCF BIOLOGICAL SAFETY OFFICER (BSO) | | | |
| I declare that the above information (vebatim) has been submitted to the University H&S office and where appropriate the local GMO committee and approval given for the generation of this GMO.  BSO PRINT NAME:……………………………………BSO Signature………………………………………………… | | | |

**SECTION 4: SHUTTLES AVAILABLE:** Information for customers.

The versions of MVA shuttle vector available permit the production of recombinant virus that either retain the fluorescent marker gene or result in a marker-free virus. The latter shuttle plasmids have the fluorescent marker gene present outside the insertion site flanks and permit the selection, during production, of recombinant virus via a transient-dominant selection method: virus is initially selected based on dual fluorescence but in subsequent rounds of purification, the recombination event resolves and the marker genes are excluded.

1. **Antigen insertion at TK locus:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Plasmid** | **Plasmid number** | **Marker in final virus** | **Gene insertion site** | **Gene Promoter** |
| **MVA- GFP-p7.5** | **p434** | **GFP** | **tk locus** | **p7.5** |
| **MVA- GFP-mH5** | **p1863** | **GFP** | **tk locus** | **mH5** |
| **MVA- GFP-ssp** | **p1864** | **GFP** | **tk locus** | **ssp** |
| **MVA- mcherry-p7.5** | **p3376** | **mcherry** | **tk locus** | **p7.5** |
| **MVA-TD-GFP-p7.5** | **p856** | **None** | **tk locus** | **p7.5** |
| **MVA-TD-GFP-mH5** | **p2821** | **None** | **tk locus** | **mH5** |

1. **Antigen insertion at F11 locus:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Plasmid** | **Plasmid number** | **Marker in final virus** | **Gene insertion site** | **Gene Promoter** |
| **MVA-GFP** | **p5586**  **(p4759 no longer to be used)** | **GFP** | **F11 locus** | **F11 native** |
| **MVA-mcherry (promoter of choice)** | **p4419** | **mcherry** | **F11 locus** | **As required (F11 not native seq – see comment in vector file)** |
| **MVA-TD-GFP** | **p4719** | **None** | **F11 locus** | **F11 native** |
| **MVA-TD-GFP-mH5+ssp** | **p4774** | **None** | **F11 locus** | **mH5 and ssp (bivalent)** |

1. **Antigen insertion at B8 locus:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Plasmid** | **Plasmid number** | **Marker in final virus** | **Gene insertion site** | **Gene Promoter** |
| **pMVA-B8-FP4-BFP** | **p4260** | **None** | **B8 locus** | **B8** |

MVA retaining fluorescence marker genes are quicker to produce, easier to titre and ideal for pre-clinical work. Marker-less MVA is required for GMP work. We are happy to discuss your requirements to assist in making the decision as to which format you require.

Maximum gene size is approx. 10kb, genes do not require a polyA tail.

TTTTNT motif must be absent from your antigen sequence

We would recommend that you use these vectors as our high-throughput systems are designed for such production. Fully annotated plasmid maps are available upon request.