Expression en système acellulaire de germe de blé :

Une solution pour la production de protéines membranaires et/ou toxiques fonctionnelles

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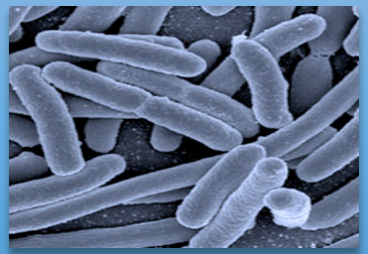
Several Hosts systems can be used to produce recombinant proteins



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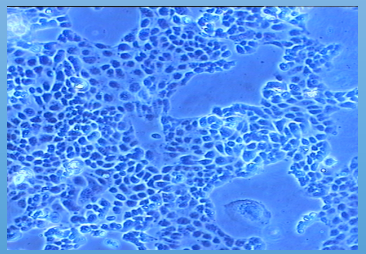


Mammalian cells
CHO, HEK

Wheat germ Cell free system

Wheat Germ Cell-free system

Main advantages over classical cell-based methods

- Expression of proteins that are toxic to the cells
- Translation is 10 times slower than in bacteria → better protein folding!!
- Proteins can be expressed in a large temperature range (4°C-30°C)
- No need for codon optimisation
- Open system: supplementation with detergents or phospholipids possible
- Effective and specific incorporation of labeled amino acids (NMR studies)
- No need for cell harvesting and cell lysis

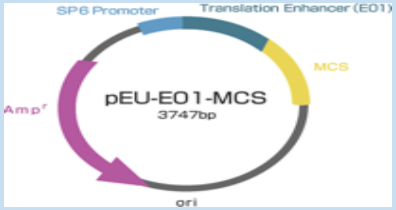
Wheat germ Cell free system

Wheat Germ Cell-free system

How does it work?

Wheat embryo extract preparation

Plasmid preparation



***in vitro* transcription**

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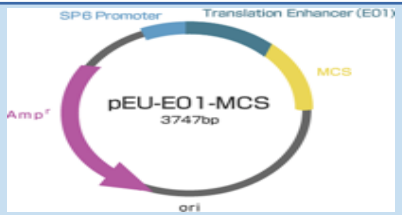
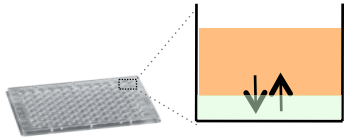


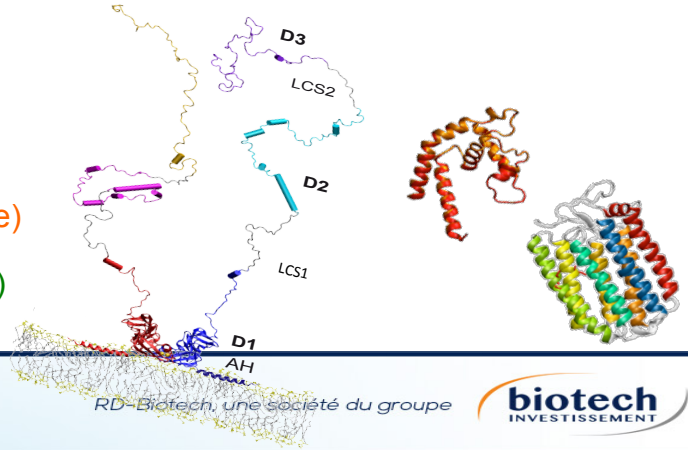
in vitro transcription

Protein synthesis



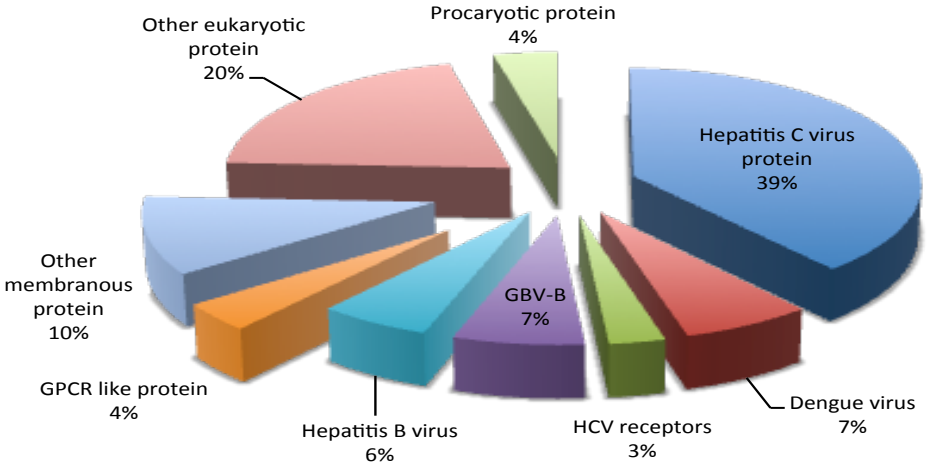
Feeding buffer (amino acids, ATP, GTP, creatin phosphate)

Reaction Mix (wheat germ extract, mRNA, creatin kinase)



Wheat Germ Cell-free system
System efficiency

More than 100 contructs tested up to now



More than 90% of proteins were successfully expressed

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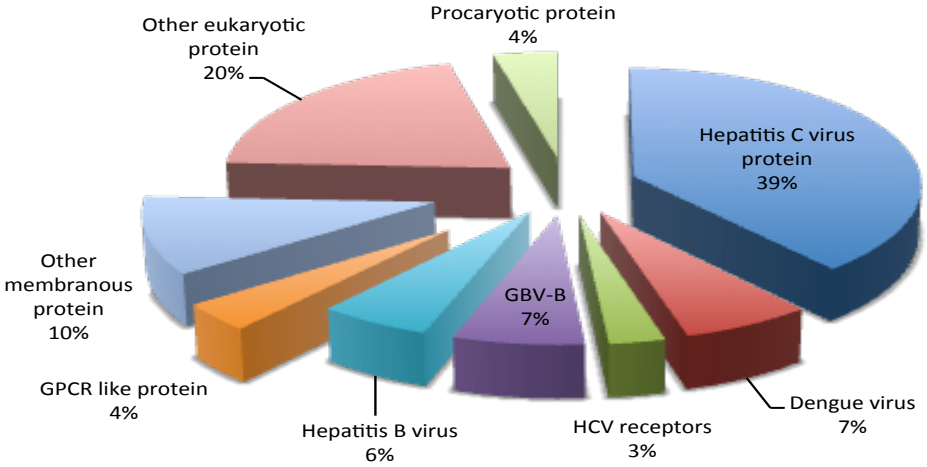
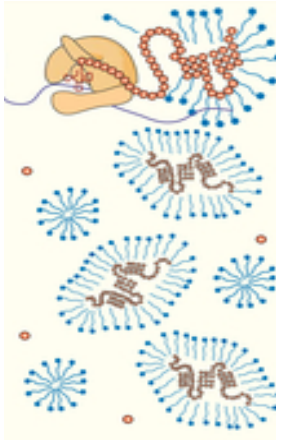


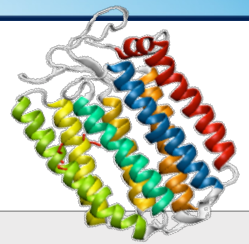


Membrane proteins are kept in soluble form by insertion into provided detergent micelles

(Protein Expr Purif. Fogeron *et al.*, 2015)

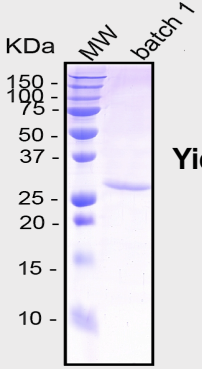
More than 90% of proteins were successfully expressed

Some examples among many others…



Membrane proteins belonging to GPCRs family structure

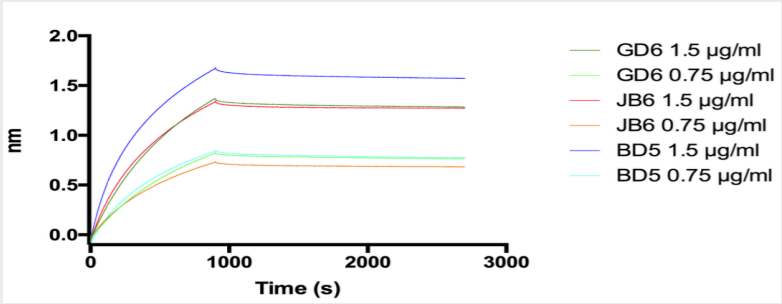
Halorhodopsin



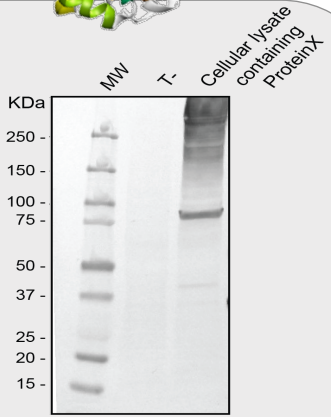
Yield: ~ 0,6 mg/ml WGE

SDS-PAGE of Halorhodopsin purified protein (in fusion with a C-terminal Strep-Tag) expressed in wheat germ cell-free system and purified on Strep-Tactin affinity column

ProteinX



Interferometry Binding assays of ProteinX to monoclonal antibodies GD6, JB6 and BD5. Sensorgrams resulting from the interaction of Protein 1 and the three mAbs covalently immobilized by amine coupling



Western-Blotting of cellular lysate containing ProteinX detected with mAb GD6 produced from recombinant ProteinX

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