

### PaintSHOP Library Structure

- 30 spots, 200 kb each
  - each spot has  $\geq 1000$  probes with  $\leq 100$  off-target score
- Structure
  - Outer F primer (forward, same for all probes)
    - “custom set”, upload seq.txt: ATCTAGCCCATACGGCAATG
  - 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:

The screenshot displays two sections of the PaintSHOP web application interface. The top section, titled "Advanced Probe Settings (optional)", includes a "Repeat:" dropdown set to "allowed", an "Off-Target Score:" slider set to 100, and a "Max K-mer Count:" slider set to 5. Below these is a "Restore Default Parameters" button. The bottom section, titled "Balance Set (optional)", includes a "Show/Hide" toggle set to "Show", a "Number of probes per target:" slider set to 1,000, and an "Enable:" dropdown set to "Trim".

4. Click the “Append Sequences” tab

## 5. Add the 5' outer primer:

Design Scheme:

☐ RNA Probe Design

☒ DNA Probe Design

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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

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

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 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 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:

3' 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

Select Sequence Set  
Custom Set

Upload Custom Set (optional)  
Browse... 3\_outer\_primer\_custom.txt  
Upload complete

Append

## 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

Used Appending:  
☒ Appended Sequences  
☐ Appended Barcodes  
☐ No

Design Scheme:  
☐ RNA Probe Design  
☒ DNA Probe Design

Choose a file to download:  
Order File

Download