**Tobacco Etch Virus Proteinase test cleavage**  
Wednesday, October 8, 2003

10 × mTEVP buffer (20 mL made):
- 500 mM Tris, pH 8.0
- 5 mM EDTA [ethylenedinitrilato] tetraacetic acid disodium salt (mw 372.2, 0.0372 g in 20 mL)
- 10 mM DTT (mw 154.25, 0.038 g in 20 mL)

NEB usBiological mTEVP, cat. # T7950, lot # 21071301M
This mutant has reduced self-cleavage and contains a His tag.

Two 60-μL reactions were set up both at room temp.  
and (in the heating block) at 30°C.

a: 20 μL (10 μg) 0.5 mg/μL protein
   25 μL sterile water
   5 μL 10 × mTEVP buffer
   0 μL mTEVP

b: 20 μL (10 μg) protein soln.
   25 μL sterile water
   5 μL 10 × mTEV proteinase buffer
   0.4 μL mTEVP (8 units/μL) 3 units

\[ 24°C \]

\[ 24°C \]

C: as "a" at 30°C

D: as "b" at 30°C

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**Image:**

- Anti-FLAG

<table>
<thead>
<tr>
<th>Lane</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10 μL ladder</td>
</tr>
<tr>
<td>2</td>
<td>9 μL a (c), t=0</td>
</tr>
<tr>
<td>3</td>
<td>9 μL b (d), t=0</td>
</tr>
<tr>
<td>4</td>
<td>9 μL a (c), t=1 h</td>
</tr>
<tr>
<td>5</td>
<td>9 μL b (d), t=1 h</td>
</tr>
<tr>
<td>6</td>
<td>9 μL a (c), t=2 h</td>
</tr>
<tr>
<td>7</td>
<td>9 μL b (d), t=2 h</td>
</tr>
<tr>
<td>8</td>
<td>9 μL a (c), t=3.75 h</td>
</tr>
<tr>
<td>9</td>
<td>9 μL b (d), t=3.75 h</td>
</tr>
</tbody>
</table>

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1  2  3  4  5  6  7  8  9  

\[ a + b \text{ (24°C)} \]

\[ c + d \text{ (30°C)} \]