# A cell-surface expression system for the display of heterologous gene products using chimeric flagellin fusions of a *Bacillus halodurans* isolate

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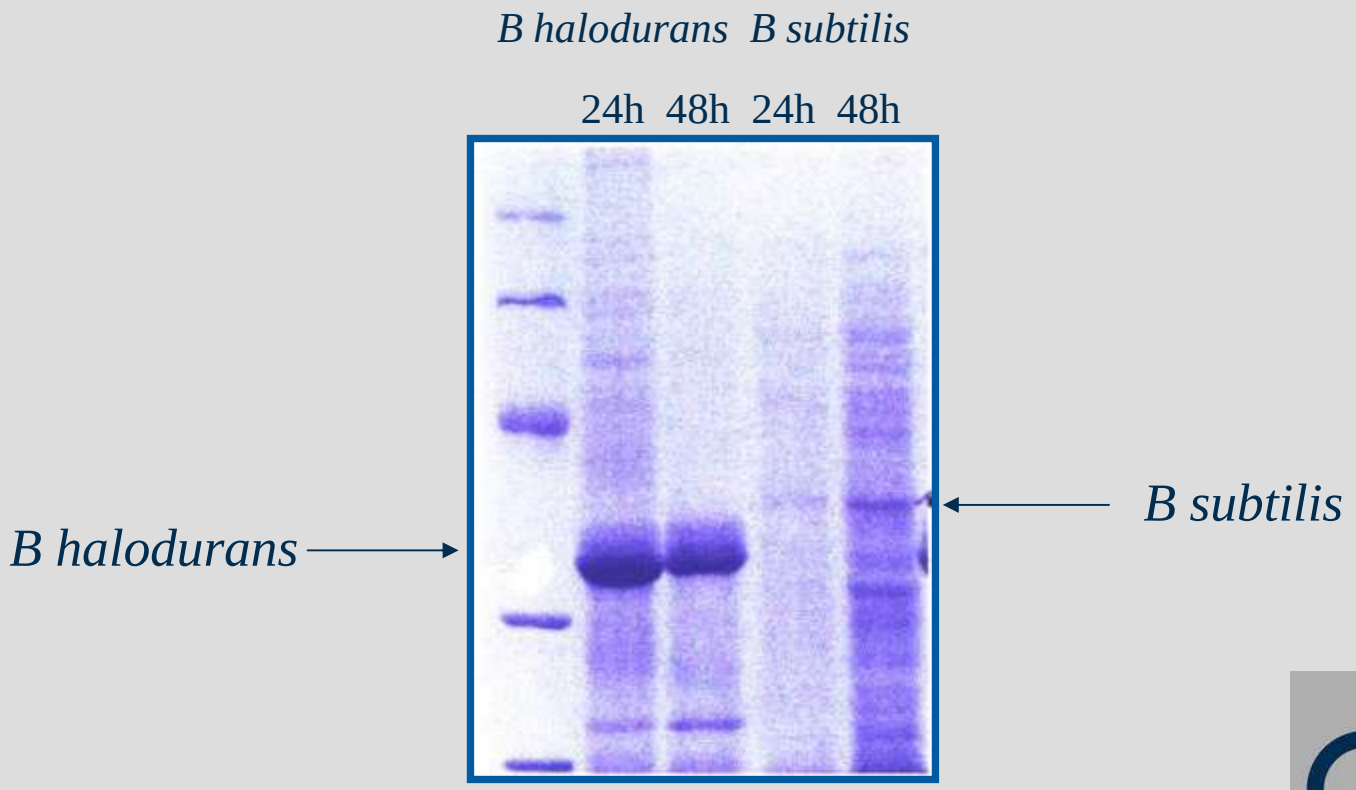
# *Bacillus halodurans* Alk 36

- Ability to over-produce cell surface protein continuously for up to 144 hours
- Ability to grow over a wide pH range: pH 7.5 to 10.5
- Ability to grow over a wide temperature range: 30°C - 55°C.
- Isolate identified by 16S rRNA homology study.

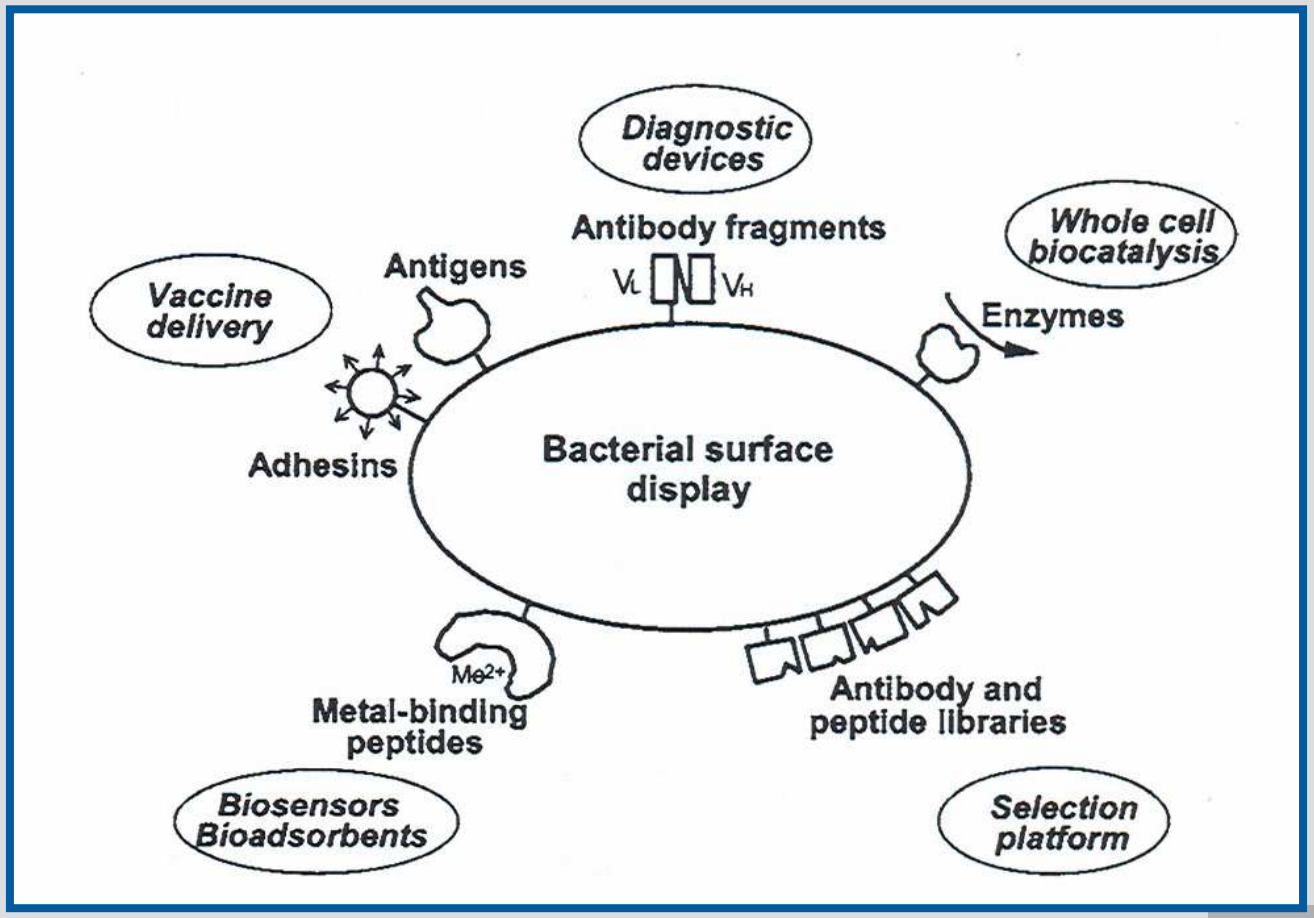
# PAGE gel showing over-production of cell surface protein by *B halodurans* Alk36



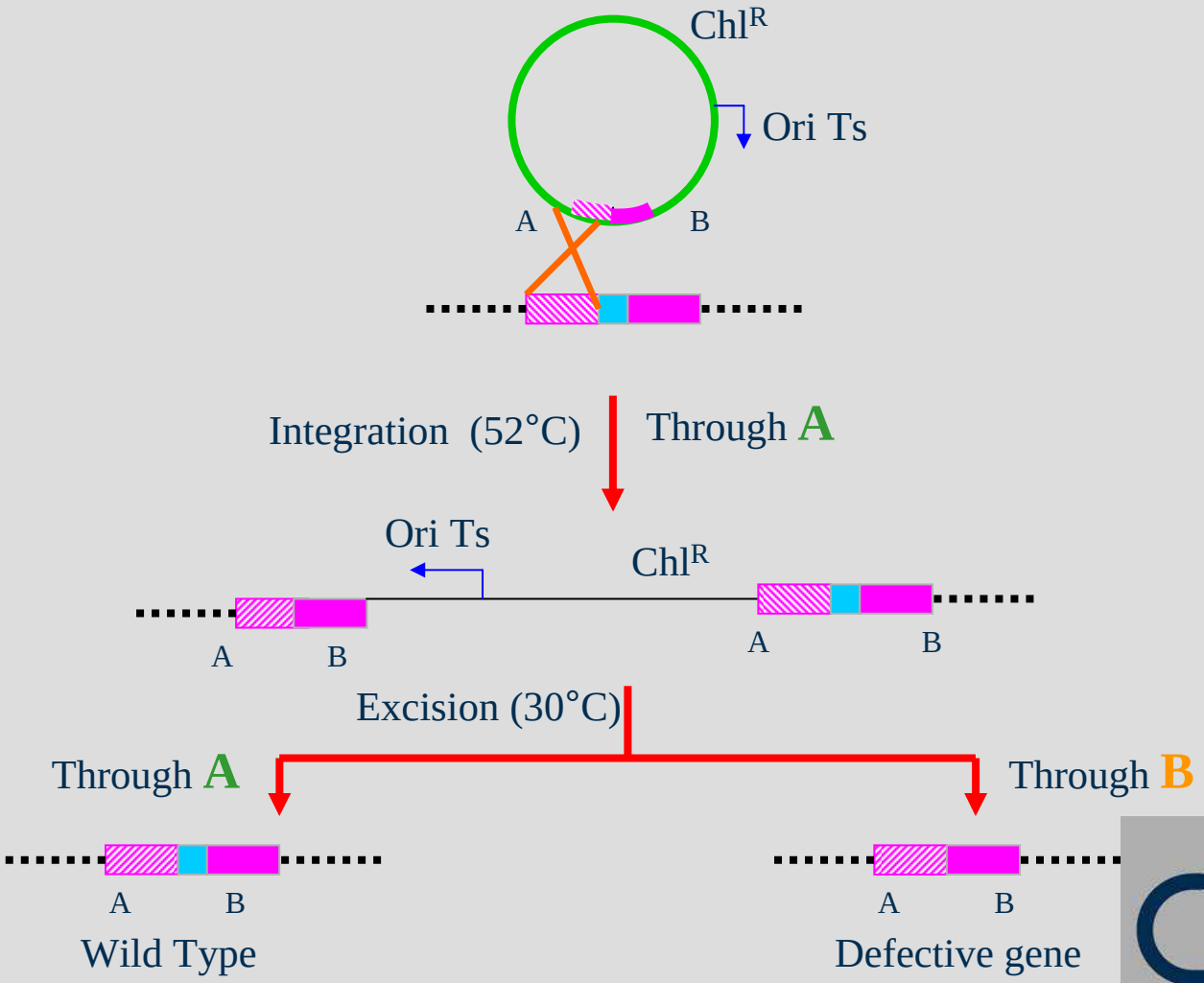
# Identification of cell surface protein

- N-terminal sequencing gave rise to homology to flagellin protein, product of the *hag* gene
- Gene was cloned by using degenerate primers and inverse PCR
- The gene sequence as well as the up- and down- stream regions was found to be 100% homologous at the nucleotide level to *B halodurans* C125.
- The entire genome of *B halodurans* C125 is sequenced.

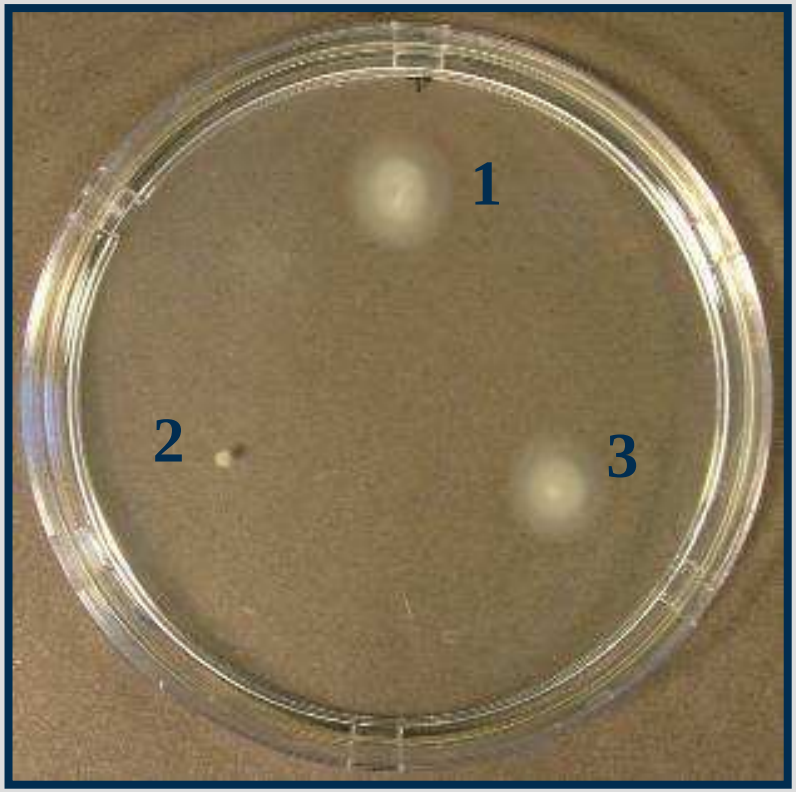
# Applications of Surface display



# Gene targeted disruption



# Motility plates showing *B halodurans* Alk 36 mutants




Motility plates of the different *B.halodurans* Alk36 strains.

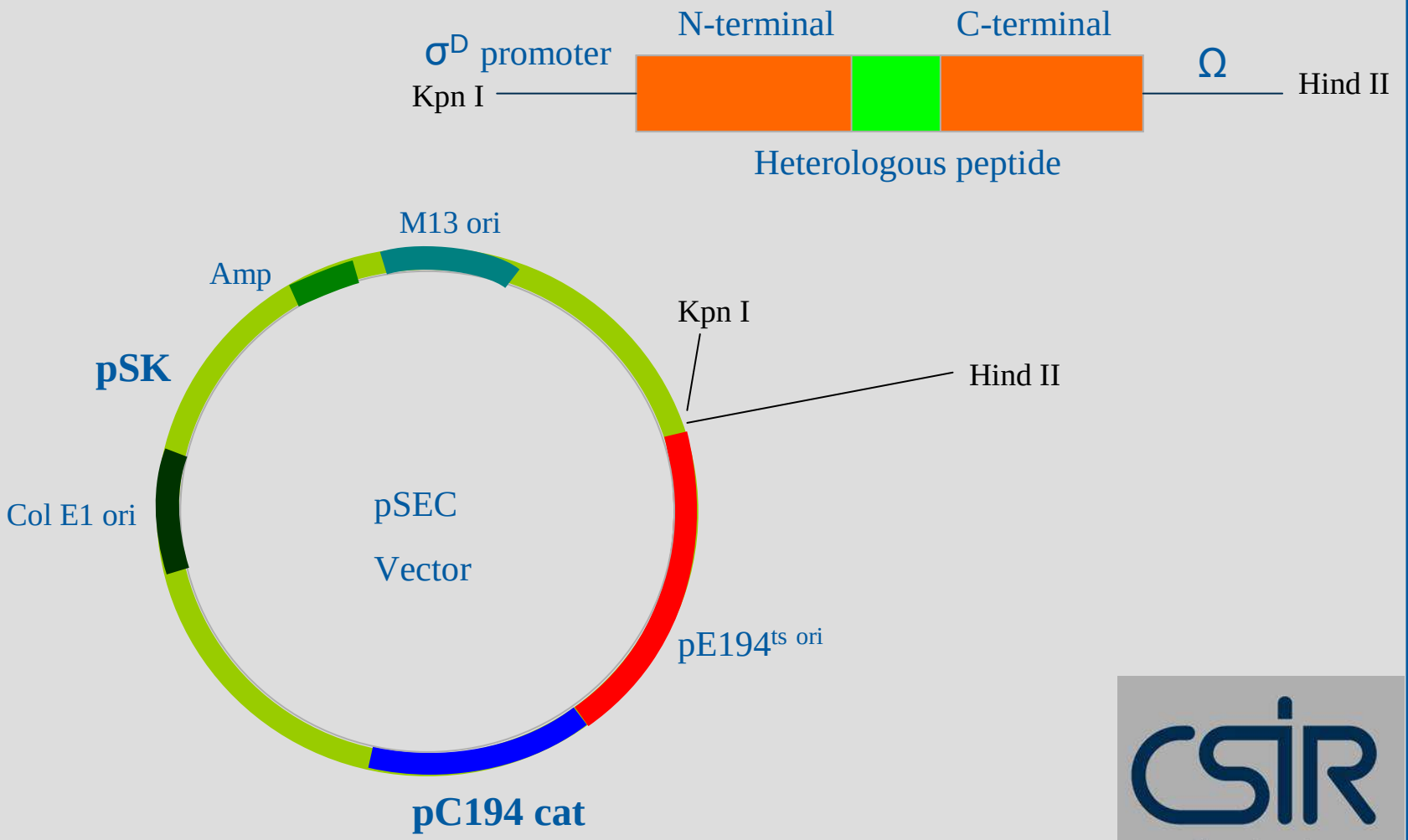
Colony

(1); *B. halodurans* Alk36 wild type,

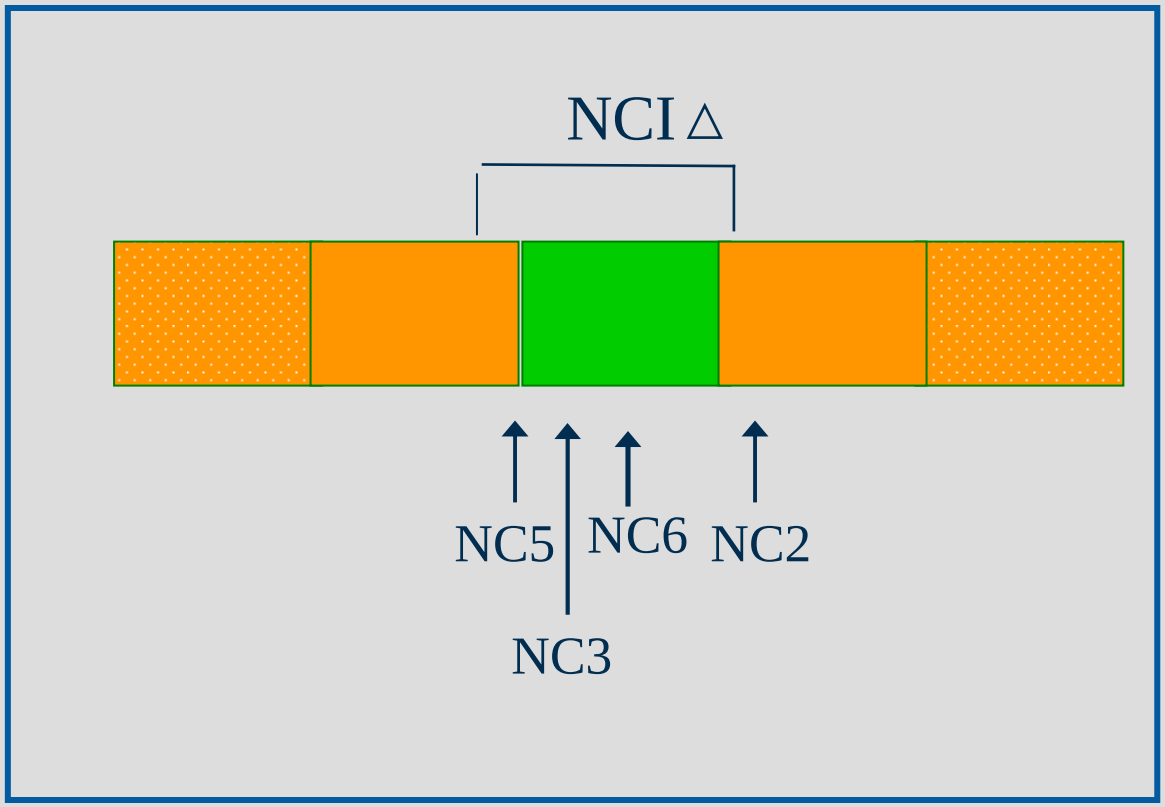
(2) BhFC01 (Δ*hag*),

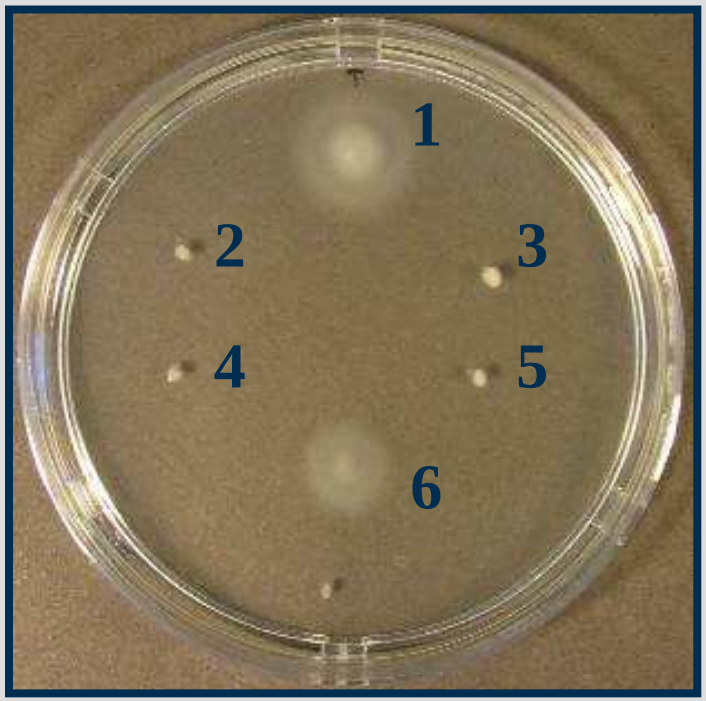
(3); BhFC01 (Δ*hag*) + pSEC194FliC.

# Construct for flagellin sandwich fusion



# Diagrammatic representation of a flagellin protein showing linker insertion at different sites



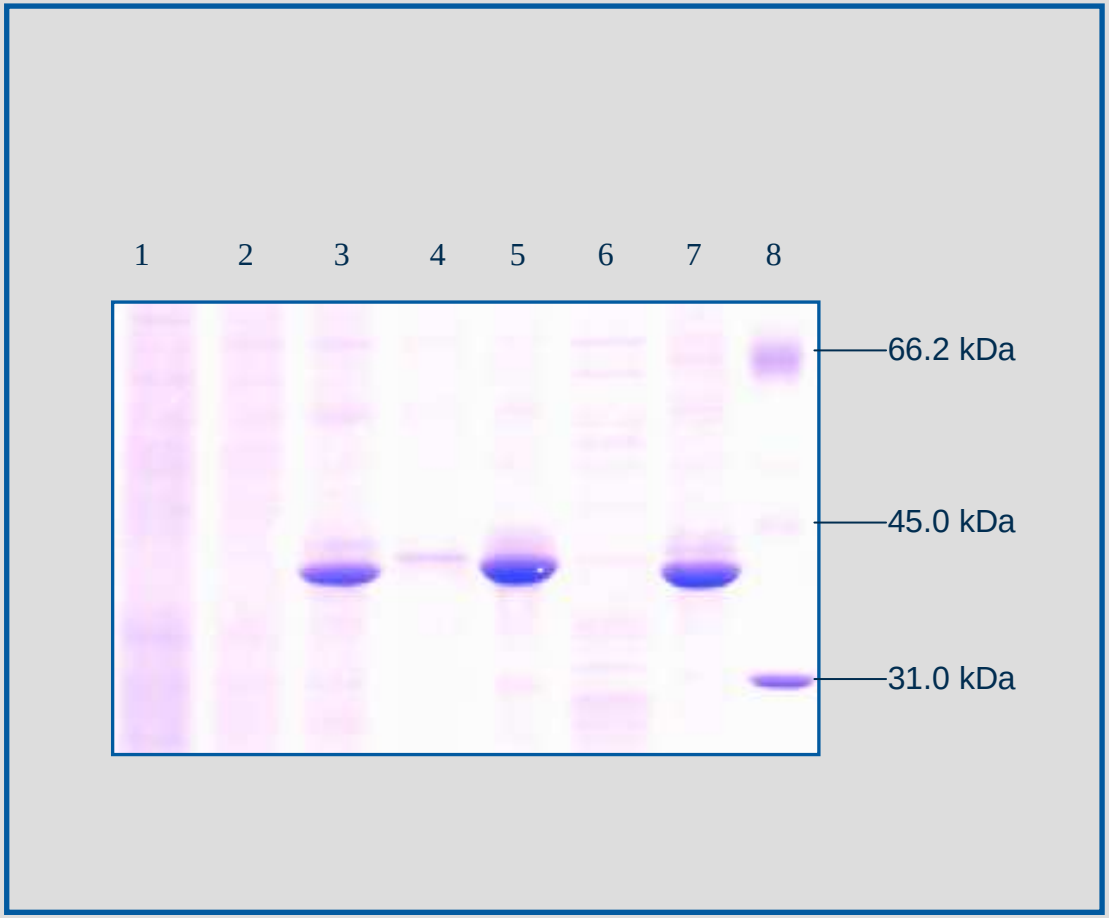



## Motility plates of the different *B. halodurans* Alk36 strains.

Colony
(1) B. *halodurans* Alk36 wild type,

Colonies 2-6 BhFC04 containing:
(2) pSECNC1,
(3) pSECNC2,
(4) pSECNC3,
(5) pSECNC5,
(6) pSECNC6

# PAGE gel showing cell surface proteins produced by the different constructs

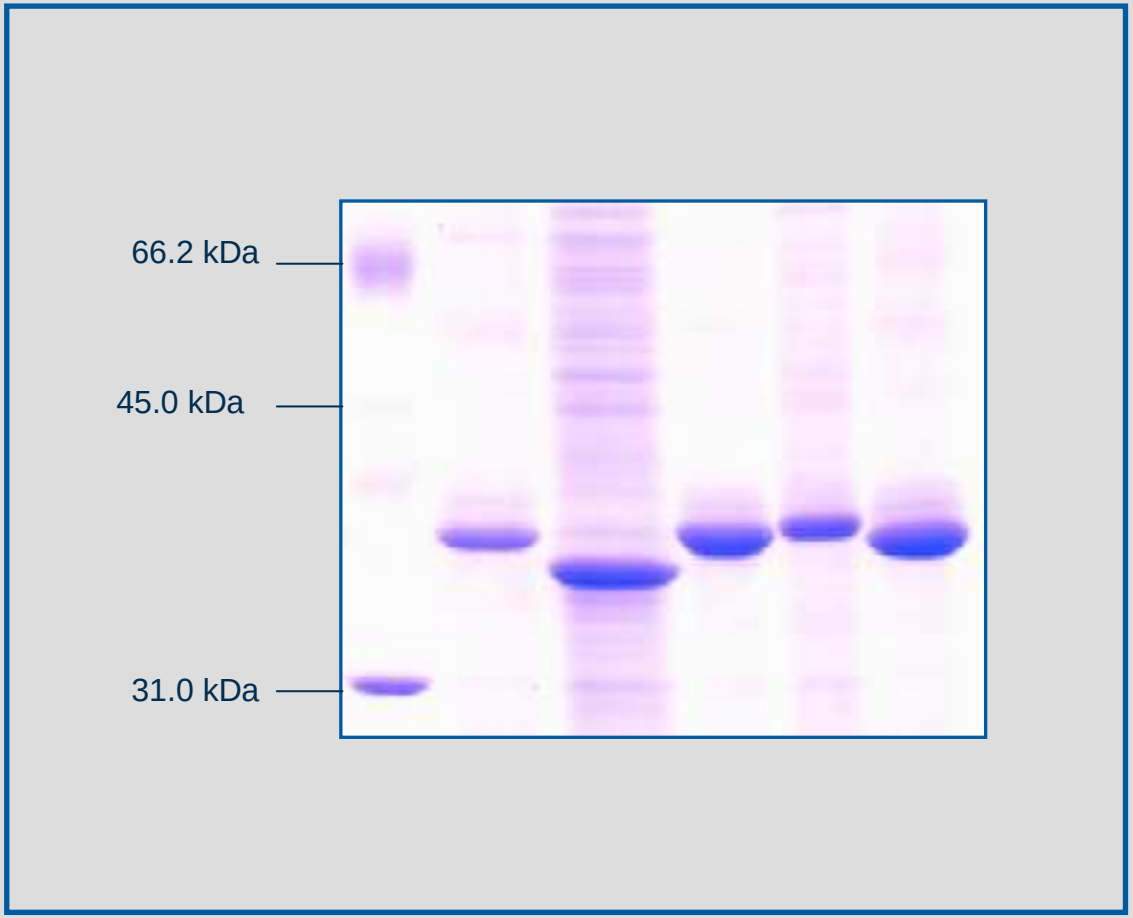



1. NC1
2. NC2
3. NC3
4. NC5
5. NC6
6. BhFC04
7. *B. halodurans*

# Surface display for bioremediation/ biomining

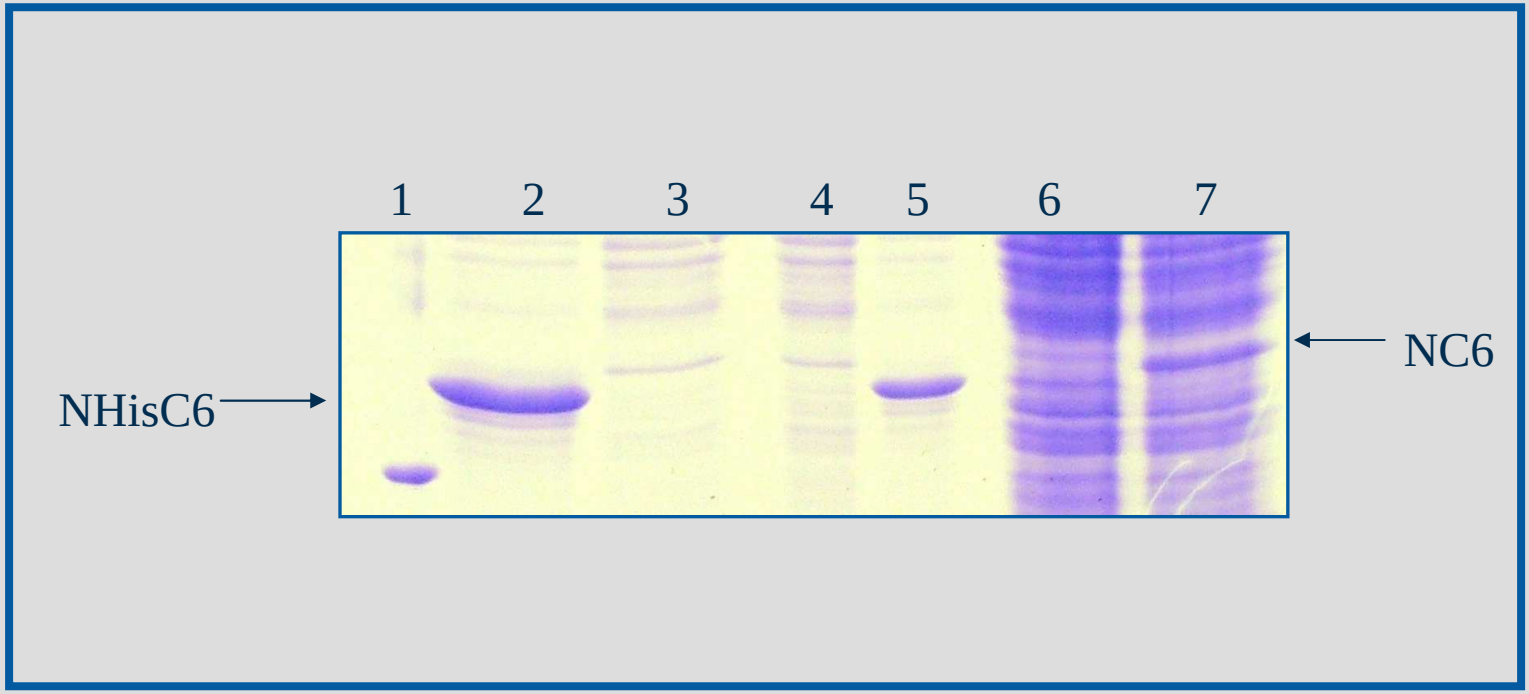
- A poly-His peptide which is able to bind $Cd^{2+}$ and $Ni^{2+}$ ions was synthesized and inserted as in-frame fusions into both the NC3 and NC6 sites.
- This poly-His tag resulted in a 15 amino acid peptide insertion within the flagellin protein.
- Construct checked for over-production of fusion protein.
- Construct checked for functionality of the His-tag for metal binding.

# PAGE gel showing over-production of chimeric poly-His flagellin proteins




1. LMW ladder
2. NC3
3. NHisC3
4. NC6
5. NHisC6
6. *B. halodurans*

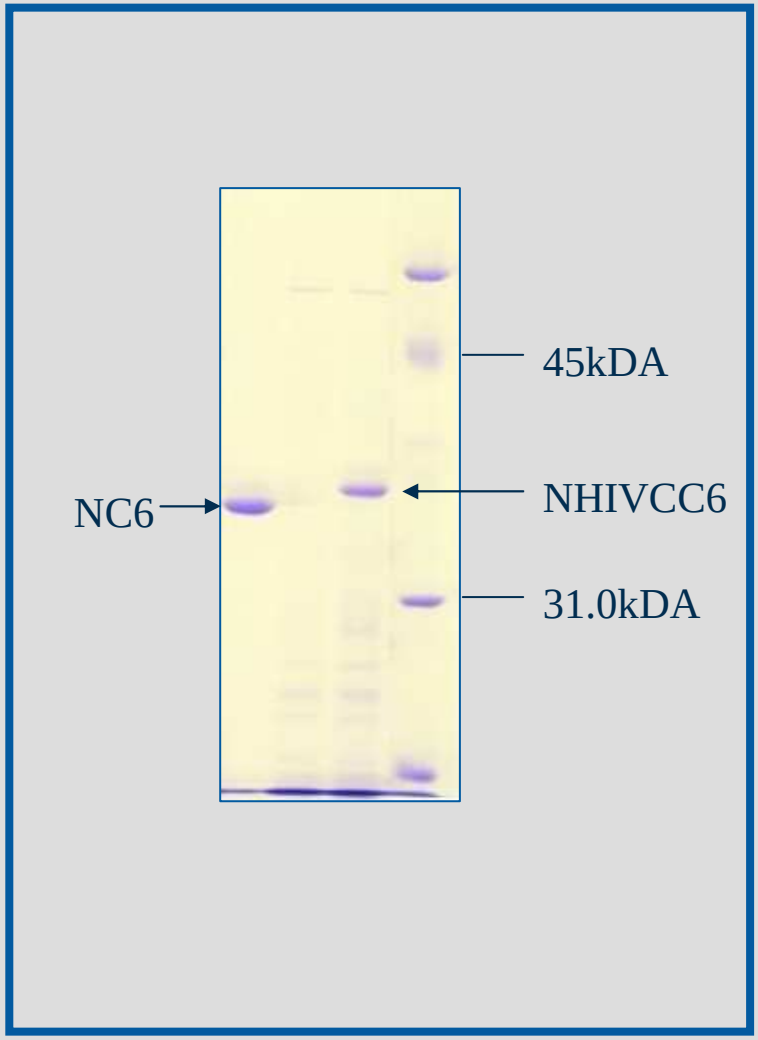
# Evaluation of the functionality of the poly-His tag for metal binding

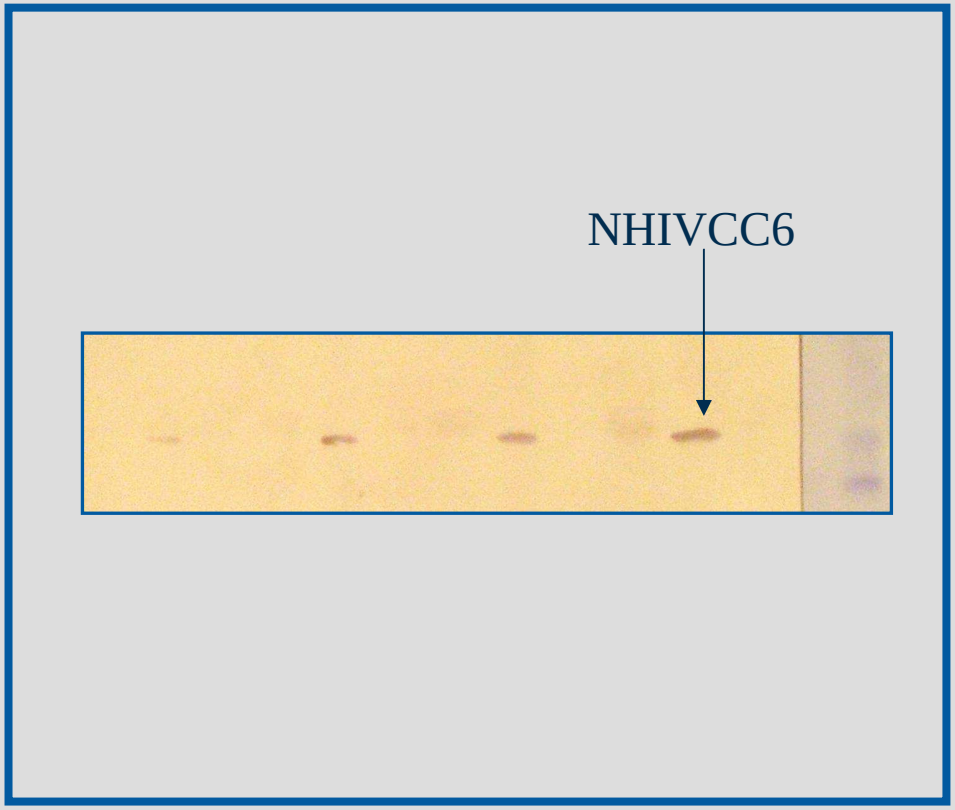


# Expression of an HIV antigenic peptide as a chimeric flagellin fusion

- Synthetic peptide sequence used was based on a consensus sequence of the variable region of all HIV-1 subtype C V3 South African isolates.
- Peptide size is 24 amino acids and full insert size is 29 amino acids.
- Peptide cloned as an in-frame fusion into the pSEC vector.

# Expression of antigen on cell surface and evaluation of immunogenicity

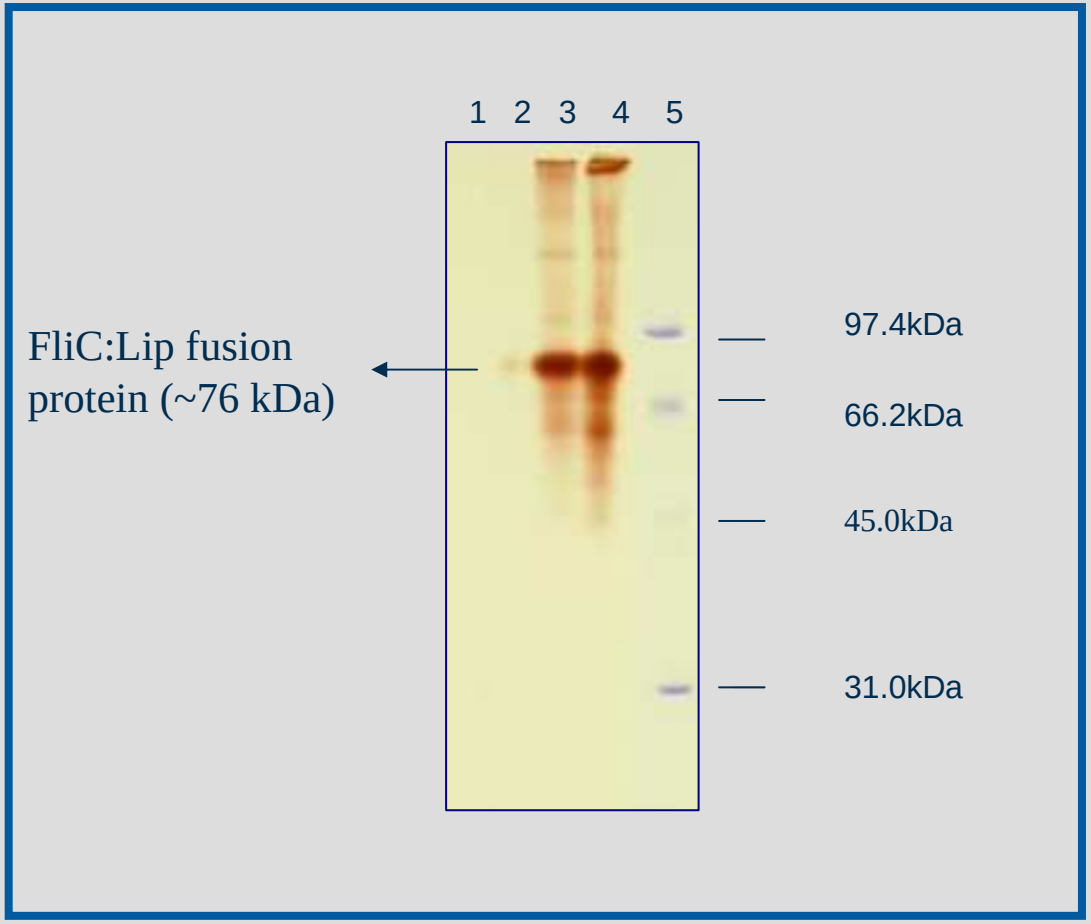


# Application of the expression system in a biotransformation

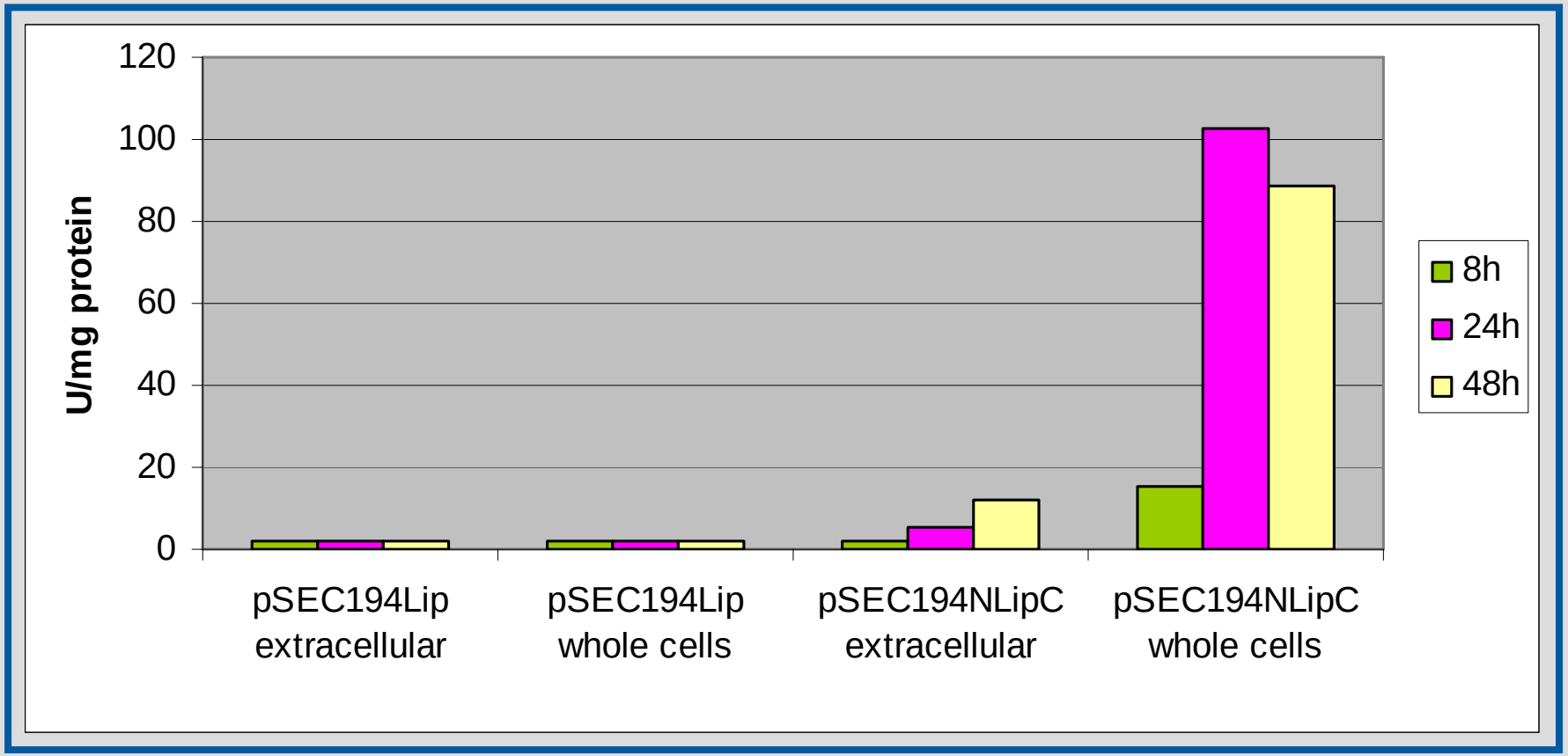
- Lipase is an important enzyme in chiral resolution of many key pharmaceutical intermediates.
- A thermostable lipase originally from *Geobacillus thermolovorans* was cloned into pSEC as an in-frame flagellin fusion.
- Enzyme activity was determined from whole cell bioassays using p-nitrophenyl palmitate as a substrate.

# Zymogram showing location of lipase activity




1. Extracellular fraction
2. Cell surface Fraction
3. Cell wall
4. Intracellular
5. LMW marker

# Enzyme activities of whole cell bio-assays



# Advantages of this expression system

- Gram-positive bacteria are robust and cell growth is not impaired by the production of chimeric flagellin fusions
- Chimeric flagellin production is continuous and an inducible promoter system is not necessarily required for over-expression.
- Versatility of the expression system: custom designed peptides can be displayed.

# Advantages of this expression system

- Small peptides are over-produced on the cell surface, in shake flask studies 50 to 60mg/L chimeric fusion proteins are produced.
- Ease of isolation of chimeric flagella for further applications.
- Integration of the flagellin/peptide fusions into the host chromosome thereby ensuring that no heterologous plasmid DNA or antibiotic markers are present in the production strain.

# Acknowledgements

The project team who made it all happen:

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