Sortase A

Week 1

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click Summary in the footer.

Summary

1. Testing conjugation between spider silk and a peptide

2
1 Testing conjugation between spider silk and a peptide

Responsible
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Protocols used
Sortase A Conjugation
SDS-PAGE

Modifications and comments to protocols
The reaction proceeded for 30 minutes at 37 °C instead of 3 hours at room temperature.

Experimental Data

Samples: 10 ml of 521 µM Z\textsubscript{Q32C}-SR-H\textsubscript{6} was dissolved in 1x Sortase A binding buffer.
25 ml 10x Sortase A binding buffer
2 tubes with 50 µl of 1mM Sortase A in Sortase A storage buffer.
30 mg/ml Spider silk

Calculations
Total volume = 550 µl.
Amount of Z\textsubscript{Q32C}-SR-H\textsubscript{6} = 230 µl ⇒ (c_2*V_2)/(c_1)=(n_2)/(c_1)=V_1=120nmol/521µl = 230 µl.
Amount of Sortase A Binding buffer= 55 µl ⇒ 1x in final volume, 10x in stock, (1x/10x)*Total volume= 0.1*550= 55 µl
Amount of Sortase A = 2.75 µl ⇒ V_1=(5µM*550µl)/1mM=2.75 µl
Amount of Spider silk with N-terminal= 231µl ⇒ M_w=22.997kDa
Concentration = 3 mg/ml = 130 nmol/ml
Volume = (30nmol)/(139nmol/ml) = 231 µl
Results and Conclusions

Table 1: Order of the samples in Figure 1.

<table>
<thead>
<tr>
<th>Well</th>
<th>Sample</th>
<th>Date</th>
<th>Temperature for conjugation(°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ladder</td>
<td>28/6</td>
<td>37</td>
</tr>
<tr>
<td>2</td>
<td>Negative Control</td>
<td>28/6</td>
<td>37</td>
</tr>
<tr>
<td>3</td>
<td>Spidersilk + Z</td>
<td>28/6</td>
<td>37</td>
</tr>
<tr>
<td>4</td>
<td>Negative Control</td>
<td>7/7</td>
<td>37</td>
</tr>
<tr>
<td>5</td>
<td>Spidersilk + Z</td>
<td>7/7</td>
<td>37</td>
</tr>
<tr>
<td>6</td>
<td>Negative control</td>
<td>7/7</td>
<td>RT</td>
</tr>
<tr>
<td>7</td>
<td>Spidersilk + Z</td>
<td>7/7</td>
<td>RT</td>
</tr>
<tr>
<td>8</td>
<td>Ladder</td>
<td>7/7</td>
<td>RT</td>
</tr>
</tbody>
</table>

Figure 1: SDS-PAGE with samples described in Table 1.

Discussion and Troubleshooting

The sample in well 4-7 does not look good and has probably failed. The conjugation from 28/6 in well 3 looks as well a bit strange and have probably not worked either. The size should be SIZE and no clear band at that size can be seen.