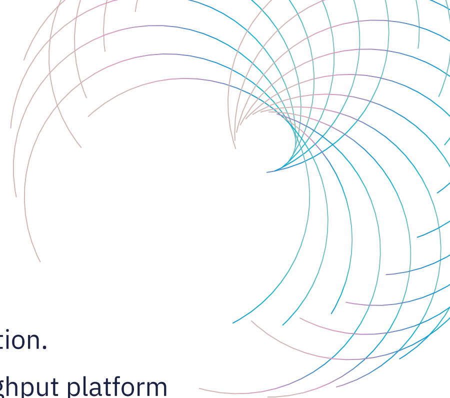
proteus

BY SEQENS

SEQENZYM® Customized Kits

for Enzyme Hit Detection

The first step toward your biocatalytic process !



We offer Additional Core-Services

In-House Screening :

Unleash the full potential of your project!

- **Maximize Success:** Tap into our team's expertise to ensure flawless execution.
- **Swift Screening:** Accelerate your research with our automated high-throughput platform
- **Increase your Odds:** Improve your success rate by screening diversity: explore additional relevant enzymes from proprietary and public databases.

Micriobology

Screen our natural strains and associated metabolites that match your application.

Proteins & Strains engineering

Take your enzyme's activity to the next level (random and/or semi-rational approaches)

Precision Fermentation

Enhance your customized compound production by microorganism tailoring and process optimization.

Development and Optimization of Biocatalytic Processes

Benefit from Protéus scale-up by Design of Experiment (DoE) expertise and Seqens industrialization capabilities.

Biocatalysis Training

Elevate your expertise by enrolling in a comprehensive theory classes and hands-on enzyme handling workshops.

SEQENZYM COLLECTION LIST

- **650+ enzymes**, including 50+ commercial-scale options.
- **Available in various formats**: microplates, microplate strips, or vials.
- **Custom-Made Solutions**: Mix different enzyme families on a single plate.
- **Flexible Quantity Options**: Order from 10 mg to 10 g of enzyme extracts.
- **Precise enzyme-specific protocols** included in each delivery.

For more informations contact us at: seqenzym@seqens.com

BIOMASS TREATMENT ENZYMES

Amylases	6
β-glucosidases	5
Cellulases	5
Xylanases	6

COFACTOR RECYCLING ENZYMES

NAD(P)H oxidases	2
Formate dehydrogenases	2
Glucose dehydrogenase	1
L-lactate dehydrogenase	1
L-alanine dehydrogenase	1

HYDROLASES

Dehalogenases	4
Epoxide hydrolases	11
Lipases	263
Nitrilases	14
Proteases	3
Phytases	2

LYASES

Hydratases	15

OXIDOREDUCTASES

Alcohol dehydrogenases	69
Amine dehydrogenases	18
Bayer-Villiger MonoOxygenases	10
Cytochromes P450	4
Ene-reductases	129
Hydroxy Steroid Dehydrogenases	13
Imine reductases	41
Laccases	21

TRANSFERASES

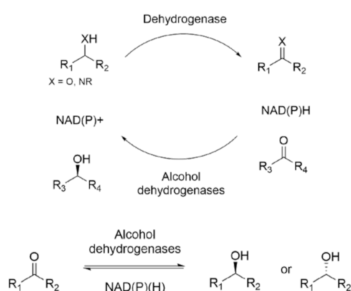
Threonine aldolases	4
Sulfotransferases	3
Transaminases	10

The numbers of enzymes per family are subject to change.

Protéus offers the capabilities to elevate your enzyme production to meet the requirements of large-scale industrial applications.

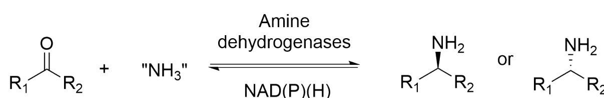
ALCOHOL DEHYDROGENASES
COFACTOR RECYCLING ENZYME

Alcohol Dehydrogenases (ADH also called ketoreductases, KRED) are NAD(P)H-dependent enzymes that catalyze the oxidation of alcohol into ketones and also the reversible reduction reaction to afford chiral alcohols. Cofactor regeneration can be carried out by the addition of an excess of a cheap co-substrate (like i-PrOH for reduction reactions or acetone for oxidation reactions). ADH can also be used in combination with other dehydrogenases or oxidoreductases for the recycling of the cofactors NAD(P)(H).



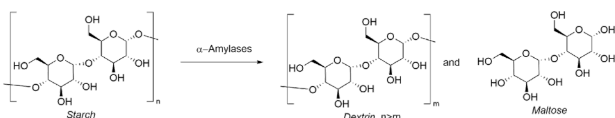
AMINE DEHYDROGENASES

The amine dehydrogenases (AmDH) catalyze the enantioselective amination of ketones. First, the ketone is converted into imine in the presence of an ammonium salt. The imine is then reduced at the expense of NAD(P)H into the corresponding chiral amine. Efficient cofactors recycling procedures can be implemented by coupling amines dehydrogenases with a formate dehydrogenase. The use of an ammonium formate buffer delivers substrates for both enzymes.



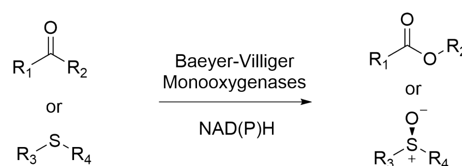
α-AMYLASES

α-amylases are glycosidases with a hydrolytic activity towards α(1→4) bonds of starch generating dextrin, maltose, and ultimately glucose.



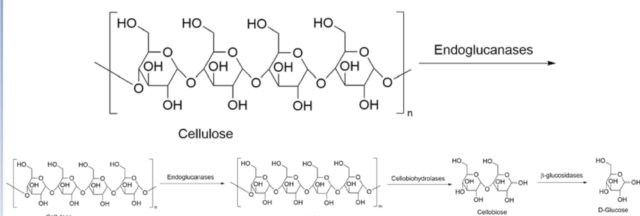
BAEYER VILLIGER MONOOXYGENASE (BVMO)

Baeyer-Villiger monooxygenases (BVMO) are flavin-dependent enzymes that catalyze the oxidation of ketones into the corresponding esters as well as the oxidation of sulfides into chiral sulfoxides. Upon NAD(P)H reduction, the flavin cofactor reacts with dioxygen to form a reactive hydroperoxide responsible for the observed reactions. A NAD(P)H-recycling system must be implemented.



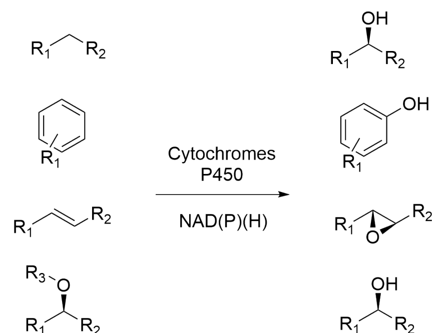
CELLULASES: ENDOGLUCANASES, CELLOBIOHYDROLASES AND β-GLUCOSIDASES

Cellulases are hydrolytic enzymes acting on cellulose; they hydrolyze the β-(1→4) bonds to form cellulose oligomers (endoglucanases) or cellobiose (cellobiohydrolases). β-Glucosidases hydrolyze cellobiose resulting in the formation of D-glucose.



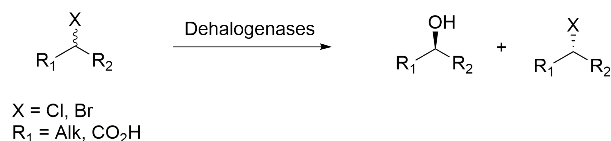
CYTOCHROMES P450

Cytochromes P450 (CYP450) catalyze diverse oxidation reactions like hydroxylation of alkyl positions or aromatic rings, dealkylation of aryl methyl ethers, epoxidation of alkenes, oxidation of sulfides... The reactions rely on the activation of dioxygen through a hemic iron center. This kind of catalytic center receives electrons from a NAD(P) H-dependent reductase partner. The proposed cytochromes are self-sufficient: the reductase partner is fused to the cytochrome part. A NAD(P)H recycling system must be implemented.



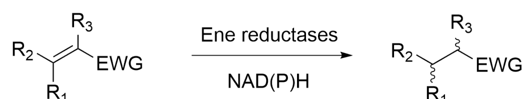
DEHALOGENASES

The 2-haloacid dehalogenases catalyze the hydrolytic dehalogenation of 2-haloalkanoic acids to produce 2-hydroxyalkanoic acids. They are only active onto compounds exhibiting halogen at the C2 position. Haloalkane dehalogenases catalyze the hydrolysis of halogenated compounds into the corresponding alcohols. These enzymes are active towards halogenated alkanes, cycloalkanes, alkenes, ethers, alcohol, ketones, or cyclic dienes.



ENE REDUCTASES

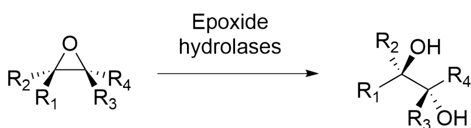
Ene-reductases (ERED) are flavin-dependent enzymes catalyzing the stereoselective reduction of activated C=C bonds at the expense of a nicotinamide cofactor NAD(P) H. Different substitution patterns are tolerated onto the alkene; however the alkene must be conjugated ideally to a ketone, an aldehyde or a nitro group eventhough in some cases, nitrile, ester, imide groups are sufficiently activating. A NAD(P)H-recycling system must be implemented.



EWG = ketone, aldehyde, nitro, imide...
In some cases, ester and nitrile can be accepted

EPOXYDE HYDROLASES

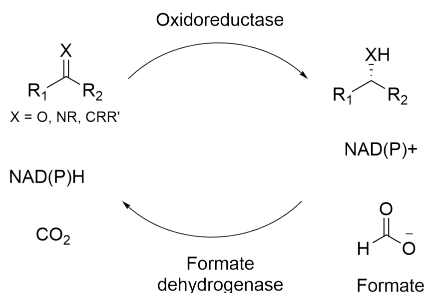
Epoxide hydrolases catalyze the opening of oxirane rings by water, generating vicinal diols as products. When the starting epoxide is racemic, the epoxide hydrolase can selectively recognize one of the two enantiomers of the substrate allowing the kinetic resolution of the racemic mixture. Note that different substitution patterns could be tolerated and therefore different regioselectivity could be observed.



FORMATE DEHYDROGENASES

COFACTOR RECYCLING ENZYME

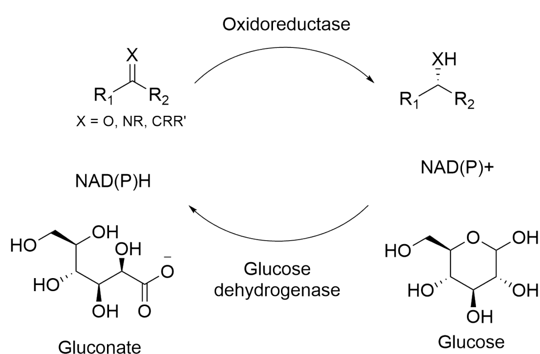
Formate dehydrogenases FDH are used as coenzymes for recycling NAD(P)H cofactors. The FDH can oxidize formate to carbon dioxide at the expense of NAD(P)+ resulting in an elegant recycling system where the by-product is easily eliminated from the reaction mixture (degassing). Two enzymes exhibiting complementary dependency to NADP+ and NAD+ are proposed.



GLUCOSE DEHYDROGENASE

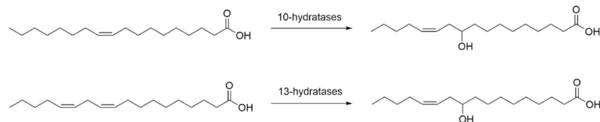
COFACTOR RECYCLING ENZYME

Glucose dehydrogenase is a very efficient coenzyme for NAD(P)H cofactors recycling. This enzyme oxidizes D-glucose into gluconolactone at the expense of NAD(P)+. The proposed enzyme tolerates both cofactors and exhibits good activity even at 50°C. Despite these interesting features, the processes relying on this recycling system suffer from the formation of one equivalent of gluconolactone. The latter gets spontaneously hydrolyzed into gluconic acid resulting in a drop in the pH.



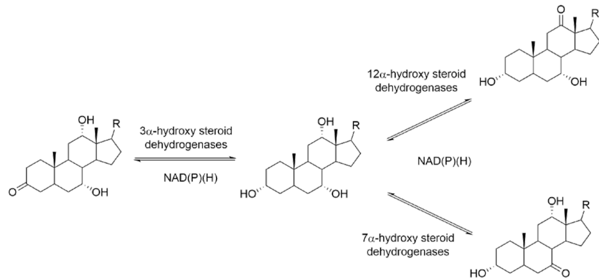
HYDRATASES

Hydratases are flavin-dependent enzymes that catalyze the hydration of unsaturated fatty acids. Enzymes exhibiting selectivity for position 10 or 13 are available.



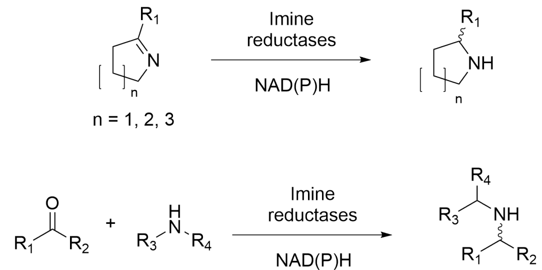
Hydroxy Steroid Dehydrogenases

Hydroxy Steroid Dehydrogenases (HSDH) are affiliated with the alcohol dehydrogenases family. These NAD(P)-dependent enzymes catalyze the interconversion of hydroxy and keto groups on steroid compounds. Different regio- and stereoselectivity can be achieved depending on the HSDH nature: 3α, 7α, 7β, and 12α. A NAD(P)H-recycling system must be implemented.



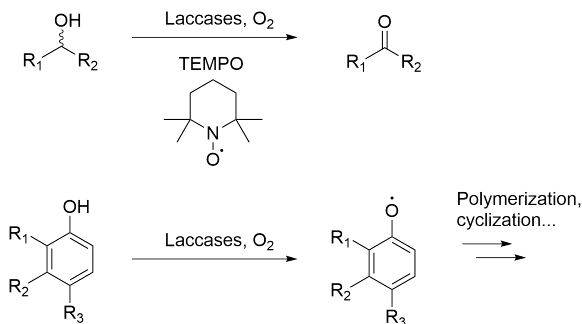
Imine Reductases

Imine reductases (IRED) are NAD(P)H-dependent enzymes that catalyze the asymmetric reduction of substituted imines affording chiral amines. Furthermore, the enzyme family mediates also the reductive amination of ketones/aldehydes with amines. A NAD(P)H-recycling system must be implemented.



Laccases

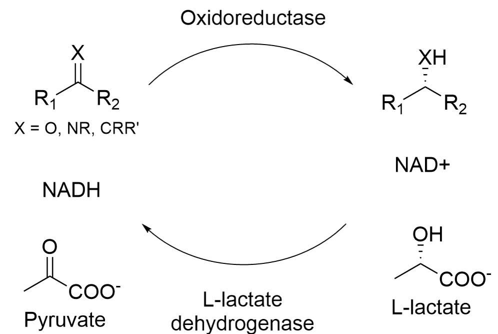
Laccases are Cu-dependent enzymes that catalyze oxidation reactions at the expense of oxygen. The use of a mediator (like TEMPO) permits the oxidation of alcohols into ketones. Another important part of their reactivity is the oxidation of phenol derivatives that result in oligomerization. Most of the substrates and catalyzed reactions are related to lignin-based compounds. SEQENZYM™ Laccases are made of **14** enzymes as lyophilized cell-free extracts.



L-Lactate Dehydrogenase

Cofactor recycling enzyme

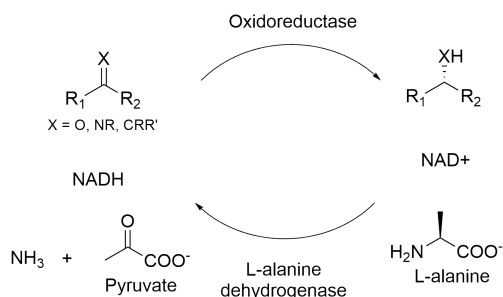
L-lactate dehydrogenase selectively catalyzes the interconversion between L-lactate and pyruvate at the expense of NAD(H). This enzyme can be considered as a coenzyme for NAD(H) cofactor recycling



L-ALANINE DEHYDROGENASE

Cofactor recycling enzyme

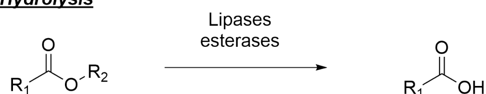
L-alanine dehydrogenase is a NAD-dependent aminoacid dehydrogenase that catalyze the amination of pyruvate into L-alanine in the presence of ammonium ions. This enzyme can be used in combination with transaminases to recycle L-alanine as the amine donor. The enzyme may also be used as a coenzyme for NAD(H) cofactor recycling.



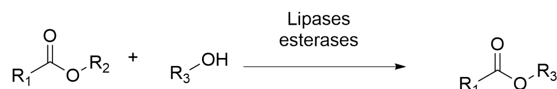
LIPASES AND ESTERASES

Lipases and esterases catalyze the hydrolysis of esters into the corresponding acids. When the reactions are run in anhydrous conditions, the enzymes can promote the esterification of acids and the transesterification of esters. They do not require any cofactor, are generally stable, and tolerate high ratios of organic solvents.

Hydrolysis



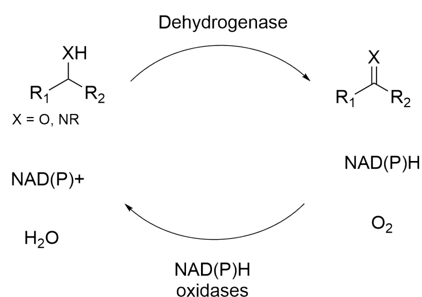
Transesterification



NAD(P)H OXIDASES

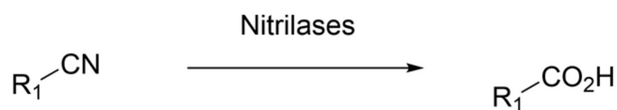
Cofactor recycling enzyme

NAD(P)H oxidases are flavin-dependent enzymes that catalyze the oxidation of NAD(P)H at the expense of oxygen resulting in the formation of NAD(P)+ and H2O. Two NAD(P)H oxidases are proposed for their complementary dependency to NADP+ and NAD+.



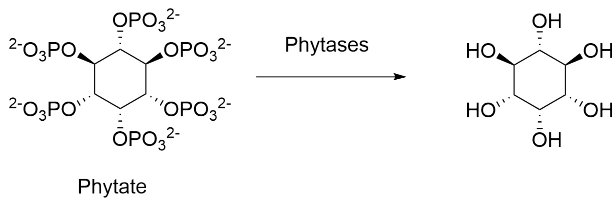
NITRILASES

Nitrilases catalyze the hydrolysis of nitriles to carboxylic acids. They can promote the kinetic resolution of racemic mixtures resulting in the formation of enantio-enriched products.



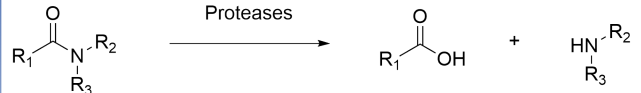
PHYTASES

Phytases are a kind of phosphatases that hydrolyze phytate into inositol.



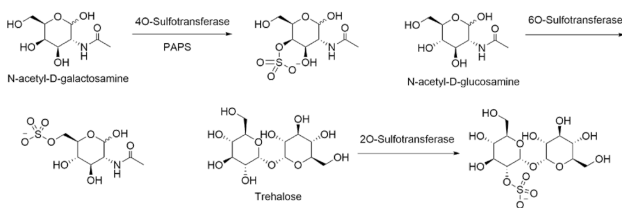
PROTEASES

Proteases are hydrolytic enzymes active on amide bonds.



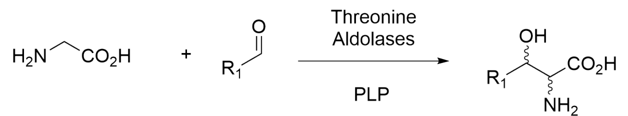
SULFOTRANSFERASES

Sulfotransferases catalyze the sulfation reaction of hydroxy groups at the expense of the cofactor 3'- phosphoadenosine-5'-phosphosulfate (PAPS). The 3 available enzymes promote the sulfation of different substrates, namely *N*-acetyl-D-galactosamine, N-acetyl-D-glucosamine and trehalose.



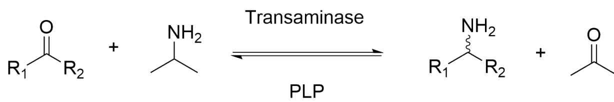
THREONINE ALDOLASES

Threonine aldolases are PLP-dependent enzymes that catalyze the condensation between glycine and aldehydes generating the corresponding amino acids with the formation of 2 stereogenic centers. Enzymes with both L- and D-selectivity are provided. Very good selectivity are generally observed at the α position, however, the selectivity at the β-position depends on the involved aldehyde.



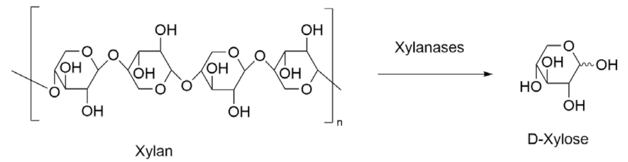
TRANSAMINASES

Transaminases are PLP-dependent enzymes that catalyze the transfer of an amino group from an amine donor to an amine acceptor, the target substrate. The substrate is generally a prochiral ketone leading to a chiral amine at the expense of a cheap amine donor (for instance, *iso*-propylamine).



XYLANASES

Xylanases catalyze the hydrolysis of β-(1→4) bonds of xylans resulting in the formation of D-xylose.



GENERAL INSTRUCTIONS

- Enzymes are delivered as freeze-dried cell-free extracts stored in vials or microplates. Additional remaining substances may include endogenous proteins and buffer salts, which can contribute to enzyme's stability. Once you find a hit, if necessary, a high purity enzyme production process can be developed at an additional cost. However, cell-free extracts are suitable for most industrial processes. Product **might be stored at -20°C** when received. The powders might be freshly resuspended in aqueous buffers for every activity assay.

- As a rule of thumb, **enzymatic assays** are generally implemented with **5 to 10 mg of extract in 1 mL of reaction mixture**.

- In most of the cases, enzymes are used in **aqueous conditions**. However, if the substrate is not soluble, DMSO could be used as a generally well-tolerated cosolvent if the proportion is not above **10% v/v.** Alternative cosolvents (alcohols, alkanes, ethers) can be considered but their tolerance is less general.

- For first trials, the substrate concentration should not be above **20 mM**. If the addition of 10% v/v DMSO does not permit the total solubilization of the substrate, the reactions can be run anyway and might allow for the identification of active enzymes.

- When **NAD(P)(H) cofactors** are required, a cofactor recycling system might be implemented to use a **catalytic amount of cofactor.** They might also switch chemical equilibrium in somes cases. Different co-enzymes are available in this catalog depending on the reaction direction (oxidation or reduction) and the application.

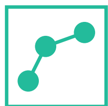
- In a first attempt, enzymatic assays can be performed at **30°C in 2 mL-vials, eppendorf tubes or in microplates**. The reaction mixtures are then stirred gently with orbital or magnetic devices accordingly to the considered vessel.

- From an analytical point of view, the reactions can be monitored over time or be analyzed after one night according to any **chromatographic method**. The reaction can be stopped by heat, extreme pH or by dilution into an organic solvent compatible with the analytical method. Once deactivated, the enzymes generally get precipitated in the reaction mixtures. Appropriate analytical sample preparation procedure might be set-up to get rid of the precipitated enzymes (filtration, centrifugation, liquid-liquid extraction...).

About Protéus by Seqens

Protéus, a wholly subsidiary of Seqens Group, is the French leader in protein engineering technologies and associated industrial biocatalytic processes :

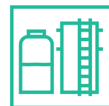
- **5 000+** exclusive and diversified **microorganism collection** including micro-algae and thousands of bacteria and archaebacteria extremophiles
- **1 500 fully sequenced strains** combined with data mining tools, offering a unique pool of million enzymes
- **650+ enzyme portfolio** for tailor-made kits
- **Patented protein evolution technologies** (EvoSight™ & L-Shuffling™) to optimize enzymes performances
- ***In silico* design** of smart libraries & molecular dynamic analysis
- A multidisciplinary skilled team dedicated to **custom bioprocess development and scale-up** for client's applications
- Seqens capabilities enabling in-house **implementation of large-scale biotransformation processes**



Created in **1998** and within **SEQENS** since 2017

20 Research scientists & experts

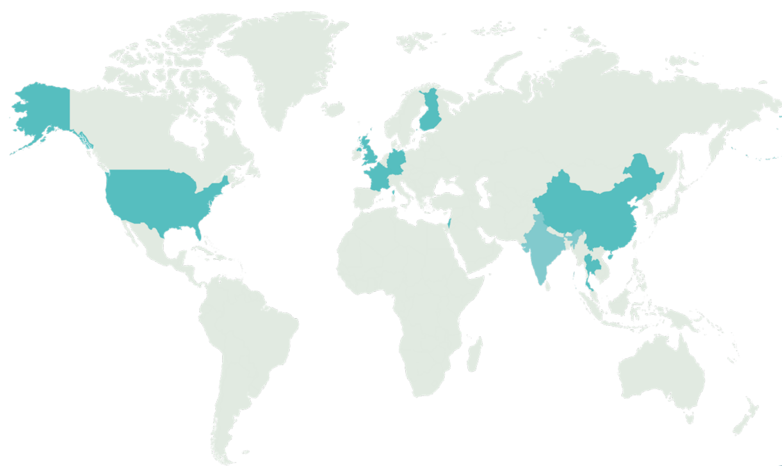
Development of tailored enzymes & biocatalytic processes



Fermentation scale-up from Lab to **300L bioreactors**

The only EU-based company offering both **directed evolution** services and **large reaction capabilities**, for fine chemicals & cosmetics ingredients

Seqens, an integrated global leader in pharmaceutical synthesis and specialty ingredients



3, 300
people

9
R&D centers

9
Countries

300
scientists, experts and engineers

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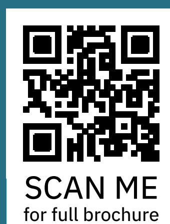

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