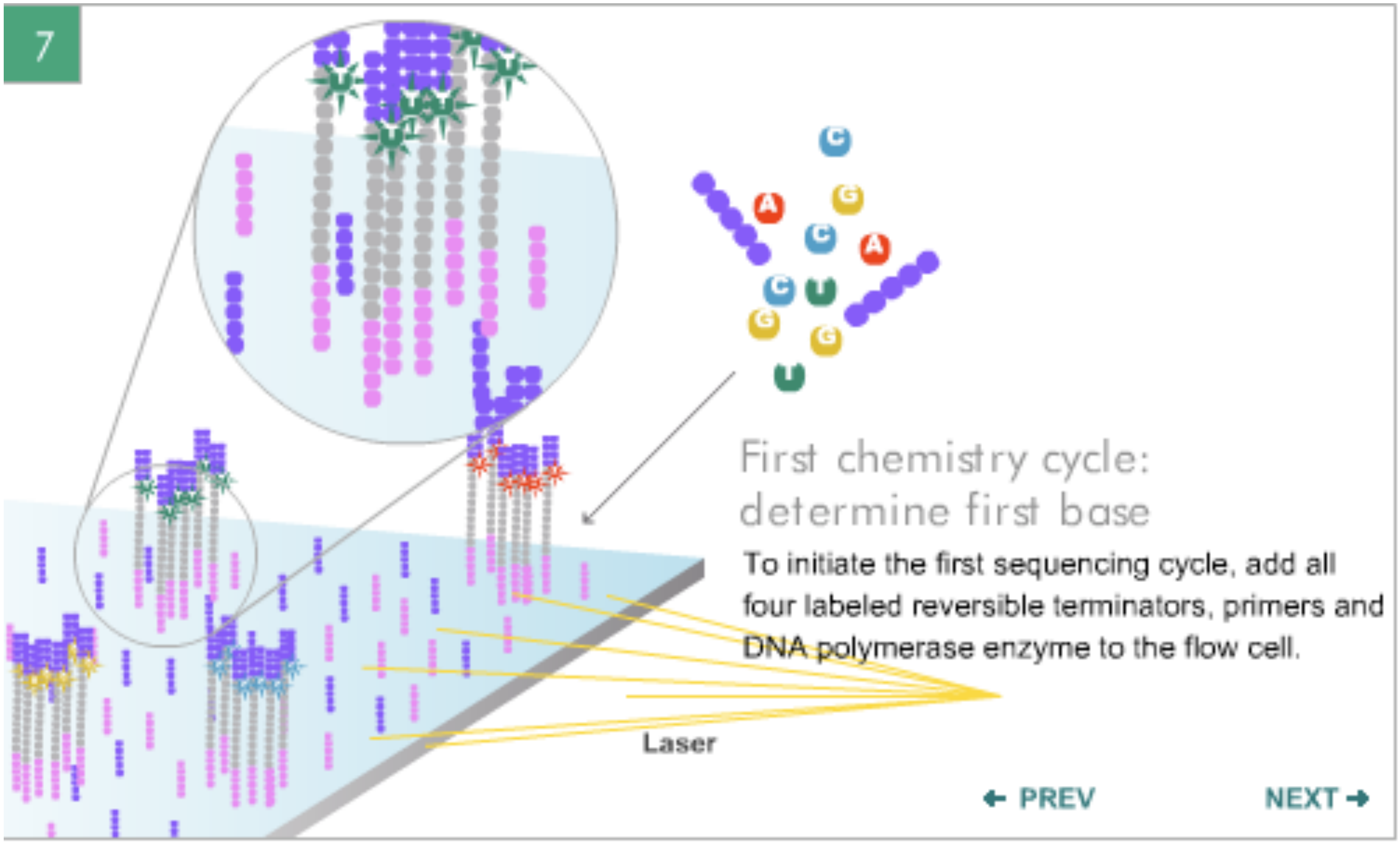
GNUMap: Unbiased Probabilistic Mapping of Next-Generation Sequencing Reads

Nathan Clement
Computational Sciences Laboratory
Brigham Young University
Provo, Utah, USA

GNUMap

Next-Generation Sequencing (Solexa/Illumina)



First chemistry cycle: determine first base

To initiate the first sequencing cycle, add all four labeled reversible terminators, primers and DNA polymerase enzyme to the flow cell.

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Problem Statement

- Map next-generation sequence reads with variable nucleotide confidence to a model reference genome that may be different from the subject genome.
  - Speed
    - Tens of millions of reads to a 3Gbp genome
  - Accuracy
    - Mismatches included?
    - Repetitive regions
  - Visualization

Workflow

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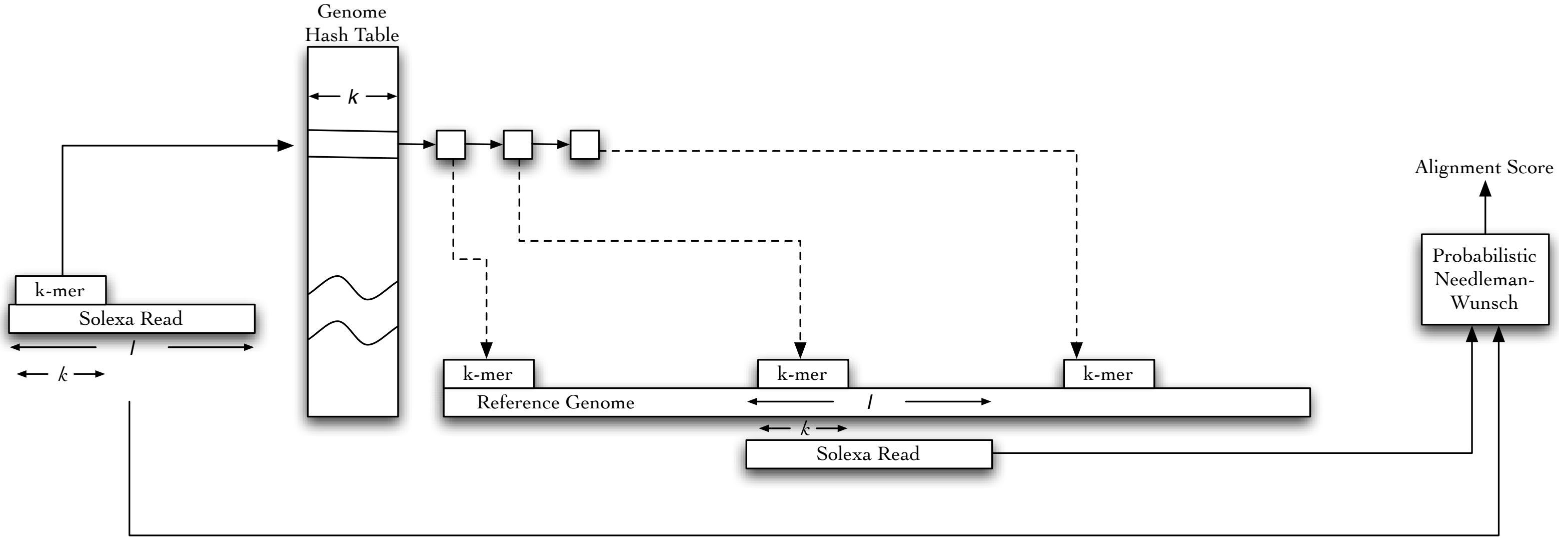

Indexing the genome

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- Fast lookup of possible hit locations for the reads
    - Hashing groups locations in the genome that have similar sequence content
        - k-mer hash of exact matches in genome can be used to narrow down possible match locations for reads
    - Sorting genome locations provides for content addressing of genome
- GNUMAP uses indexing of all 10-mers in the genome as seed points for read mapping

Building the Hash Table

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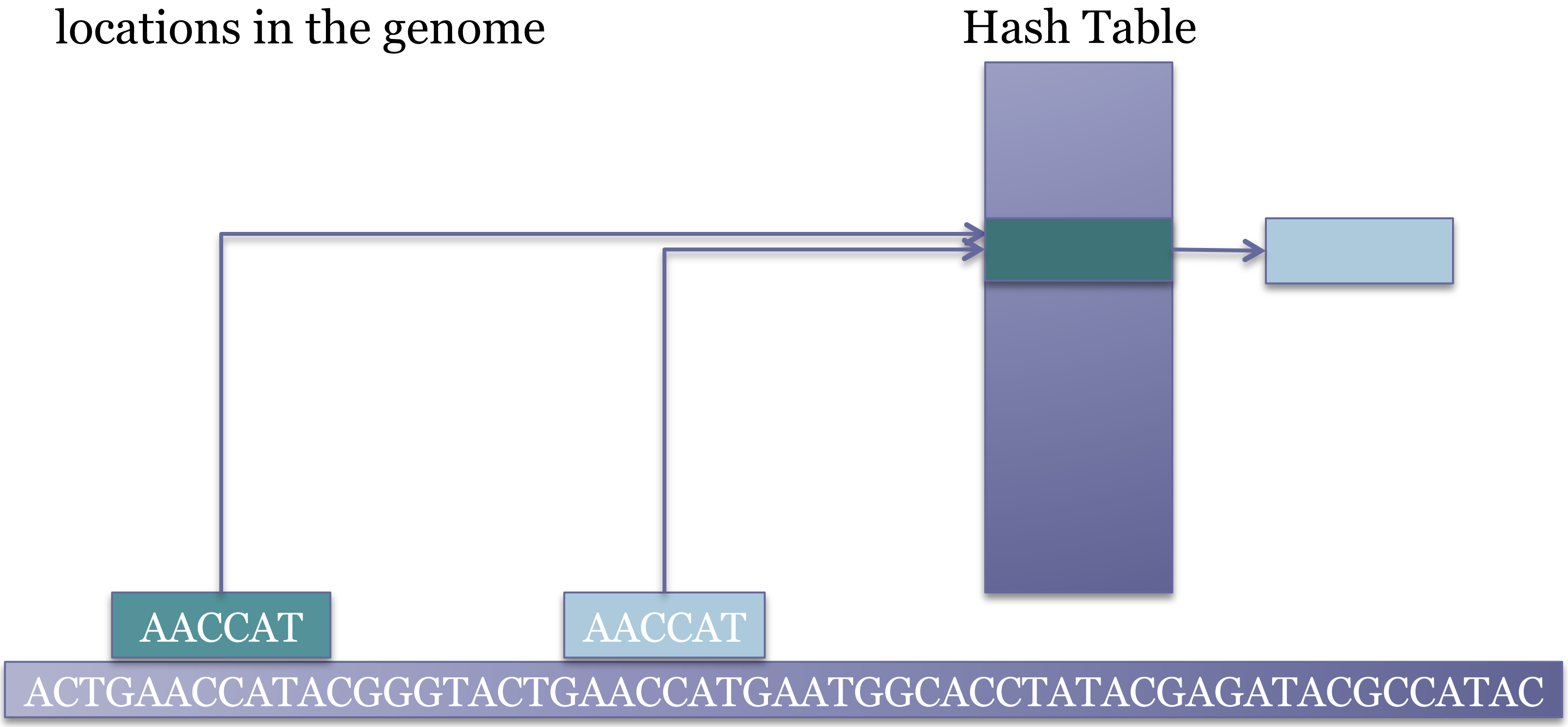
Sliding window indexes all locations in the genome



Alignment

- Given a possible genome match location, determine the quality of the match
- If you call bases in the read
  - Every base gets the same weight in the alignment, no matter what the quality
  - Later bases in the read that have lower quality have equal weight in the alignment with high quality bases at the start of the read
- GNUMap uses a Probabilistic Needleman-Wunsch to align reads found with seed points from the genome hash

Probabilistic Needleman Wunsch

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j	0	1	2	3	4	5
PWM	*A*	0.059	0.000	0.172	0.271	0.300
	C	0.108	0.320	0.136	0.209	0.330
	G	0.305	0.317	0.317	0.164	0.045
	T	0.526	0.578	0.375	0.356	0.325
NW		T	T	T	T	C
	0	-2	-4	-6	-8	-10
T	-2	**0.052**	-1.948	-3.948	-5.948	-7.948
T	-4	-1.844	**0.208**	-1.792	-3.792	-5.792
C	-6	-3.844	-1.792	**-0.520**	-2.448	-4.448
A	-8	-5.844	-3.792	-2.374	**-0.978**	-2.978
C	-10	-7.844	-5.792	-4.131	-2.774	**-1.318**

- Uses PWM in calculation of alignment score
- Allows for probabilistic mismatches and gaps
- Greater ability to map reads of variable confidence

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Assignment

- Given a read that has matches to possibly multiple locations in the genome, assign the read to locations where it matches
  - Repeat Masking– Discard reads that match to repeat regions.
    - Half of the human genome contains repeat regions, so you are not able to map to those regions
    - Many regulatory regions are repeated in the genome
  - Map to all locations – Repeat regions will be over-represented since one read will generate multiple hits
  - Pick a random location – Biased if there are small numbers of reads
- GNUMap uses probabilistic mapping to allocate a share of the read to matching locations in the genome according to the quality of the match

Equation for probabilistic mapping

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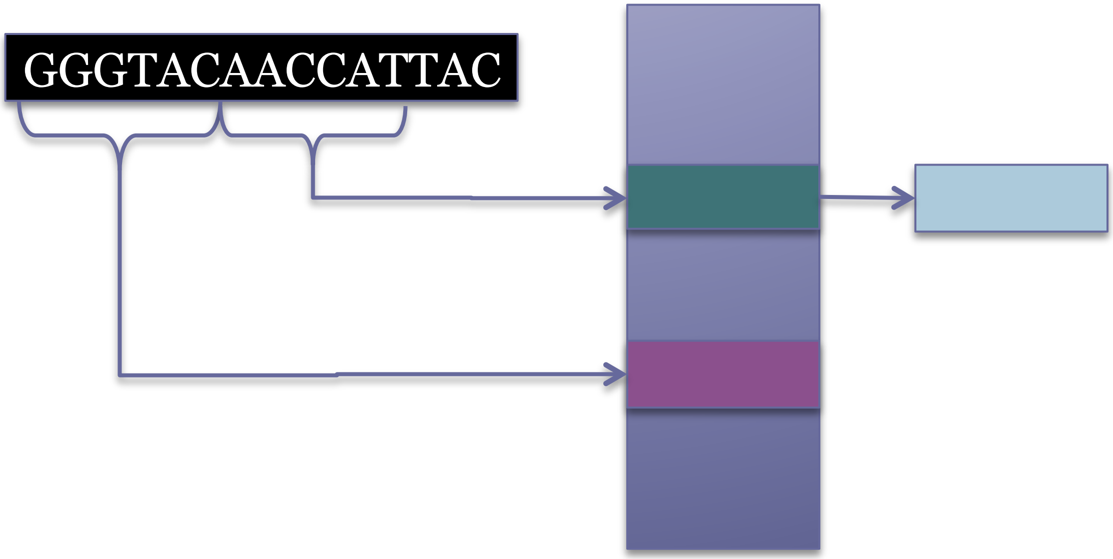
$$G_{M_j} = \frac{Q_{M_j}}{n_{M_j} Q_{M_j} + \sum_{k \neq j}^{n} n_{M_k} Q_{M_k}}$$

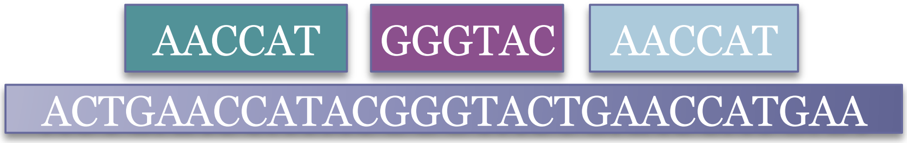
- Allows for multiple sequences of different matching quality.
- Includes probability of each read coming from any genomic position.

Alignment

Read from sequencer



Read is added to both repeat regions proportionally to their match quality



Which Program to Use?

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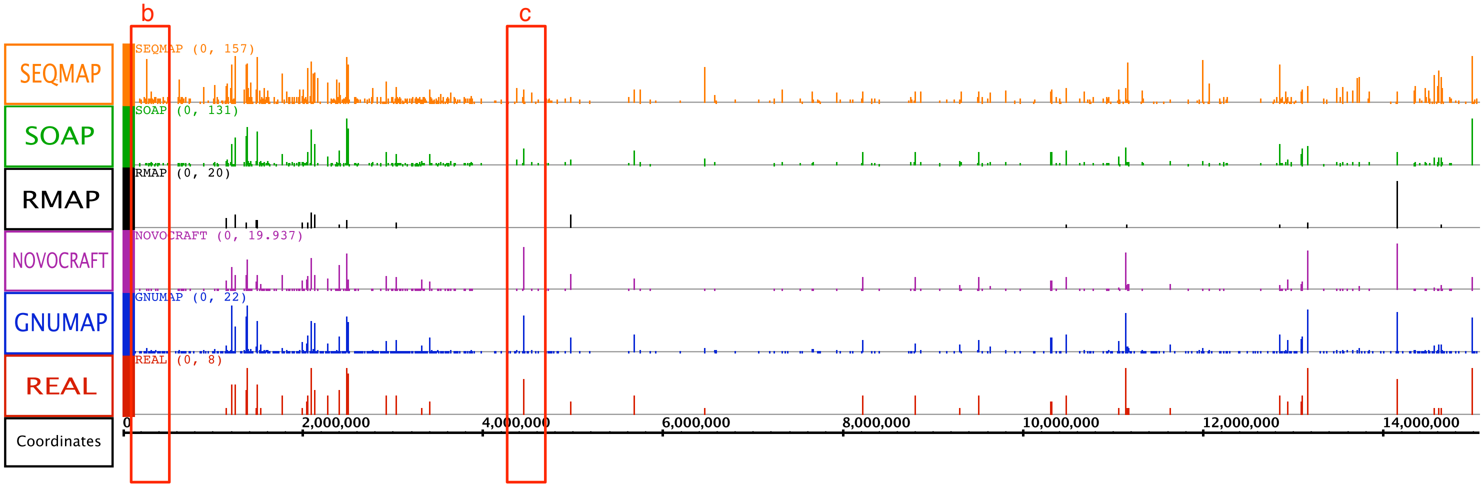
- Many different programs. How do they relate?
  - ELAND (included with Solexa 1G machine)
  - RMAP (Smith et al., BMC Bioinformatics 2008)
  - SOAP (Li et al., Bioinformatics 2008)
  - SeqMap (Jiang et al., Bioinformatics 2008)
  - Slider (Malhis et al., Bioinformatics 2008)
  - MAQ (Unpublished, http://maq.sourceforge.net/)
  - Novocraft (Unpublished, http://www.novocraft.com)
  - Zoom (Lin et al., Bioinformatics 2008)
  - Bowtie (Langmead et al., Genome Biology 2009)
  - …

Simulation Studies



- Ambiguous reads cause:
  1. Missed (unmapped) regions
  2. Too many mapped regions (noise)

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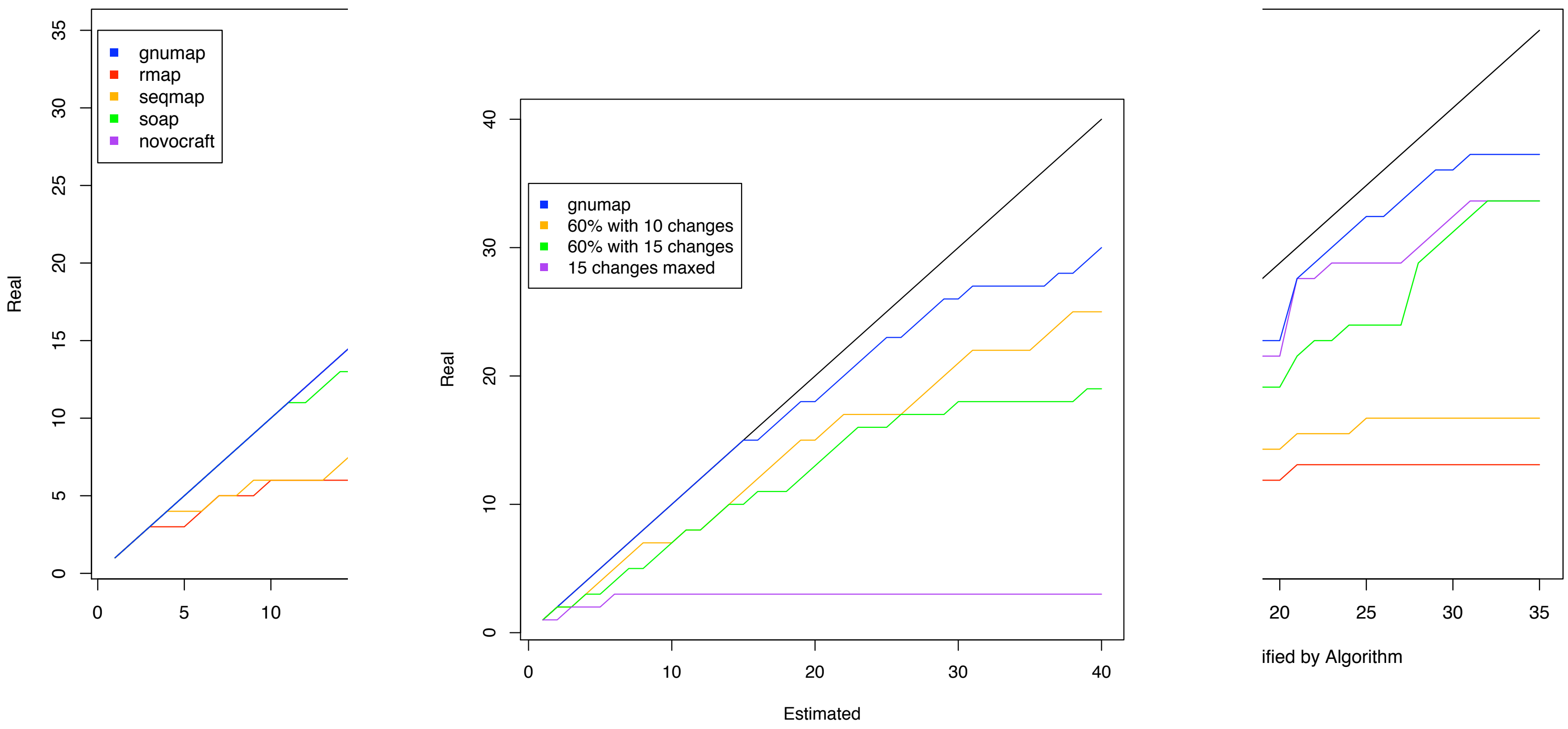
Simulation Studies



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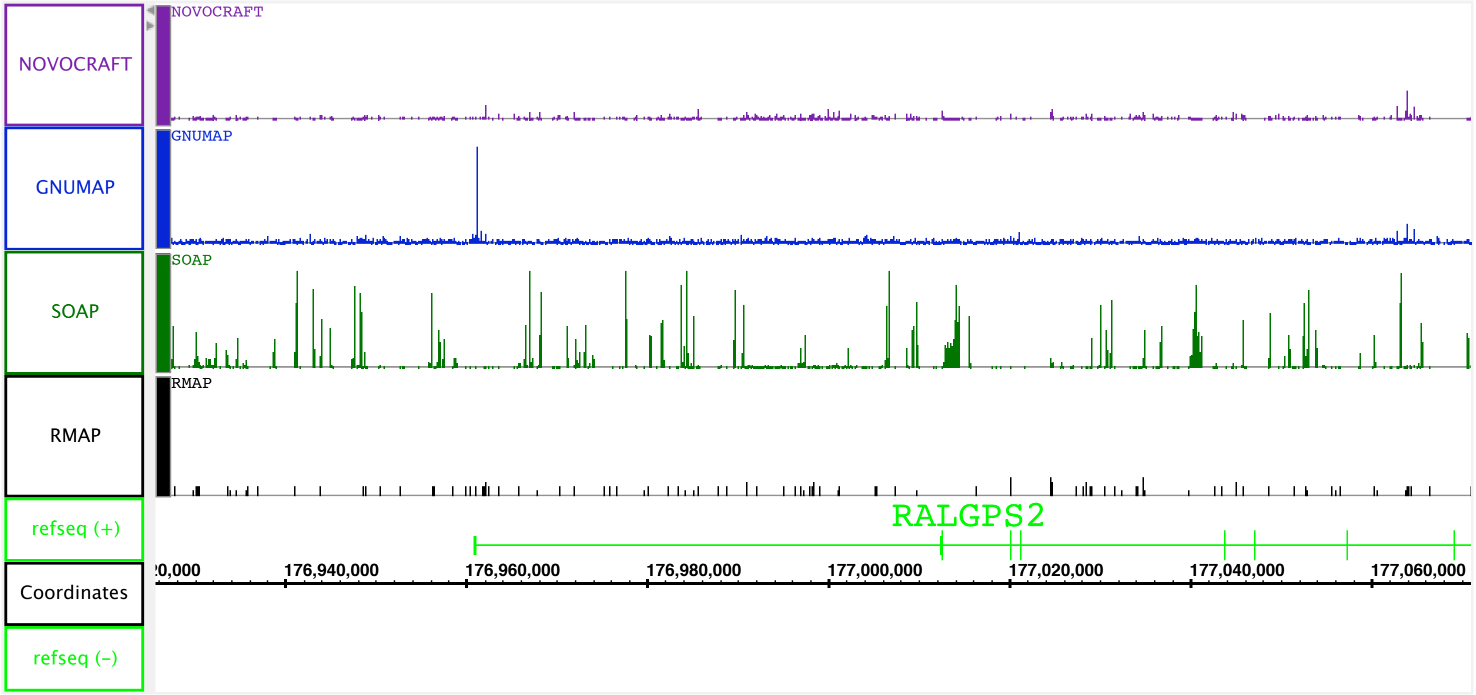
Actual Data



- ETS1 binding domain
- Repetitive region

Future Plans

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- Removal of adaptor sequences
- Methylation analysis
- Paired-end reads
- SOLiD color space

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