The Role of LincRNA in B Cells
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Background
- LincRNA is capped, multi-exonic, tissue specific, and developmentally regulated.
- shRNA makes a tight hairpin turn that silences gene expression by RNA interference.
- Research on lincRNA may explain organism diversity.
- Since cell lines can be created with Pre-ProB and ProB Cells, these two types are used in research on lincRNA.

Methods

Plasmid Growth
(Figure 3. LB/Amp plate)
- Allow E.coli bacteria to take up plasmid on a LB/Ampicilin plate.

Figure 1. Overall Project Procedure.

Part of the project that is currently focused on:
- Design several shRNA constructs against each lincRNA chosen and determine which shRNA is most effective in lincRNA’s expression knockdown.

Plasmid (blue) is longer than LMP (red). Therefore, shRNA is successfully incorporated in the plasmid.

Transfection
- Allow 293T, human kidney cells, to take up and grow the plasmid plus packaging plasmid.
- Overnight, cells will secrete plasmid-contained virus into the liquid media.

Spin Infection
- Mix B Cells with the liquid media that contains virus.
- Infection the same B Cells again by mixing them with the liquid media that contains virus.

Results

Table 1. Amount of DNA after Mini-Prep.

<table>
<thead>
<tr>
<th>Purified</th>
<th>Amount (ng/µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB9b</td>
<td>619.6</td>
</tr>
<tr>
<td>PB9c</td>
<td>723.3</td>
</tr>
<tr>
<td>PB10a</td>
<td>550.2</td>
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<td>PB10b</td>
<td>730.5</td>
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<tr>
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<tr>
<td>PPB7b</td>
<td>512.4</td>
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<tr>
<td>PPB8a</td>
<td>513.8</td>
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- Determine how well B Cells were infected by flow cytometry and expression of CD25.

Discussion and Conclusion
- There are variances in the amount of transcript after shRNA infection illustrated in Figure 12. Overall, shRNA knockdown was more effective in the IgH1-lincRNA than it was in IgH2-lincRNA as shown in Figure 13. b and c presented the most knockdown; better constructs shall be examined to demonstrate a more efficient knockdown. For IgH2-lincRNA, there was a complication before RT-PCR in 2b, with hardly any RNA found in purification for that sample. Clearly, our shRNA constructs against IgH2-lincRNA were ineffective. In future projects, new constructs will be created to successfully knockdown the expression in IgH2.
- After the most effective shRNAs are determined for each lincRNA, changes and differentiation in B Cells can be analyzed.

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