You have been given a target pool of transcripts (each color corresponds to a different sequence). Your job is to find the abundances of these transcripts using SQUICH.

In each round of SQUICH, you will expose the target pool to a mixture of coding and blocking oligos. These mixtures have been prepared ahead of time. The coding and blocking oligos are sequence-specific.

If same-sequence coding oligos and/or blocking oligos are present, the target transcript will duplex randomly with one of them (indicated by linking the target transcript paperclip and the labeled "coding" or "blocking" paperclip of the same color).

If there are no coding or blocking oligos available, then the transcript will remain single-stranded. A target transcript that duplexes (i.e. linked paperclips) with a coding oligo can be read out at the end by
sequencing (indicated by placing the linked paperclips in the "coded" pile). A target transcript that duplexes with a blocking oligo will not be read out (indicated by placing the linked paperclips in the "blocked" pile). If a transcript is coded or blocked in one round, it drops out of the pool and is no longer available in subsequent rounds.

Running SQUICH:
You will perform three rounds of SQUICH. Each round has a specific set of bags containing coding and blocking oligos (since the codes are round-specific). For each round,
1. Get all the bags for the current round. Randomly draw clips from each bag of mixed coding/blocking oligos and link them to the same-color targets. Continue until you run out of either target paperclips or coding/blocking paperclips.
2. Place all the coded duplexes in a common “coded” pile, and all the blocked duplexes in a common “blocked” pile. All duplexes, regardless of color or round, should end up in one of these two piles.
3. Set any unused coding/blocking oligos aside. These don’t get read out in sequencing.
4. Use the single-stranded (unlinked) remainder of the target pool for the next round.

Sequencing:
The coding oligos contain a “barcode” (code #) indicating at which round of SQUISH they were added. So, the duplexes in the “coded” pile contain both transcript and round information. To “sequence” your SQUICH products, fill out the following table:

<table>
<thead>
<tr>
<th>Color</th>
<th>Code 1 counts:</th>
<th>Code 2 counts:</th>
<th>Code 3 counts:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blue</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pink</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purple</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gray</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion questions:
1. Looking only at this table, what can you tell about the relative abundances of the paperclips?
2. Can you calculate the number of each sequence (color) present in the initial target pool?
3. Would performing another round be useful in this case? Why or why not? What determines how many rounds you need?
4. To get the same information and precision, how many reads would you need in traditional sequencing?