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**[17–17]**

**Supporting document 1**

Risk and technical assessment – Application A1135

Beta-galactosidase as a Processing Aid (Enzyme)

# Executive summary

Application A1135 seeks approval for the use of β-galactosidase (lactase) from a genetically modified strain of *Bacillus licheniformis* as a processing aid. The stated purpose of this enzyme, namely the hydrolysis of lactose in cow’s milk to produce reduced-lactose or lactose-free milk and milk products, is clearly articulated in the Application.

The evidence presented to support the proposed uses provides adequate assurance that the enzyme, in the form and prescribed amounts, is technologically justified and is effective in achieving its stated purpose. The enzyme preparation meets international purity specifications for enzymes used in the production of food.

There are no public health and safety issues associated with the use of the β-galactosidase preparation produced by genetically modified *B. licheniformis* (strain PP3930) as a food processing aid on the basis of the following considerations:

* The production organism *B. licheniformis*, is neither toxigenic, pathogenic or sporogenic and is not present in the final enzyme preparation proposed to be used as a food processing aid. Furthermore, *B. licheniformis* has a history of safe use as the production organism for a number of enzyme processing aids that are already permitted in the Code.
* Residual enzyme is expected to be present in the final food but would be inactivated by heat-treatment or non-active because of the lack of lactose, and susceptible to digestion like any other dietary protein.
* Bioinformatic analyses indicated that the enzyme has no biologically relevant homology to known protein allergens or toxins.
* The enzyme preparation caused no observable effects at the highest tested doses in a 90-day toxicity study in rats. The No Observable Adverse Effect Level was 0.672 g enzyme solid/kg bw/d, which was the highest dose tested.
* The enzyme preparation was not mutagenic *in vitro*.

Based on the reviewed toxicological data, FSANZ concludes that in the absence of any identifiable hazard, an Acceptable Daily Intake (ADI) ‘not specified’ is appropriate for β-galactosidase from *B. licheniformis.* A dietary exposure assessment is therefore not required.

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# 1 Introduction

FSANZ received an application from Novozymes Australia Pty Ltd seeking approval for the enzyme β-galactosidase (EC 3.2.1.23), also known as lactase, to be used as a processing aid. The enzyme is sourced from a genetically modified strain of *Bacillus licheniformis* expressing a lactase gene from *Bifidobacterium bifidum*. Lactase will be produced from this bacterial species by a fermentation process.

The Applicant states the enzyme will be used during the processing of cow’s milk and other lactose-containing products to produce lactose-free or reduced-lactose dairy products. The use of lactase results in improvement of organoleptic properties (taste and flavour), physicochemical properties (texture and freezing point) and nutritional properties (digestibility).

## 1.1 Objectives of the risk and technical assessment

Currently, there are no permissions for the enzyme lactase from *B. bifidum* in the Code. Therefore, any application to amend the Code to permit the use of this enzyme as a food processing aid requires a pre-market assessment.

The objectives of this risk assessment are to:

* determine whether the proposed purpose is clearly stated and that the enzyme achieves its technological function in the quantity and form proposed to be used as a food processing aid
* evaluate any potential public health and safety concerns that may arise from the use of lactase as a processing aid.

# 2 Food technology assessment

## 2.1 Characterisation of β-galactosidase (lactase)

### 2.1.1 Identity of the enzyme

Information regarding the identity of the enzyme provided by the Application has been verified using the appropriate internationally accepted reference for enzyme nomenclature, the International Union of Biology and Molecular Biology ([IUBMB](http://www.enzyme-database.org/)[[1]](#footnote-2)). Additional information located from the IUBMB has also been included.

Generic common name β-galactosidase

Accepted IUBMB name β-D-galactoside galactohydrolase

EC[[2]](#footnote-3) number 3.2.1.23

CAS number 9031-11-2

Commercial name Saphera

Other names lactase, β-lactosidase, maxilact, lactozyme, S 2107, trilactase, hydrolact, β-D-galactanase, oryzatym and sumiklat

Reaction: Hydrolysis of terminal non-reducing β-D-galactose residues in β-D-galactosides

### 2.1.2 Enzymatic properties

Lactase catalyses the hydrolysis of terminal non-reducing β-D-galactose residues in β-D-galactosides. The most common reaction is the hydrolysis of the disaccharide D-lactose, resulting in the generation of the monosaccharides, D-glucose and D-galactose.

### 2.1.3 Physical properties

The commercial enzyme preparation is supplied in two forms, based on two proposed uses:

1. Saphera 900 LS – a sterile, light yellow liquid preparation with approximately 0.8% (w/w) enzyme, 60% (w/w) glycerol and 39.2% (w/w) water
2. Saphera 2600 L – a light yellow liquid preparation with approximately 2.3% (w/w) enzyme, 60% (w/w) glycerol and 37.7% (w/w) water

The enzyme preparations are standardised to an activity of 900 and 2600 lactase activity units (LAU) per gram of Saphera. The maximum recommended amount for food manufacturing is 7500 LAU per kg of lactose. This corresponds to 2.88 g of Saphera 2600 L per kg lactose, which is equivalent to 66 mg of the enzyme product per kg lactose.

## 2.2 Production of the enzyme

The enzyme is produced by a submerged fed-batch pure culture fermentation process which is common for the production of many food-grade enzymes.

The production steps can be summarised as a fermentation process, a purification process, formulation of the final commercial enzyme preparation, and a quality control process. The raw materials used are food grade and the enzyme preparations are stated by the Applicant to be made according to Good Manufacturing Processes for food.

The fermentation process involves two steps, the initial inoculum fermentations to produce enough of the microorganism for the production fermentation and then the main fermentation. The downstream processing steps taken after the main fermentation to produce the enzyme preparation consist of: removal of the production strain and other solids, ultrafiltration and/or evaporation to concentrate and further purify the enzyme, preservation and stabilisation and then a final filtration. More detail of the individual steps is provided in the Application.

All the raw materials used in the production of the enzyme preparation are permitted food additives and processing aids in the Code (as detailed in the Application) and are appropriate for their purpose.

### 2.2.1. Potential presence of allergens

Soybean meal is one of the potential vegetable protein raw material sources for the fermentation. Starch hydrolysates, which can be produced from wheat starch, may also be used as a fermentation raw material. The Applicant has provided evidence that soy proteins and gluten are not present in the final enzyme preparation.

## 2.3 Specifications

There are international specifications for enzyme preparations used in food production.

These have been established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA; [Food Safety and Quality](http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/enzymes/en/)[[3]](#footnote-4)) and the Food Chemicals Codex (USP, 2014). Both of these specification sources are primary sources listed in Schedule 3 (Identity and Purity), specifically section S3—2. Enzyme preparations must meet these purity specifications.

The Application provides analytical results on a lactase batch (OFFR 6-7) confirming the enzyme preparation meets the required international specifications (Table 2.1).

Table 2.1: Enzyme preparation compared to the JECFA and Food Chemicals Codex (FCC) enzyme specifications

|  |  |  |  |
| --- | --- | --- | --- |
| Analysis | Specifications | | |
| Product | JECFA | FCC |
| Lead (mg/kg) | ND† | ≤5 | ≤5 |
| Coliforms (cfu/g) | ≤10 | ≤30 | ≤30 |
| *E. coli* (in 25 g) | ND | ND |  |
| *Salmonella* (in 25 g) | ND | ND | ND |
| Antibiotic activity | ND | ND |  |

† ND: not detected

## 2.4 Technological function

Milk and milk products contain lactose, to which some consumers are intolerant. The amount of lactose in a food may be reduced by hydrolysis, the products of which are glucose and galactose. Hydrolysis can be achieved using acids or by enzymatic digestion with the enzyme lactase. Generally the use of lactase is a simpler process and it is commonly used in food manufacturing for lactose reduction. The lactose-free or lactose-reduced products may be milks or milk products used as ingredients in other foods.

Commercial preparations of lactase have been derived from a number of sources, including bacteria and yeast. The Applicant claims that their lactase is superior to these other preparations, especially yeast-based lactases, for the following reasons:

(i) absence of invertase and amylase side-activity, eliminating the likelihood of oligosaccharide formation

(ii) with reduced oligosaccharide formation, there is increased stability in sweetness and taste

(iii) with increased stability of sweetness, there is little need to add extra sugars to the milk and thus a reduction in calorie intake

(iv) the optimal temperature and pH of the lactase enzyme from *B. bifidum* makes this enzyme more suitable for yogurt production

Depending on the formulation of the lactase preparation, it may be used for traditionally pasteurised or UHT dairy products.

## 2.5 Food technology conclusion

The evidence presented in the Application supports the proposed use of the β-galactosidase (lactase) preparation in food production to hydrolyse lactose. The Applicant has provided adequate assurance that the enzyme is technologically justified and the enzyme preparation meets international purity specifications.

# 3 Hazard assessment

## 3.1 Background

### 3.1.1 Chemistry

Details of the chemistry of the lactase produced by *B. licheniformis* including relevant physicochemical and enzymatic properties, and product specifications, are provided in the Food technology assessment (Section 2).

### 3.1.2 Description of the genetic modification processes

The β-galactosidase, also known as lactase, is produced by a genetically modified (GM) strain of *B. licheniformis* (production strain PP3930).

The introduced lactase gene, designated *galT1*, was based on the coding sequence for the lactase (*bbgIII*) gene from *B. bifidum* (strain NCIMB 41171). The sequence has been modified to express the signal peptide from an alkaline protease (*aprH*) gene of *Bacillus clausii*, which ensures secretion of the enzyme product. A hybrid promoter containing elements from *B. licheniformis*, *B. amyloliquefaciens* and *B. thuringiensis* drives the *galT1* gene and a terminator sequence from *B. licheniformis* is used to ensure termination of transcription. The synthetic lactase gene was transferred into plasmid pE194 (Horinouchi and Weisblum, 1982) to create the expression plasmid pPP2771.

The expression plasmid pPP2771 was transformed into the *B. licheniformis* strain AEB1763 to generate the production strain PP3930. A site-specific integration method mobilised the cassette into four specific loci in AEB1763. Thus PP3930 contains four copies of the *galT1* gene. DNA sequence analysis and Southern blotting confirmed there are no other coding sequences, including antibiotic resistance genes, from the plasmid present in the final production strain.

Full details of the genetic modification to the production organism were provided as “Confidential Commercial Information”.

### 3.1.3Scope of the hazard assessment

The hazard of lactase derived from *B. licheniformis* strain PP3930 was evaluated by considering the:

* hazard of the production organism, including any history of safe use in food production processes;
* hazard of the encoded protein, including potential allergenicity and toxicity; and
* toxicity studies on the enzyme preparation intended for commercial use.

## 3.2 Hazard of the production organism – *B. licheniformis PP3930*

*B. licheniformis* is a soil and plant living saprophyte. The production strain PP3930 has been engineered from a non-toxigenic isolate (Pedersen *et al*, 2002) to prevent sporulation, which minimises this organism’s virulence and allows designation as a Risk Group 1 (RG1) agent, not associated with disease in healthy adult humans (NIH, 2016).

Although toxigenic and spore-forming strains of *B. licheniformis* have been isolated from raw milk, milk products and commercially-produced baby food (Gopal *et al*, 2015) the incidence of human infections and pathogenicity is rare and tends to be limited to immune-compromised individuals (Haydushka *et al*, 2012; Logan, 2012; EPA, 1997).

Industrialstrains of *B. licheniformis* are widely used to produce food-grade enzymes. FSANZ has previously assessed the safety of *B. licheniformis* as the source organism for a number of food processing aids and the following enzymes derived from *B. licheniformis* (both GM and non-GM) are listed in Schedule 18 as permitted in Standard 1.3.3 of the Code: α-amylase, chymotrypsin, endo-1,4-β-xylanase, glycerophospholipid cholesterol acyltransferase, maltotetraohydrolase, pullulanase and serine proteinase.

Data submitted by the Applicant indicated that *B. licheniformis* PP3930is not detectable in the final enzyme preparation to be used as a food processing aid. The organism is removed during a multi-step recovery of the purified enzyme following submerged fed-batch pure culture fermentation. The final stage involves filtrations at defined pH and temperature intervals that result in an enzyme concentrate solution free of the production strain.

The Applicant also provided data from Southern blot analyses to demonstrate that the introduced DNA has been stably integrated into the production strain’s genome.

Full details on the genetic stability of the insert in the production organism were provided as “Confidential Commercial Information”.

## 3.3 Hazard of the encoded protein – β-galactosidase (E.C. 3.2.1.23)

### 3.3.1 History of use

β-galactosidase is an enzyme used as a processing aid for the production of reduced lactose or lactose-free dairy products. This enzyme catalyses the hydrolysis of β-D-galactosides, such as lactose, into glucose and galactose. FSANZ has previously accessed the safety of β-galactosidase from a variety of microorganisms and these are listed in Schedule 18 as permitted in Standard 1.3.3 of the code: *Aspergillus niger, A. oryzae, Bacillus circulans, Kluyveromyces marxianus and K. lactis*.

The commercial enzyme preparation from the Applicant, known as Saphera, is available as a liquid product containing 60% (w/w) glycerol, to stabilise the enzyme, and water. There are two strengths of the enzyme available, based on the activity of the lactase. These are Saphera 900 LS and Saphera 2600 LS, containing lactase with an activity of 900 and 2600 lactase activity units (LAU)/g respectively.

The U.S. Food & Drug Administration (FDA) has granted Generally Recognised as Safe (GRAS) status to the enzyme preparation under the intended conditions of use (FDA, 2015). The Danish Veterinary and Food Administration have also approved the enzyme for the intended use (DVFA, 2015). The Applicant has stated that the Mexican Federal Committee for Protection from Sanitary Risks (COFEPRIS) has approved the enzyme product for food applications but the Applicant did not provided evidence of this acceptance.

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### 3.3.2 Bioinformatic analysis for potential allergenicity

The Applicant has provided the results of an *in silico* analysis comparing the lactase amino acid sequence from *B. licheniformis* to known allergenic proteins in the Food Allergy Research and Resource Program dataset, which is available through [AllergenOnline](http://www.allergenonline.org)[[4]](#footnote-5) (University of Nebraska).

Four types of analyses were performed for this comparison:

(a) 35% identity over 80 amino acids to examine potential IgE cross-reactivity:- alignment was performed using a ClustalW program with the FASTA v34 algorithm (Pearson and Lipman, 1988).

(b) The same as a) but with scaling enabled in order to find any matches that may have high identity over windows shorter than 80 amino acids.

(c) 100% identity over eight contiguous amino acids: - alignment was performed using a ClustalW program with the FASTA v34 algorithm.

(d) 35% identity over full length of allergen or 100% identity over any eight amino acid window: - the [EMBOSS Needle](http://www.ebi.ac.uk/Tools/psa/emboss_needle)[[5]](#footnote-6) program was used to determine the optimal global alignment, based on the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970).

There were no hits when the lactase protein was aligned to any of the known allergens in the four analyses performed. This indicates that the lactase protein produced by *B. licheniformis* is highly unlikely to be allergenic.

### 3.3.3 Bioinformatic analysis for potential toxicity

The Applicant has provided results from an *in silico* comparison of the lactase amino acid sequence from *B. licheniformis* to known protein toxins identified in the [UNIPROT database](http://www.uniprot.org)[[6]](#footnote-7). A total of 50,251 proteins were identified in UNIPROT (database date: 11-Feb-2016) using the keyword *toxin* but excluding the word *fragment*. The comparison method used a [ClustalW 2.0.10](http://www.clustal.org/clustal2)[[7]](#footnote-8) sequence alignment program (Larkin *et al*, 2007).

The greatest homology found was 15.0%, indicating that the lactase protein produced by *B. licheniformis* is highly unlikely to be toxic.

### 3.3.4 Bioinformatic analysis for pepsin digestibility

To confirm the digestibility of lactase, potential cleavage sites were investigated by FSANZ using the amino acid sequence of lactase and the [PeptideCutter tool](http://web.expasy.org/peptide_cutter)[[8]](#footnote-9) in the ExPASy Proteomics Site. Lactase has multiple cleavage sites for pepsin (136 sites at pH 1.3 and 248 sites at pH >2), trypsin (114 sites), chymotrypsin (110 high-specificity sites, 179 low-specificity sites) and endopeptidases (274 sites). On this basis, lactase is considered likely to be as susceptible to digestion as the vast majority of dietary proteins.

## 3.4 Evaluation of toxicity studies of the enzyme product

|  |
| --- |
| **Study submitted**  Nielsen LA (2014) Summary of toxicity data: Lactase, batch PPL34637 from *Bacillus licheniformis*. File # 2014-11425-01, Novozymes A/S (unpublished). |

The Applicant provided data for a range of unpublished toxicity studies on a representative lactase preparation, which was independently evaluated by FSANZ. These studies comprised:

* Two mutagenicity assays - an Ames test conducted in accordance with OECD test Guideline 471 (OECD 1997) and an *in vitro* micronucleus test conducted in accordance with OECD test Guideline 487 (OECD 2010)
* A 90-day oral toxicity study in rats conducted in accordance with OECD Test Guideline 408 (OECD 1998).

The test substance was a lactase preparation batch PPL34537, supplied in liquid form and dissolved in water. The characterisation of toxbatch PPL34537 is presented in Table 3.1.

Table 3.1: Characterisation data of lactase batch PPL34537

|  |  |
| --- | --- |
| **Characteristic** | **PPL34537** |
| Activity (LAU†/g) | 6730 |
| Total Organic Solids (% w/w) | 6.4 |
| Water (% w/w) | 90.7 |
| pH | 7.5 |
| Ash at 600ºC (% w/w) | 2.9 |
| Specific gravity | 1.050 |
| Dry Matter (% w/w) | 9.3 |

† LAU - lactase activity units as determined by hydrolysis of *o*-nitrophenyl β-D-galatopyranoside

### 3.4.1 Mutagenicity

|  |
| --- |
| **Studies submitted**  Pedersen PB (2013) Lactase, batch PPL34637: Test for mutagenic activity with strains *Salmonella typhimurium* and *Escherichia coli*. Report: Study No. 20138037. Novozymes A/S, (unpublished)  Whitwell J (2014) Lactase, PPL34637: Induction of micronuclei in cultured human peripheral blood lymphocytes. Study No. 8292559 / NZ 20136071. Covance Laboratories Ltd, (unpublished). |

The outcome of these mutagenicity studies are summarised in Table 3.2. Appropriate controls were tested in these studies and gave the expected results. Treatments were performed with lactase in the absence or presence of Aroclor-1254 activated S9 mix. Both studies gave negative results.

**Table 3.2: Summary of mutagenesis assay results**

| **Test** | **Test system** | **Test article** | **Concentration or dose range** | **Result** |
| --- | --- | --- | --- | --- |
| Bacterial Reverse Mutation Assay  (Ames test) | *Salmonella typhimurium* strains TA1535, TA100, TA1537 & TA98  *Escherichia coli* strain WP2 *uvrA pKM101* | Lactase PPL34537  Vehicle deionised water | 156–5000 µg/ml ± S9# or 0.2M phosphate buffer | Negative |
| *In vitro* Micronucleus Assay  (Cytokinesis-block method) | Human lymphocytes prepared from the pooled blood of 2 healthy, non-smoking female donors | As above | 1st experiment (3 h exposure)  3000-5000 µg/ml ± S9  2nd experiment (24 h exposure)  500-5000 µg/ml - S9 | Negative |

#S9 = rat liver preparation treated with Aroclor 1254 to activate enzymes in the liver homogenate.

### 3.4.2 Subchronic toxicity

|  |
| --- |
| **Study submitted**  HLS (2013) Lactase, batch PPL34637: Toxicity study by oral gavage administration to Sprague-Dawley rats for 13 weeks. Report number LKG0074. Huntington Life Sciences (unpublished). |

The toxicity study was performed over 90 days on groups of ten male and ten female Crl:CD(SD) rats (Charles River UK Ltd, UK). At the beginning of the study, the rats were 6-7 weeks old, with weight ranges of 231-292 g for males and 178-222 g for females. Five animals of the same gender were housed in each cage. Food and water were unrestricted during the treatment.

A total volume of 10 ml/kg bw (Lactase; toxbatch PPL34637; activity 6730 LAU/g, sourced from the Sponsor) was administered daily by gavage. The dose range administered was 0, 7067, 23319 and 70665 LAU/kg bw/day. To achieve this range, control animals

(0 LAU/kg bw) received reverse osmosis water (vehicle), while the treatment groups received diluted enzyme at 10% (v/v), 33% (v/v) or 100% (undiluted) respectively.

Observations for mortality were made twice daily and detailed clinical symptoms and behaviour were assessed daily prior to drug administration. Sensory reactivity, grip strength and motor activity were assessed in Week 12. Bodyweight was recorded one week prior to treatment, the day of commencement of treatment and then weekly thereafter. Food consumption was recorded weekly, starting one week prior to treatment. Water uptake was assessed by visual examination. Blood was collected from the sublingual vein while under light general anaesthesia and after animals had fasted overnight during Week 12 for assessment of clinical chemistry parameters (electrolytes, liver and renal function) and haematology parameters (complete blood count, cell morphology and coagulation test) . Ophthalmological examinations were performed on all animals prior to treatment and then in Week 12, for the control and 100% lactase treated groups only. Necropsy was performed on days 91 – 92, when tissue examination and collection was performed. Necropsy was by carbon dioxide asphyxiation and exsanguination.

There were no treatment-related deaths, clinical symptoms or ophthalmic abnormalities reported in this study. Female rats in the 33% and 100% groups did show a decreasing trend in hind limb grip strength (motor activity) but the values fell within the range of normal variation seen in the control and 10% dosed animals. Bodyweight gain, food consumption and water uptake were comparable across all groups.

There was no treatment-related effect on any haematology or clinical chemistry parameters. There were no treatment-related macroscopic abnormalities, differences in organ weights or histopathological findings. The NOAEL (No Observed Adverse Effect Level) for both males and females was 0.672 g enzyme solid/kg bw/d or 70665 LAU/kg bw/d, which was the highest dose tested.

These results are in agreement with previous studies examining animal toxicity of commercial enzyme preparations. In these studies, rats were treated with either Tilactase (10,000 ONPG units/g) from *Penicillium multicolor* over 35 days (Flood and Kondor, 2004) or Neutralact (11,400 neutral lactase units/g) from *K. lactis* over 28 days. There were no deaths or treatment-related adverse effects seen in the animals and the NOAELS reported for the Tilactase and Neutralact preparations were 4 g/kg bw/d and 10 g/kg bw/d respectively. A more recent study investigating a more highly active recombinant form of the *Aspergillus oryzae* lactase gene expressed in *Pichia pastoris* also reported no treatment-related adverse effects in rats treated for 90 days (Zou et al, 2014).

## 3.5 Risk Assessment Conclusions

There are no public health and safety issues associated with the use of the product Saphera containing the enzyme lactase from *B. licheniformis* when used as a food processing aid on the basis of the following considerations:

* The production organism *B. licheniformis* is not toxigenic, pathogenic or sporogenic and is absent in the final enzyme preparation proposed to be used as a food processing aid. Further, *B. licheniformis* has a history of safe use as the production organism for a number of enzyme processing aids that are already permitted in the Code.
* Residual enzyme is expected to be present in the final food product but would be inactivated by heat-treatment or pasteurisation, or non-active because of a lack of lactose, and susceptible to digestion like any other dietary protein.
* Bioinformatic analysis indicated that the enzyme has no biologically relevant homology to known protein allergens or toxins.
* The enzyme preparation caused no observable effects at the highest tested doses in a 90-day toxicity study in rats. The NOAEL was 0.672 g enzyme solid/kg bw/d, the highest dose tested.
* The enzyme preparation was not mutagenic *in vitro*.

Based on the reviewed toxicological data, it is concluded that, in the absence of any identifiable hazard, an Acceptable Daily Intake (ADI) ‘not specified’ is appropriate. A dietary exposure assessment is therefore not required.

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1. <http://www.enzyme-database.org/> [↑](#footnote-ref-2)
2. EC: Enzyme Commission, internationally recognised number that provides a unique identifier for the enzyme [↑](#footnote-ref-3)
3. <http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/enzymes/en/> [↑](#footnote-ref-4)
4. [www.allergenonline.org](http://www.allergenonline.org) [↑](#footnote-ref-5)
5. [www.ebi.ac.uk/Tools/psa/emboss\_needle](http://www.ebi.ac.uk/Tools/psa/emboss_needle) [↑](#footnote-ref-6)
6. [www.uniprot.org](http://www.uniprot.org) [↑](#footnote-ref-7)
7. [www.clustal.org/clustal2](http://www.clustal.org/clustal2) [↑](#footnote-ref-8)
8. <http://web.expasy.org/peptide_cutter/> [↑](#footnote-ref-9)