Re-transformation of the SPLINTR libraries

<table>
<thead>
<tr>
<th>SPLINTR library</th>
<th>Total number of barcodes</th>
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<tbody>
<tr>
<td>SPLINTR GFP</td>
<td>200,000</td>
</tr>
<tr>
<td>SPLINTR BFP</td>
<td>700,000</td>
</tr>
<tr>
<td>SPLINTR mCHERRY</td>
<td>1,200,000</td>
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1) Thaw Endura electrocompetent cells on ice immediately prior to use (Lucigen, #60242-2). Chill the 0.1 cm electroporation cuvettes on ice. Warm Lucigen recovery media to 37 degrees.

2) Add 200 ng (dilute in water to 10 uL maximum volume) of SPLINTR library to 50 uL of competent cells on ice. Mix gently.

3) Electroporate the 50 uL of transformation mix in the chilled cuvette using the following settings:

<table>
<thead>
<tr>
<th>Optimal Settings</th>
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<tbody>
<tr>
<td>0.1 cm cuvette</td>
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<tr>
<td>10 µF</td>
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<tr>
<td>600 Ohms</td>
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<tr>
<td>1800 Volts</td>
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4) Immediately add 950 uL of pre-warmed Recovery Media (Lucigen, arrives with the cells) to the cuvette. Transfer to a bacterial culture tube and recover at 37 °C for 1 hour on a shaking platform at 250 RPM.

5) Once cells are recovered, perform the following serial dilutions to estimate transformation efficiency. Incubate plates for 16 hours at 37 °C. This will enable estimation of transformation efficiency to ensure final library barcode representation is maintained. Aim for ~50-200 colonies per unique barcode in the final library.

6) Put the remaining 990 uL of the transformation in 500 mL of LB broth supplemented with ampicillin (100 ug/mL) and grow at 37 for 16 hours °C.

7) If the library efficiency is good based on the colony number determined by the serial dilutions, proceed with maxi-prep using preferred kit.
8) Conduct 2-step barcode PCR on 10ng of the final plasmid pool using the population-based barcode PCR protocol. Conduct this in technical duplicate and use separate, compatible indices on the second step PCR. Sequence at 100M paired end reads per replicate. See additional protocols for reference library construction.