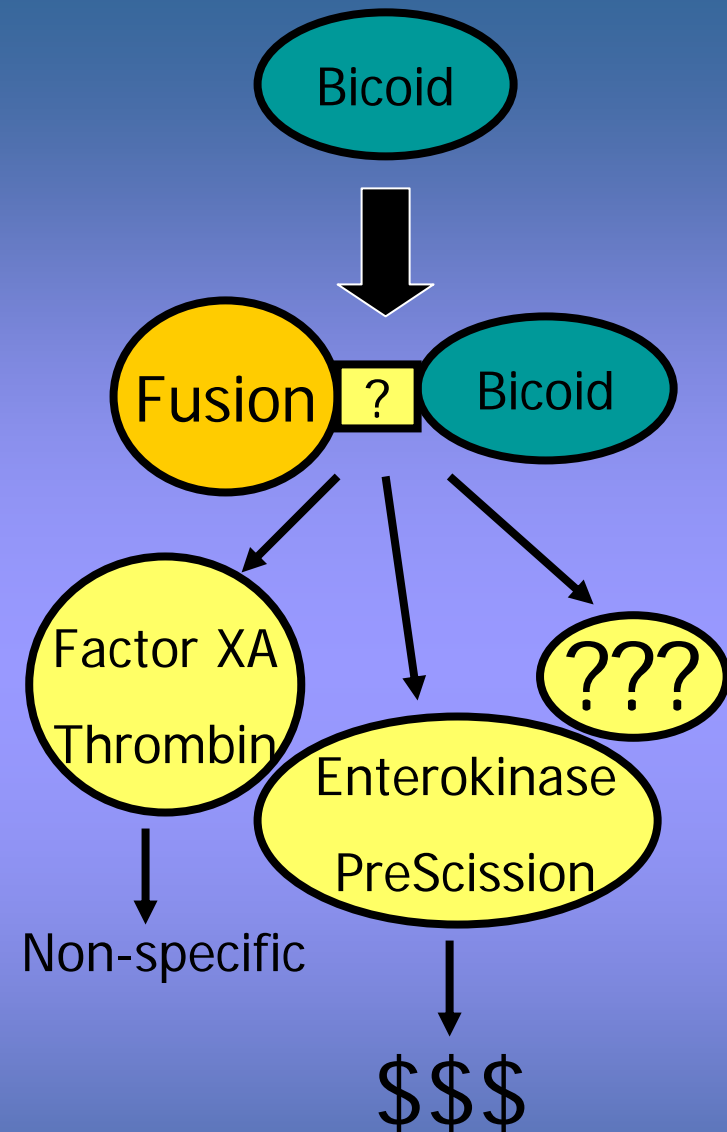


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MACROMOLECULAR CRYSTALLOGRAPHY LABORATORY

NATIONAL  
CANCER  
INSTITUTE

David S. Waugh, Ph.D.  
Protein Engineering Section

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Position Available

Dr. Waugh earned his Ph.D. in Biochemistry from Indiana University in 1989 under the direction of Dr. Norman Pace. He was a Postdoctoral Fellow in Dr. Robert Sauer's laboratory at the Massachusetts Institute of Technology before becoming Director of the Macromolecular Engineering Laboratory at Hoffmann-La Roche in 1991. In 1996, Dr. Waugh established the Protein Engineering Section at the NCI-Frederick Cancer Research and Development Center.

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Tobacco Etch Virus

Tobacco Vein Mottling Virus

<http://mcl1.ncifcrf.gov/waugh.html>

(1) Why TEV/TVMV?

(2) Obtaining the TEV or TVMV Proteases

(3) Production

(4) Cleavage of your fusion protein

(5) Novel Applications/ Just For Fun


- Easy to Purify in Soluble form in Large Quantities
- Highly Specific Cleavage Sites (7aa)
- Inexpensive to Produce
- Leaves only one non-native N-terminal glycine on your protein of interest
- Easy to remove from your protein after cleavage is complete

# How to Obtain the TEV and/or TVMV Proteases

# Obtaining the TEV/TVMV Proteases for your Lab

<http://addgene.org>


Addgene: Plasmid Repository for Life Sciences http://www.addgene.org/pgvcc1

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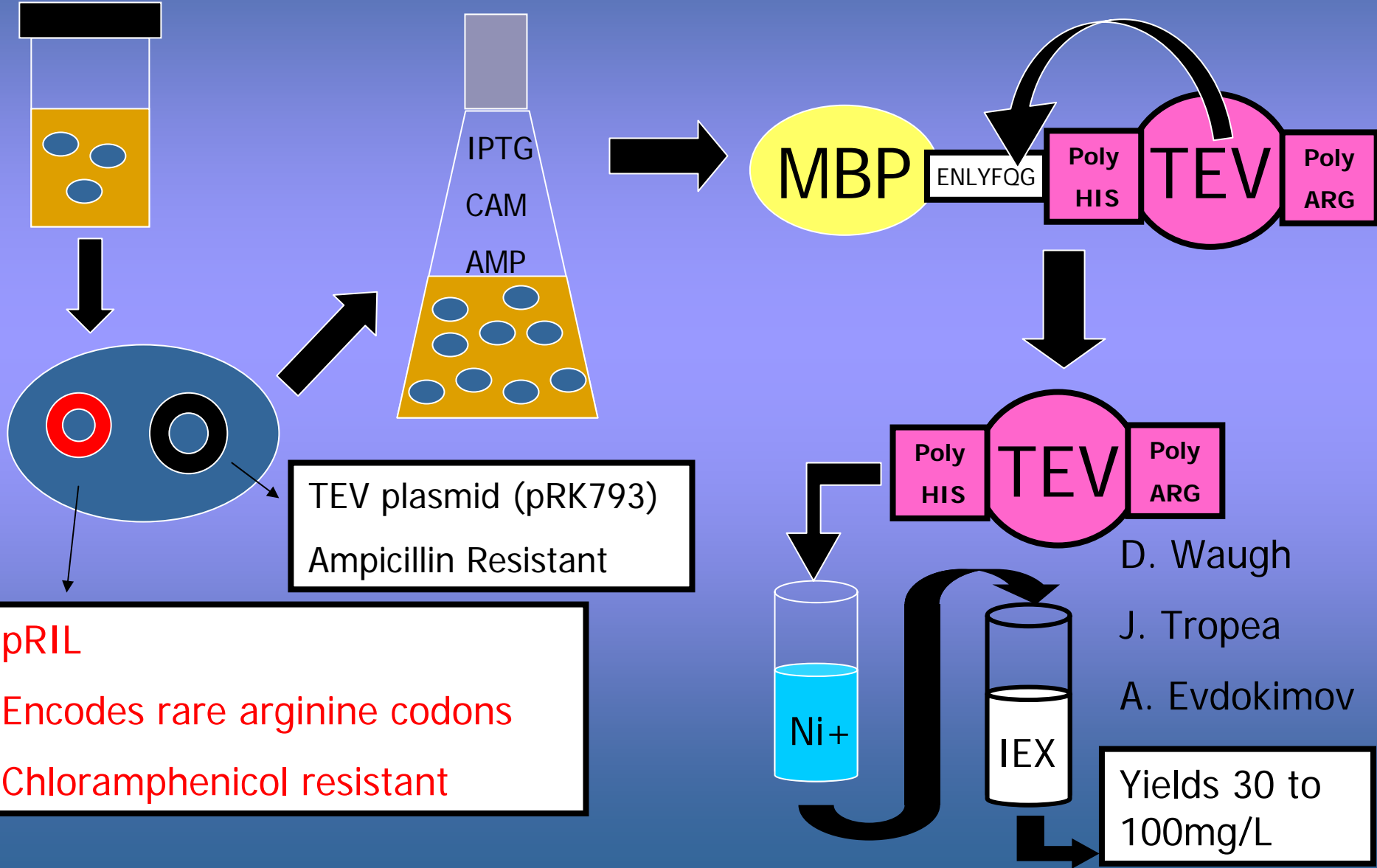
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*Science* 25 March 2005: Vol. 307. no. 5717, p. 1877

# Production of the Protease in your Lab

# Production of the TEV/TVMV Protease



TEV plasmid (pRK793)  
Ampicillin Resistant

pRIL  
Encodes rare arginine codons  
Chloramphenicol resistant

D. Waugh  
J. Tropea  
A. Evdokimov  
Yields 30 to 100mg/L

# Special Notes on TEV Production

- Sonication of the cell pellets containing the TEV protease may result in inactivation
- Recommended reagents, buffers, and more detailed protocols can be found on the website of Dr. Waugh
- Most buffers contain minimum 5% glycerol; TEV is stored in buffer containing 40% glycerol
- TEV is stable at  $-80^{\circ}\text{C}$  for 1 year



Incorporation of the TEV  
cleavage site into your system

5'- EcoRI\_TEV Site\_*5' end of the Protein of Interest* 3'-

5'- GCAC GAA TTC GAA AAC CTG TAT TTT CAG GGT CCA CGT CGC ACC CG 3'-  
E N L Y F Q G

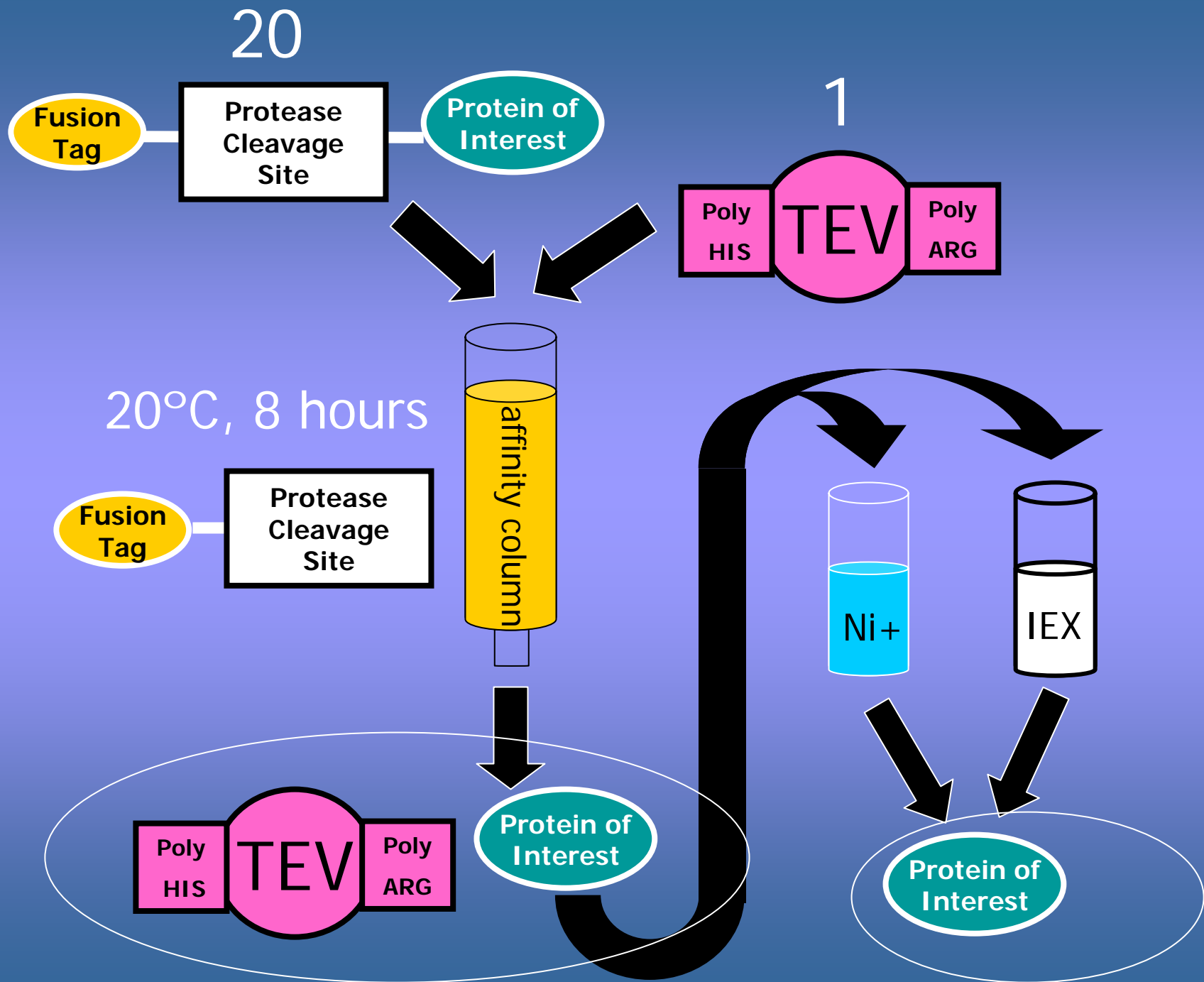
3'- *3' End of the Protein of Interest*\_STOPSTOP\_HindIII 5'-

3'- GTC GTG TTC CTG GTC AGG ATT ATT TTC GAA CGG CGC 5'-

5' EcorI/TEV Site/Protein of Interest/STOPSTOP/HindIII 3'

3' 5'

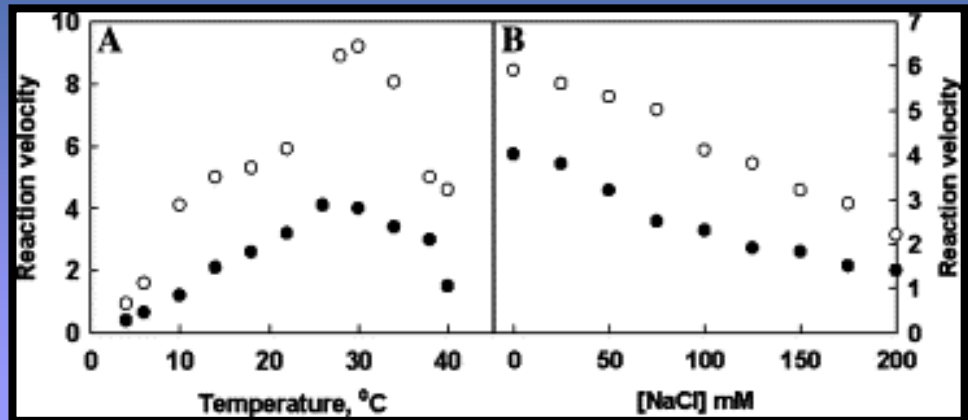
Using the TEV or TVMV Protease in  
your System



## Reaction Optimization

Optimum T = 30°C

Optimum [salt] = 0mM



## TEV/TVMV Cross-reactivity

### And Kinetic Parameters

Kinetic parameters for TVMV and TEV proteases

Substrate	Enzyme	$K_M$ (mM)	$k_{cat}$ ( $s^{-1}$ )	$k_{cat}/K_M$ ( $mM^{-1}s^{-1}$ )
TENLYFQ/SGTRR	TEV	$0.061 \pm 0.010$	$0.16 \pm 0.01$	$2.62 \pm 0.46$
TETVRFQ/SGTRR	TVMV	$0.065 \pm 0.009$	$0.07 \pm 0.01$	$1.08 \pm 0.17$
TENLYFQ/SGTRR	TVMV	Not cleaved		
TETVRFQ/SGTRR	TEV	Not cleaved		

# Just For Fun

