

# Séquençage

Applications au laboratoire de  
Microbiologie clinique

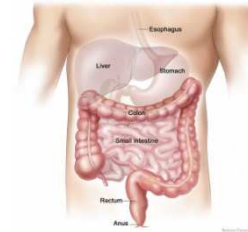
Cécile Meex

# Caractérisation des BLSE produites par les entérobactéries

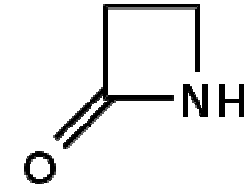
# Entérobactéries



- Morphologie: Bactéries de type bacille Gram négatif
- Habitat: intestin de l'homme et des animaux
- Résistance aux AB: naturelle ou acquise
- Pathogénicité: nombreuses maladies de gravité variable chez l'homme (infection urinaire, fièvre typhoïde, dysenterie bacillaire, peste,...).
- Classification: ~30 genres dont *Escherichia spp.*, *Klebsiella spp.*, *Enterobacter spp.*...



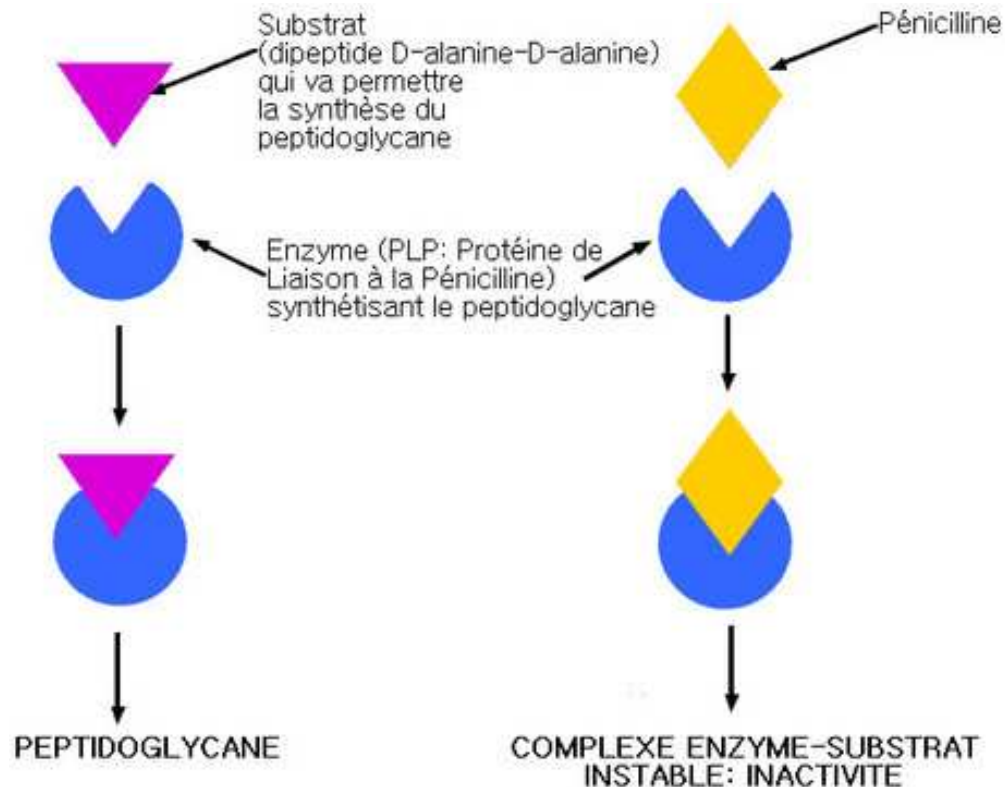
# Bêta-lactamines



Familles	Sous-classes	Principales molécules
<b>Pénicillines</b>	Groupe de la pénicilline G.	<i>Pénicilline G, pénicilline V.</i>
	Pénicillines résistantes aux bêta-lactamases.	<i>Oxacilline, cloxacilline, flucloxacilline.</i>
	Aminopénicillines.	<i>Ampicilline, amoxicilline.</i>
	Carboxypénicillines.	<i>Témocilline, ticarcilline.</i>
	Uréido-pénicillines.	<i>Pipéracilline</i>
	Inhibiteurs de bêta-lactamase.	<i>Acide clavulanique, tazobactam, sulbactam.</i>
<b>Céphalosporines</b>	1 <sup>ère</sup> génération	<i>Céfadroxil, céfalexine, céfalotine,...</i>
	2 <sup>ème</sup> génération	<i>Céfuroxime, céfamandole,...</i>
	3 <sup>ème</sup> génération	<i>Céfotaxime, ceftazidime, ceftriaxone,...</i>
	4 <sup>ème</sup> génération	<i>Céfépime.</i>
<b>Carbapénèmes</b>		<i>Méropénème, imipénème,...</i>
<b>Monobactames</b>		<i>Aztréonam.</i>

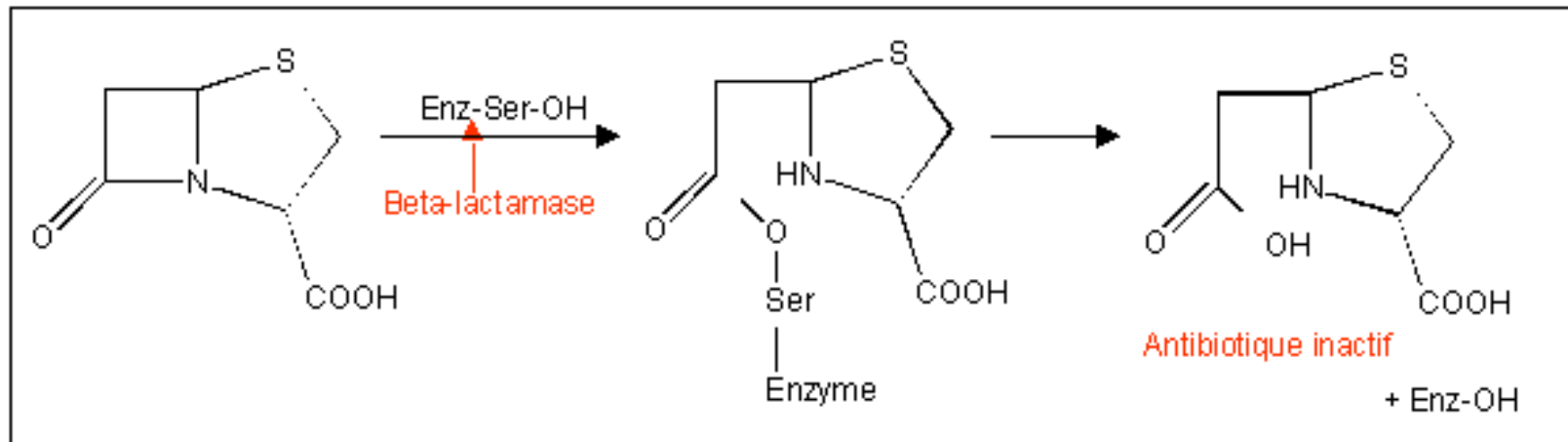
# Bêta-lactamines

- Mode d'action: inhibition de la synthèse de la paroi bactérienne



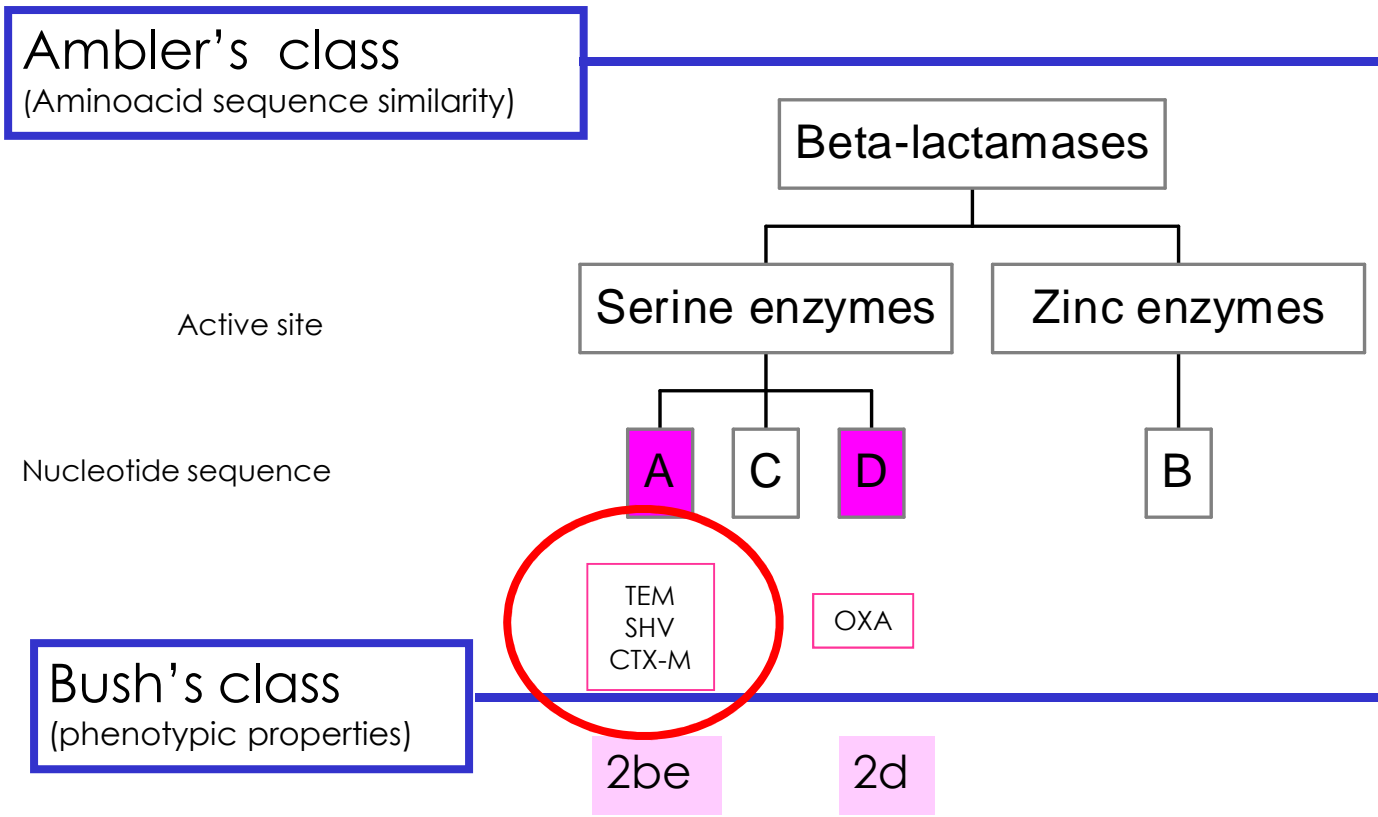
# Bêta-lactamases

- Enzymes inactivatrices des bêta-lactamines
- Principal mécanisme de résistance des entérobactéries aux bêta-lactamines.



# Bêta-lactamases

- Classification



# Bêta-lactamases à spectre étendu (BLSE)

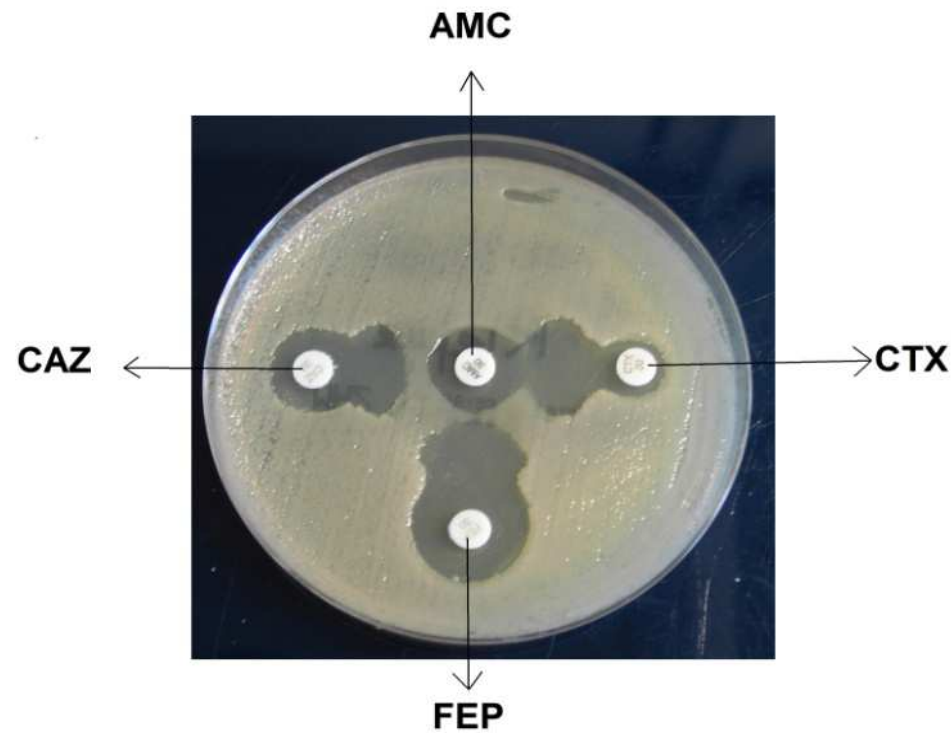
- Bêta-lactamases hydrolysant:
  - l'ensemble des pénicillines
  - les céphalosporines de 1ère, 2ème, 3ème et 4ème génération
  - l'aztréonam
- Inhibées par les inhibiteurs de bêta-lactamases tels que l'acide clavulanique

→ **résistance à l'ensemble des bêta-lactamines  
à l'exception des céphamycines et des  
carbapénèmes**



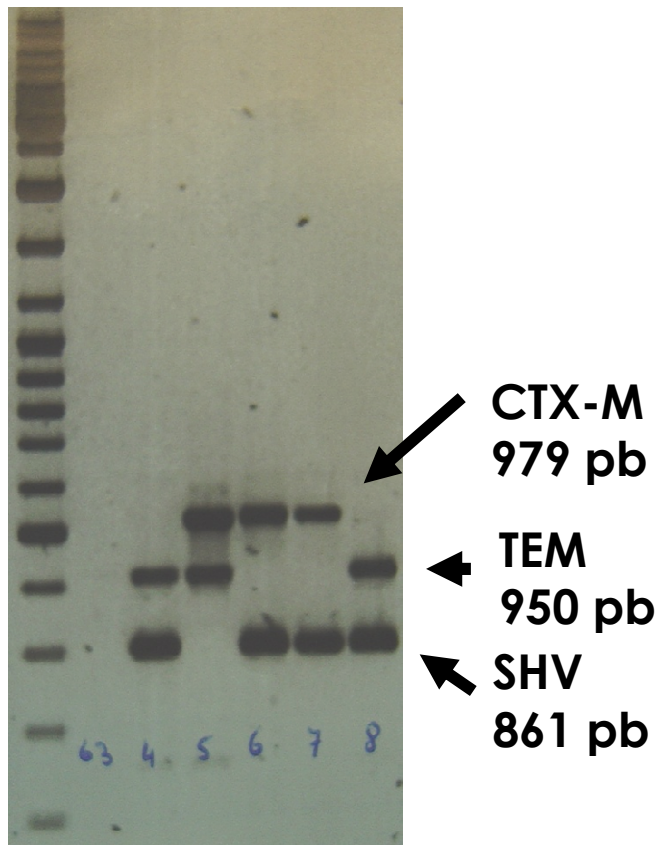
# BLSE: Détection phénotypique

- Test de synergie



# BLSE: détection génotypique

- Extraction de l'ADN < colonies bactériennes
- PCR: gènes TEM, SHV, CTX-M



# BLSE: Séquençage

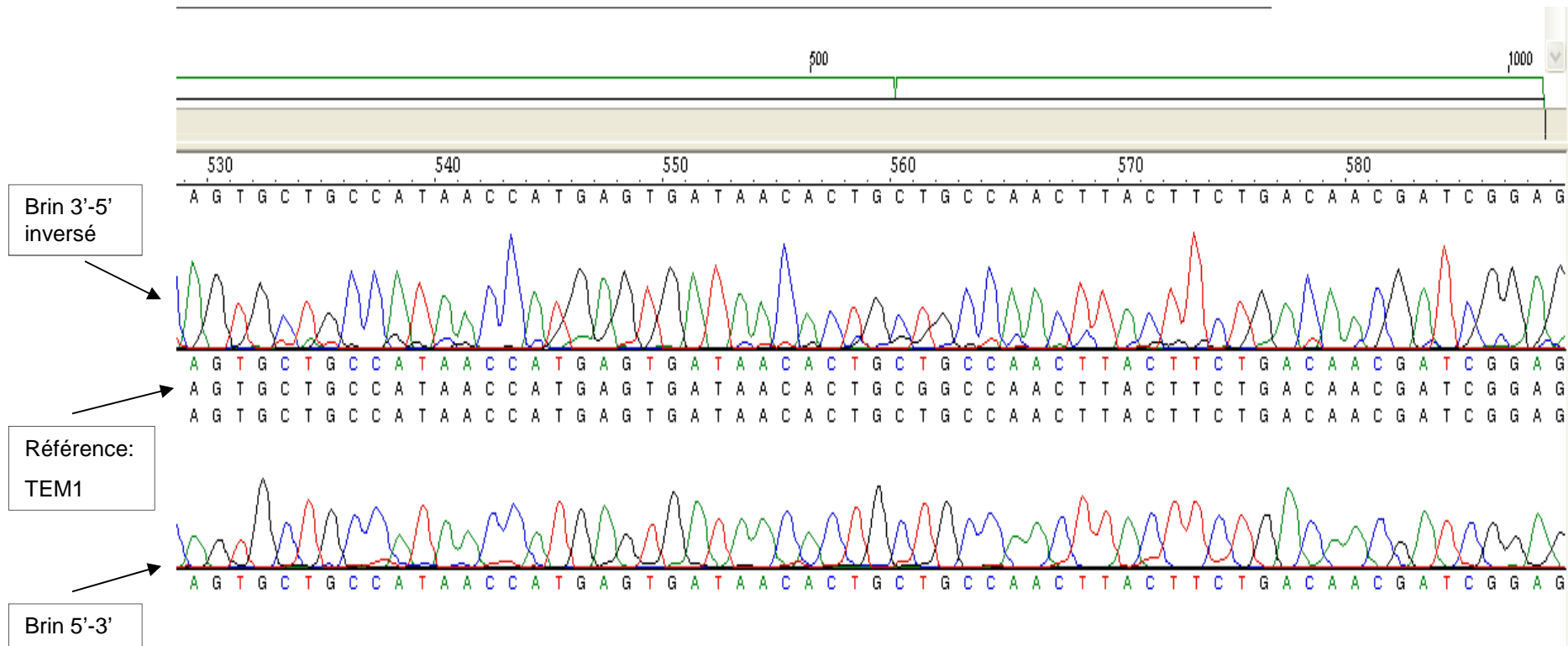
- But: Obtenir l'identification précise de la BLSE
    - ~ 140 BLSE de type TEM
    - ~ 70 BLSE de type SHV
    - ~ 50 BLSE de type CTX-M
      - NB: TEM-1, TEM-2 et SHV-1 ne sont pas des BLSE
- **Confirmation de la production de BLSE et études épidémiologiques**

# BLSE: Séquençage

- Purification de l'ADN amplifié
- Séquençage selon la méthode de Sanger:
  - Vidéo

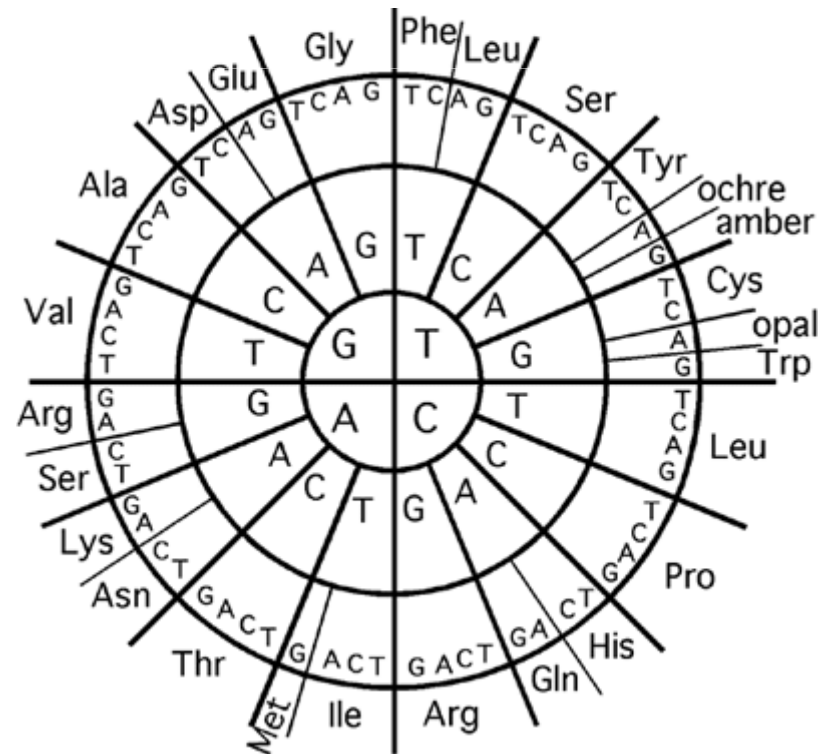
# Démonstration pratique

- Utilisation du logiciel Vector NTI pour l'analyse des séquences



# Démonstration pratique

- Traduction de la séquence nucléotidique en séquence d'acides aminés sur le site Expasy du SIB



# Démonstration pratique

- Introduction de la séquence d'acides aminés sur le site Protein Blast du NCBI : recherche de la séquence en acides aminés la plus comparable dans la base de données et identification précise de la bêta-lactamase.

1<sup>er</sup> exemple

Séquence TEM



# http://web.expasy.org/translate/

The screenshot shows a Windows Internet Explorer browser window with the title "ExPASy - Translate tool - Windows Internet Explorer". The address bar contains the URL "http://web.expasy.org/translate/". The browser's menu bar includes "Fichier", "Edition", "Affichage", "Favoris", and "Outils". The search bar features the Google logo and the text "Rechercher" and "Autres >>". The favorites bar shows "Favoris" and several links, including "Aurore boréale photos, vid...", "Sites suggérés", "CHU de Liège Bienvenue", "Accéder à plus de mo...", and "Hotmail". The main content area has a header with the ExPASy logo (SIB and ExPASy Bioinformatics Resource Portal) and the word "Translate". Below the header, the text reads "Translate tool" followed by a description: "Translate is a tool which allows the translation of a nucleotide (DNA/RNA) sequence to a protein sequence." A prompt asks the user to "Please enter a DNA or RNA sequence in the box below (numbers and blanks are ignored)." Below this is a large, empty text input box with a vertical scrollbar. At the bottom, there is a dropdown menu for "Output format:" set to "Verbose ('Met', 'Stop', spaces between residues)", and two buttons: "Reset" and "TRANSLATE SEQUENCE".

# http://web.expasy.org/translate/

ExPASy - Translate tool - Windows Internet Explorer

http://web.expasy.org/translate/

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SIB ExpASY Bioinformatics Resource Portal Translate

**Translate tool**

Translate is a tool which allows the translation of a nucleotide (DNA/RNA) sequence to a protein sequence.

Please enter a DNA or RNA sequence in the box below (numbers and blanks are ignored).

```
CATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGT
TTATTTTTCTAAATACATTCAAATATGATCCGCTCATGAGACAATAACCCCTGATAAATGCTTCAATAAT
ATTGAAAAAGGAAGAGTATGAGTATTC AACATTTTCGTGTGCGCCCTTATCCCTTTTTTGCGGCATTTTG
CCTTCCTGTTTTGCTCACCCAGAAACGCTGGTGAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGA
GTGGGTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTTC
CAATGATGAGCACTTTTAAAGTTCTGCTATGTGGTGGGTATTATCCCGTGTGACGCCGGGCAAGAGCA
ACTCGGTGCGCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAAGTACAGAAAAGCATCTT
ACGGATGGCATGACAGTAAGAGAATTATGCAATGCTGCCATACCATGAGTGATAACACTGCTGCCAAT
TACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGACACAACATGGGGGATCATGTAAC
TCGCCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCT
GCAGCAATGGCAACAACGTTGCGCAAACTATTAACGGGCAACTACTTACTCTAGCTTCCCGGCAACAAT
TAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCCTTCTGCGCTCGGCCCTCCGGCTGGCTGGTT
TATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGT
AAGCCCTCCGCTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGA
TCGCTG
```

Output format:

or

Please select one of the following frames:

5'3' Frame 1

HDNNGFLDVRWHFSGKCARNPYLFIFLNTFKYVSAHETITLINASIILKKEEYEYESTFSCRPYSLFCGILPSCFCSPRNAGESKRC  
Stop RSVGCTSGLHRTGSQQR Stop DP Stop EFSRRTFSNDEHF Stop SSA Met WCGIIPC Stop RRRARATRSPTLTFSE Stop LG Stop VL  
TSHRKASYGWHDSKRIMet QCCNHHE Stop Stop HCCQLTSDNDRRTEGANRFFAQHGGSCNSP Stop SLGTGAE Stop SHTKRA  
Stop HHDACSNGNVAQTINWRTTYSSFPATINRLDGGG Stop SCRTTSALGPSGLWLVYC Stop Stop IWSR Stop AWVSRVHCSTGAR  
W Stop ALPYRSYLHDGESGNYG Stop TK Stop TDR

5'3' Frame 2

Met II Met V Stop TSGGTFRGNVRGTPICLFF Stop IHSN Met YPL Met RQ Stop P Stop Stop Met LQ Stop Y Stop KRK S Met S IQHFRVALIPFFAA  
FCLPFAHPETLVKVKDAEDQLGARVGYIELDLNSGKILESFRPEERFP Met Met STFKVLLCGAVLSRVDAEQEQLGRRIHYSQN  
DLVEYSPVTEKHLTDG Met TVRELCSAAIT Met SDNTAANLLLTIGGPKELTAF LHN Met GDHVTRLDRWEP LNEAIPNDRDTT  
Met PAA Met ATTLRKLTLTGELLTLASRQQLIDW Met EADKVGAPLLRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYT  
TGSQAT Met DERNRQIA

5'3' Frame 3

Stop Stop Stop WFLRRQVALFGE Met CAEPLFVYFSKYIQICIRS Stop DNNPDKCFNIEKGRV Stop VFNIFVSPLFPFLRHFAFLFLT  
QKRW Stop K Stop K Met LKISWVHEWVTSNWISTAVRSLRVFAPKNVFQ Stop Stop ALLKFCYVVRYPVLTGKSNSVAAYTILR Met T  
WLSTHQSQKSILR Met A Stop Q Stop ENYAVLP Stop P Stop VITLLPTYF Stop QRSEDRRS Stop PLFCTTWGIMet Stop LALIVGNRS Stop  
Met KPYQTTSVTPRCLQQWQQRCANY Stop LANYLL Stop LPGNN Stop Stop TGWRRIKLQDHF CARPFRLAGLLLINLEPVSVGLAVS  
LQHWGQ Met VSPPVS Stop LSTRRGVRQLW Met NEIDRSL

3'5' Frame 1

QRSVYFVHP Stop LPDSPSCR Stop LRYGRAYHLAPVLQ Stop YRETHAHLRQIQQ Stop TSQPEGPSAEVVLQLYPPPSLLIVAGK  
LE Stop VVRQLIVCATLLPLLQASWCHARRLVWLHSAPVPNDQGELHDPCCAKKRLAPSVLRSLSVSWQQCYHSWLWQHCH  
LLLSCHP Stop DAFL Stop LVSTQPSHSENSVCGDRVALARRQHGIIPHHIAEL Stop KCSSLENVLRGENSQQGSYRC Stop DPVRCNP  
LVHPTDLQHLLLSPAFLGEQKQEGK Met PQKRE Stop GRHENVEYSYSSFFNIIAFIRVIVS Stop ADTYLNVFRKINK Stop GFRAHF  
PEKCHLTSKKPLLS

3'5' Frame 2

SDLISFIHSCLTPRRVDNYDTGGLTIWPQCCNDTARPTLTGSRFISNKPASRKGRAQKWSCNFIRLHPVY Stop LLPGS Stop SK  
Stop FAS Stop Stop FAQRCCCHCRHRGVTLVVWYGFILRFPTIKASY Met IPHVVQKSG Stop LLRSSDRCQK Stop VGS SVITHGYGS  
TA Stop FSYCHAIRK Met LFCDW Stop VLNQVILRIVYAATELLLPVNTG Stop YRTT Stop QNFKSAHHWKTFFGAKTLKDLTAVEIQF  
DVTHSCTQLIFSIFYFHQRFWVSKNRKAKCRKKGKGDTK Met LNHTLPLFSILLKHL SGLLSHERIHI Stop Met YLEK Stop TNRGSA  
HISPKSAT Stop RLRNHYYH

3'5' Frame 3

AICLFRSSIVA Stop LPVV Stop ITTIREGLPSGPSAA Met IPRDRSPAPDLSAINQPAGRAERRSGPATLSASIQSINCCREARVSSS  
PVNSLRNVVAIAAGIVVSRSSFG Met ASFSSGSQRSRRVT Stop SP Met LCKKAVSSFGPIVVRSKLA AVL SL Met V Met AALHNSLT  
V Met PSVRCF SVTGEYSTKSF Stop E Stop C Met RRPSCSCPASTRDNTAPHSRTLKVLIIKGRSSGRKLSRILPLLSSS Met Stop PTR  
APN Stop SSASFTFTSVSG Stop AKTGRQNAAKK GIRATRKC Stop ILILFLFQYY Stop SIYQGYCL Met SGYIFECI Stop KKNKQIGVPRTF  
PRKVPPDV Stop ETIII Met

# http://web.expasy.org/translate/

ExpASY Translate Tool - Select an initiator methionine - Windows Internet Explorer

http://web.expasy.org/cgi-bin/translate/dna\_sequences?/work/expasy/tmp/http/seqdna.7701,2

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### Translate Tool

Please select one of the "Methionine" or one of the highlighted residues following a **Stop** codon (or the beginning of the sequence).

This will create a virtual Swiss-Prot entry, comprising the residues from your chosen start position up to the following **Stop** codon.

MIIMVS **Stop** TSGGTFRGNVRGTPICLFF **Stop** IHSNMYPLMRQ **Stop** P **Stop** \_ MLQ **Stop** Y **Stop** KRK**SMS** QHFRVALIPFFAAFCLPVFAH  
PETLVKVKDAEDQLGARVGYIELDLNSGKILESFRPEERFPMMSTFKVLLCGAVLSRVDAQQEQLGRRIHYSQNDLVEYSPVTEK  
HLTDG **M**TVRELCSAAIT **M**SDNTAANLLLTIGGPKELTAFLHN **M**GDHVTRLDRWEPNELNEAIPNDERDTT **M**PAAMATTLRKLTTGE  
LLTLASRQQLIDW **M**EADKVVAGPLLRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQAT **M**DERNRQIA

# http://web.expasy.org/translate/

ExPASy Translate Tool - Virtual entry - Windows Internet Explorer

http://web.expasy.org/cgi-bin/translate/dna\_sequences?/work/expasy/tmp/http/seqdna.7701,2,53

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```
ID VIRT7737 Unreviewed; 276 AA.
AC VIRT7737;
DE Translation of nucleotide sequence generated on ExPASy
DE on 24-Mar-2012 by 139.165.88.87.
CC -!- This virtual protein sequence will automatically be deleted
CC from the server after a few days.
DR SWISS-2DPAGE; VIRT7737; VIRTUAL.
SQ SEQUENCE 276 AA; 8057BFCFCD4BCE1F CRC64.
MSIQHFRVAL IPFFAAFCCLP VFAHPETLVK VKDAEDQLGA RVGYIELDLN SGKILESFRP
EERFPMSTF KVLICGAVLS RVDAGQEQLG RRIHYSQNDL VEYSPVTEKH LTDGMTVREL
CSAAITMSDN TAANLLTTI GGPKELTAFI HNMGDHVTIL DRWEPELNEA IPNDRDITM
PAAATTLRK LLTGELLTLA SRQLIDWME ADKVAGPLLR SALPAGWFIA DKSGAGERGS
RGIIAALGPD GKPSRIVVIY TTGSQATMDE RNRQIA
//
```

Sequence in FASTA format

[BLAST](#) BLAST submission on ExPASy/SIB

 Sequence analysis tools: ProtParam, ProtScale, Compute pI/Mw, PeptideMass, PeptideCutter,

 ScanProsite

 Direct Submission to SWISS-MODEL

# http://web.expasy.org/translate/

ExPASy Translate Tool - Virtual entry - Windows Internet Explorer

http://web.expasy.org/cgi-bin/translate/dna\_sequences?work/expasy/tmp/http/seqdna.7701,2,53

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```
ID VIRI7737          Unreviewed;          276 AA.
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DE Translation of nucleotide sequence generated on ExPASy
DE on 24-Mar-2012 by 139.165.88.87.
CC -!- This virtual protein sequence will automatically be deleted
CC from the server after a few days.
DR SWISS-2DPAGE; VIRI7737; VIRTUAL.
SQ SEQUENCE 276 AA; 8057BFCFC4BCE1F CRC64.
MSIQHFRVAL IPFFAFCPLP VFAHPETLVK VKDAEDQLGA RVGYIELDLN SGKILESFRP
EERFPMSTF KVLLCGAVLS RVDAGQEQLG RRIHYSQNDL VEYSPVTEKH LTDGMTVREL
CSAAITMSDN TAANLLTTI GGPKELTAFI HNMGDHVTRL DRWPELNEA IPNDERDTM
PAAMATTLRK LLTGELLTLA SRQLIDWME ADKVAGPLLR SALPAGWFIA DKSGAGERGS
RGIIAALGPD GKPSRIVVIY TTGSQATMDE RNRQIA
//
```

Sequence in FASTA format

[BLAST](#) BLAST submission on ExPASy/SIB

Sequence analysis tools: ProtParam, ProtScale, Compute pI/Mw, PeptideMass, PeptideCu

ScanProsite

Direct Submission to SWISS-MODEL

# http://www.ncbi.nlm.nih.gov/blast/

The screenshot shows the NCBI BLAST web interface in a Windows Internet Explorer browser. The browser's address bar displays the URL: [http://www.ncbi.nlm.nih.gov/blast/Blast.cgi?PAGE=Proteins&PROGRAM=blastp&BLAST\\_PROGRAMS=blastp&PAGE\\_TYPE=BlastSearch&SHOW\\_DEFAULTS=on](http://www.ncbi.nlm.nih.gov/blast/Blast.cgi?PAGE=Proteins&PROGRAM=blastp&BLAST_PROGRAMS=blastp&PAGE_TYPE=BlastSearch&SHOW_DEFAULTS=on). The browser's menu bar includes 'Fichier', 'Edition', 'Affichage', 'Favoris', and 'Outils'. The search bar contains the text 'Rechercher' and 'Autres >>'. The browser's toolbar shows several open tabs, including 'ExpASY Translate Tool - Virtu...' and 'Protein BLAST: search pr...'. The BLAST interface is titled 'Standard Protein BLAST' and features a navigation bar with 'Home', 'Recent Results', 'Saved Strategies', and 'Help'. The 'blastp' tab is highlighted with a red circle. The main content area includes a 'Query subrange' section with 'From' and 'To' input fields, and a 'Choose Search Set' section with a 'Database' dropdown set to 'Non-redundant protein sequences (nr)'. The 'Job Title' field is empty, and the 'Align two or more sequences' checkbox is unchecked.

# http://www.ncbi.nlm.nih.gov/blast/

Protein BLAST: search protein databases using a protein query - Windows Internet Explorer

http://www.ncbi.nlm.nih.gov/blast/Blast.cgi?PAGE=Proteins&PROGRAM=blastp&BLAST\_PROGRAMS=blastp&PAGE\_TYPE=BlastSearch&SHOW\_DEFAULTS=on

Home Recent Results Saved Strategies Help

NCBI/ BLAST/ blastp suite **Standard Protein BLAST**

blastn **blastp** blastx tblastn tblastx

BLASTP programs search protein databases using a protein query. [more...](#) [Reset page](#) [Bookmark](#)

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) [Clear](#) Query subrange [From](#) [To](#)

IPNDERDTIM  
PAAAMATTLRK LLIGELLTLA SRQQIDWME ADKVAGPLLR SALPAGWFIA  
DKSGAGERGS  
RGIIAALGPD GKPSRIVVIY TTGSQATMDE RNRQIA

Or, upload file  [Parcourir...](#)

Job Title   
Enter a descriptive title for your BLAST search

Align two or more sequences

PHI-BLAST (Pattern Hit Initiated BLAST)

DELTA-BLAST (Domain Enhanced Lookup Time Accelerated BLAST)

Choose a BLAST algorithm

**BLAST** Search database Non-redundant protein sequences (nr) using Blastp (protein-protein BLAST)

show results in a new window

[Algorithm parameters](#)

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NCBI



# http://www.ncbi.nlm.nih.gov/blast/

NCBI Blast:Protein Sequence (276 letters) - Windows Internet Explorer

http://www.ncbi.nlm.nih.gov/blast/Blast.cgi

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Home Recent Results Saved Strategies Help

NCBI/ BLAST/ blastp suite/ Formatting Results - PVD06TAX012 [\[Formatting options\]](#)

**Job Title: Protein Sequence (276 letters)**

Putative conserved domains have been detected, click on the image below for detailed results.

Query seq. 50 100 150 200 250 276

Superfamilies Transpeptidase superfamily

Multi-domains PRK15442

Request ID	PVD06TAX012
Status	Searching
Submitted at	Sat Mar 24 07:46:14 2012
Current time	Sat Mar 24 07:46:16 2012
Time since submission	00:00:02

This page will be automatically updated in 12 seconds

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# http://www.ncbi.nlm.nih.gov/blast/

NCBI Blast: Protein Sequence (276 letters) - Windows Internet Explorer

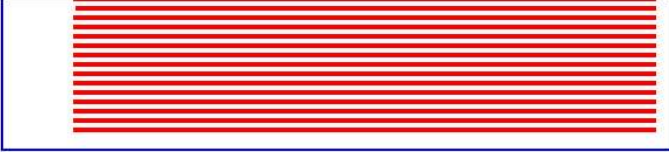
http://www.ncbi.nlm.nih.gov/blast/Blast.cgi

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**Descriptions**

Legend for links to other resources: UniGene GEO Gene Structure Map Viewer PubChem BioAssay

Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
<a href="#">AAS4684.1</a>	TEM-1 beta-lactamase [Serratia marcescens] >qb AAS46846.1  TEM-1	571	571	100%	0.0	100%	
<a href="#">AAS46845.1</a>	TEM-1 beta-lactamase [Serratia marcescens]	570	570	100%	0.0	100%	
<a href="#">ACT97477.1</a>	TEM-1b beta-lactamase [mixed culture bacterium CA_qf1DD01_19]	571	571	100%	0.0	100%	
<a href="#">ABY81258.1</a>	beta-lactamase [Escherichia coli] >qb ACK75718.1  beta-lactamase	570	570	100%	0.0	100%	
<a href="#">CAL26907.1</a>	beta-lactamase protein [Acetobacter pasteurianus]	570	570	100%	0.0	100%	
<a href="#">ACZ02437.2</a>	extended spectrum beta-lactamase TEM-1 [Providencia rettgeri]	570	570	100%	0.0	100%	
<a href="#">AAQ57123.1</a>	beta-lactamase TEM-128 [Acinetobacter baumannii]	570	570	100%	0.0	100%	
<a href="#">NP_052173.1</a>	beta-lactamase [Neisseria gonorrhoeae] >ref NP_569411.1  beta-lac	570	570	100%	0.0	100%	
<a href="#">ABP57022.1</a>	beta lactamase [Cloning vector pTrueBlue]	569	569	100%	0.0	99%	
<a href="#">AAD13350.1</a>	beta-lactamase [Expression vector pPK113] >qb AAD29959.1  beta-	569	569	100%	0.0	99%	
<a href="#">ACF32746.1</a>	putative inhibitor-resistant TEM-163 beta-lactamase [Escherichia co	569	569	100%	0.0	99%	
<a href="#">AEF15432.1</a>	beta-lactamase TEM-1 [Escherichia coli]	568	568	99%	0.0	100%	
<a href="#">ACN82383.1</a>	TEM-116 beta-lactamase [Aeromonas hydrophila] >qb ACN82384.1	569	569	100%	0.0	99%	
<a href="#">ADL40245.1</a>	TEM-1 beta-lactamase [Pseudomonas aeruginosa] >qb ADR29904.1	569	569	100%	0.0	99%	
<a href="#">AAF74292.1</a>	extended-spectrum beta-lactamase [Escherichia coli]	568	568	100%	0.0	99%	
<a href="#">CAG30723.1</a>	hypothetical protein [Hordeum vulgare subsp. vulgare] >qb ACT9842	568	568	100%	0.0	99%	
<a href="#">YP_003829050.1</a>	beta-lactamase class A [Escherichia coli] >qb ADL13944.1  TEM-33	568	568	100%	0.0	99%	
<a href="#">CAA74912.2</a>	beta-lactamase class A [Capnocytophaga ochracea]	568	568	100%	0.0	99%	
<a href="#">AAA72841.1</a>	b-lactamase [unidentified cloning vector] >qb AFB73755.1  beta-lac	568	568	100%	0.0	99%	
<a href="#">AAM61953.1</a>	beta-lactamase TEM-105 [Escherichia coli]	568	568	100%	0.0	99%	
<a href="#">ACX83572.1</a>	extended spectrum beta-lactamase TEM-1 [Morqanella morqanii]	568	568	99%	0.0	100%	
<a href="#">CAJ66089.1</a>	beta-lactamase TEM-150 [Escherichia coli] >qb ACT32331.1  extend	568	568	100%	0.0	99%	

# 2<sup>ème</sup> exemple

Séquence SHV

# http://web.expasy.org/translate/

The screenshot shows a web browser window titled "ExPASy - Translate tool - Windows Internet Explorer". The address bar displays "http://web.expasy.org/translate/". The browser's menu bar includes "Fichier", "Edition", "Affichage", "Favoris", and "Outils". A search bar with the Google logo is present, along with a search button labeled "Rechercher" and a link "Autres >>". The browser's favorites bar shows several items, including "Aurore boréale photos, vid...", "Sites suggérés", "CHU de Liège Bienvenue", "Accéder à plus de mo...", and "Hotmail". The main content area of the browser displays the ExPASy website interface. At the top, there is a logo for "SIB" and "ExPASy Bioinformatics Resource Portal" on the left, and the word "Translate" on the right. Below this, the heading "Translate tool" is followed by a description: "Translate is a tool which allows the translation of a nucleotide (DNA/RNA) sequence to a protein sequence." A text input box contains a long DNA sequence: "ATGCGTTATATTCGCTGTGTATTATCTCCCTGTTAGCCACCCTGCGGCTGGCGGTACAGCCAGCCCGCAGCCGCTTGAGCAAATTAACAAGCGAAGCCAGCTGTTCGGCCCGCGTAGGCATGATAGAAATGGATCTGGCCAGCGGCCGACGCTGACCGCCTGGCCGCGCGATGAACGCTTCCCATGATGAGCACCTTTAAAGTATGTCTCTGCGGCGCAGTGCTGGCGCGGGTGGATGCCGGTGACGAACAGCTGGAGCGAAAAGATCCACTATCGCCAGCAGGATCTGGTGGACTACTCGCCGGTCAGCGAAAAACACCTTGCCGACGGCATGACGGTTCGGCGA ACTCTGCGCCCGCCGATTACCATGAGCGATAACAGCGCCGCAATCTGCTGCTGGCCACCGTCGGCGGC CCGCAGGATTGACTGCCTTTTGGCCAGATCGGGGACAAACGTCACCCGCTTGACCGCTGGGAAACGG AACTGAATGAGGCGCTTCCGGGGACGCGCGGACACCCTACCCGGCCAGCATGGCCGCGACCCTGGG CAAGCTGCTGACCAGCCAGCGTCTGAGCGCCCGTTCCGCAACGGCAGCTGCTGCACTGGATGGTGGACGAT CGGGTCGCGGACCGTTGATCCGCTCCGTGCTGCCGGCGGGCTGGTTTATCGCCGATAAGACCGGAGCTA GCAAGCGGGGTGCGCGCGGGATTGTCCGCCTGCTTGCCCGGAATAACAAAGCAGAGCGCATTGTGGTGAT TTATCTGCGGGATACGCGCGGAGCATGGCCGAGCGAAATCAGCAAATCGCCGGGATCGGCGCGCGTGA TCGAG". Below the input box, there is a dropdown menu for "Output format" set to "Verbose ('Met', 'Stop', spaces between residues)". At the bottom, there are two buttons: "Reset" and "TRANSLATE SEQUENCE".

# http://web.expasy.org/translate/

Translate Tool - Results of translation - Windows Internet Explorer

http://web.expasy.org/cgi-bin/translate/dna\_aa

Translate

Translate Tool - Results of translation

Please select one of the following frames:

**5'3' Frame 1**  
Met RYIRLCIISV L AT L P L A V H A S P Q P L E Q I K Q S E S Q L S G R V G Met IE Met D L A S G R T L T A W R A D E R F P Met Met S T F K V V L C G A V L A R V  
S A G D E O L E R K I H Y R Q Q D L V D Y S P V S E K H L A D G Met T V G E L C A A A I T Met S D N S A A N L L L A T V G G P A G L T A F L R Q I G D N V T R L D R W E  
T E L N E A L P G D A R D T T T P A S Met A A T L R K L L T S Q R L S A R S Q R Q L L Q W Met V D D R V A G P L I R S V L P A G W F I A D K T G A S K R G A R G I V A L  
L G P N N K A E R I V V I Y L R D T P A S Met A E R N Q Q I A G I G A A S Q R S

**5'3' Frame 2**  
C V I F A C V L S P C Stop P P C R W R Y T P A R S R L S K L N K A K A S C R A A Stop A Stop Stop K W I W P A A A R Stop P P G A P Met N A F P Stop Stop A P L K  
Stop C S A A Q C W R G W Met P V T N S W S E R S T I A S R I W W T T R R S A K N T L P T A Stop R S A N S A P P L P Stop A I T A P P I C C W P P S A A P Q D Stop  
L P F C A R S A T T S P A L T A G K R N Stop Met R R R F P A T P A T P L P R P A W P R P C A S C Stop P A S V Stop A P V R N G S C C S G W W T I G S P D R Stop S A  
P C C R R A G L S P I R P E L A S G V R A G L S P C L A R I T K Q S A L W Stop F I C G I R R R A W P S E I S K S P G S A R R D R

**5'3' Frame 3**  
A L Y S P V Y Y L P V S H P A A G G T R Q P A A A Stop A N Stop T K R K P A V G P R R H D R N G S G Q R P H A D R L A R R Stop T L S H D E H L Stop S S A L R R S A  
G A G G C R Stop R T A G A K D P L S P A G S G G L L A G Q R K T P C R R H D G R R T L R R R H Y H E R Stop Q R R Q S A A G H R R R P R R I D C L F A P D R R Q R  
H P P Stop P L G N G T E Stop G A S R R R P R H H Y P G Q H G R D P A Q A A D Q P A S E R P F A T A A A A V D G G R S G R R T V D P L R A A G G L V Y R R Stop D  
R S Stop Q A G C A R D C R P A W P E Stop Q S R A H C G D L S A G Y A G E H G R A K S A N R R D R R G V I E

**3'5' Frame 1**  
L D H A A P I P A I C Stop F R S A Met L A G V S R R Stop I T T Met R S A L L F G P S R A T I P R A P R L L A P V L S A I N Q P A G S T E R I N G P A T R S S T I H C S S C  
R C E R A L R R W L V S S L R R V A A Met L A G V V V S R A S P G S A F S S V S Q R S R R V T L S P I W R K K A V N P A G P P T V A S S R L A A L L S L Met V Met  
A A A Q S S P T V Met P S A R C F S L T G E Stop S T R S C W R Stop W I F R S S C S S P A S T R A S T A P Q S T T L K V L I Met G K R S S A R Q A V S V R P L A R S I  
S I Met P T R P D S W L S L C I C S S G C L A C T A S G R V A N R E I I H R R I Stop R

**3'5' Frame 2**  
S I T P R R S R R F A D F A R P C S P A Y P A D K S P Q C A L L C Y S G Q A G R Q S R A H P A C Stop L R S Y R R Stop T S P P A A R S G S T V R R P D R P P S T A A  
A A V A N G R S D A G W S A A C A G S R P C W P G Stop W C R G R R R E A P H S V P F P S G Q G G Stop R C R R S G A K R Q S I L R G R R R W P A A D W R R C Y  
R S W Stop W R R R R V R R P S C R R Q G V F R Stop P A S S P D P A G D S G S F A P A V R H R H P P A P A L R R R A L L Stop R C S S W E S V H R R A R R S A  
C G R W P D P F L S C L R G P T A G F R F V Stop F A Q A A A G W R V P P A A G W L T G R Stop Y T G E Y N A

**3'5' Frame 3**  
R S R R A D P G D L L I S L G H A R R R I P Q I N H H N A L C F V I R A K Q G D N P A R T P L A S S G L I G D K P A R R Q H G A D Q R S G D P I V H H P L Q Q L P L R T G  
A Q T L A G Q Q L A Q G R G H A G R G S G V A G K R L I Q F R P A V K A G D V V A D L A Q K G S Q S C G A A D G G Q Q I G G A V I A H G N G G G A E F A D  
R H A V G K V F F A D R R V V H Q I L L A I V D L S L Q L F V T G I H P R Q H C A A E H Y F K G A H H G K A F I G A P G G Q R A A A G Q I H F Y H A Y A A R Q L A F A L F  
N L L K R L R A G V Y R Q R Q G G Stop Q G D N T Q A N I T H

# http://web.expasy.org/translate/

ExPASy Translate Tool - Select an initiator methionine - Windows Internet Explorer

http://web.expasy.org/cgi-bin/translate/dna\_sequences?/work/expasy/tmp/http/seqdna.10965,1

Fichier Edition Affichage Favoris Outils ?

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ExPASy Translate Tool - ... Protein BLAST: search protei...

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STB EXPASY Biinformatics Resource Portal Translate Home | Contact

**Translate Tool**

Please select one of the "Methionine" or one of the highlighted residues following a **Stop** codon (or the beginning of the sequence).

This will create a virtual Swiss-Prot entry, comprising the residues from your chosen start position up to the following **Stop** codon.

MRVIRLCIISLLATLPLAVHASPQPLEQIKQSESQLSGRVGMIEMDLASGRTLTAWRADERFPM MSTFKVVLCGAVLARVDAGDE  
QLERKIHRYRQQDLVDYSPVSEKHLADGMTV GELCAAAITMSDNSAANLLLATVGGPAGLTAFLRQIGDNVTRLDRWETELNEALP  
GDARDTTTPASMAATLRKLLTSQRLSARSQRQLLQW MVDDR VAGPLIRS VLPAGWFIADKTGASKR GARGIVALLGPNNKAERIV  
VIYLRDTPASMAERNQQIAGIGAA Stop S

# http://web.expasy.org/translate/

ExPASy Translate Tool - Virtual entry - Windows Internet Explorer

http://web.expasy.org/cgi-bin/translate/dna\_sequences?/work/expasy/tmp/http/seqdna.10965,1,1



Fichier Edition Affichage Favoris Outils ?

Google QLSGRV GMIEMDLASG R.LTLAWRADE RFPMMSTFKV VLCGAVLARV Rechercher Autres >>

Favorites Aurere boréale photos, vid... Sites suggérés CHU de Liège Bienvenue Accéder à plus de mo... Hotmail

ExpASY Translate Tool - ... Protein BLAST: search protei...

Google Cette page est en anglais. La traduire à l'aide de la barre d'outils Google ? [En savoir plus](#) Pas en anglais ? [Aidez-nous à améliorer notre service](#) Traduire Désactiver la traduction des


  **ExpASY**  
Bioinformatics Resource Portal


## Translate


```
ID VIRT11018 Unreviewed; 279 AA.  
AC VIRT11018;  
DE Translation of nucleotide sequence generated on ExpASY  
DE on 24-Mar-2012 by 139.165.88.87.  
CC -!- This virtual protein sequence will automatically be deleted  
CC from the server after a few days.  
DR SWISS-2DPAGE; VIRT11018; VIRTUAL.  
SQ SEQUENCE 279 AA; 6D2CA5B70421CC7F CRC64.  
MRYIRLCIIS LLATLPLAVH ASPQPLEQIK QSEQLSGRV GMIEMDLASG RLTAWRADE  
RFPMMSTFKV VLCGAVLARV DAGDEQLERK IHYRQQDLVD YSPVSEKHLA DGMTVGELCA  
AAITMSDNSA ANLLLATVGG PAGLTAFLRQ IGDNVTRLDR WETELNEALP GDARDITTPA  
SMAATLRKLL TSQRLSARSQ RQLLQNMVDD RVAGPLIRSV LPAGWFIADK TGASKRGARG  
IVALLGPNNK AERIVVIYLR DTPASMAERN QQIAGIGAA  
//
```

Sequence in FASTA format

[BLAST](#) BLAST submission on ExpASY/SIB

 Sequence analysis tools: ProtParam, ProtScale, Compute pI/Mw, PeptideMass, PeptideCutter,

 ScanProsite

 Direct Submission to SWISS-MODEL

# http://www.ncbi.nlm.nih.gov/blast/

The screenshot shows the NCBI Blast web interface in a Windows Internet Explorer browser. The browser's address bar displays the URL <http://www.ncbi.nlm.nih.gov/blast/Blast.cgi>. The page title is "NCBI Blast:Protein Sequence (279 letters)". The browser's menu bar includes "Fichier", "Edition", "Affichage", "Favoris", and "Outils". The search bar contains the text "Rechercher" and "Autres >>". The browser's toolbar shows various icons for navigation and printing. The main content area displays a table of search results. A red box highlights the first row of the table, which is circled in red. The table has the following columns: Accession, Description, Max score, Total score, Query coverage, E value, Max ident, and Links. The first row is: CAI76\_27.1, beta-lactamase SHV-12 [Escherichia coli], 566, 566, 100%, 0.0, 100%. The second row is: ABD93867.1, SHV-12 extended-spectrum beta-lactamase [Klebsiella pneumoniae], 566, 566, 100%, 0.0, 100%. The third row is: AAS09882.1, SHV-5 extended spectrum beta-lactamase [Escherichia coli], 566, 566, 100%, 0.0, 100%. The fourth row is: YP\_001338660.1, putative beta-lactamase SHV-12 [Klebsiella pneumoniae subsp. pneu], 565, 565, 100%, 0.0, 100%. The fifth row is: AAU84950.1, SHV beta-lactamase [Klebsiella pneumoniae], 565, 565, 100%, 0.0, 99%. The sixth row is: AAR97951.1, beta-lactamase SHV-5 [Escherichia coli], 564, 564, 100%, 0.0, 99%. The seventh row is: ABA06588.1, extended-spectrum beta-lactamase SHV-66 [Klebsiella pneumoniae], 564, 564, 100%, 0.0, 99%. The eighth row is: ADM25824.1, extended spectrum beta-lactamase SHV-134 [Klebsiella pneumoniae], 564, 564, 100%, 0.0, 99%. The ninth row is: AAS09880.1, SHV-5 extended spectrum beta-lactamase [Escherichia coli], 564, 564, 100%, 0.0, 99%. The tenth row is: ABA06586.1, mutant extended-spectrum beta-lactamase SHV-64 [Klebsiella pneu], 564, 564, 100%, 0.0, 99%. The eleventh row is: ACN72587.1, beta-lactamase SHV-12-like protein [Salmonella enterica subsp. entr], 564, 564, 100%, 0.0, 99%. The twelfth row is: POA928.1, RecName: Full=Beta-lactamase SHV-2; AltName: Full=SHV-2A; Flags, 564, 564, 100%, 0.0, 99%. The thirteenth row is: AAS09884.1, SHV-5 extended spectrum beta-lactamase [Escherichia coli], 563, 563, 100%, 0.0, 99%. The fourteenth row is: AAP20890.1, extended-spectrum beta-lactamase SHV-48 [Acinetobacter baumani], 563, 563, 100%, 0.0, 99%. The fifteenth row is: ABC25482.1, beta-lactamase SHV-71 [Acinetobacter baumannii], 563, 563, 100%, 0.0, 99%. The sixteenth row is: AEO09376.1, beta-lactamase, partial [Enterobacter cloacae], 563, 563, 99%, 0.0, 100%. The seventeenth row is: AAP33454.2, extended spectrum beta-lactamase [Klebsiella pneumoniae] >qb|AC, 563, 563, 100%, 0.0, 99%. The eighteenth row is: AAQ55480.1, beta-lactamase SHV-56 [Acinetobacter baumannii], 563, 563, 100%, 0.0, 99%. The nineteenth row is: CAB37325.2, Beta-lactamase [Escherichia coli], 563, 563, 100%, 0.0, 99%. The twentieth row is: ABC58727.1, extended-spectrum beta-lactamase SHV-86 [Klebsiella pneumoniae], 563, 563, 100%, 0.0, 99%.

Legend for links to other resources: [U](#) UniGene [E](#) GEO [G](#) Gene [S](#) Structure [M](#) Map Viewer [B](#) PubChem BioAssay

Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
CAI76_27.1	beta-lactamase SHV-12 [Escherichia coli]	566	566	100%	0.0	100%	
ABD93867.1	SHV-12 extended-spectrum beta-lactamase [Klebsiella pneumoniae]	566	566	100%	0.0	100%	
AAS09882.1	SHV-5 extended spectrum beta-lactamase [Escherichia coli]	566	566	100%	0.0	100%	
YP_001338660.1	putative beta-lactamase SHV-12 [Klebsiella pneumoniae subsp. pneu]	565	565	100%	0.0	100%	<a href="#">G</a>
AAU84950.1	SHV beta-lactamase [Klebsiella pneumoniae]	565	565	100%	0.0	99%	
AAR97951.1	beta-lactamase SHV-5 [Escherichia coli]	564	564	100%	0.0	99%	
ABA06588.1	extended-spectrum beta-lactamase SHV-66 [Klebsiella pneumoniae]	564	564	100%	0.0	99%	
ADM25824.1	extended spectrum beta-lactamase SHV-134 [Klebsiella pneumoniae]	564	564	100%	0.0	99%	
AAS09880.1	SHV-5 extended spectrum beta-lactamase [Escherichia coli]	564	564	100%	0.0	99%	
ABA06586.1	mutant extended-spectrum beta-lactamase SHV-64 [Klebsiella pneu]	564	564	100%	0.0	99%	
ACN72587.1	beta-lactamase SHV-12-like protein [Salmonella enterica subsp. entr]	564	564	100%	0.0	99%	
POA928.1	RecName: Full=Beta-lactamase SHV-2; AltName: Full=SHV-2A; Flags	564	564	100%	0.0	99%	
AAS09884.1	SHV-5 extended spectrum beta-lactamase [Escherichia coli]	563	563	100%	0.0	99%	
AAP20890.1	extended-spectrum beta-lactamase SHV-48 [Acinetobacter baumani]	563	563	100%	0.0	99%	
ABC25482.1	beta-lactamase SHV-71 [Acinetobacter baumannii]	563	563	100%	0.0	99%	
AEO09376.1	beta-lactamase, partial [Enterobacter cloacae]	563	563	99%	0.0	100%	
AAP33454.2	extended spectrum beta-lactamase [Klebsiella pneumoniae] >qb AC	563	563	100%	0.0	99%	
AAQ55480.1	beta-lactamase SHV-56 [Acinetobacter baumannii]	563	563	100%	0.0	99%	
CAB37325.2	Beta-lactamase [Escherichia coli]	563	563	100%	0.0	99%	
ABC58727.1	extended-spectrum beta-lactamase SHV-86 [Klebsiella pneumoniae]	563	563	100%	0.0	99%	



<http://www.lahey.org/studies/>

- $\beta$ -Lactamase Classification and Amino Acid Sequences for TEM, SHV and OXA Extended-Spectrum and Inhibitor Resistant Enzymes
- Contrôle manuel du résultat du Blast



# Contrôle de la séquence en aa

SHV1 MRYIRLCIIS LLATLPLAVH ASPQPLEQIK LSESQLSGRV GMIEMDLASG  
RTLTAWRADE RFPMMSTFKV VLCGAVLARV DAGDEQLERK IHYRQQDLVD  
YSPVSEKHLA DGMTVGELCA AAITMSDNSA ANLLLATVGG PAGLTAFLRQ  
IGDNVTRLDR WETELNEALP GDARDTTTPA SMAATLRKLL TSQRLSARSQ  
RQLLQWMVDD RVAGPLIRSV LPAGWFIADK TGA GERGARG IVALLGPNNK  
AERIVVIYLR DTPASMAERN QQIAGIGAA

Exemple MRYIRLCIIS LLATLPLAVH ASPQPLEQIK QSESQLSGRV GMIEMDLASG  
RTLTAWRADE RFPMMSTFKV VLCGAVLARV DAGDEQLERK IHYRQQDLVD  
YSPVSEKHLA DGMTVGELCA AAITMSDNSA ANLLLATVGG PAGLTAFLRQ  
IGDNVTRLDR WETELNEALP GDARDTTTPA SMAATLRKLL TSQRLSARSQ  
RQLLQWMVDD RVAGPLIRSV LPAGWFIADK TGA SKRGARG IVALLGPNNK  
AERIVVIYLR DTPASMAERN QQIAGIGAA

Exemple = SHV-12

Exemples d'études  
épidémiologiques menées au  
CHU Liège

# Colonisation digestive par des E-BLSE dans la communauté

- Etude menée en 2007 en association avec des médecins généralistes
- 284 échantillons de selles prélevés chez des patients non hospitalisés
- 25 E-BLSE isolées de 20 patients (7.04%)

# Colonisation digestive par des E-BLSE dans la communauté

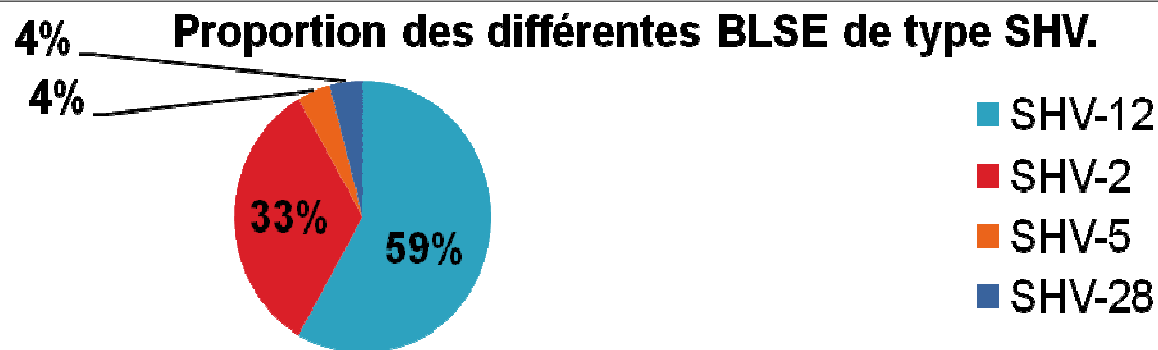
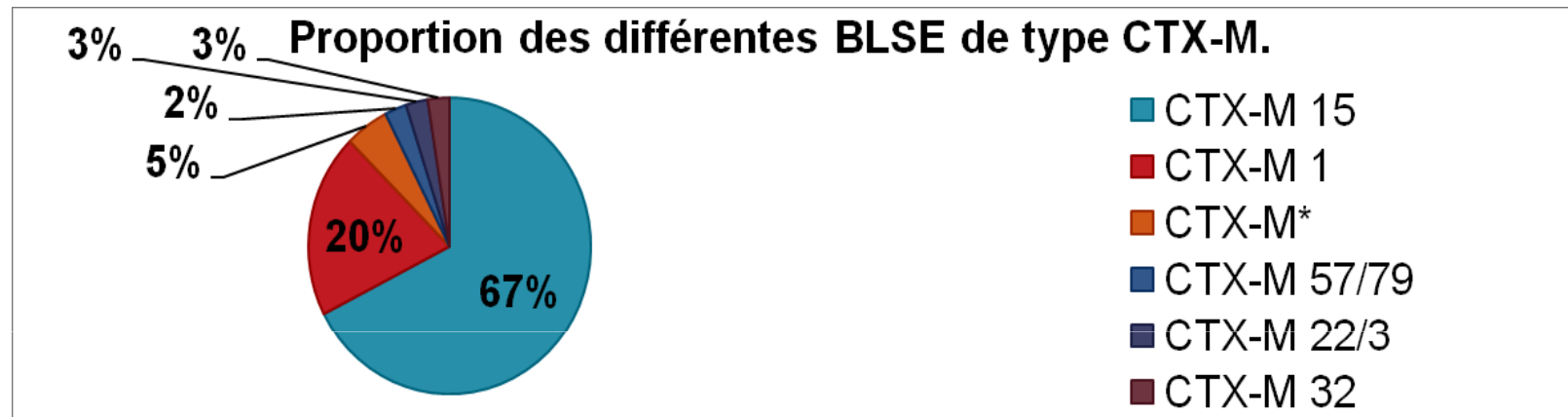
Résultat des PCR et séquençages:

Species (Number of isolates)	Type of identified beta-lactamase (Number of isolates)			
	TEM	CTX-M	TEM and CTX-M	SHV, BEL, VEB, GES or OXA 1/2/10
<b><i>E.coli</i> (19)</b>	TEM-1 (7)	CTX-M-1 (4)	TEM-1 and CTX-M-1 (1)	/
	TEM-19 (1)			
	TEM-52 (4)	CTX-M-15 (2)		
<b><i>E.aerogenes</i> (1)</b>	TEM-52 (1)	/	/	/
<b><i>P.mirabilis</i> (3)</b>	TEM-24 (3)	/	/	/
<b><i>S. fonticola</i> (1)</b>	/	/	/	/

# Epidémiologie des E-BLSE dans les HC et les prélèvements de dépistage

- CHU Liège en 2011
- Détection phénotypique
  - 32 E-BLSE < hémocultures positives
  - 67 E-BLSE < prélèvements de dépistage (frottis rectaux et selles)
- Détection génotypique
  - 74 E-BLSE confirmées par PCR et séquençage
  - 54% de CTX-M, 32% de SHV et 14% de TEM.

# Epidémiologie des E-BLSE dans les HC et les prélèvements de dépistage



**BLSE de type TEM: 100% de TEM-24**



# Proportion de E-BLSE dans la communauté au Cameroun

- Cameroun, 2009
- 358 selles collectées de patients non hospitalisés et de volontaires sains
  - 23.1% de portage de E-BLSE chez les patients
  - 6.7% de portage de E-BLSE chez les volontaires
- Caractérisation des BLSE
  - 97% de CTX-M (CTX-M-15 et CTX-M-1)
  - 3% de SHV-12

[Magoue Lonchel C](#), [Meex C](#), [Gangoue-Pieboji J](#), [Boreux R](#), [Okomo Assoumou MC](#), [Melin P](#), [De Mol P](#). Proportion of extended-spectrum  $\beta$ -lactamase-producing Enterobacteriaceae in community setting in Ngaoundere, Cameroon. [BMC Infect Dis](#). 2012 Mar 9;12(1):53. [Epub ahead of print]