Heterologous Membrane Protein Production System

(OTT ID 1006)

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Membrane Protein Production System

- **Importance**
  - Membrane proteins account for 30-50% of the most promising pharmaceutical targets
- **Problems**
  - Membrane proteins are difficult to synthesize in large quantities
  - Current systems produce small amounts that are often misfolded, inactive, or toxic to host cells
- **Solution**
  - The bacterium *Rhodospirillum rubrum* forms an intracytoplasmic membrane (ICM) in response to membrane protein synthesis
  - The ICM is non-essential for growth and can incorporate foreign and over-expressed membrane proteins without disrupting normal cellular function
  - Gene expression is regulated in a non-toxic and inexpensive fashion by adjusting oxygen levels
  - Active and correctly folded membrane proteins can be expressed with a high yield

Market Potential

Applications:

- Therapeutic protein production
- Vaccine development
- Antibody production
- Production of high value proteins
- Commercial Protein Expression Kits
  - Crystallographic preparations
  - NMR preparations
  - Ligand/inhibitor assays
  - High through-put screening assay materials
  - Basic research use

Market:

- Global Industry Analysts has predicted that the global market for protein drugs is forecast to reach $158 billion by 2015.
- The life science tools market has continued to show steady growth over the last few years and is currently valued at more than $42 billion (BCC Research 2011)
- The market is predicted to grow to $81 billion by 2016. Protein research-related tools are projected to rise to $9.1 billion in 2016.

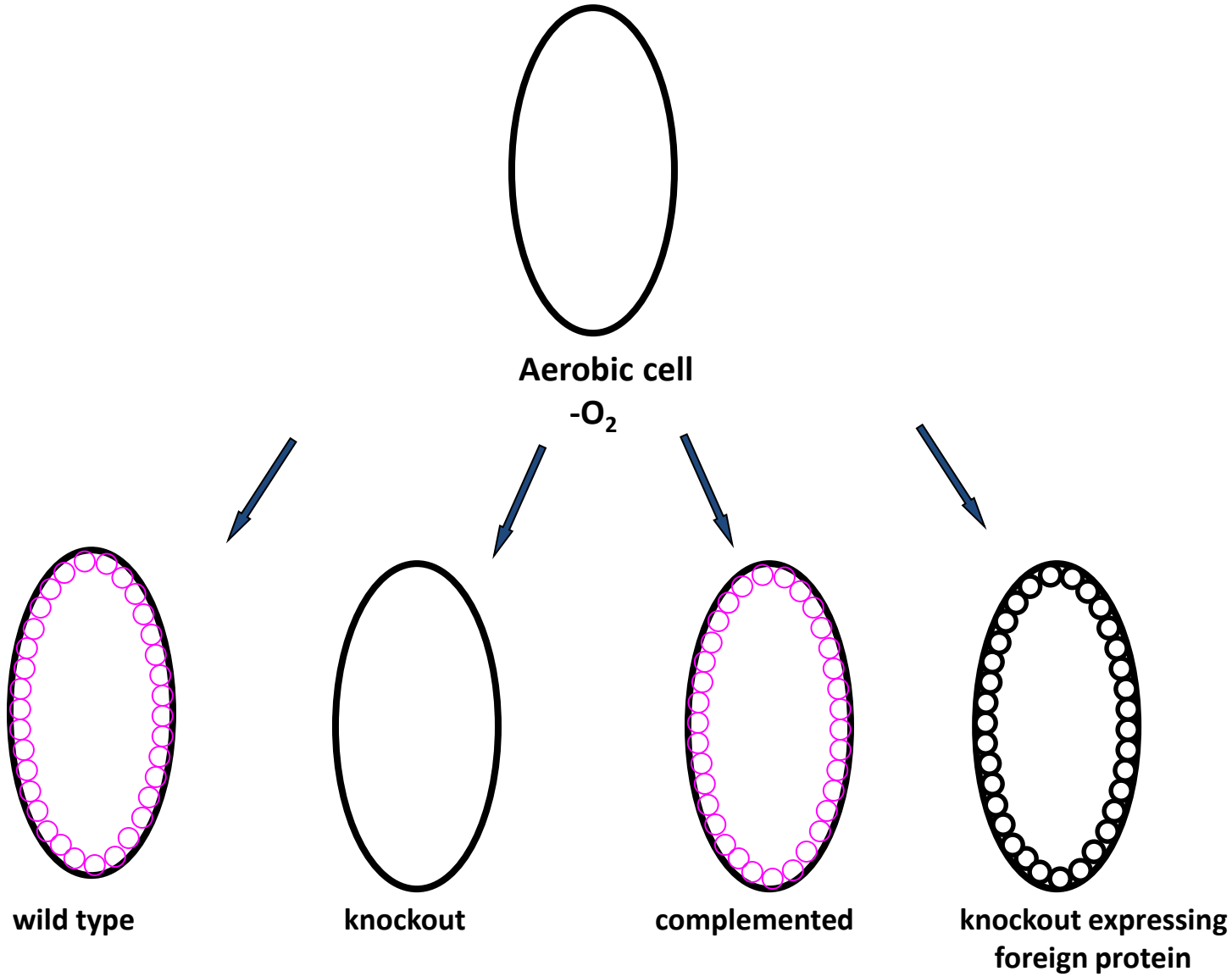
Intellectual Property

- Issued U.S. Patent 6,680,179
- Issued U.S. Patent 6,951,741
- Issued U.S. Patent 8,481,287
- This technology is available for licensing
- We are looking for partners to aid in finalizing the development of the expression kit into a marketable product for both scientific research and industrial use.
- The kit has been further developed to work using electrocompetent bacterial cells for electroporation of the vector containing the gene of interest.
- We are also looking for partners interested in utilizing the expression system internally for protein production for pharmaceutical products (i.e. vaccines, therapeutics, etc.)

Testing Performed

- The kit is has been transferred through material transfer agreements to several universities and companies
- Type of proteins being tested or already tested:
  - Efflux transporter protein (*P. aeruginosa*)
  - Chemokine proteins for testing structure-function relationships
  - Inner mitochondrial membrane transport proteins (human)
  - Rice protein involved in low temperature signaling
  - Magnetosome proteins
  - Human ETF-QO
  - *M. tuberculosis* drug target candidates
  - Integral membrane proteins
  - Anion channel proteins

Intracytoplasmic Membrane (ICM) formation



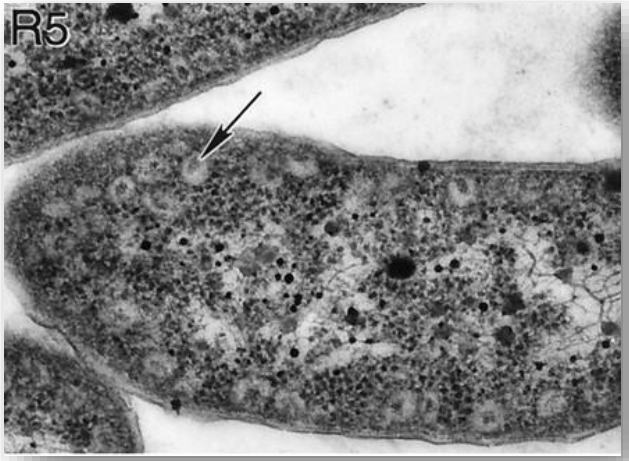
Rhodospirillum rubrum host/vector system

- **Mutants lacking the structural proteins of the photochemical apparatus do not form ICM but retain the capacity to do so when a native or foreign membrane protein is synthesized**

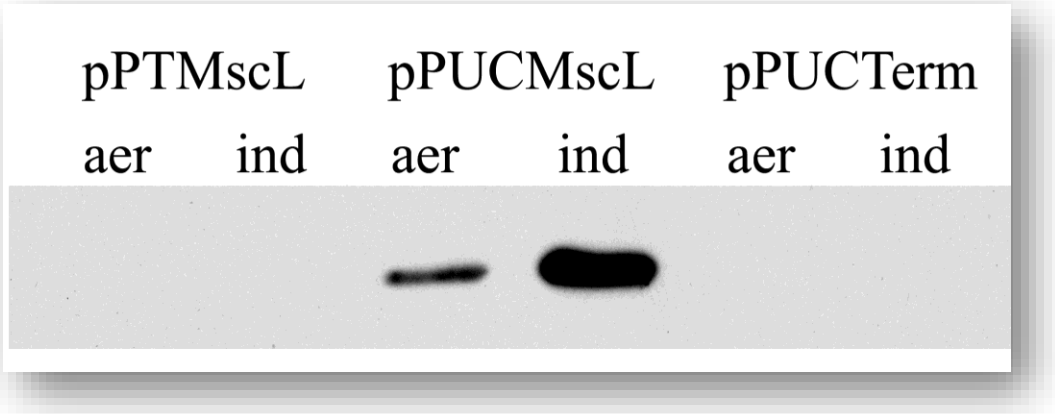
Host: *R. rubrum* H2 (*puhA, pufBALM* mutant)

Vector: pPUCTerm

Rhodobacter capsulatus puc promoter regulated by oxygen



Production of MscL with reduced oxygen



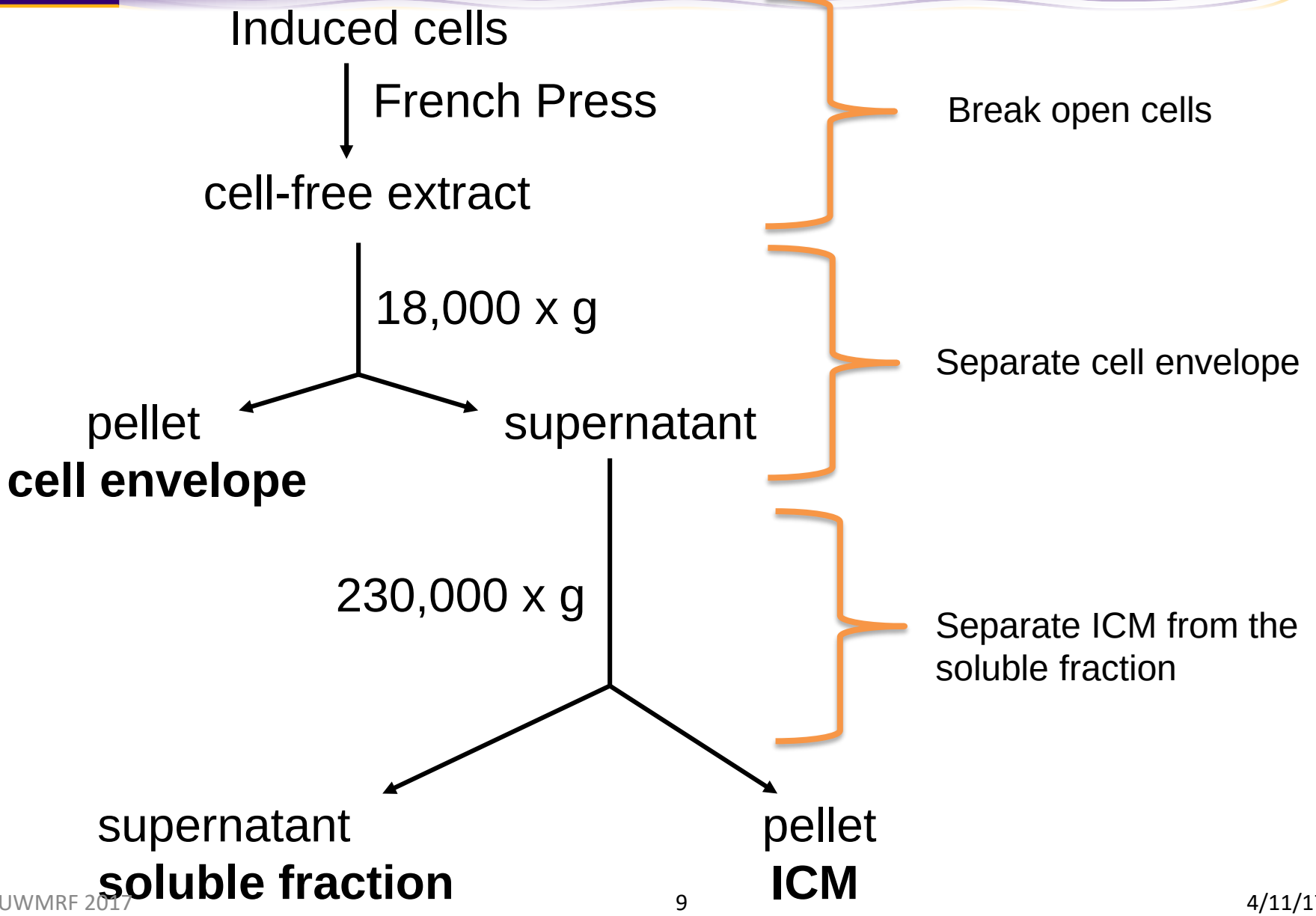
No promoter

Expression vector

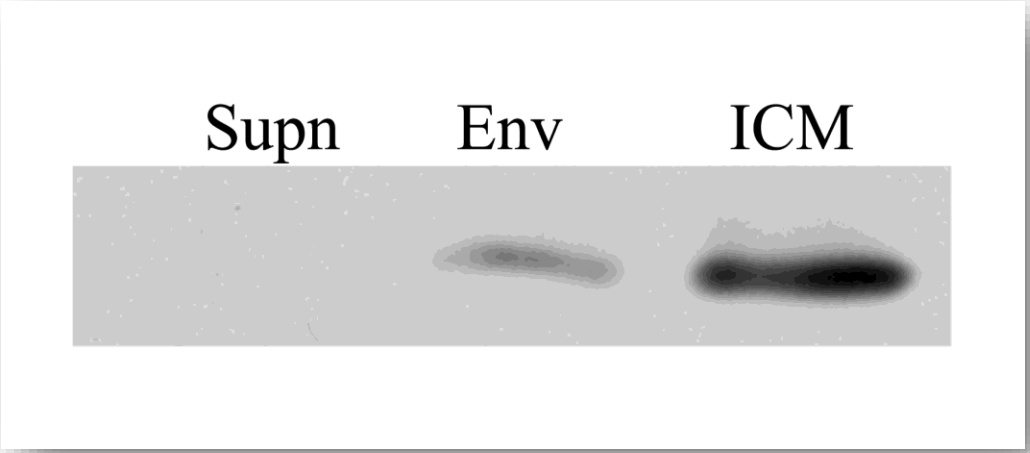
No *mscL* gene

MscL = 14 kDa *Pseudomonas* transport protein with 2 transmembrane domains
aer = aerobic conditions
ind = reduced oxygen conditions

Method for Harvesting from ICM



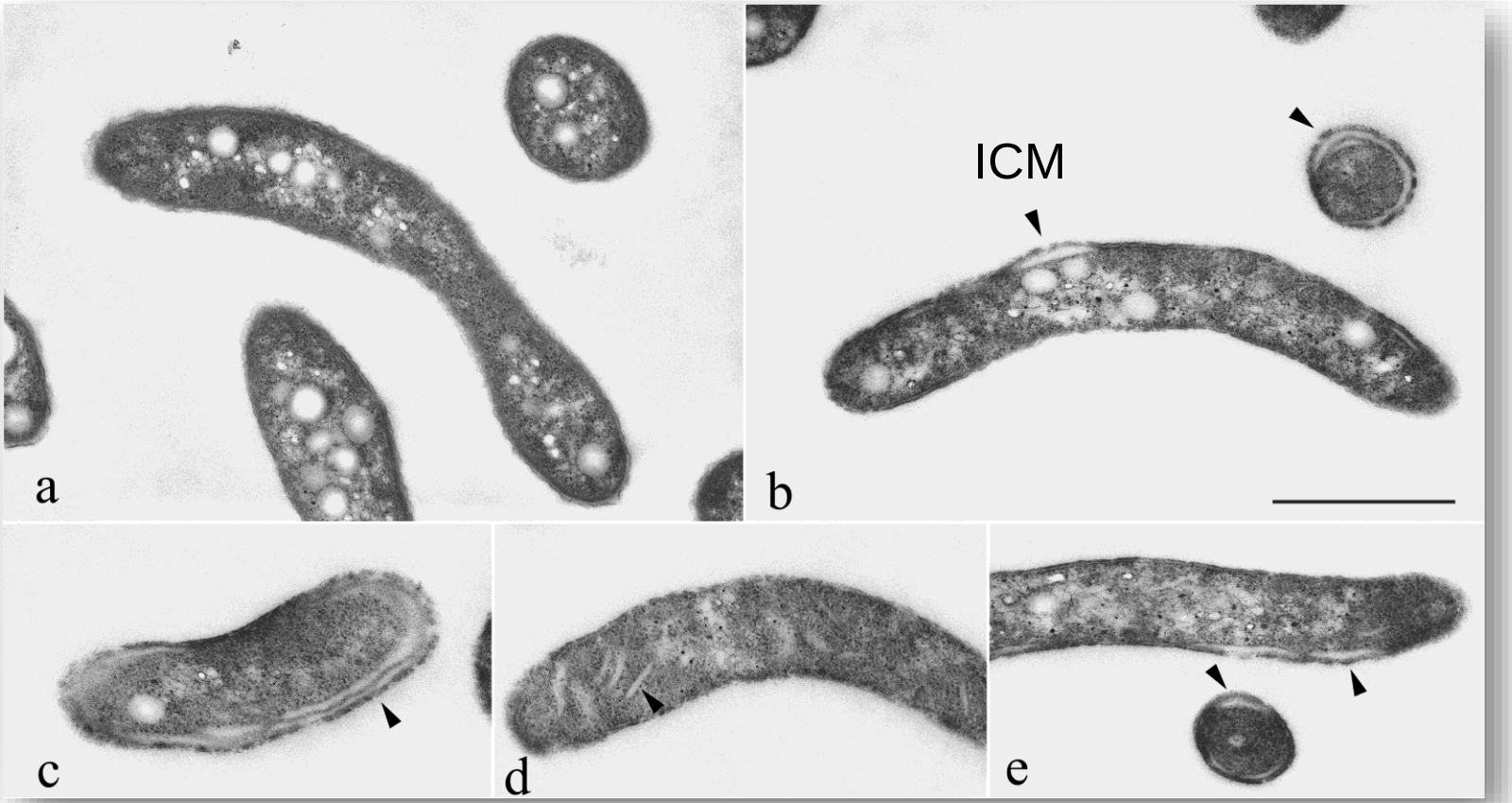
MscL localization in *R. rubrum* H2



•MscL is enriched in the intracytoplasmic membrane fraction compared to the cell envelope

MscL stimulates ICM formation in *R. rubrum* (b – e)

Expression vector control



Method for Protein Production

300 ml cultures in Fernbach flasks

↓ **incubation with reduced aeration**

induced cultures

↓ **harvest, wash, French Press**
230,000 x g

pellet (crude membrane fraction)

↓ **dodecylmaltoside (DDM)**
ultracentrifugation

soluble extract

↓ **IMAC**
(immobilized metal affinity chromatography)

assess purity and recovery

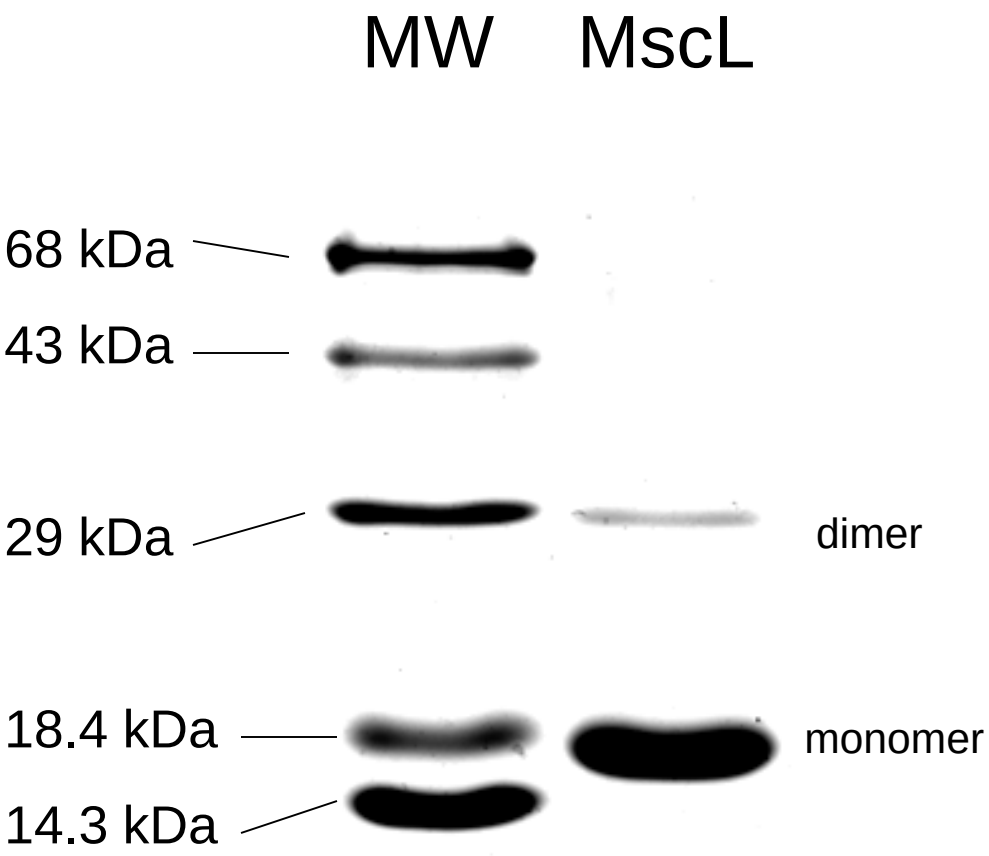
Grow and induce cells

Harvest and break open cells

Use of detergent to solubilize membrane proteins

Purification of tagged protein of interest

Recovery and Purification of MscL



R. rubrum vs. *E. coli* expression

***R. rubrum*:**

- **23.4 mg/L**
- **5.6 mg/g cell paste**

***E. coli* C43 (DE3):**

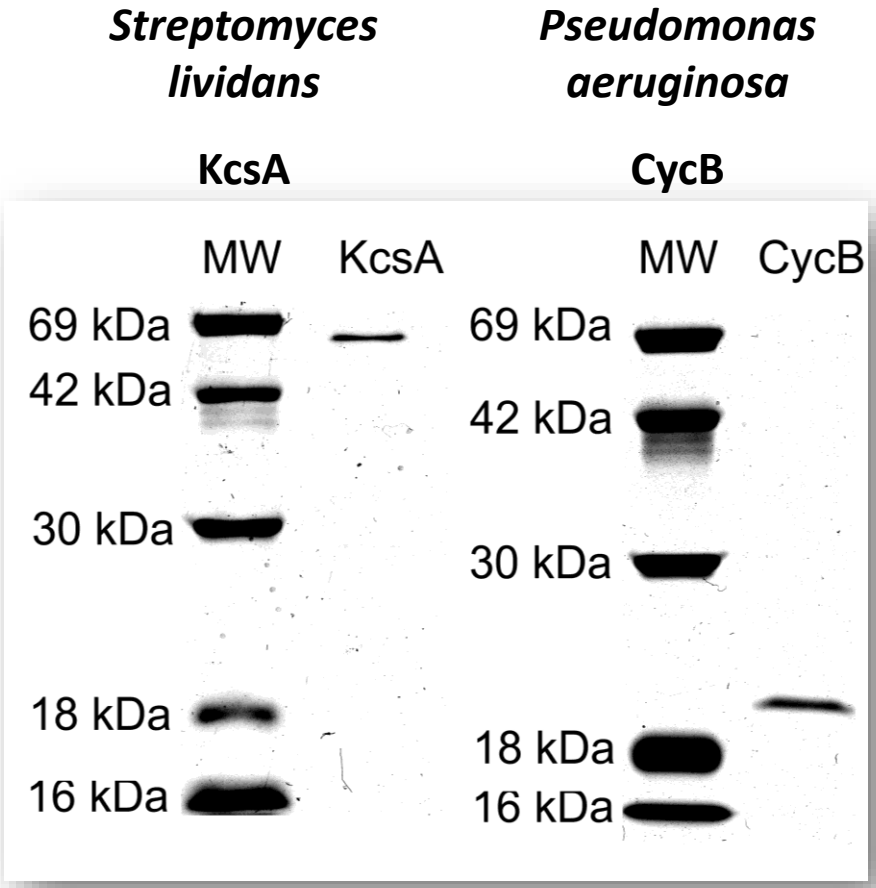
- **No detectable MscL recovered using same protein recovery system**
- **MscL appears to be in inclusion bodies**

Direct comparison in *E. coli*

E. coli				H2
2500pellet	Supn	CMF	SMP	CMF
				
500X	**500X**	**500X**	**250X**	**1X**

- **Supn is 230,000 x g supernatant fraction**
- **CMF is crude membrane fraction (230,000 x g pellet)**
- **SMP is DDM solubilized membrane protein**

Other Proteins Expressed in *R. rubrum* H2



KcsA in *R. rubrum*

- 13.7 – 14.4 mg/L
- 2.19-2.55 mg/g cell paste
- Purified to homogeneity

KcsA in *E. coli* C41

- 4.5 mg/L
- 75% pure
- Ron Viola, personal communication

Protein Recovery in *R. rubrum* H2

Protein	mg/L culture	mg/g cell paste	mg/mg crude membrane protein
MscL	22.8 – 23.4	5.53 – 5.60	0.81 – 0.106
KcsA	13.7 – 14.4	2.19 – 2.55	0.042 – 0.081
CycB	6.57 – 7.36	1.12 – 1.21	0.038 – 0.065

Proteins produced in native conformation

- CycB difference spectrum and heme peroxidase activity
- Purified MscL and KcsA are present as oligomers

Summary

- This protein expression kit allows for the expression of foreign membrane proteins where other expression systems may fail
- Potential for efficient, large-scale production of proteins for multiple uses such as vaccines, therapeutics, enzyme therapies and basic research
- Purification of the membrane protein is simple
- Expression of the system is simple and inexpensive using low oxygen levels
- The bacterium *R. rubrum* is easy to work with and non-pathogenic

Next Steps

- Needs a multiple cloning site in vector; currently only 2 sites
- Carry out further side by side expression testing with *E. coli* to evaluate
- Optimize competent cells to be use with kit

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