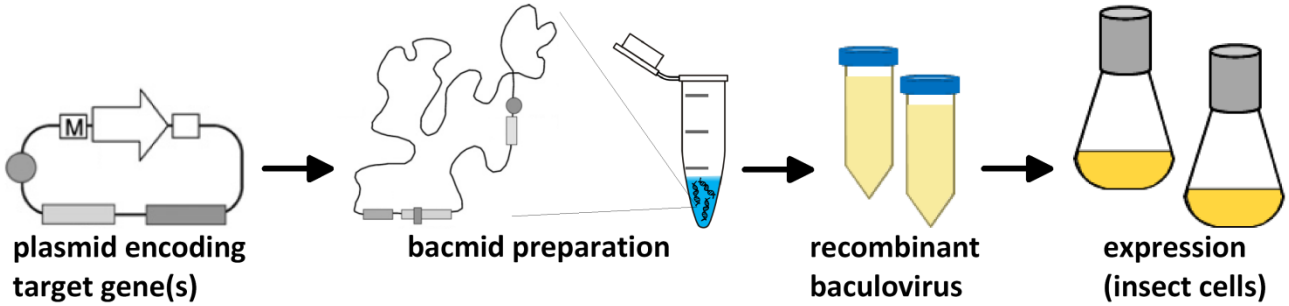
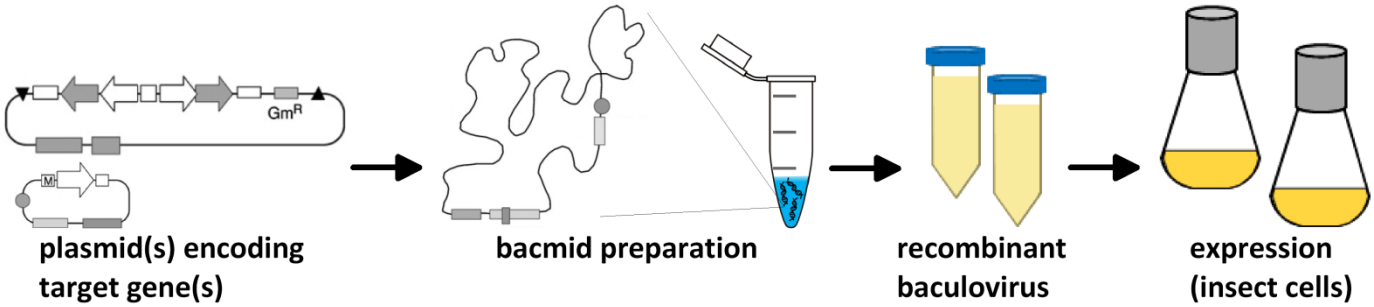
Expression in insect cells (Sf21, Hi5)

Recombinant protein expression in insect cells offers a lot of advantages. In contrast t *E.coli*, insect cells offer mammalian-like systems for protein secretion and processing, including Golgi-apparatus and ER. Furthermore, insect cells are capable of providing nearly all mammalian post translational modifications, like for example phosphorylations and palmitoylation. Therefore, most mammalian proteins and protein complexes recombinantly produced in insect cells are correctly folded and active. Solely N-glycosylations produced by insect cells differ from their mammalian counterpart: N-glycosylation in insect cells is simpler and more homogeneous. This rarely has an influence on the activity of recombinant proteins but oftentimes makes protein crystallization easier.
The following methods for recombinant protein expression in insect cells are offered by our facility:

1. **Baculovirus-mediated expression (BEVS)**
   This method makes use of the fact that Lepidoptera insects (as well as cell lines derived from such organisms, like for example Sf21 or Hi5) are naturally susceptible for infections by baculoviruses. For baculovirus-mediated protein expression, the desired open reading frames are incorporated into a recombinant baculovirus. The recombinant viruses are then used for infection of insect cells growing in suspension, inducing the expression of the proteins on interest. This expression system is particularly well-suited for the high-scale production of intracellular proteins.



1b. Expression using the MultiBac system
The MultiBac system is a vector system specifically developed for the baculovirus-mediated expression of multi-protein complexes. The system allows for (occasionally complex) cloning strategies to include open reading frames of many target proteins into one recombinant baculovirus. The target proteins are then co-expressed in insect cells, assemble to native protein complexes *in vivo* and eventually can be purified as fully-assembled protein complexes.

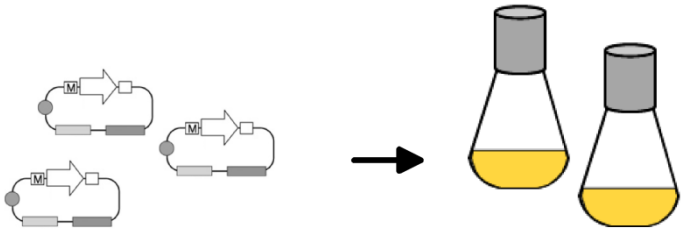


Advantages: - good yields for intracellular proteins

Disadvantages: - relatively time consuming, due to multi-step virus production (3 to 4 weeks)
-lysis of insect cells upon infection (might result in reduces protein quality)

2. Plasmid-based expression in insect cells (PEI-based transfection)

For plasmid-based protein expression in insect cells, a plasmid containing the desired open reading frame(s) is directly transduced into insect cells via complexation with the polycation polyethylenimine (PEI). The plasmid gets diluted with every cell division, resulting in a likewise limited timeframe for protein expression. The facility offers small-scale test expressions (4 or 30 ml), as well as large-scale expressions (up to one liter).Furthermore, small-scale split-GFP screens [Bleckmann *et al.*, 2016] can be used to check for the expression of soluble proteins with up to 46 samples in parallel. For this and other purposes, the facility possesses the micro cultivation system "BioLector" from M2P labs.

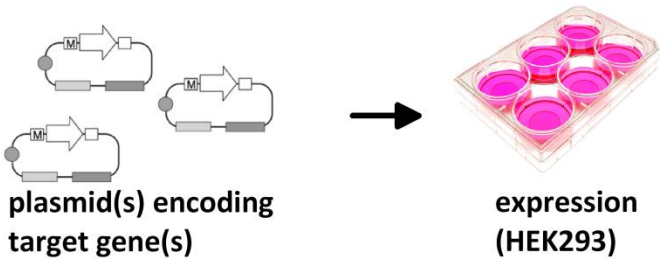


Advantages:
- fast expression (approximately one week)
- high viability of insect cells is maintained
- high yields for intracellular and secreted proteins

Disadvantages:
- large amount of DNA needed for transfections
- yields are oftentimes less, compared to baculovirus-mediated expression

Expression in adherent HEK293 cells

For authentic production of human target genes, human cell lines – like for example HEK293 cells – are obviously a good choice. HEK293 cells grow adherent and are well known for their reliable growth, as well as for being relatively easy to transfect with corresponding expression vectors. Our facility offers transient expression of proteins in HEK293 cells, as well as the establishment of stable cell lines.

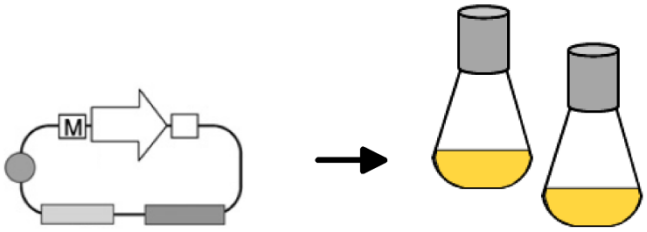


Advantages:
- relatively fast expression (transient transfection)
- selection of single clones possible (stable cell lines)
- native environment for expression of human proteins

Disadvantages:
- large amount of DNA needed for transfections
- yields are limited
- high costs for cultivation of human cells (medium, handling, etc.)

Expression in *E.coli*

E.coli is the standard system for production of recombinant proteins. It resembles by far the most cost-effective way to produce large amounts of recombinant protein. Thy system is limited however due to its prokaryotic nature. The **Recombinant Protein Expression Facility** has access to most commonly used *E.coli* expression strains and offers small-scale test expressions as well as production-scale expressions.



plasmid encoding target gene(s)

expression (*E.coli*)