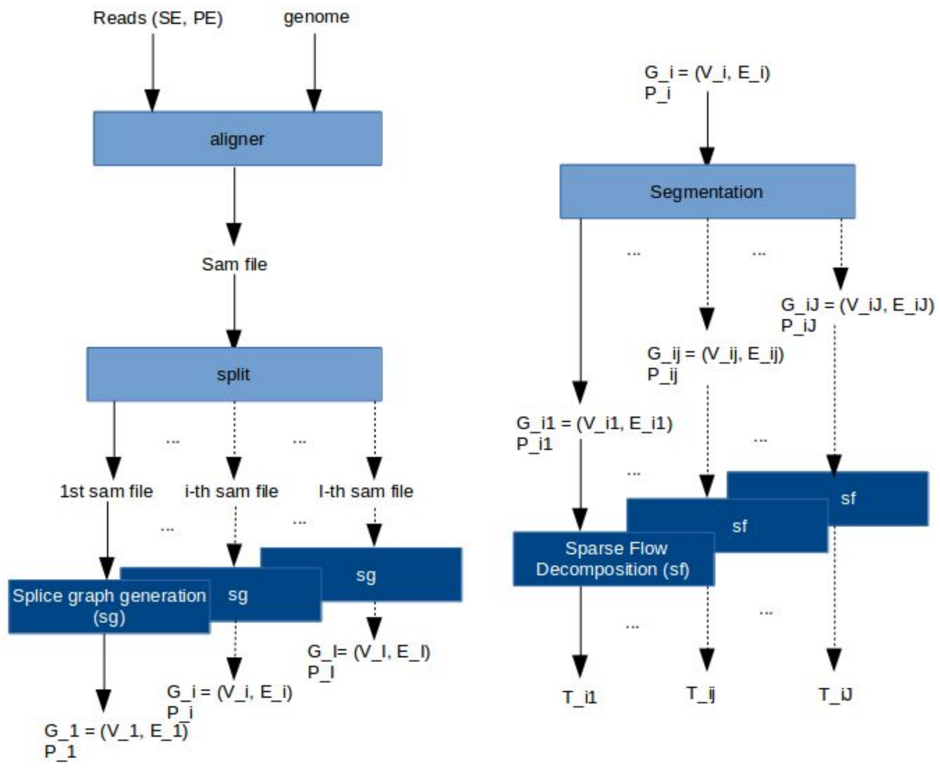
Supplementary File 2: RefShannon Algorithm Details

1 Overall Flow

Figure 1 shows a more implementation-based (rather than concept-based) overall flow of RefShannon.

Figure 1: **Overall Flow of RefShannon**. Note SE means single end, PE means pair end. sam file is a file format to store read alignment. P_i is set of known paths associated with splice graph G_i, which can be further segmented into sub splice graphs G_{ij} with associated known paths P_{ij}. T_{ij} is the reconstructed transcriptome for G_{ij}. Dark blue blocks are where parallization applies.



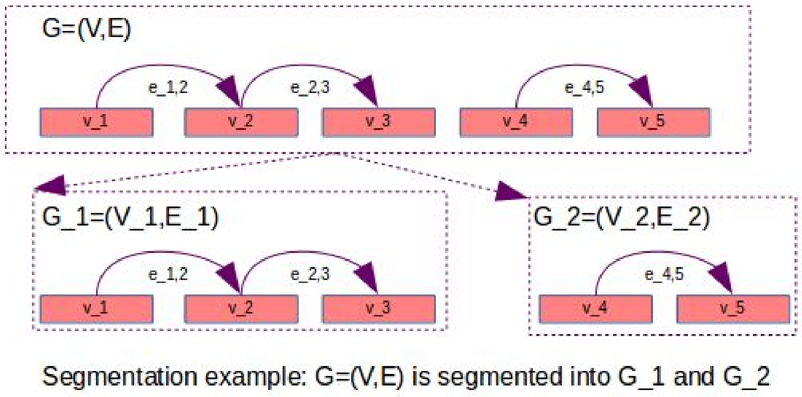
We first use alignment softwares to align reads that are either single end (SE) or pair end (PE) reads onto genome to obtain read alignment that is usually stored in sam file format (`https://samtools.github.io/hts-specs/SAMv1.pdf`). A genome can contain multiple chromosomes where DNA sequences are packed in (e.g. human genome hg19 contains chromosomes chr1, ..., chr15,...,etc).

We then split read alignment based on target chromosomes. For each smaller alignment (e.g. i-th sam file) targeted at a particular chromosome (e.g. i-th chromosome), we build a splice graph (e.g. $G_i = (V_i, E_i)$). We segment it further (as illustrated in Figure 1 and Figure 2) into (sub) splice graphs (smaller connected components) $G_{i1} = (V_{i1}, E_{i1}), ..., G_{iJ} = (V_{iJ}, E_{iJ})$. We could consider each (sub) splice graph G_{ij} as j-th gene region on i-th chromosome, and the transcripts recovered from G_{ij} (i.e. denoted as T_{ij} in Figure 1) are j-th gene's isoforms.

We then apply a sparse flow decomposition algorithm on every (sub) splice graph G_{ij} for transcript recovery.

Parallelization (e.g. multiprocess in Python) is used in splice graph generation for different chromosomes and also used in sparse flow decomposition for different G_{ij}s within each i-th chromosome.

Figure 2: **Segmentation Example**. G is segmented into two connected components G_1 and G_2.



2 Splice Graph Generation

Algorithm 1 is a pseodo code of our splice graph generation method based on read alignment using pair end reads. Modification to use single end reads is straight forward.

Algorithm 1: Splice Graph Generation for Pair End Reads

```
Input: Set of read alignments $R^A = \{r_i^A\}$ and $R^B = \{r_i^B\}$ where
       $(r_i^A, r_i^B)$ form i-th pair read alignments, as well as ROC tuning
       parameter $F \in [0, 1]$.
Output: Splice graph $G = (V, E)$ and known paths $P_{known}$. $v \in V$
        refers to a genomic region (e.g. exon or a sub part of exon),
        $e = (v_1, v_2) \in E$ means there's flow from $v_1$ to $v_2$. Edge
        weight is also recorded and described by $w_e$. $p \in P_{known}$ is a
        tuple of triple nodes that is traversed by some known flow.
1  Infer read coverage $c$ from read alignment $R^A \cup R^B$.
2  Infer splicing information $s$ (splice junctions, splice donors and
     acceptors) from $R^A \cup R^B$. This could be inferred from the CIGAR
     pattern in sam file format of each read alignment.
3  Build initial set of (sub) exon regions $V^{(0)} = \{v^{(0)}\}$ from $c$ and $s$.
4  Merge (sub) exon regions from $V^{(0)}$ to get $V$ (i.e. node set $V$ in splice
     graph $G = (V, E)$):
5  Init $V = \emptyset$
6  for $v_i^{(0)} \in V^{(0)}$ do
7      merge $v_{i-1}^{(0)}$ and $v_i^{(0)}$ into $v_i^{(0)}$ if necessary:
8      if (1) $Gap(v_{i-1}^{(0)}, v_i^{(0)}) \leq T_{small\_gap}$ or (2)
         $Gap(v_{i-1}^{(0)}, v_i^{(0)}) \in (T_{small\_gap}, T_{mid\_gap}]$ and $v_{i-1}^{(0)}$ and $v_i^{(0)}$ have
         coverage sum $\in [T_a, T_b(F)]$ then
9          merge $v_{i-1}^{(0)}$ and $v_i^{(0)}$ into $v_i^{(0)}$
10     otherwise, $V \leftarrow V \cup \{v_{i-1}^{(0)}\}$
11 $V \leftarrow V \cup \{v_{remained}^{(0)}\}$
12 Build edge set $E$ and known path set $P_{known}$ as following:
13 Init $E = \emptyset, P_{known} = \emptyset$
14 for pair $r_i^A \in R^A, r_i^B \in R^B$ do
15     find $r_i^A$ goes through regions $v_{a_1}, ..., v_{a_m}$
16     find $r_i^B$ goes through regions $v_{b_1}, ..., v_{b_n}$
17     $E \leftarrow E \cup \{e_j = (v_{a_j}, v_{a_{j+1}})\}$, also update $w_{e_j}$ for $j \in \{1, ..., m-1\}$
18     $E \leftarrow E \cup \{e_k = (v_{b_k}, v_{b_{k+1}})\}$, also update $w_{e_k}$ for $k \in \{1, ..., n-1\}$
19     $P_{known} \leftarrow P_{known} \cup \{(v_{a_j}, v_{a_{j+1}}, v_{a_{j+2}})\}$ for $j \in \{1, ..., m-2\}$
20     $P_{known} \leftarrow P_{known} \cup \{(v_{b_k}, v_{b_{k+1}}, v_{b_{k+2}})\}$ for $k \in \{1, ..., n-2\}$
21     (assume $v_{a_1} < v_{b_1}$, or we can swap $r_i^A$ and $r_i^B$)
22     if there is no $v \in V$ s.t. $v$ is between $v_{a_m}$ and $v_{b_1}$ then
23         $E \leftarrow E \cup \{e = (v_{a_m}, v_{b_1})\}$, also update $w_e$
24         $P_{known} \leftarrow P_{known} \cup \{(v_{a_{m-1}}, v_{a_m}, v_{b_1}), (v_{a_m}, v_{b_1}, v_{b_2})\}$
```

3 Sparse Flow Decomposition

To reconstruct transcripts from splice graph, there are both global sparse flow decomposition as well as local sparse flow decomposition. Global sparse flow decomposition iteratively does local sparse flow decomposition at each node in a topological order, while the local sparse flow decomposition tries to recover minimum number of flows under the node's inedge and outedge weight constraints. Algorithm 2 and Algorithm 3 describe the global and local sparse flow decompositions respectively.

Algorithm 2: Global Sparse Flow Decomposition

```
Input: A splice graph described in a direct acyclic graph $G = (V, E)$.
       $V$ includes special "start" node $v_s$ and "end" node $v_e$. A set of
       known paths $P_{known}$.
Output: A set $T$. $\forall t \in T$ is a sequence of nodes starting from $v_s$ and
        ending at $v_e$ to represent a transcript.
1  sort $v \in V$ in topological order
2  for $v \in V \setminus \{v_s, v_e\}$ in topological order do
3      if $v$ has only 1 in-edge and 1 out-edge then
4          skip processing $v$
5      else
6          Prepare weights of in-edges $\{w_i, i \in InEdges(v)\}$ and weights
             of out-edges $\{w_j, j \in OutEdges(v)\}$ for $v$, and known paths
             for $v$ from $P_{known}$
7          Use them to find local sparse flow decomposition of $v$:
             $\{f_{i,j} | i \in InEdges(v), j \in OutEdges(v)\}$ (see Algorithm 3)
8          for $(i, j) \in InEdges(v) \times OutEdges(v)$ do
9              if $f_{i,j} > 0$ then
10                 create $v_{i,j}$ with same region information of $v$
11                 add $v_{i,j}$ into $V$
12                 create new edge $(InNode(i), v_{i,j})$ of weight $f_{i,j}$ into $E$
13                 create new edge $(v_{i,j}, OutNode(j))$ of weight $f_{i,j}$ into $E$
14         remove $v$ from $V$ and its incident edges from $E$
15     for $j \in OutEdges(v_s)$ do
16         add $t_j$ into $T$
```

Algorithm 3: Local Sparse Flow Decomposition

```
  Input: Edge weights $\{w_i | i \in InEdges(v)\}$ and
         $\{w_j | j \in OutEdges(v)\}$, known paths for $v$:
         $P_{known,v} = \{p | p = (v_a, v, v_b) \in P_{known}, (v_a, v) \in InEdges(v), (v, v_b) \in OutEdges(v)\}$, as well as the ROC
         tuning parameter $F \in [0, 1]$
  Output: A flow matrix $\{f_{i,j} | i \in InEdges(v), j \in OutEdges(v)\}$ that
          has the minimum number of $f_{i,j} > 0$ under constraint of
          $\sum_i f_{i,j} = w_j, \sum_j f_{i,j} = w_i$. $f_{i,j} > 0$ means there's a flow of
          weight $f_{i,j}$ going through $i$-th inedge of $v$ (i.e.
          $InNode(i)$),$v$,and $j$-th outedge of $v$ (i.e. $OutNode(j)$).
1 Init solution for output as $f^{best}$
2 for n-th trial in N trials do
3     generate a coefficient matrix $r = \{r_{i,j}\}$ with $r_{i,j} = 0$ if
        $(InNode(i), v, OutNode(j)) \in P_{known,v}$
4     get n-th solution $f^n = \{f^n_{i,j}\}$ for the Relaxed Sparse Flow Problem
        using linear programming:
5     find $argmin_f \sum_{i,j} f_{i,j} r_{i,j}$ such that
        $\sum_{i \in InEdges(v)} f_{i,j} = w_j, \forall j \in OutEdges(v)$ and
        $\sum_{j \in OutEdges(v)} f_{i,j} = w_i, \forall i \in InEdges(v)$ and $f_{i,j} \geq 0, r_{i,j} > 0$
6     trim $f^n$ (e.g. $f_{i,j} \leftarrow 0$ if $f_{i,j} < 0.001 \times min(w_i, w_j)$)
7     $f^{best} \leftarrow f^n$ if $f^n$ is sparser than $f^{best}$
8 ROC tuning: Set $f_{ij} \in f^{best}$ to be 0 if $f_{ij}/max_{f_{ij} \in f^{best}} f_{ij} < F$
9 return $f^{best}$
```

3.1 Intuition of Sparse Solution

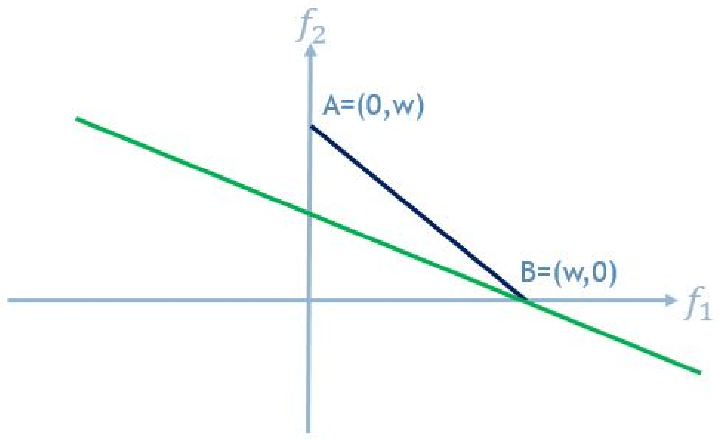
Figure 3 has given a simplified example to illustrate why the Relaxed Sparse Flow Problem (see Algorithm 3) brings sparse solution. The constraint of the example is $f_1 + f_2 = w, f_1 \geq 0, f_2 \geq 0$, which leads to the line AB in the figure. Given coefficients $r = \{r_1, r_2\}$, let the objective function be $c = r_1 f_1 + r_2 f_2$. The minimum of c is reached either at point A or point B depending on the angle of the line $r_1 f_1 + r_2 f_2$. When we try multiple coefficients r, a sparse solution thus always tends to be returned.

Figure 3: **An Example for Sparse Solution Illustration**

$$argmin_f \; f_1 r_1 + f_2 r_2$$

s.t.

$$f_1 + f_2 = w$$
$$f_1 \geq 0, f_2 \geq 0$$



4 ROC Tuning

ROC tuning is mainly used to check the sensitivity and false positive trade off when we evaluate assemblers' performance using simulated datasets, where the ground truth reference transcripts are known.

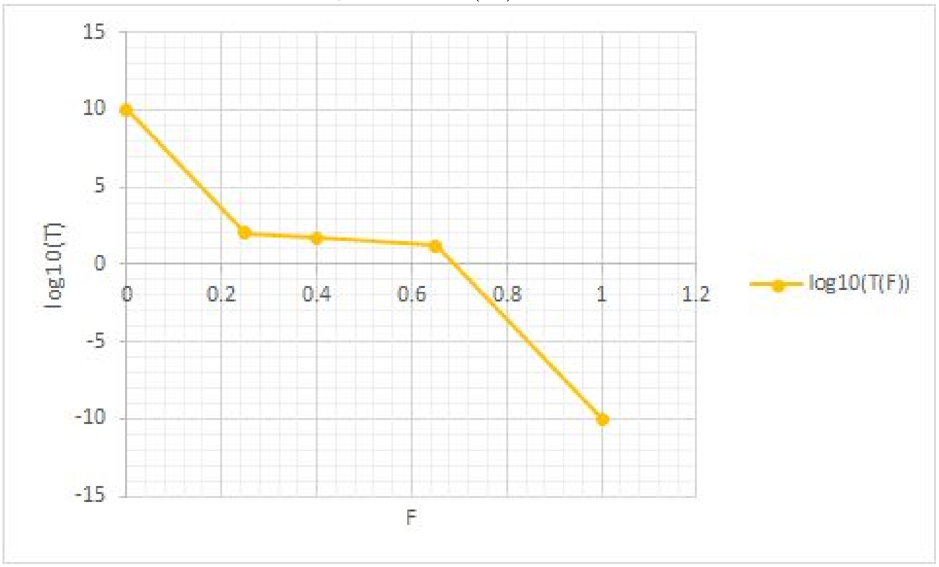
To begin with, We notice that the merge step in splice graph generation can fill the exonic gaps but risk to increase the chances of introducing false positives. We also notice that the local sparse flow decomposition may produce noisy flows: when the sparsity of the flow set (e.g. $||f||_0$) is high, there may also be more false positive flows.

To control the sensitivity and false positive trade off, we use a threshold $F \in [0, 1]$ to adaptively adjust the merge step of splice graph generation as well as the local sparse flow decomposition output.

Specifically, when we do merge step during splice graph generation, the criteria $T_b(F)$ (below line 7 in Algorithm 1) for two regions to merge is affected by F. When F increases, $T_b(F)$ decreases to reduce region merge operations in order to reduce false positives, accompanied by decreased sensitivity. The $T_b(F)$ is empirically determined as shown in Figure 4. When we do local sparse flow decomposition at node v and have obtained the local sparse flow solution $f = \{f_{ij} | i \in InEdges(v), j \in OutEdges(v)\}$, we set $f_{ij} \in f$ to be zero if $f_{ij}/max_{i,j} f_{ij} < F$ (see line 8 in Algorithm 3). Therefore, when F is zero, no f_{ij} is post-processed to be set as zero and max sensitivity is reached. When F is one, only f_{ij} of max flow value is returned and thus min false positive is reached. As F increases from zero to one, sensitivity decreases, resulting in reduced false positives.

The F values we use for ROC curves in main paper are selected as $0, 0.25, 0.96$.

Figure 4: $T_b(F)$ versus F


log10(T)
F
log10(T(F))