Assembly by restriction enzyme of Lh Masp 1 Type 2
Summary
Another approach to address polymerization of spider silk monomers was performed through restriction enzyme cloning, in spite of additional scars that this technique.

MaSp1 type2 PCR product gel purification
Mireia
350 uL of MaSp1 type 2 PCR product were run on a 0.8% agarose gel (80 V, ~45 min)
09/04 - 09/09

Mireia

MaSp1 type2 restriction cloning into pJP22

Mireia

Transformation of DH5α with pJP22-MaSp1-2X

09/10

Alkaline lysis of DH5α transformed with pJP22-MaSp1-2X

Mireia

09/13
Alkaline lysis PCR of DH5α pJP22-MaSp1-2X

Mireia

Digestion of pJP22-MaSp1-2X with BamHI and Scal (A) / BglII and Scal (B)

Mireia

A: 3531 bp + 1570 bp
B: 3333 bp + 1768 bp

**Bold** number fragments contain a copy of the MaSp1 insert and are to be ligated to duplicate that insert and regenerate the Amp resistance gene.

Mireia and Felipe
As the PRC done on 9/19 showed the previous ligation wasn't successful, we decided to digest the 2X plasmid once again to try another ligation later. Digestion electrophoresis in agarose gel showed bands in the right spots:

OBS: band curviness due to putting too much DNA in the wells, what makes middle DNA travel faster through gel. Ends of the bands travel correctly and should be used for reference.

**Ligation of pJP22-MaSp1-2X fragments A and B (2nd time)**

Mireia and Felipe

<table>
<thead>
<tr>
<th>Item</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂O</td>
<td>1 uL</td>
</tr>
<tr>
<td>A (55,5ng/uL)</td>
<td>4 uL (~222 ng)</td>
</tr>
<tr>
<td>B (43ng/uL)</td>
<td>3 uL (~132 ng)</td>
</tr>
<tr>
<td>T4 Buffer 10X</td>
<td>1 uL</td>
</tr>
<tr>
<td>Ligase</td>
<td>1 uL</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>10 uL</strong></td>
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</table>

Reaction conditions:
<table>
<thead>
<tr>
<th>TEMP</th>
<th>TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td>30X</td>
<td>22°C</td>
</tr>
<tr>
<td></td>
<td>37°C</td>
</tr>
<tr>
<td></td>
<td>4°C</td>
</tr>
</tbody>
</table>

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**PCR of MaSp1 4-mer ligation (2nd time)**

Mireia

Result: 2-mer amplicon band.

Hypothesis: The primers can attach at the ends of the 4mer but also at the center, so probably we are obtaining only the 2mer amplicon. All primers attach and the polymerase is not able to amplify through the whole 4mer, because it finds an attached primer on its way. So: transform and check for colony growth, miniprep and digest for 4mer confirmation.

**Transformation of DH10B with the 2nd 4-mer ligation**

Mireia

Electroporation of 50 uL DH10B with 2 uL ligation. (1 mm cuvette, 1800 V)

Result: 5 colonies grew on the LBCb plate.

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Alkaline lysis of 5 MaSp1t2 4mer colonies
Mireia

Digestion of pJP22 MaSp1t2 4mer with PstI and KpnI for 4mer confirmation
Felipe and Mireia

As we couldn’t see the insert bands due to RNA on the XhoI/BamHI digestion, we decided to try again with PstI/KpnI because that would form more discernable bands, at ~1300 (2mer) and ~1500 (4mer)

<table>
<thead>
<tr>
<th>Item</th>
<th>Volume for 1 reaction</th>
<th>Volume for 7 reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>PstI NdeI</td>
<td>1uL</td>
<td>7 uL (used 5)</td>
</tr>
<tr>
<td>KpnI</td>
<td>1uL</td>
<td>7 uL (used 5)</td>
</tr>
<tr>
<td>H₂O</td>
<td>11uL</td>
<td>77 uL (used 77+4)</td>
</tr>
<tr>
<td>Buffer</td>
<td>2uL</td>
<td>14 uL</td>
</tr>
<tr>
<td>Total</td>
<td>15uL</td>
<td>105 uL</td>
</tr>
</tbody>
</table>
Result: A ~1500 band showed we have the 4mer insert.

**Digestion of pJP22-MaSp1-4X with BamHI and Scal (A) / BglII and Scal (B)**

Mireia

A: BamHI + Scal (Fast Digest Thermo) 1h ~37°C  
B: BglII + Scal (Pharmacia) overnight ~37°C

Result:
A: **3759** and 1570 (Not completely cut after 1h at ~37°C, after the gel it was left for 30 min at 37°C)  
B: 3333 and **1966**

**bold = contains the 4X MaSp1t2 insert.**

5 uL restriction check. 45 uL were run afterwards to purify the bold fragments:
Ligation of pJP22-MaSp1-4X fragments A and B

Colony PCR of the pJP22-MaSp1 8X transformation
Mireia

No 8mer band (~950b) was obtained.

Colony PCR 2 of the pJP22-MaSp1 8X transformation
Mireia

PCR done with more colonies this time (26) to confirm 8mer plasmid absence
No 8mer band (~950b) was obtained.

Digestion of pJP22-MaSp1-4X with BamHI and Scal (A) / BglII and Scal (B) (2nd time)
Mireia

Ligation of pJP22-MaSp1-4X fragments A and B (2nd time)