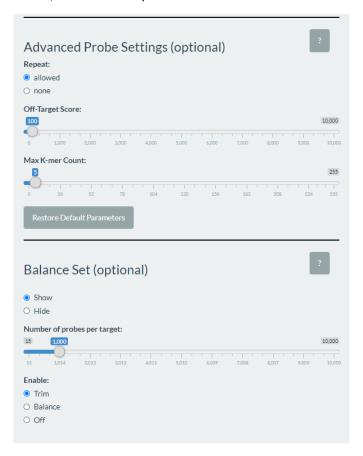**PaintSHOP Library Structure**

- 30 spots, 200 kb each
    - each spot has >= 1000 probes with <= 100 off-target score
- Structure
    - Outer F primer (forward, same for all probes)
        - "custom set", upload seq.txt: ATCCTAGCCCATACGGCAATG
    - 5' Bridge
    - Inner primer
    - 3' Outer primer
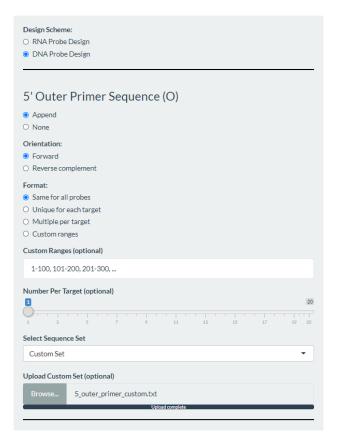        - "custom set", upload seq.txt: GTATCGTGCAAGGGTGAATGC

**PaintSHOP Workflow**

1. Go to https://oligo.shinyapps.io/paintshop/ and click "DNA Probe Design"

2. Set mode to "file" and upload BED file with regions
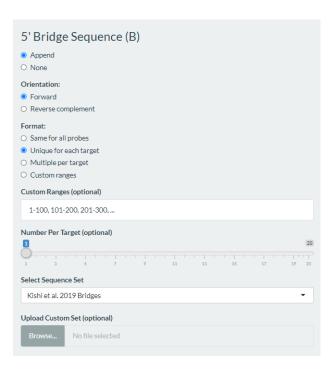
3. Once probes are loaded, filter to these params:



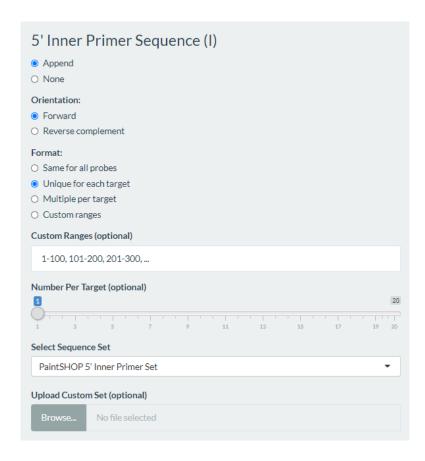4. Click the "Append Sequences" tab
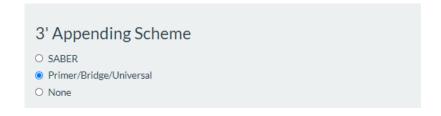
5.  Add the 5' outer primer:

    **Design Scheme:**
    ○ RNA Probe Design
    ● DNA Probe Design

    ## 5' Outer Primer Sequence (O)

    ● Append
    ○ None

    **Orientation:**
    ● Forward
    ○ Reverse complement

    **Format:**
    ● Same for all probes
    ○ Unique for each target
    ○ Multiple per target
    ○ Custom ranges

    **Custom Ranges (optional)**

    1-100, 101-200, 201-300, ...

    **Number Per Target (optional)**
    1                                                                    20
    1      3       5      7       9      11      13      15      17     19  20

    **Select Sequence Set**

    Custom Set                                                            ▼

    **Upload Custom Set (optional)**

    Browse...    5_outer_primer_custom.txt
    Upload complete

6.  Add the Kishi 2019 5' bridge sequences:

    ## 5' Bridge Sequence (B)

    ● Append
    ○ None

    **Orientation:**
    ● Forward
    ○ Reverse complement

    **Format:**
    ○ Same for all probes
    ● Unique for each target
    ○ Multiple per target
    ○ Custom ranges

    **Custom Ranges (optional)**

    1-100, 101-200, 201-300, ...

    **Number Per Target (optional)**
    1                                                                    20
    1      3       5      7       9      11      13      15      17     19  20

    **Select Sequence Set**

    Kishi et al. 2019 Bridges                                             ▼

    **Upload Custom Set (optional)**

    Browse...    No file selected

7.  Add the PaintSHOP 5' inner primer per-target:

## 5' Inner Primer Sequence (I)
○ Append
○ None

**Orientation:**
● Forward
○ Reverse complement

**Format:**
○ Same for all probes
● Unique for each target
○ Multiple per target
○ Custom ranges

**Custom Ranges (optional)**

    1-100, 101-200, 201-300, …

**Number Per Target (optional)**

`1`                                                                                    `20`

1      3      5      7      9      11      13      15      17      19   20

**Select Sequence Set**

    PaintSHOP 5' Inner Primer Set                                              ▼

**Upload Custom Set (optional)**

    Browse…      No file selected

8.  Choose the "Primer/Bridge/Universal" 3' appending scheme:

## 3' Appending Scheme
○ SABER
● Primer/Bridge/Universal
○ None

9. Add the custom 3' outer primer:



10. Click "Append" to append specified sequences to probes

11. Click the "Download" tab

12. Select DNA probe design and download the order file with appended sequences