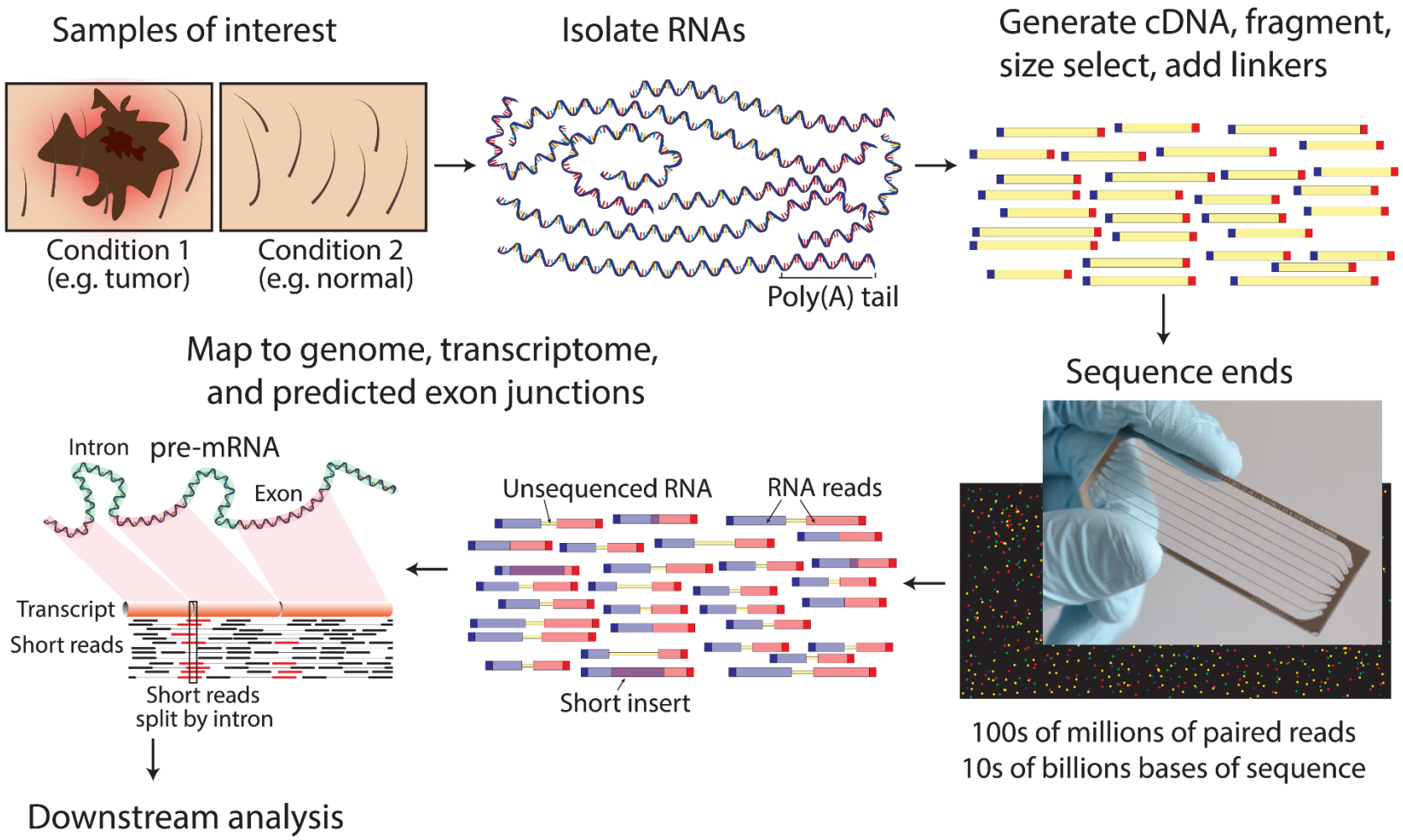
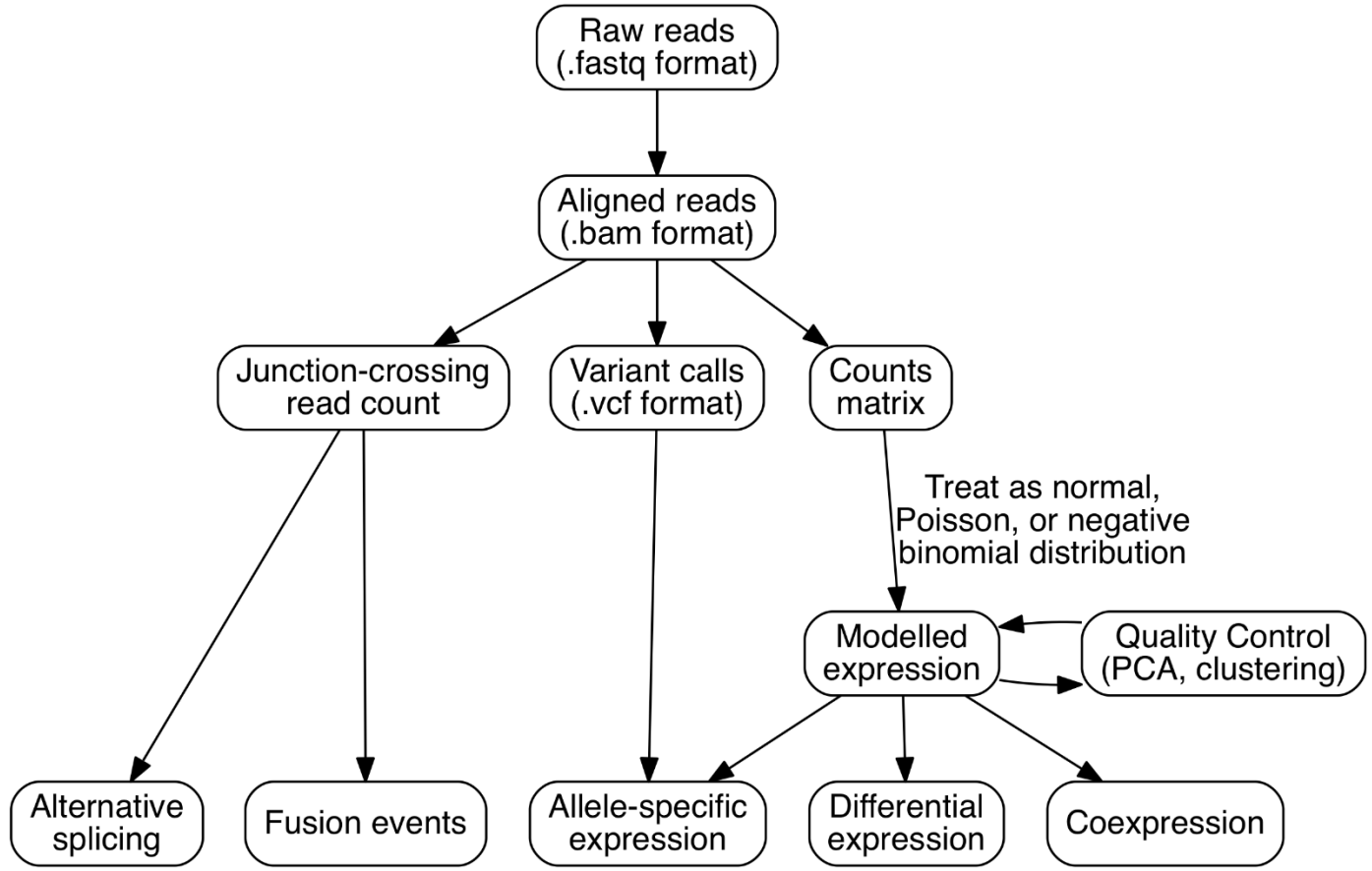
RNA-Seq

RNA-Seq Experimental Workflow



https://en.wikipedia.org/wiki/RNA-Seq

RNA-Seq Data Analysis



https://en.wikipedia.org/wiki/RNA-Seq

Li & Homer '2010: A survey of sequence alignment algorithms for next-generation sequencing

- Fast alignment algorithms use auxiliary data indices
  - For short reads,
  - For reference genomes
  - For both
- Indexing algorithms
  - Hash tables
  - Suffix trees
  - BWT

Hash Table indexing

- Seed-extend paradigm
  - E.g., BLAST
  - Use hash table to find locations of k-mers (k=11 for DNA) and extend using variation of Smith-Waterman
- Improvements to BLAST to handle short reads against long genomes
  - Seed using non-consequtive matches, aka "spaced seed".
  - Specific locations vs. any k-mismatch hit (with a bound on k<=2 to limit possibilities)
  - Minimum number of seeds for given read length, sensitivity requirement and memory usage.

Hash Table Indexing

- Memory requirement is problematic
- One Solution: Two-level indexing.
  - Hash table for j-long keys (j<q)
  - To find q-long keys, first search in j-long prefix hash table, then binary search in the bucket.

Allowing gaps

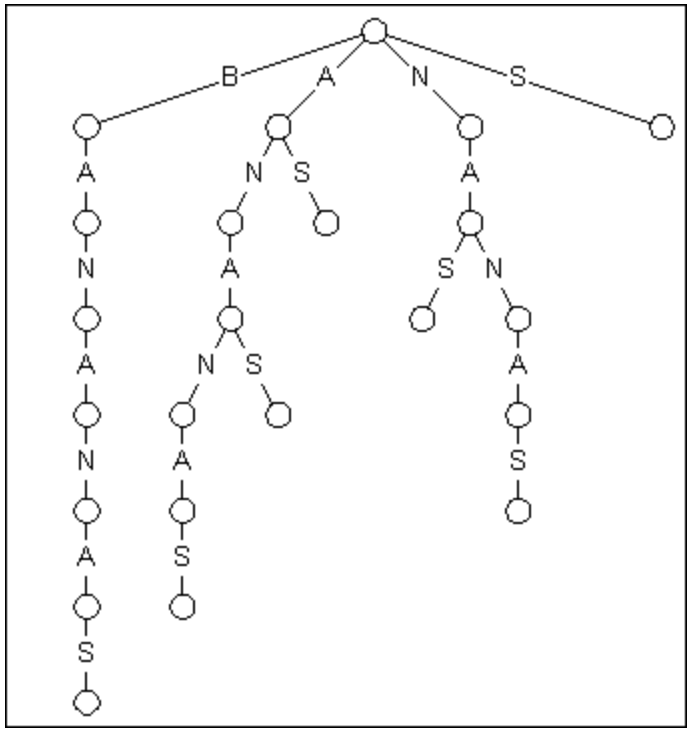
- Hash table doesn't allow for gaps in seeds
- Solution: q-gram filter (BLAT)
  - With at most k differences, the w-long query and the w-long database substring share at least (w+1) – (k+1)q common substrings of length q.
- Spaced seeds: extend on long seed match. vs. q-gram filter: initiates extension with multiple shorter seed matches.

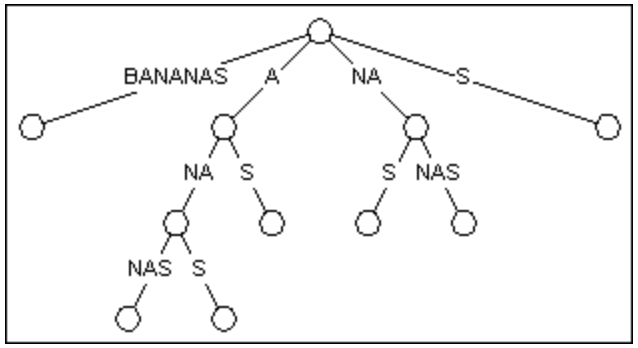
Improvements in Seed-Extension

- Seed extension is typically unnecessary with long spaced seeds
- Most only extend without gaps.
- Improvements to seed extension
  - Constrain dynamic programming around seeds
  - Vectorized code

Suffix tries

- A suffix trie is a data structure that stores all suffixes of a text.





http://marknelson.us/1996/08/01/suffix-trees/

Burrows Wheeler Transform

Transformation				
Input	All rotations	Sorted into lexical order	Taking last column	Output last column
^BANANA|	^BANANA| |^BANANA A|^BANAN NA|^BANA ANA|^BAN NANA|^BA ANANA|^B BANANA|^	**A**NANA|^B **A**NA|^BAN **A**|^BANAN **B**ANANA|^ **N**ANA|^BA **N**A|^BANA ^BANANA| |^BANANA	ANANA|^**B** ANA|^BA**N** A|^BANA**N** BANANA|^ NANA|^B**A** NA|^BAN**A** ^BANANA| |^BANAN**A**	BNN^AA|A