Supplemental Material

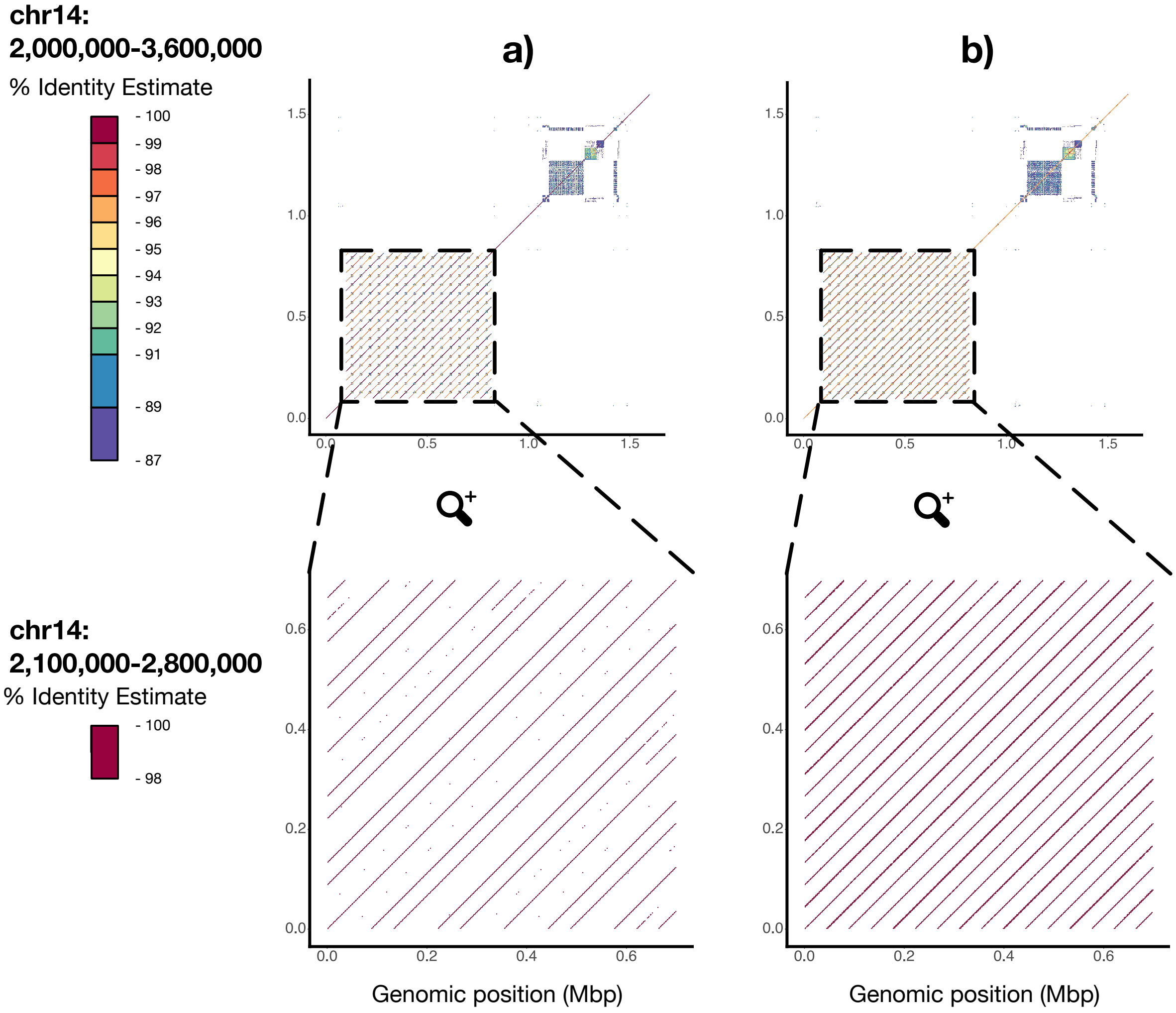
Algorithm 1 Partition Sequence S into sets of modimizers

```
    Input: List of hashed $k$-mers $S_k[x_1, x_2, ...x_n]$, window size $w$, sparsity $s$
    Output: List of modimizer sets $MOD_s = [A_1, A_2, ..., A_r]$
function BaseLayer($S_k$,$w$,$s$)
    $n \leftarrow$ length of $S_k$
    $r \leftarrow \lceil \frac{n}{w} \rceil$                         ▷ Set number of windows (resolution) based on $n$ and $w$
    $m \leftarrow \frac{w}{s}$                               ▷ Set the expected modimizer density
    $MOD_s \leftarrow$ list of size $r$
    for $i \leftarrow 0$ to $r$ do                         ▷ Retrieve all modimizers within each interval
        $MOD_s[i] \leftarrow \{\}$
        $start \leftarrow wi$                          ▷ Set non-overlapping interval boundaries
        $end \leftarrow \min((start + w), n - 1)$
        $MOD_s[i] \leftarrow$ GetModimizers$(S_k[start : end], s, d)$        ▷ Populate list with sets of modimizers
    end for
    return $MOD_s$
end function

function GetModimizers($S_k, s, m$)
    $A \leftarrow \forall x \in \{S_k[A]\} : x \equiv 0 \bmod s$        ▷ Gather the set of unique modimizers per interval
    $\hat{s} \leftarrow \frac{s}{2}$
    while $|A| < \frac{m}{2}$ and $\hat{s} > 1$ do     ▷ Resample at higher density if number of modimizers is under threshold
        $A \leftarrow \forall x \in \{S_k[A]\} : x \equiv 0 \bmod \hat{s}$
        $\hat{s} \leftarrow \frac{\hat{s}}{2}$
    end while
    return $A$
end function
```

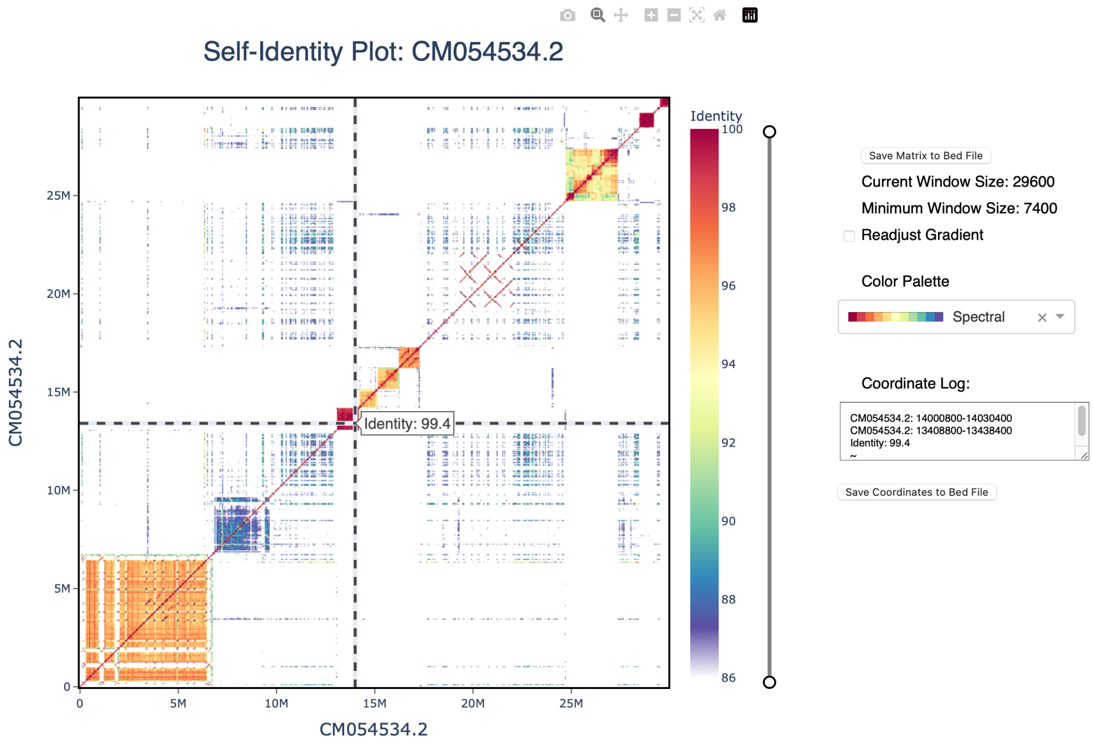
Algorithm 2 Partition Sequence S into a modimizer hierarchy H

```
    Input: List of hashed $k$-mers $S_k[x_1, x_2, ...x_n]$, minimum window size $\hat{w}$, sparsity $\hat{s}$, resolution $r$
    Output: List $H = [MOD_{\hat{s}}, MOD_{2\hat{s}}, ..., MOD_{2^{l-1}\hat{s}}]$
function BuildHierarchy($S_k$,$\hat{w}$,$\hat{s}$,$r$)
    $n \leftarrow$ length of $S_k$
    $l \leftarrow \lfloor \log_2 (\frac{n}{\hat{w}r}) \rfloor$          ▷ Initialize number of layers based on min. window size and resolution
    $H \leftarrow$ list of size $l$
    $H[0] \leftarrow$ BaseLayer$(S_k, \hat{w}, \hat{s})$                    ▷ Compute bottom layer
    for $i \leftarrow 1$ to $l$ do               ▷ Iteratively compute subsequent layers from previous layer
        $\hat{r} \leftarrow 2^{l-1-i}r$                   ▷ Halve the resolution when building subsequent layer
        $H[i] \leftarrow$ AddLayer$(H[i-1], 2^i\hat{s}, 2^i\hat{w}, n, \hat{r})$
    end for
    return $H$
end function

function AddLayer($MOD_{\hat{s}}[A_1, A_2, ..., A_r], s, w, n, \hat{r}$)
    $MOD_s \leftarrow$ list of size $\hat{r}$                              ▷ Initialize current layer
    $m \leftarrow \frac{w}{s}$                          ▷ Update expected sketch size for current layer
    for $i \leftarrow 0$ to $\hat{r}$ do       ▷ Retrieve modimizers from matching intervals within the previous layer
        $MOD_s[i] =$ GetModimizers$((MOD_{\hat{s}}[A_{2i}] \cup MOD_{\hat{s}}[A_{2i+1}]), s, m)$
    end for
    return $MOD_s$
end function
```

References



Supplementary Figure 1. Screenshots of ModDotPlot run on a human acrocentric short arm (CHM13 chr14:2,000,000-3,600,000), highlighting the rDNA array with and without ModDotPlot's interval extension activated. **a)** With no interval extension, the full 16 copy rDNA array is visible; however, when zoomed in (chr14:2,100,000-2,800,000) and filtered for >98% sequence identity, some rDNA copies disappear from the plot due to registration artifacts. **b)** With intervals extended (i.e. when computing similarity for the cell M(A,B), interval B is extended by $w/2$ in both directions), all rDNA copies appear at all zoom levels.



Current Window Size: 29600

Minimum Window Size: 7400

□ Readjust Gradient

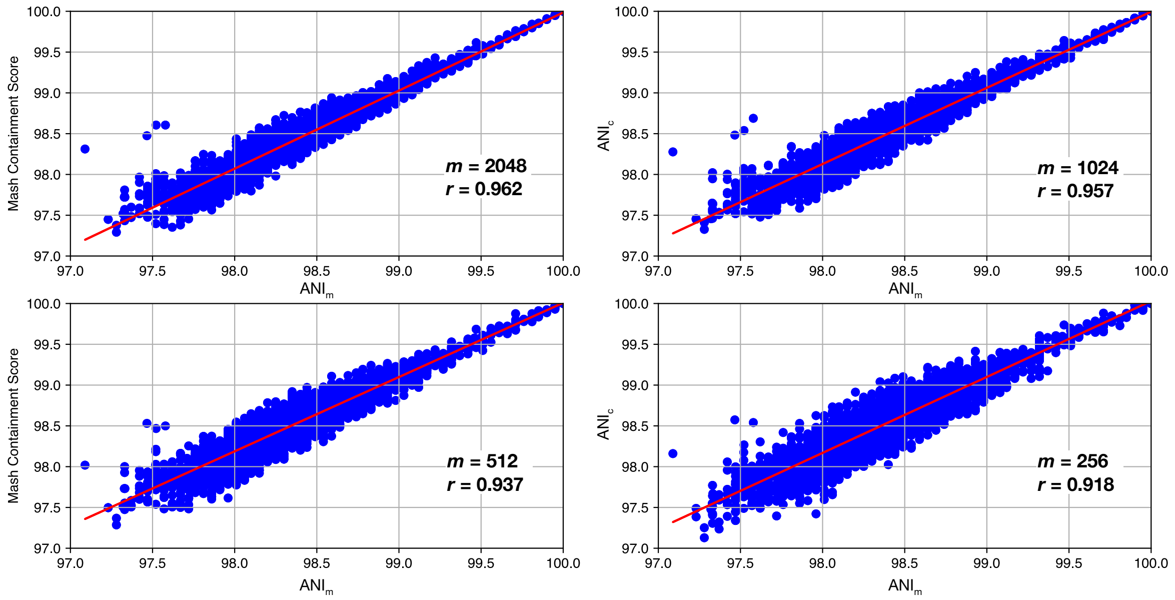
Color Palette

Spectral

Coordinate Log:

```
CM054534.2: 14000800-14030400
CM054534.2: 13408800-13438400
Identity: 99.4
~
```

Supplementary Figure 2. Screenshot of ModDotPlot's interactive mode, showcasing the entire Y chromosome of a gibbon (mSymSyn1, Makova *et. al* 2024)). Despite spanning almost 30 Mbp, ModDotPlot was able to create 3 matrices in under 2 minutes, with around 2.5GB of memory. Screenshot was taken using ModDotPlot version v0.8.0 (git commit `ed190c7`).



Supplementary Figure 3. Scatterplot of ANI_m against the ANI_c distances under various sketch sizes. The 10-mer non-canonical HOR region from Figure 4 was excluded from analysis.