Mapping of viral epitopes with prokaryotic expression products

Brief Review

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Summary. Several systems are available for the expression of foreign gene sequences in Escherichia coli. We describe the use of prokaryotic expression products of viral gene fragments in order to identify the regions that specify the binding sites of antibodies. This approach is particularly successful if the antigenicity does not depend on the native protein, but only on the amino acid sequence, i.e., if the epitope is sequential. Combining prokaryotic expression with the use of synthetic peptides often permits a fast and accurate mapping of an epitope. The occurrence of immunodominant sequential epitopes on the surface of viruses seems to be a widespread phenomenon.

Introduction

As in most areas of biology and biochemistry, the study of viruses and their interactions with the immune system has been revolutionized by the recombinant-DNA technology. This review describes a new method for studying the antigenicity of viral proteins which involves the insertion of fragments from the gene coding for the protein into one of the many available expression vectors. By testing the antigenicity of the expression product, the protein segment involved in antibody binding can then be identified.

After the initial studies of Rüther et al. [98] with lysozyme, this approach led to the localization of several B-cell and T-cell epitopes. Recombinant antigens are most suitable for identifying the same category of epitopes that are detected by the use of synthetic peptides, namely the so-called linear or sequential epitopes. Expression products of viral genes are also useful for serodiagnosis and for studying virus neutralization.
Definitions and operational criteria

The antigenicity of proteins has been a controversial issue for many years [7, 10, 11, 129–131]. One source of confusion lies in the use of several terms that have overlapping meanings. Therefore, we will briefly define these terms as they are used in this review.

An epitope is the part of the antigen that is involved in the binding of a particular antibody. This definition implies that an epitope is specified by the antibody; and that an antigen may have as many epitopes as there are different antibodies recognizing the antigen [130]. Further, it should be noted that an epitope also depends on the method of localization. For instance, the use of peptides cross-reacting with the antigen may delineate an epitope that corresponds to part of the antigen-antibody interface observed by X-ray crystallography [131].

Not all residues within an epitope need to be essential for binding. A residue or a group of residues may be denoted as an antigenic determinant to indicate its measurable contribution to the binding of the antibody, rather than a passive presence in the epitope. Antigenic determinants can be defined by the analysis of escape mutants [84, 94, 137] or by the systematic replacement or deletion of residues [40, 73, 74].

Monoclonal antibodies that have identical, overlapping or adjacent epitopes will compete for binding to the antigen. We use the term antigenic site to denote the part of the antigen that is the target of a group of mutually competing antibodies. Although the whole surface of a protein may be potentially antigenic [10, 11], the number of antigenic sites seems to be limited.

Epitopes can be classified as sequential (or linear) or as conformational [106, 128]. Epitopes are sequential, if the antigenicity only depends on the primary structure of the protein antigen. Operationally, this is indicated if the antigenicity is retained after denaturation of the protein or if the epitopes can be mimicked by a peptide with the same sequence as the protein segment, but without any stable native-like conformation. Conversely, antibodies with conformational epitopes only bind to the antigen in its native conformation. The terms conformation-dependent or conformation-independent can be regarded as synonyms of conformational and sequential, respectively, ‘conformation’ referring to the native structure of the antigen.

Presumably, any epitope will have a defined conformation when bound to the antibody. Conceivably, conformational epitopes depend on the same intramolecular interactions that stabilize the structure of the antigen, while the conformation of a sequential epitope is mainly stabilized by intermolecular interaction with the antibody. This agrees with the notion that sequential epitopes correspond to those parts of the surface that are relatively mobile [121, 129, 136] or even disordered [2].

An independent criterion distinguishes between continuous and discontinuous epitopes [7]. In most cases, conformational epitopes will be discontinuous, i.e., formed by residues that are not contiguous in the amino acid sequence but are
brought together by the folding of the protein. However, continuous epitopes may also depend on the protein conformation [7]. Further, since replacement analysis [40, 73] has demonstrated that within an sequential epitope antigenic residues can be interspersed by non-antigenic ones, the distinction between continuous and discontinuous seems somewhat arbitrary [129]. Another reason for our preference of the original terms of Sela [106] is that a simple experiment—testing the effect of denaturation on antigenicity—decides on the classification.

**Expression systems**

In order to be expressed as a functional gene in a prokaryotic cell, a coding sequence must be flanked by a number of signals [16, 65, 101]: a promotor, a transcription termination site, a ribosome binding site, an ATG start codon and a stop codon. Except for the start and stop codons, these signals are specific for the host cell. Normally, all these signals are provided by the expression vector.

Three main factors determine the yield of an expression product:

*The strength of the promotor*

In most cases, a strong promotor, such as $P_{tac}$ or $\lambda P_L$, is preferred for a high level of gene expression. Further, to prevent continuous accumulation of the expression products leading to growth retardation or cell death, an inducible promotor is needed.

*Factors governing the initiation of protein synthesis*

These factors are the distance between the ribosome-binding site and the start codon, local mRNA secondary structure and other features of the sequence [104]. The problem of optimizing the initiation rate can be circumvented by fusing the foreign sequence to a well-expressed bacterial gene downstream of the bacterial start codon, leading to the synthesis of a hybrid protein.

*The level of proteolytic degradation*

This is probably the most unpredictable and critical factor. Another advantage of fusing the sequence to a bacterial gene is that hybrid proteins are often relatively stable, particularly when the accumulated expression product precipitates inside the cell [65, 69, 115].

Many applications require posttranslational processing and correct folding of the polypeptide [12]. If eukaryotic processing such as phosphorylation or glycosylation is necessary for antigenic activity, prokaryotic expression systems are of no use at all. Intracellular precipitation competes with correct folding of the protein and necessitates the use of elaborate in vitro renaturation procedures [76].
| Vector | Carrier protein or fusion gene | Insertion site | Promotor | Restriction enzyme sites for insertion | Reference |
|--------|--------------------------------|----------------|----------|--------------------------------------|-----------|
| Phage λ vectors |
| λgt 11 | lacZ                           | #1007-1008     | \( P_{lac} \) | EcoRI                                 | [142]     |
| λgt 22 | lacZ                           | #1007-1008     | \( P_{lac} \) | EcoRI, SalI, XbaI, NotI\(^c\)         | [47]      |
| λJK 2  | lacZ                           | N-terminal     | \( P_{lac} \) | several                               | [109]     |
| λJK 4  | lacZ                           | C-terminal     | \( P_{lac} \) | several                               | [109]     |
| LacZ fusion plasmids |
| pEX    | cro-lacI-lacZ                  | C-terminal     | \( \lambda P_{R} \) | as pUC8\(^d, e\)                     | [116]     |
| pEX11-13 | cro-lacI-lacZ                 | C-terminal     | \( \lambda P_{R} \) | as pUC8 + KpnI, SpeI, SphI\(^d\)     | [61 a]    |
| pUEX\(^g\) | cro-lacI-lacZ              | C-terminal     | \( \lambda P_{R} \) | as pEX\(^b, e\)                       | [13]      |
| pEX627 | cro-lacI-lacZ                  | C-terminal     | \( \lambda P_{R} \) | as pEX2                                | [117]     |
| pEX3407 | cro-lacI-lacZ                  | C-terminal     | \( \lambda P_{R} \) | BamHI, SalI, PstI                     | [57]      |
| pHK 413 | cro-lacI-lacZ                  | between        | \( P_{lacUV5} \) | BglII, HindIII, Smal, and             | [112]     |
| pHK 414 | cro-lacI-lacZ                  | \( cro \) and  | \( P_{lacUV5} \) | Smal, and                             | [112]     |
| pTC413 | cro-lacI-lacZ                  | lacI           | \( P_{lac} \) | BamHI                                 | [55]      |
| pMR\(^h\) | cI (#1-157)-lacI-lacZ          | between cI     | \( P_{lac} \) | HindIII, BamHI, Smal, BamHI\(^d\)    | [45]      |
| and lacI|                                 |                |          |                                       |           |
| pORF\(^b\) | ompF (#1-37)-lacZ              | between ompF    | \( P_{omp} \) | SalI, BamHI, Smal, BamHI or BglII, BamHI, Smal, BamHI\(^d\) | [134, 135]|
| and lacZ|                                 |                |          |                                       |           |
| pUL\(^l\) | lacZ                           | N-terminal     | \( P_{lac} \) | StuI, Dral, or Smal (blunt)           | [120]     |
| pUK    | lacZ                           | #5-6           | \( P_{lac} \) | PstI, BamHI, EcoRI                   | [59]      |
| Plasmid | Description | Fusion Location | Promoter | Restriction Sites |
|---------|-------------|----------------|-----------|------------------|
| pUR    | lacZ        | C-terminal     | $P_{lac}$ | BamHI, SalI, XbaI, HindIII or BamHI, SalI, PstI, HindIII<sup>d</sup> |
| pWS50  | (cII #1-12)-lacZ | between cII and lacZ | $\lambda P_L$ | NruI<sup>110</sup> |
| pWR590 | lacZ #1-590 | C-terminal     | $P_{lac}$ | several<sup>64</sup> |

**Plasmids with fusion to other sequences**

| Plasmid   | Description | Fusion Location | Promoter | Restriction Sites |
|-----------|-------------|----------------|-----------|------------------|
| pATH     | trpE (336 codons) | C-terminal | $P_{trp}$ | several<sup>139</sup> |
| pCG150   | malE (#28-392)-lacZ<sup>k</sup> | between malE and lacZ | $P_{lac}$ | several<sup>29</sup> |
| pCG806   | malE (#1-392)-lacZ<sup>k</sup> | between malE and lacZ | $P_{lac}$ | several<sup>29</sup> |
| pEx30    | MS2 polymerase #1-99 | C-terminal | $\lambda P_L$ | EcoRI, Clal, HindIII, PstI, XbaI, HindIII<sup>d</sup> |
| pEx31    | MS2 polymerase #1-99 | C-terminal | $\lambda P_L$ | EcoRI, BamHI, PstI, BglII, XbaI, HindIII<sup>d</sup> |
| pFS2.2   | E. coli enterotoxin subunit B | C-terminal | $P_{lac}$ | SmaI, BamHI, EcoRV<sup>103</sup> |
| pHE6     | N-protein #1-33 | C-terminal | $\lambda P_L$ | SmaI, BamHI, SalI, PstI, HindIII<sup>75</sup> |
| pIN-I    | outer       | #3-4          | $P_{tpp}$ | EcoRI, HindIII, BamHI<sup>d</sup> |
| pIN-II   | membrane    | #3-4          | $P_{tpp} + P_{lac}$ | EcoRI, HindIII, BamHI<sup>d</sup> |
| pINIII-A<sup>nm</sup> | pro-           | #3-4          | $P_{tpp} + P_{lac}$ | EcoRI, HindIII, BamHI<sup>d</sup> |
| pINIII-B<sup>nm</sup> | lipo-         | #20-21        | $P_{tpp} + P_{lac}$ | EcoRI, HindIII, BamHI<sup>d</sup> |
| pINIII-C<sup>nm</sup> | protein     | #27-28        | $P_{tpp} + P_{lac}$ | EcoRI, HindIII, BamHI<sup>d</sup> |
| pIN-III- OmpA<sup>1</sup> | signal peptide of OmpA | C-terminal | $P_{tpp} + P_{lac}$ | EcoRI, HindIII, BamHI<sup>d</sup> |
| pJL6     | cII #1-13   | C-terminal    | $\lambda P_L$ | Clal or HindIII<sup>63</sup> |
| pJLA16   | cII #1-13   | C-terminal    | $\lambda P_L$ | Clal or HindIII<sup>63</sup> |
| pNGS     | artificial #1-15<sup>n</sup> | C-terminal | $P_{lac}$ | as pUC8 or pUC18<sup>118</sup> |
| pRIT<sup>2x</sup> | staphylococcal protein A<sup>k</sup> | C-terminal | $\lambda P_R$ | EcoRI, SmaI, BamHI<sup>81</sup> |
| pRIT<sup>5, o</sup> | staphylococcal protein A<sup>k</sup> | C-terminal | $P_{pvo}$ | SmaI, PstI, and HindIII<sup>1</sup> |
| pRX      | trpE #1-18  | C-terminal    | $P_{trp}$ | several<sup>d</sup> |

<sup>1</sup>Mapping of viral epitopes with prophage expression products
Table 1 continued

| Vector | Carrier protein or fusion gene<sup>a</sup> | Insertion site<sup>a</sup> | Promotor<sup>a</sup> | Restriction enzyme sites for insertion<sup>b</sup> | Reference |
|--------|---------------------------------|-----------------|----------------|---------------------------------|-----------|
| Plasmids allowing cleavage of hybrid protein | | | | | |
| pCG806fX<sup>q</sup> | as pCG806 | as pCG806 | $P_{lac}$ | several | [68] |
| pEAP8<sup>q</sup> | penicillinase signal sequence | C-terminal | from penicillinase gene | $HindIII$ | [54] |
| pGEX<sup>k, r</sup> | glutathione S-transferase<sup>s</sup> | C-terminal | $P_{lac}$ | $BamHI$, $SmaI$, $EcoRI$ | [113] |
| pJC264<sup>l</sup> | $cheY$ | C-terminal | $P_{lac}$ | $PstI$, $EcoRI$ | [38] |
| pJG200<sup>u</sup> | pro-α-2 collagen (60 residues)-lacZ #9-end | N-terminal | $\lambda P_R$ | $BamHI$ | [39] |
| pLcIl-(nic-0)<sup>q</sup> | cII #1-31 | C-terminal | $\lambda P_L$ | $BamHI$, $XbaI$, $SalI$, $PstI$, $HindIII$ | [79] |
| Plasmids without gene fusion | | | | | |
| pANK, pANH, pPL2 | — | — | $\lambda P_L$ | $KpnI$, $HpaI$, or $BamHI$ | [107] |
| pKC30<sup>v</sup> | — | — | $\lambda P_L$ | $BamHI$ | [93] |
| pAS | — | — | $\lambda P_L$ | $BamHI$, $Asp718$, $SalI$, $NcoI$, $NdeI$, $SmaI$, $XmaI$, $NruI$, $HpaI$, or $EcoRV$ | [108] |
| pOTS<sup>v</sup> | — | — | $\lambda P_L$ | $BamHI$ | [26] |
| pRC23<sup>v</sup> | — | — | $\lambda P_L$ | $EcoRI$ | [23] |
| pEV-vrf<sup>v</sup> | — | — | $\lambda P_L$ | $EcoRI$, $BamHI$, $ClaI^d$ | [23] |
| pRDB8 | — | — | T4 gene 32 | several | [33] |
| pRDB9<sup>x</sup> | — | — | | | |
| Vector | Description |
|--------|-------------|
| ptac-12 | $P_{tac}$ (PvuII) |
| pKK233-2 | $P_{trc}$ (NcoI, PstI, HindIII) |
| pXmnATG | $P_{tac}$ (XmnI) |

a Amino acid residues or codons. Abbreviations in italics indicate genes from which fragments have been used either as part of the fusion gene or (preceded by ‘P’) as inducible promoter. cI: phage λ repressor gene, cII: phage λ regulator gene, cro: phage λ antirepressor gene, galK: E. coli galactokinase gene, lacI: E. coli lac repressor gene, lacZ: E. coli β-galactosidase gene, malE: E. coli maltose binding protein, ompF: E. coli outer membrane porin gene, tac, trc: trp-lacZ hybrid promoter, trp: E. coli anthranilate synthetase

b From 5' to 3'
c λ gt22 contains no SacI site (Promega Notes 18, 1989), as originally reported [47]
d Available for expression in all three reading frames
e EcoRI site only present in one of the reading frames
f Essentially identical to pEX, but containing the c1857 gene coding for the temperature-sensitive repressor
g Synthesis of β-galactoside only if the insertion restores the reading frame
h Broad-host-range vectors
i Allows affinity purification of the hybrid protein
j Secretion of expression product
k Plasmid carrying lacI coding for the lac repressor
l Containing an Asn sequence to stabilize the expression product
m Shuttle vector for secretion in periplasmatic space in E. coli and for extracellular secretion in gram-positive hosts
n The sequence Ile-Glu-Gly-Arg at the C-terminus of the carrier protein allows specific cleavage by the blood coagulation protease Factor X
o Excretion vector; signal sequence cleaved off after transport of product through inner membrane; outer membrane made permeable by the expression of the kil gene on the plasmid
p Expression products of pGEX-2T and pGEX-3X contain C-terminal of the carrier protein the recognition sequences of thrombin, and factor Xa, respectively, allowing specific cleavage of the hybrid protein
q Expression products reported to be both soluble and stable
r A methionine C-terminal of the cheY protein allows specific cleavage of the hybrid protein by CNBr
s The collagen sequence allows specific cleavage by collagenase
t No start codon provided by the vector
u Derivative of pAS1 containing the λ t0 terminator
v Proteolytic degradation inhibited by infection with phage T4

Other expression vectors are described in [25] and [89]
A number of prokaryotic expression vectors are listed in Table 1. Indicated are the bacterial gene used to generate a hybrid protein (setting the compromise between solubility and degradation), the promoter and the unique restriction sites available for inserting the foreign sequence. The first expression system used to map viral antigenic determinants was the phage $\lambda$ Charon 16 [82]. This phage is comparable to $\lambda$ gt 11 [142], one of the most popular expression vectors. In both phage systems, the foreign gene fragment is inserted in the EcoRI site near the 3' end of the $\text{lacZ}$ gene. After adding the synthetic inducer isopropyl-$\beta$-D-thiogalactopyranoside, the foreign sequence is expressed as part of a $\beta$-galactosidase hybrid protein. Direct immunoscreening allows the selection of recombinant plaques synthesizing an antigenic sequence. The main advantages of phage $\lambda$ systems are the high efficiency of transfection, the possibility of screening plaques at a high density and the availability of worked-out, reliable protocols. The main disadvantage of $\lambda$ gt 11 is the availability of only an EcoRI site for insertion; this has been eliminated in a new variant $\lambda$ gt 22 [47].

Like $\lambda$ gt 11, several of the plasmid expression systems have been devised originally for the construction of cDNA expression libraries. Other systems have been constructed to investigate the products of open reading frames (pORF, pMR) or to produce native-like proteins. An advantage of plasmids is that the procedures for plating out, growing and DNA isolation are very simple. Furthermore, the new technique of electroporation allows efficiencies of transformation that are at least comparable to those of the packaging and transfection with $\lambda$ DNA.

The most popular system for epitope mapping is the pEX system [116]. These plasmids contain the strong $\lambda P_R$ promotor, regulated by a temperature-sensitive repressor, and a polylinker region at the end of a cro- lacZ fusion gene, available in the three different readings frames. During the development of this vector [115], it was found that insertions at the 3' end of the fusion gene gave more stable expression products than insertions at the 5' end. The pEX expression products are quite insoluble, ensuring that the product of virtually any foreign sequence is protected effectively against degradation. Further, lysis in SDS and transfer to nitrocellulose filters allow a direct immunoscreening of colonies.

To increase the versatility of the pEX system, the plasmids pEX 11, pEX 12, and pEX 13 were constructed by incorporating a polylinker with 7 different sites [61 a]. Incorporation in the plasmid of the c1857 gene coding for the temperature-sensitive $P_R$ repressor yielded the pUEX plasmids, which can be propagated in normal host strains [13].

**Mapping strategies**

A prerequisite for epitope mapping via heterologous gene expression is the availability of recombinant DNA clones containing the relevant coding information. Different strategies have been used to generate subgenomic fragments
which, after insertion in a expression vector, direct the synthesis of an antigenic expression product.

Results can be obtained rapidly by using restriction enzymes, but the accuracy of localization in this case obviously depends on the presence of suitable cleavage sites. More accurate localization may be obtained by constructing a library of small DNase I fragments and selecting epitope-producing clones by immunoscreening of colonies [31, 25, 58, 66, 72, 82].

A third approach is the construction of a series of deletion clones with Bal31 [30, 100, 125], exonuclease III [17, 52] or restriction enzymes [41, 132]. However, epitope delineation by progressive deletions from only one side may lead to erroneous interpretations. Since the antigenicity of the expression product is destroyed as soon as one essential antigenic determinant is deleted, it is this determinant that is mapped and not the complete epitope [17, 41, 125].

Finally, a delineation with a resolution of a single amino acid residue can be obtained by expressing synthetic oligonucleotides [20, 61].

Viral epitopes synthesized in E. coli

Table 2 compiles the use of recombinant antigens for the mapping of viral epitopes.

With only a few exceptions [17, 55, 80] fusion proteins were solubilized in buffers containing SDS and a reducing agent. Subsequently, the products are fractionated by gel electrophoresis, transferred to nitrocellulose and incubated with antibodies. However, this procedure is only suitable for antibodies that are capable of recognizing the viral protein after Western blotting [9, 17, 20, 31, 36, 55, 61, 66, 67, 111, 123]. Conversely, negative results have been reported with monoclonal antibodies that recognize denaturation-sensitive epitopes [17, 36, 41, 61, 66]. An interesting exception is the conformational site IV on the G2 protein of Rift Valley fever virus [55], which could be localized within 20-residues by immunoprecipitation of an expression product.

Are epitopes synthesized in E. coli always sequential?

To what extent is an expression product inside the E. coli cell or immobilized on a blotting membrane able to fold to a native-like structure? In most cases the antigenicity appears to depend only on a small subsequence that can be flanked by any bacterial or viral sequence. In such cases formation to a stable native conformation is not likely and the epitope is evidently sequential. Indeed, several epitopes could be delineated further by testing synthetic peptides [44, 52, 56, 58, 61, 67, 82, 88, 100, 132, 141]. So, we may consider a prokaryotic expression product as antigenically equivalent to denatured protein. This does not exclude a local native-like structure, but only in the afore-mentioned case [55], this was substantiated by the negative effect of denaturation on antigenicity.
### Table 2. Epitope mapping via expression in *E. coli*

| Virus                  | Protein       | Vector | Mapping strategy       | Antibodya | Antigenic sequence(s)b | Reference |
|------------------------|---------------|--------|------------------------|------------|------------------------|-----------|
| **Herpesviruses**      |               |        |                        |            |                        |           |
| Human cytomegalovirus  | gp58          | pEX    | restriction enzymes    | NMAb, MAb  | 548-614-645c           | [124]     |
| Herpes simplex virus 1 | gD            | pEX    | restriction enzymes    | NMAb       | 1-55d                  | [56]      |
| Herpes simplex virus 1 | gD            | pEX    | DNase library          | human      | 288-327, 355-369d      | [58]      |
| Epstein-Barr virus     |               |        |                        |            |                        |           |
| type A                 | EBNA 2        | pLCII  | restriction enzymes    | human, type specific | 378-435 | [95]      |
| type B                 | EBNA 2        | pLCII  |                        |            | 390-454                |           |
| **Hepadnaviruses**     |               |        |                        |            |                        |           |
| Hepatitis B virus      | HBcAgc        | pEx31b | *Bal31* 3' deletions,  | human, anti-(HBe + HBc) | 74-83-89 | [100]    |
|                        |               |        | restriction enzymes    | MAb, anti-HBe2 | 2-79-138, 2-138-140f |           |
|                        |               |        |                        | MAb, anti-HBc | 74-83, (2-73)-(83-89) |           |
|                        |               |        |                        | MAb, other HBe sites | 2-63, |           |
|                        |               |        |                        |            | 2-64-89                |           |
| **Adenoviruses**       |               |        |                        |            |                        |           |
| Adenovirus 2           | E1A protein   | pKRS101g | restriction-enzyme deletions | MAb, rabbit | 23-120h | [123]    |
| Adenovirus 2           | fibre polypeptide | pUC    | restriction enzymes    | MAb, NMabs, three sites | 383-583 | [133]    |
| **Papovaviruses**      |               |        |                        |            |                        |           |
| Human papilloma virus 6b | L1 ORF product | pATH   | restriction enzymes    | human, rabbit | 420-433, 197-222 | [52]      |
| Virus Type                        | Protein / Antigen | Vector | Deletions / Enzymes | MAbs / Other Data |
|----------------------------------|-------------------|--------|---------------------|-------------------|
| **Human papilloma virus 1**      | E4 gene product   | pEX    | Bal31 3' deletions  | MAbs 15-28, 15-35-37, 15-61-63, 15-77-82 [30] |
| **SV40**                         | T-antigen         | pUR    | restriction enzymes | NMAbs 446-708 [77] |
| **Reoviridae**                   |                   |        |                     |                   |
| Reovirus 3                       | σ1                | pUC13  | restriction-enzyme  | NMAbs 5-125-226-368 (125-225)-(226-368) 226-368-455 [80] |
| **Coronaviruses**                |                   |        |                     |                   |
| Mouse hepatitis virus M          | pEX               |        | Bal31 5' deletions  | rabbit, immunodominant site^k^ 217-227 213-217-227 [122] |
| Mouse hepatitis virus S          | pEX               |        | restriction enzymes | NMAb site A 839-862^l^ [67] |
| Infectious bronchitis virus S    | pEX               |        | restriction enzymes, DNase library, oligonucleotides | rabbit, mouse chicken 549-570-582^m^ 549-566 549-570-582 (553-555)-(565-567) |
|                                 |                   |        |                     | chicken 549-570-582 555-556-561^l^ 555-565-567^l^ |
|                                 |                   |        |                     | MAb site G 549-570-582 (553)-(554)-563 |
|                                 |                   |        |                     | NMAb site G (549-554)-(567-569)-(570-574) |
| Transmissible gastroenteritis    | pEX               |        | restriction enzymes | MAb site B 1-325-378 1-325-378 [36, 88] |
| virus, feline infectious         |                   |        |                     | NMAb site C 1-325-378 |
| peritonitis virus                |                   |        |                     | MAb site D 326-379-529-558 |
| **Paramyxoviruses**              |                   |        |                     | NMAb COR 5 607-629-651-660 [36, 88] |
| Measles virus                    | fusion protein    | pEX    | Bal31 3' deletions  | MAb 1-17-306 1-330-513^l^ [132] |
| Virus                     | Protein    | Vector | Mapping strategy          | Antibody<sup>a</sup>         | Antigenic sequence(s)<sup>b</sup> | Reference |
|--------------------------|------------|--------|---------------------------|-------------------------------|-----------------------------------|-----------|
| Measles virus            | nucleoprotein | pRIT2  | restriction enzymes, Exo III, 3' deletions<sup>i</sup> | MAb, site I, MAb, site II, MAb, site III | 23-122-150, 457-475, 457-519-525 | [17]      |
| Sendai virus             | nucleoprotein | pRC23  | 5' deletions with restriction enzymes | MAb                           | 1-456-517, 1-426-455, 1-290-425, 1-290-295 | [41]      |
| Newcastle disease virus  | HN         | pUC19  | restriction enzymes oligonucleotides | NMAb                          | 346-353                           | [20]      |
| **Rhabdoviruses**        |            |        |                           |                               |                                   |           |
| Infectious hematopoietic necrosis virus |           |        |                           |                               |                                   |           |
| **Bunyaviridae**         |            |        |                           |                               |                                   |           |
| Rift Valley fever virus  | G          | pATH3  | Sau3AI                    | rabbit                        | 335-450                           | [42]      |
| **Retroviruses**         |            |        |                           |                               |                                   |           |
| Feline leukemia virus    | gp70       | λ Charon 16 | DNase library | NMAb cI.25                    | 213-226                           | [82]      |
| Human immunodeficiency virus I | gp120 | λ gt11 | DNase library | MAb, site a, MAb, site b<sup>o</sup>, MAb, site c, MAb, site d, MAb, site e | 187-276, 210-274, 393-408, 403-457, 387-449, 409-499, 21-85, 34-55 | [31]      |
| Species                          | Species line | Antigenic site | Description                                      | References |
|---------------------------------|--------------|----------------|--------------------------------------------------|------------|
| Human immuno-deficiency virus I | gp120        | pOTS           | restriction enzymes                              | chimpansee, immuno-dominant site | [44]       |
| Human immuno-deficiency virus   | gp41         | \(\lambda_{gt11}\) | DNase library                                   | human MAb  | 83-93       | [9]        |
| Human immuno-deficiency virus I | gp41         | pATH           | restriction enzymes                              | human, immuno-dominant site      | 76-127     | [139]      |
| Human immuno-deficiency virus   | p24          | pUC            | restriction enzymes                              | MAb        | 1-177-244   | [60]       |

**Picornaviruses**

| Species                          | Species line | Antigenic site | Description                                      | References |
|---------------------------------|--------------|----------------|--------------------------------------------------|------------|
| Poliovirus 1                    | VP1          | pBR.322        | restriction-enzymes and Bal31 deletions          | NMAb, site 1 | 1-96-104   | [125, 141] |
| Human rhinovirus 2              | VP2          | pEx34          | Bal31 deletions                                  | NMAb       | 1-153-164  | [111]      |

| Notes                            |              |                |                                                  |            |
|---------------------------------|--------------|----------------|--------------------------------------------------|------------|
| **a** Species names denote polyclonal antisera |              |                |                                                  |            |
| **b** Numbers amino acid or codon numbers. In italics, sequences of residues that have been shown to contain antigenic determinants |              |                |                                                  |            |
| **c** The reported epitopes boundary 608-625 is only based on an assumed epitope length of 6 and 9 residues |              |                |                                                  |            |
| **d** More accurate localizations by testing synthetic peptides |              |                |                                                  |            |
| **e** HBc, HBe1, and HBe2 denote three antigenic sites on the core antigen HBcAg or on its antigenic variant HBeAg, both products of the C gene. HBc has been mapped with monoclonal antibodies, but is also the most immunodominant site recognized by human polyclonal antisera |              |                |                                                  |            |
| **f** Evidence cited that residues 2-77 contain essential determinants of site HBe2 |              |                |                                                  |            |
| **g** pBR.322 derivative with a \(\text{BgII}\) cloning site near the \text{3'} end of the \text{trp} gene |              |                |                                                  |            |
| **h** Localized more accurately by testing antibody binding of protein fragments or adenovirus-SV40 protein fragments |              |                |                                                  |            |
| **i** Exo III, exonuclease III   |              |                |                                                  |            |
| **k** Affinity purification via adsorption to the expression products yielded two fractions that recognized the two different epitopes |              |                |                                                  |            |
| **l** Localized more accurately by PEPSCAN peptide analysis |              |                |                                                  |            |
| **m** Residue numbering according to [61] |              |                |                                                  |            |
| **n** Epitope sensitive to denaturation by boiling in SDS and dithiothreitol and localized by immunoprecipitation |              |                |                                                  |            |
| **o** MAbs capable of blocking the interaction with the cell receptor CD4 |              |                |                                                  |            |
| **p** Same epitope localized with peptides [43, 105] |              |                |                                                  |            |
| **q** Epitope localization confirmed by analysing escape mutants [84] |              |                |                                                  |            |
| **r** Similar to pEx30 and pEx31 |              |                |                                                  |            |
As mentioned before, a sequential epitope may represent a component of the complete epitope as would be observed by X-ray crystallography. This is exemplified by two discontinuous epitopes of foot-and-mouth disease virus [87]. A similar situation may exist for site IV or D of transmissible gastroenteritis virus [88].

**T-cell epitopes**

All epitopes discussed so far are the targets of the soluble immunoglobulins, which are relevant for the humoral immune response. The cellular response is mediated by the T-cell receptor of T-lymphocytes. According to the current consensus, T-cell antigens are processed inside the antigen-presenting cells. This generates antigen fragments, which are bound on the cell surface by class-I (for cytotoxic T-cells) or class-II (for helper T cells) major histocompatibility antigens [for a review, see 24]. As a consequence of this process, T-cell epitopes are inherently sequential and can be mimicked by peptides [96, 97] or expression products [62]. In two recent reports, pEX expression products have been used to localize T-cell epitopes of a viral protein, the F protein of measles virus [27] or the E2 protein of Semliki Forest virus [114].

**Comparison with other methods of epitope mapping**

The suggestion that the antigenicity of bacterial expression products is almost exclusively limited to sequential epitopes implies that the same epitopes can also be mimicked by synthetic peptides. However, expression of gene fragments in *E. coli* should be considered as an approach complementary to the use of synthetic peptides, rather than as an alternative [61, 66]. Expression products can localize an antigenic sequence within 20 to 100 residues, depending on the available restriction sites and the mapping strategy. Within such a region, peptides can then be used for an exact localization. The combination of expression in pEX and PEPSCAN peptide synthesis has been applied successfully to measles virus [132], different coronaviruses [61, 66, 88] and to a T-cell epitope of *Mycobacterium tuberculosis* [126, 127].

A number of epitopes delineated by using expression products could not be detected by PEPSCAN analysis [61, 88]. This is most likely explained by the length of these epitopes: 11 and more than 17 residues for two epitopes of infectious bronchitis virus [61] and more than 21 residues for an epitope of feline infectious peritonitis virus [88].

Recently [87], the use of combinations of peptides to delineate discontinuous epitopes of foot-and-mouth disease virus was reported. This method, if generally applicable, would be a useful alternative to the analysis of MAb-resistant or non-binding mutants, which in principle only gives information about antigenic determinants.

A few reports [21, 34, 51, 70, 77, 123] describe the use of eukaryotic expression for epitope mapping. Three epitopes on the gD protein of herpes
simplex virus I were sensitive to reduction and alkylation, but not to 0.1% SDS [21], suggesting that these epitopes were partially conformational. However, other epitopes localized by eukaryotic expression could also be mimicked by prokaryotic products [77, 123] or peptides [28, 51]. It seems unlikely, therefore, that eukaryotic expression of gene fragments is a general method to localize conformational epitopes.

**Serological studies**

Polyclonal antisera are likely to contain antibodies which, by their specificity for linear epitopes, recognize prokaryotic expression products. This then allows the use of such products for serodiagnosis. The sera of AIDS patients appeared to recognize the bacterial expression products of env [18, 19, 22, 50, 53, 139], *pol* [83], or *gag* [140] fragments from human immunodeficiency virus (HIV). In addition, differences between individual sera could be defined by expression of the HIV *tat* gene [3] or fragments of the *env* [139] or *gag* [140] genes. Similar studies have been carried out with human T-cell leukemia virus-I [53, 86, 102]. A fusion protein containing the sequence coded by the BMRF1 open reading frame of Epstein-Barr virus was considered as serodiagnostic indicator protein [92].

**Immunogenicity of expression products**

Antisera raised against bacterial expression products that cross-react with the native antigen will have, like anti-peptide sera, a predetermined specificity. Studying the neutralization of viral infection by such antisera could be of relevance for vaccine development. Despite negative results with canine parvovirus [112], infectious bursal disease virus [8] and bovine rotavirus [37], there are several reports about expression products that did induce in vitro neutralizing sera. Examples are the gp120 sequence from HIV [90], the VP7 sequence from simian rotavirus [6], the VP7c sequence from bovine rotavirus [71], the major antigenic site on VP1 from foot-and-mouth-disease virus [14, 15, 138], the VP1 regions 52-302 and 24-129 from poliovirus I [49], the VP2 sequence from infectious pancreatic necrosis virus [64], and the N-terminal gD sequence from herpex simplex virus I [56, 58]. More spectacular is the induction of protective immunity. This was observed with a recombinant immunogen containing the core-antigen sequence of hepatitis B virus in one of two chimpanzees [78], with the G sequence from hematopoietic necrosis virus in fish [42] and with E2 sequences from Semliki Forest virus in mice [46]. In the latter case, the sequences eliciting partial or complete protection were localized within residues 114-149 and 216-288, respectively. Remarkably, no in vitro neutralization was observed.

**A view on viral antigenicity**

The available information on the location of sequential epitopes allows a few generalization to be made.
The distinction between antigenic sites, recognized by a group of mutually competing MAbs, and the epitopes of individual MAbs has now be substantiated [31, 61, 66, 88, 100]. The number of these sites found on a viral protein is usually limited. Often, one of the sites is immunodominant and is recognized by the majority of polyclonal antisera and/or monoclonal antibodies [43, 44, 61, 66, 84, 85, 94, 100, 105, 140]. All these sites appear to be sequential. Therefore, the preference of the immune system for certain sites may be explained by the location of regions that by their segmental mobility can conform to the paratopes of the antibody [136]. This does not exclude, however, the presence of conformational sites on other parts of the accessible surface. So, the concept of an antigenic structure, specifying a limited number of antigenic sites [7], can be reconciled partially with the notion that the whole surface of the protein is potentially antigenic [10, 11].

By their location, viral surface proteins are likely to be involved in molecular recognition processes and to interact with the host immune system. Conceivably, flexible regions on the surface are a typical feature of this category of proteins.

References
1. Abrahamsen L, Moks T, Nilsson B, Uhlen M (1985) Secretion of heterologous gene products to the culture medium of Escherichia coli. Nucleic Acids Res 14: 7487-7501
2. Acharya R, Fry E, Stuart D, Fox G, Rowlands D, Brown F (1989) The three-dimensional structure of foot-and-mouth disease virus at 2.9 Å resolution. Nature 337: 709-716
3. Aldovini A, Debouck C, Feinberg MB, Rosenberg M, Arya SK, Wong-Staal F (1986) Synthesis of the complete trans-activation gene product of human T-lymphotropic virus type III in Escherichia coli: demonstration of immunogenicity in vivo and expression in vitro. Proc Natl Acad Sci USA 83: 6672-6676
4. Amann E, Brosius J, Ptashne M (1983) Vectors bearing a hybrid trp-lac promoter useful for regulated expression of cloned genes in Escherichia coli. Gene 25: 167-178
5. Amann E, Brosius J (1985) ‘Atg vectors’ for regulated high-level expression of clones genes in Escherichia coli. Gene 40: 183-190
6. Arias CF, Ballado T, Plebanski M (1986) Synthesis of the outer-capsid glycoprotein of the simian rotavirus SA11 in Escherichia coli. Gene 47: 211-219
7. Atassi MZ (1984) Antigenic structures of proteins. Eur J Biochem 145: 1-20
8. Azad AA, Fahey KJ, Barrett SA, Erny KM, Hudson PJ (1986) Expression in Escherichia coli of cDNA fragments encoding the gene for the host-protective antigen of infectious bursal disease virus. Virology 149: 190-198
9. Banapour B, Rosenthal K, Rabin L, Sharma V, Young L, Fernandez J, Engleman E, McGrath M, Reyes G, Lifson J (1987) Characterization and epitope mapping of a human monoclonal antibody reactive with the envelope glycoprotein of human immunodeficiency virus. J Immunol 139: 4027-4033
10. Benjamin DC, Berzofsky JA, East JJ, Gurd FRN, Hannum C, Leach SJ, Margoliash E, Michael JG, Miller A, Prager EM, Reichlin M, Sercarz EE, Smith-Gill SJ, Todd PE, Wilson AC (1984) The antigenic structure of proteins: a reappraisal. Ann Rev Immunol 2: 67-101
11. Berzofsky JA (1985) Intrinsic and extrinsic factors in protein antigenic structure. Science 229: 932-940
12. Bialy H (1987) Recombinant proteins: virtual authenticity. Biotechnology 5: 883-891
13. Bressan GM, Stanley KK (1987) pUEX, a bacterial expression vector related to pEX with universal host specificity. Nucleic Acids Res 15: 10056

14. Broekhuijsen MP, Blom T, Kottenhagen M, Pouwels PH, Meloen RH, Barteling SJ, Enger Valk RE (1986) Synthesis of fusion proteins containing antigenic determinants of foot-and-mouth disease virus. Vaccine 4: 119–124

15. Broekhuijsen MP, Blom T, van Rijn J, Pouwels PH, Klasen EA, Fasbender MJ, Enger-Valk BE (1986) Synthesis of fusion proteins with multiple copies of an antigenic determinant of foot-and-mouth disease virus. Gene 49: 189–197

16. Brown TA (1986) Gene cloning, an introduction. Van Nostrand Reinold, Wokingham, UK

17. Buckland R, Giraudon P, Wild F (1989) Expression of measles virus nucleoprotein in Escherichia coli: use of deletion mutants to locate the antigenic sites. J Gen Virol 70: 435–441

18. Cabradilla CD, Groopman JE, Lanigan J, Renz M, Lasky LA, Capon DJ (1986) Serodiagnosis of antibodies to the human AIDS retrovirus with a bacterially synthesized env polypeptide. Biotechnology 4: 128–133

19. Certa U, Bannwarth W, Stueber D, Gentz R, Lanzer M, le Grice S, Guillot F, Wendler I, Hunsmann G, Buja H, Mous J (1986) Subregions of a conserved part of the HIV gp41 transmembrane protein are differentially recognized by antibodies of infected individuals. EMBO J 5: 3051–3056

20. Chambers P, Nesbit M, Yusoff K, Millar NS, Samson ACR, Emmerson PT (1988) Location of a neutralizing epitope for the haemagglutinin-neuraminidase glycoprotein of Newcastle disease virus. J Gen Virol 69: 2115–2122

21. Cohen GH, Isola VJ, Kuhns J, Berman PW, Eisenberg RJ (1986) Localization of discontinuous epitopes of herpes simplex virus glycoproteins D: use of a nondenaturing ("native" gel) system of polyacrylamide gel electrophoresis coupled with Western blotting. J Virol 60: 157–166

22. Crowl R, Glassy K, Gordon M, Conroy R, Schaber M, Kramer R, Shaw G, Wong-Staal F, Reddy EP (1985) HLTV-III env gene products synthesized in E. coli are recognized by antibodies present in the sera of AIDS patients. Cell 41: 979–986

23. Crowl R, Seamans C, Lomedica P, McAndrew S (1985) Versatile expression vectors for high-level synthesis of cloned gene products in Escherichia coli. Gene 38: 31–38

24. Davis M, Bjorkman PJ (1988) T-cell antigen receptor genes and T-cell recognition. Nature 334: 395–401

25. Denhardt DT, Colasanti J (1987) A survey of vectors for regulating expression of cloned DNA in E. coli. In: Rodriguez RL, Denhardt DT (eds) Vectors. Butterworths, Stoneham, MA, pp 179–204

26. Devare SG, Shatzman A, Robbins KC, Rosenberg M, Aaronson SA (1984) Expression of the PDGF-related transforming protein of simian sarcoma virus in E. coli. Cell 36: 43–49

27. De Vries P, Versteeg-van Oosten JPM, Visser IKG, van Binnendijk RS, Langeveld SA, Osterhaus ADME, Uytdehaag FGCM (1989) Measles virus-specific murine T cell clones: characterization of fine specificity and function. J Virol 142: 2841–2846

28. Dietzschold B, Eisenberg RJ, Ponce de Leon M, Golub E, Hudecz F, Varriichio A, Cohen GH (1984) Fine structure analysis of type-specific and type-common antigenic sites of herpes simplex virus glycoprotein D. J Virol 52: 431–435

29. Di Guan G, Li P, Riggs PD, Inouye H (1988) Vectors that facilitate the expression and purification of foreign peptides in Escherichia coli by fusion to maltose-binding protein. Gene 67: 21–30

30. Doorbar J, Evans HS, Coneron I, Crawford LV, Gallimore PH (1988) Analysis of HPV-1 E4 expression using epitope-defined antibodies. EMBO J 7: 825–833
31. Dowbenko D, Nakamura G, Fennie C, Shimasaki C, Riddle T, Harris R, Gregory T, Lasky L (1988) Epitope mapping of the human immunodeficiency virus type 1 gp120 with monoclonal antibodies. J Virol 62: 4703–4711
32. Duffaud GD, March PE, Inouye M (1987) Expression and secretion of foreign proteins in *Escherichia coli*. Methods Enzymol 153: 492–507
33. Duvoisin RM, Belin D, Krisch HM (1986) A plasmid expression vector that permits stabilization of both mRNAs and proteins encoded by the cloned genes. Gene 45: 193–201
34. Eisenberg RJ, Long D, Ponce de Leon M, Matthews JT, Spear PG, Gibson MG, Lasky LA, Berman P, Golub E, Cohen GH (1985) Localization of epitopes of herpes simplex virus type 1 glycoprotein D. J Virol 53: 634–644
35. Ellis RW, Malcolm Keller P, Lowe RS, Zivin RA (1985) Use of a bacterial expression vector to map the Varicella-Zoster virus major glycoprotein gene, gC. J Virol 53: 81–88
36. Enjuanes L, Gebauer F, Correa I, Bullido MJ, Suñé C, Smerdou C, Sánchez C, Lenstra JA, Posthumus WPA, Meloen RH (1989) Location of antigenic sites of the S-glycoprotein of transmissible gastroenteritis virus and their conservation in coronaviruses. In: Cavanagh D, Brown TDK (eds) Coronaviruses. Plenum Press, New York, in press
37. Francavilla M, Miranda P, di Matteo A, Sarasani A, Gerna G, Milanesi G (1987) Expression of bovine rotavirus neutralization antigen in *Escherichia coli*. J Gen Virol 68: 2975–2980
38. Gan Z-R, Condra JH, Gould RJ, Zivin RA, Bennet CD, Jacobs JW, Friedman PA, Polokoff MA (1989) High-level expression in *Escherichia coli* of a chemically synthesized gene for [Leu-28]echistatin. Gene 79: 159–166
39. Germino J, Bastía D (1984) Rapid purification of a cloned gene product by genetic fusion and site-specific proteolysis. Proc Natl Acad Sci USA 81: 4692–4696
40. Geysen HM, Rodda SJ, Mason TJ, Tribbick G, Schoofs PG (1987) Strategies for epitope mapping using peptide synthesis. J Immunol Methods 102: 259–274
41. Gill DS, Takai S, Portner A, Kingsbury DW (1988) Mapping of antigenic domains of Sendai virus nucleocapsid protein expressed in *Escherichia coli*. J Virol 62: 4805–4808
42. Gilmore Jr RD, Engelking HM, Manning DS, Leong JC (1988) Expression in *Escherichia coli* of an epitope of the glycoprotein of infectious hematopoietic necrosis virus protects against viral challenge. Biotechnology 6: 295–300
43. Gnann Jr JW, Nelson JA, Oldstone MBA (1987) Fine mapping of an immunodominant domain in the transmembrane glycoprotein of human immunodeficiency virus. J Virol 61: 2639–2641
44. Goudsmit J, Debouck C, Meloen RH, Smit L, Bakker M, Asher DM, Wolff AV, Gibbs Jr CJ, Gajdusek DC (1988) Human immunodeficiency virus type 1 neutralization epitope with conserved architecture elicits early type-specific antibodies in experimentally infected chimpanzees. Proc Natl Acad Sci USA 85: 4478–4482
45. Gray NR, Mazzara GP, Reddy P, Rosbash M (1987) Searching for clones with open reading frames. Methods Enzymol 154: 129–156
46. Grosfeld H, Velan B, Leitner M, Cohen S, Lustig S, Lachmi B-E, Shafferman A (1989) Semliki Forest virus E2 envelope epitopes induce a nonneutralizing humoral response which protects mice against lethal challenge. J Virol 63: 3416–3422
47. Han JH, Rutter WJ (1987) Lambda gt22, an improved lambda vector for the directional cloning of full-length cDNA. Nucleic Acids Res 15: 6304
48. Hasnain SE, Manavathu EK, Leung WC (1987) pXmnATG: an *E. coli* vector for expression of unfused proteins. Nucleic Acids Res 15: 3925
49. Hoatlin ME, Kew OM, Renz ME (1987) Regions of poliovirus protein VP1 produced in *Escherichia coli* induce neutralizing antibodies. J Virol 61: 1442–1447

50. Hofbauer JM, Schulz TF, Hengster P, Larcher C, Zangerle R, Koßler H, Fritsch P, Wachter H, Dierich MP (1988) Comparison of Western blot (immunoblot) based on recombinant-derived p41 with conventional tests for serodiagnosis of human immunodeficiency virus infections. J Clin Microbiol 26: 116–120

51. Hubenthal-Voss J, Houghten RA, Pereira L, Roizman B (1988) Mapping of functional and antigenic domains of the α4 protein of herpes simplex virus 1. J Virol 62: 454–462

52. Jenison SA, Yu X-P, Valentine JM, Galloway DA (1989) Human antibodies react with an epitope of the human papillomavirus type 6b L1 open reading frame which is distinct from the type-common epitope. J Virol 63: 809–818

53. Kanner SB, Cheng-Mayer C, Geffin RB, Parks WP, Beltz GA, Arthur LO, Samuel KP, Papas TS (1986) Human retroviral env and gag polypeptides: serologic assays to measure infection. J Immunol 137: 674–678

54. Kato C, Kobayashi T, Kudo T, Furušato T, Murakami Y, Tanaka T, Baba H, Oishi T, Ohtsuka T, Kato H, Moriyama S, Horikoshi K (1987) Construction of an excretion vector and extracellular production of human growth hormone from *Escherichia coli*. Gene 54: 197–202

55. Keegan K, Collett MS (1986) Use of bacterial expression cloning to define the amino acid sequences of antigenic determinants on the G2 glycoprotein of Rift Valley fever virus. J Virol 58: 263–270

56. Kocken CHM, Geerligs HJ, Bos CA, AB G, Weijer WJ, Drijfhout J-W, Welling GW, Welling-Wester S (1988) Immunological properties of an N-terminal fragment of herpes simplex virus type 1 glycoprotein D expressed in *Escherichia coli*. Arch Virol 103: 267–274

57. Kocken CHM, Scheer JMJ, Welling GW, Welling-Wester S (1988) Purification of fusion proteins expressed by pEX3 and a truncated pEX3 derivative. FEBS Lett 236: 132–134

58. Kocken CHM (1989) Recombinant fusion proteins of herpes simplex virus type 1 glycoprotein D. Academic thesis, State University of Groningen, The Netherlands

59. Koiten M, Ruether U, Mueller-Hill B (1982) Immunoenzymatic detection of expressed gene fragments cloned in the lac Z gene of *E. coli*. EMBO J 4: 509–512

60. Koito A, Hattori T, Matsushita S, Maeda Y, Nozaki C, Sagawa K, Takatsuki K (1988) Conserved immunogenic region of a major core protein (p24) of human and simian immunodeficiency viruses. AIDS Res Hum Retroviruses 4: 409–417

61. Kusters JG, Jager EJ, Lenstra JA, Koch G, Posthumus WPA, Meloen RH, van der Zeijst BAM (1989) Analysis of an immunodominant region of infectious bronchitis virus. J Immunol 143: 2692–2698

61 a. Kusters JG, Jager EJ, van der Zeijst BAM (1989) Improvement of the cloning linker of the bacterial expression vector pEX. Nucleic Acids Res 17: 8007

62. Lamb-JR, Ivanyi J, Rees ADM, Rothbard JB, Howland K, Young RA, Young DB (1987) Mapping of T cell epitopes using recombinant antigens and synthetic peptides. EMBO J 6: 1245–1249

63. Lautenberger JA, Court D, Papas TS (1983) High-level expression in *Escherichia coli* of the carboxy-terminal sequences of the avian myelocytomatosis virus (MC29) v-myc protein. Gene 23: 75–84

64. Lawrence WR, Nagy E, Duncan R, Krell P, Dobos P (1989) Expression in *Escherichia coli* of the major outer capsid protein of infectious pancreatic necrosis virus. Gene 79: 369–374

65. Lecocq JP, Zukowski M, Lathe R (1984) Cloning and expression of viral antigens in *Escherichia coli* and other microorganisms. Methods Virol 7: 121–172
66. Lenstra JA, Kusters JG, Koch G, van der Zeijst BAM (1989) Antigenicity of the peplomer protein of infectious bronchitis virus. Mol Immunol 26: 7–15
67. Luytjes WL, Geerts D, Posthumus W, Meloen R, Spaan W (1989) Amino acid sequence of a conserved neutralizing epitope of murine coronaviruses. J Virol 63: 1408–1412
68. Maina CV, Riggs PS, Grandea III AG, Slatko BE, Moran LS, Tagliamonte JA, McReynolds LA, di Guan C (1988) An Escherichia coli vector to express and purify foreign proteins by fusion to and separation from maltose-binding protein. Gene 74: 365–373
69. Marston FAO (1986) Purification of eukaryotic polypeptides synthesized in Escherichia coli. Biochem J 240: 1–12
70. Matsushita S, Robert-Guroff M, Rusche J, Koito A, Hattori T, Hoshino H, Javaherian K, Takatsuki K, Putney S (1988) Characterization of a human immunodeficiency virus neutralizing monoclonal antibody and mapping of the neutralizing epitope. J Virol 62: 2107–2114
71. McCrae MA, McCorquodale JG (1987) Expression of a major bovine rotavirus neutralization antigen (VP7c) in Escherichia coli. Gene 55: 9–18
72. Mehra V, Sweetser D, Young RA (1986) Efficient mapping of protein antigenic determinants. Proc Natl Acad Sci USA 83: 7013–7017
73. Meloen RH, Puyk WC, Meijer DJA, Lankhof H, Posthumus WPA, Schaper WMM (1987) Antigenicity and immunogenicity of synthetic peptides of foot-and-mouth disease virus. J Gen Virol 68: 305–314
74. Meloen RH, Liskamp RM, Goudsmit J (1989) Specificity and function of the individual amino acids of an important determinant of human immunodeficiency virus type I that induces neutralizing activity. J Gen Virol 70: 1505–1512
75. Milman G (1987) Expression plasmid containing the λP1 promotor and cI857 repressor. Methods Enzymol 153: 482–491
76. Mitraki A, King J (1989) Protein folding intermediates and inclusion body formation. Biotecnology 7: 690–697
77. Mole SE, Lane DP (1985) Use of simian virus 40 large T-β-galactosidase fusion proteins in an immunochemical analysis of simian virus 40 large T antigen. J Virol 54: 703–710
78. Murray K, Bruce SA, Wingfield P, van Eerd P, de Reus A, Schellekens H (1987) Protective immunization against hepatitis B with an internal antigen of the virus. J Med Virol 23: 101–107
79. Nagai K, Thøgersen HC (1987) Synthesis and sequence-specific proteolysis of hybrid proteins produced in Escherichia coli. Methods Enzymol 153: 461–481
80. Nagata L, Masri SA, Pon RT, Lee PWK (1987) Analysis of functional domains on reovirus cell attachment protein sigma 1 using cloned S1 gene deletion mutants. Virology 160: 162–168
81. Nilsson B, Abrahamson L, Uhlen M (1984) Immobilization and purification of enzymes with staphylococcal protein A gene fusion vectors. EMBO J 4: 1075–1080
82. Nunberg JH, Rodgers G, Gilbert JH, Snead RM (1984) Method to map antigenic determinants recognized by monoclonal antibodies: localization of a determinant of virus neutralization on the feline leukemia envelope protein gp70. Proc Natl Acad Sci USA 81: 3675–3679
83. Padberg C, Nowlan S, Mermer B (1989) Recombinant polypeptides from the human immunodeficiency virus reverse transcriptase define three epitopes recognized by antibodies in sera from patients with acquired immunodeficiency syndrome. AIDS Res Hum Retroviruses 5: 61–71
84. Page GS, Mosser AG, Hogle JM, Filman DJ, Rueckert RR, Chow M (1988) Three-dimensional structure of poliovirus serotype 1 neutralizing determinants. J Virol 62: 1781–1794
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85. Palker TJ, Matthews TJ, Clark ME, Cianciolo GJ, Randall RR, Langlois AJ, White GC, Safai B, Snyderman R, Bolognesi DP, Haynes BF (1987) A conserved region at the COOH terminus of human immunodeficiency virus gp120 envelope protein contains an immunodominant epitope. Proc Natl Acad Sci USA 84: 2479-2483

86. Papas TS, Samuel KP, Kan NC, Ascione R, Wong-Staal F, Lautenberger (1985) Production of oncogene-specific proteins and human T-cell leukemia (lymphotropic) retrovirus (HTLV-I) envelope protein in bacteria and its potential for use in human cancers and seroepidemiological surveys. Cancer Res 45: 4568s-4573s

87. Parry NR, Barnett PV, Ouldridge EJ, Rowlands DJ, Brown F (1989) Neutralizing epitopes of type 0 foot-and-mouth disease virus. II. Mapping three conformational sites with synthetic peptide reagents. J Gen Virol 70: 1493-1503

88. Posthumus WPA, Meloen RH, Enjuanes L, Correa I, van Nieuwstadt AP, Koch G, de Groot RJ, Kusters JG, Luytjes W, Spaan WJ, van der Zeijst BAM, Lenstra JA (1989) Linear neutralizing epitopes on the peplomer protein of coronaviruses. In: Cavanagh D, Brown TDK (eds) Coronaviruses. Plenum Press, New York, in press

89. Pouwels PH, Enger-Valk BE, Brammar WJ (1987) Cloning vectors. A laboratory manual. Elsevier, Amsterdam

90. Putney SD, Matthews TJ, Robey WG, Lynn DL, Robert-Goroff M, Mueller WT, Langlois AJ, Ghayeb J, Petteway Jr SR, Weinhold KJ, Fischinger PJ, Wong-Staal F, Gallo RC, Bolognesi DP (1986) HTLV-III/LAV-neutralizing antibodies to an E. coli-produced fragment of the virus envelope. Science 234: 1393-1395

91. Rimm DL, Pollard TD (1989) New plasmid vectors for high level expression of eukaryotic fusion proteins in Escherichia coli. Gene 75: 323-327

92. Roecikel D, Boos H, Mueller-Lantzsch N (1987) Expression of an early Epstein-Barr virus antigen (EA-D) in E. coli. Arch Virol 97: 365-372

93. Rosenberg M, H Y, Shatzman A (1983) The use of pKC30 and its derivatives for controlled expression of genes. Methods Enzymol 101: 123-138

94. Rossmann MG, Arnold E, Erickson JW, Frankenberger EA, Griffith JP, Hecht HJ, Johnson JE, Kamer G, Luo M, Mosser AG, Rueckert RR, Sherry B, Vriend G (1985) Structure of a human common cold virus and functional relationship to other picornaviruses. Nature 317: 145-153

95. Rowe DT, Clarke JR (1989) The type-specific epitopes of the Epstein-Barr virus nuclear antigen 2 are near the carboxy terminus of the protein. J Gen Virol 70: 1217-1229

96. Rothbard JB (1986) Peptides and the cellular immune response. Ann Inst Pasteur/ Virol 137E: 518-528

97. Rothbard JB, Taylor WR (1988) A sequence pattern common to T cell epitopes. EMBO J 7: 93-100

98. Rüther U, Koenen M, Sippel AE, Müller-Hill B (1982) Exon cloning: immunoenzymatic identification of exons of the chicken lysozyme gene. Proc Natl Acad Sci USA 79: 6852-6855

99. Rüther U, Müller-Hill B (1983) Easy identification of cDNA clones. EMBO J 2: 1791-1794

100. Salfeld J, Pfaff E, Noah M, Schaller H (1989) Antigenic determinants and functional domains in core antigen and e antigen from hepatitis B virus. J Virol 63: 798-808

101. Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning. A laboratory manual, 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

102. Samuel KP, Fiordellis CS, DuBois GC, Papas TS (1985) High-level bacterial expression and purification of human T-lymphotropic virus type I (HTLV-I) transmembrane env protein. Gene Anal Techn 2: 60-66
103. Schödel F, Will H (1989) Construction of a plasmid for expression or foreign epitopes as fusion with subunit B of *Escherichia coli* heat-labile enterotoxin. Infect Immun 57: 1347–1350
104. Schoner BE, Belagaje RM, Schoner RG (1987) Expression of eukaryotic genes in *Escherichia coli* with a synthetic two-cistron system. Methods Enzymol 153: 401–416
105. Schrier RD, Gnann Jr JW, Langlois AJ, Shriver K, Nelson JA, Oldstone MBA (1988) B- and T-lymphocyte responses to an immunodominant epitope of human immunodeficiency virus. J Virol 62: 2531–2536
106. Sela M (1969) Antigenicity: some molecular aspects. Science 166: 1365–1374
107. Seth A, Lapis P, vande Woude GF, Papas T (1986) High-level expression vectors to synthesize unfused proteins in *Escherichia coli*. Gene 42: 49–57
108. Shatzman AR, Rosenberg M (1987) Expression, identification, and characterization of recombinant gene products in *Escherichia coli*. Methods Enzymol 152: 661–673
109. Sieg K, Kun J, Pohl I, Scherf A, Müller-Hill (1989) A versatile phage lambda expression vector system for cloning in *Escherichia coli*. Gene 75: 261–270
110. Sisk WP, Chirikjian JG, Lautenberger J, Jorecky C, Papas TS, Berman ML, Zagursky R, Court DL (1986) A plasmid vector for cloning and expression of gene segments: expression of an HTLV-I envelope gene segment. Gene 48: 183–193
111. Skern T, Neubauer C, Frasel L, Gruendl P, Sommergruber W, Zorn M, Kuechler E, Blaas D (1987) A neutralizing epitope on human rhinovirus type 2 includes amino acid residues between 153 and 164 of virus capsid protein VP2. J Gen Virol 68: 315–323
112. Smith S, Halling SM (1984) Expression of canine parvovirus-β-galactosidase fusion proteins in *Escherichia coli*. Gene 29: 263–269
113. Smith DB, Johnson KS (1988) Single-step purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione S-transferase. Gene 67: 31–40
114. Snijders A, Benaisa-Trouw BJ, Oosting JD, Snippe H, Kraaijeveld CA (1989) Identification of a DTH inducing T-cell epitope on the E2 membrane protein of Semliki Forest virus. Cell Immunol 123: 25–35
115. Stanley KK (1983) Solubilization and immune-detection of β-galactosidase hybrid proteins carrying foreign antigenic determinants. Nucleic Acids Res 11: 4077–4092
116. Stanley KK, Luzio JP (1984) Construction of a new family of high efficiency bacterial expression vectors: identification of cDNA clones coding for human liver proteins. EMBO J 3: 1429–1434
117. Stanley KK, Herz J, Dickson J (1987) Topological mapping of complement C9 by recombinant DNA techniques suggests a novel mechanism for its insertion into target membranes. EMBO J 6: 1951–1957
118. Stoker NG, Grant KA, Dockrell HM, Howard CR, Jouy NF, McAdam KPWJ (1989) High level expression of genes clones in λgt11. Gene 78: 93–99
119. Strebel K, Beck E, Strohmaier K, Schaller H (1986) Characterization of foot-and-mouth disease virus gene products with antisera against bacterially synthesized fusion proteins. J Virol 57: 983–991
120. Tai T-N, Havelka WA, Kaplan S (1988) A broad-host-range vector system for cloning and translational lacZ fusion analysis. Plasmid 19: 175–188
121. Tainer JA, Getzoff ED, Paterson Y, Olson AJ, Lerner RA (1985) The atomic mobility component of protein antigenicity. Annu Rev Immunol 3: 501–535
122. Tooze SA, Stanley KK (1986) Identification of two epitopes in the carboxyterminal 15 amino acids of the E1 glycoprotein of mouse hepatitis virus A59 by using hybrid proteins. J Virol 60: 928–934
123. Tsukamoto AS, Ferguson BV, Rosenberg M, Weissman IL, Berk AJ (1986) An immunodominant domain in adenovirus type 2 early region 1A proteins. J Virol 60: 312–316
124. Utz U, Britt W, Vugler L, Mach M (1989) Identification of a neutralizing epitope on glycoprotein gp58 of human cytomegalovirus. J Virol 63: 1995–2001

125. Van der Werf S, Wychowski C, Bruneau P, Blondel B, Crainic R, Horodniceanu F, Girard M (1983) Localization of a poliovirus type I neutralization epitope in viral capsid polypeptide VP1. Proc Natl Acad Sci USA 80: 5080–5084

126. Van der Zee R, van Eden W, Meleno RH, Noordzij A, van Embden JDA (1989) Efficient mapping and characterization of a T cell epitope by the simultaneous synthesis of multiple peptides. Eur J Immunol 19: 43–47

127. Van Eden W, Thole JER, van der Zee R, Noordzij A, van Embden JDA, Hensen EJ, Cohen IR (1988) Cloning of the mycobacterial epitope recognized by T lymphocytes in adjuvant arthritis. Nature 331: 171–173

128. Van Regenmortel MHV, Neurath AR (1985) Structure of viral antigens. In: van Regenmortel MHV, Neurath AR (eds) Immunochemistry of viruses. The basis for serodiagnosis and vaccines. Elsevier, Amsterdam, pp 1–11

129. Van Regenmortel MHV (1987) Antigenic cross-reactivity between proteins and peptides: new insights and applications. Trends Biochem Sci 12: 237–240

130. Van Regenmortel MHV, Muller S, Queninaux VF, Altschuh D, Briand JP (1988) Operational aspects of epitope identification: structural features of proteins recognized by antibodies. In: Kohler H, LoVerde PT (eds) Vaccines: new concepts and developments. Longman, London, pp 113–122

131. Van Regenmortel MHH (1989) Structural and functional approaches to the study of protein antigenicity. Immunol Today 10: 266–271

132. Versteeg-van Oosten J, Langeveld S, Voorma HO, Weisbeek P, de Vries P, Osterhaus A, Uytdehaag F, Meleno R (1989) Epitope mapping of the fusion protein of measles virus. In: Lerner R A, Ginsberg H, Chanock RM, Brown F (eds) Vaccines 89. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp 521–527

133. Watson G, Burdon MG, Russell WC (1988) An antigenic analysis of the adenovirus type 2 fibre polypeptide. J Gen Virol 69: 525–535

134. Weinstock GM, Ap Rhys C, Berman ML, Hampar B, Jackson D, Silhavy TJ, Weisemann J, Zweig M (1983) Open reading frame expression vectors: a general method for antigen production in Escherichia coli using protein fusions to β-galactosidase. Proc Natl Acad Sci USA 80: 4432–4436

135. Weinstock GM (1987) Use of open reading frame expression vectors. Methods Enzymol 154: 156–163

136. Westhof E, Altschuh D, Moras D, Bloomer AC, Mondragon A, Klug A, van Regenmortel MHV (1984) Correlation between segmental mobility and the location of antigenic determinants in proteins. Nature 311: 123–126

137. Wiley DC, Wilson IA, Skehel JJ (1981) Structural identification of the antibody-binding sites of Hong Kong influenza haemagglutinin and their involvement in antigenic variation. Nature 289: 373–378

138. Winther MD, Allen G, Bomford RH, Brown F (1986) Bacterially expressed antigenic peptide from foot-and-mouth disease virus capsid elicits variable immunologic responses in animals. J Immunol 136: 1835–1840

139. Windheuser MG, Wood C (1988) Characterization of immunoreactive epitopes of the HIV-1 p41 envelope protein using fusion proteins synthesized in Escherichia coli. Gene 64: 107–119

140. Windheuser MG, Tegtmeier GE, Wood C (1989) Use of trpE/gag fusion proteins to characterize immunoreactive domains on the human immunodeficiency virus type 1 core protein. J Immunol 63: 4064–4068

141. Wychowski C, Van der Werf S, Siffer O, Crainic R, Bruneau P, Girard M (1983) A poliovirus type I neutralization epitope is located within amino acid residues 93-104 of viral capsid polypeptide VP1. EMBO J 2: 2019–2024
142. Young RA, Davis RW (1983) Efficient isolation of genes by using antibody probes. Proc Natl Acad Sci USA 80: 1194–1198

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