2018 LONDON CALLING

Cas9 Targeted Enrichment for Nanopore Profiling of Methylation at Known Cancer Drivers

Timothy Gilpatrick

Lab of Winston Timp

JOHNS HOPKINS

Organised by

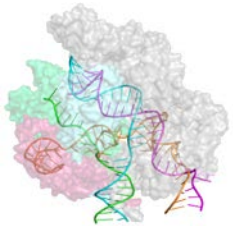
Oxford NANOPORE Technologies

- **5-10 Gb from a single MinION Flow Cell**
- **3Gb in human genome**

Goals:

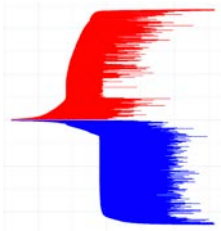
Enrich for specific loci using CRISPR/Cas9–

Higher coverage at selected loci from a single flow cell



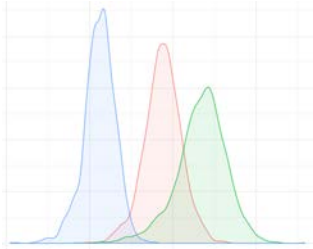
Approach

- DNA/RNP complex assembly
- Complex capture with magnetic beads



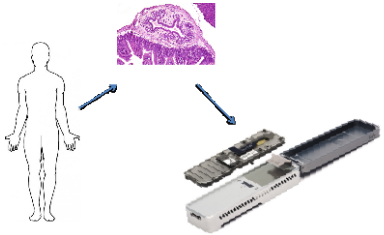
Performance

- Single bacterial and mixed microbial populations
- Human cell lines



Methylation Analysis

- Compare with illumina data
- Look at allele specific methylation



Clinical Applications

- DNA methylation
- Tandem repeats / Structural variants

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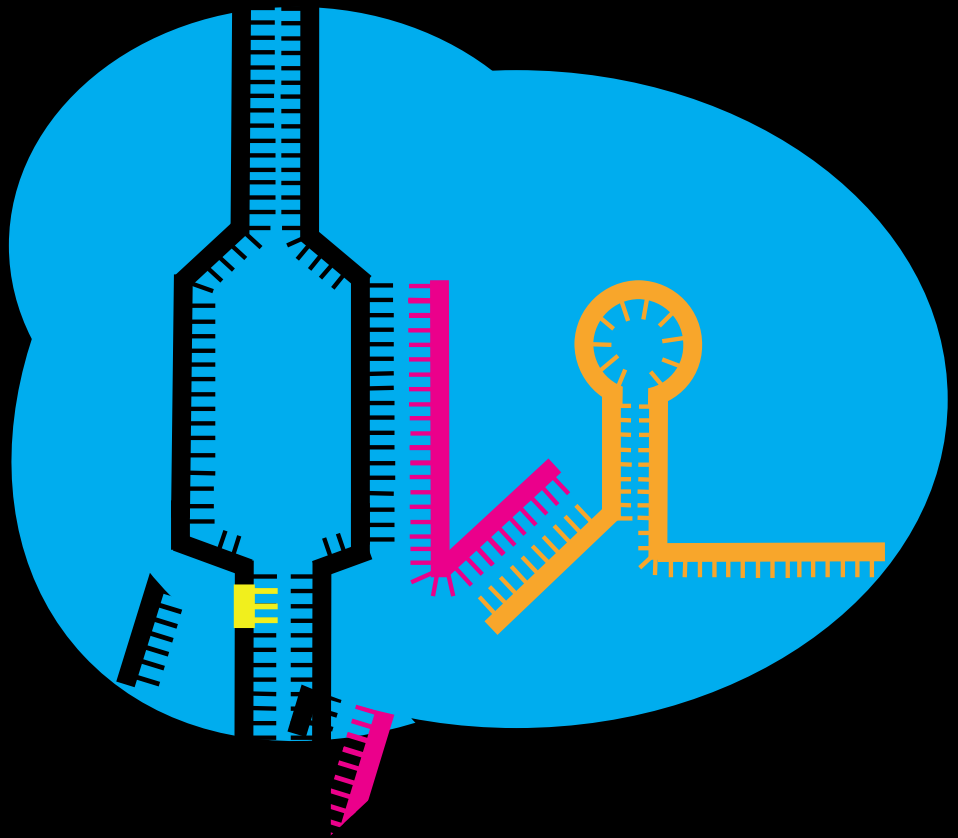
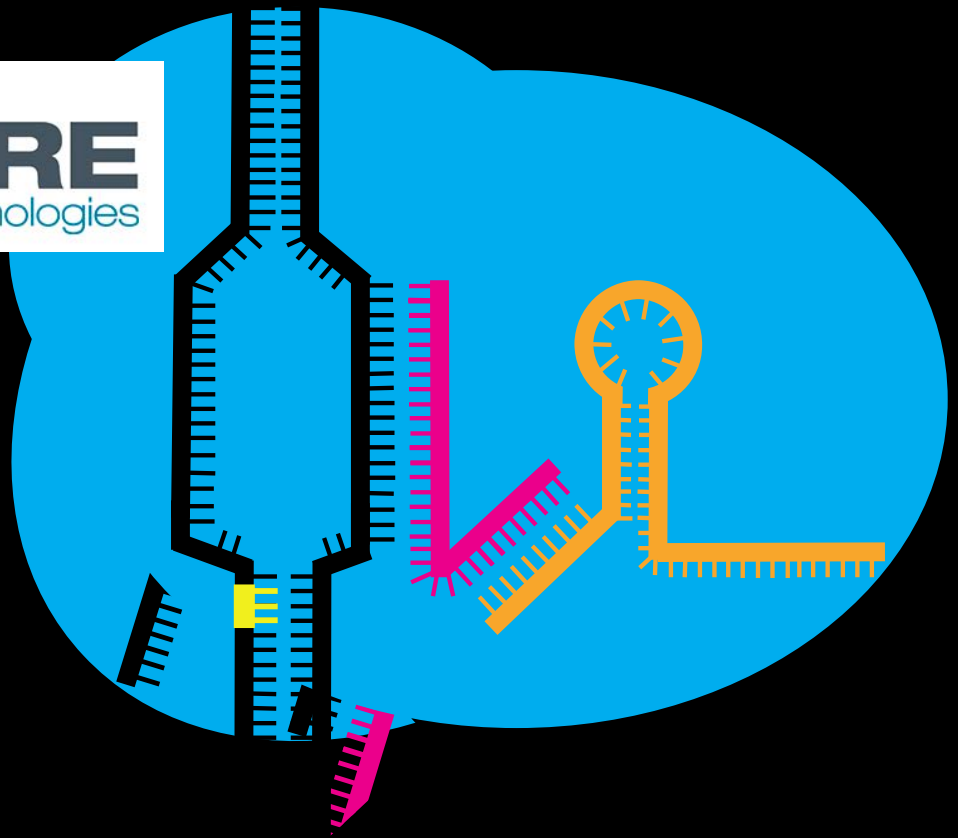
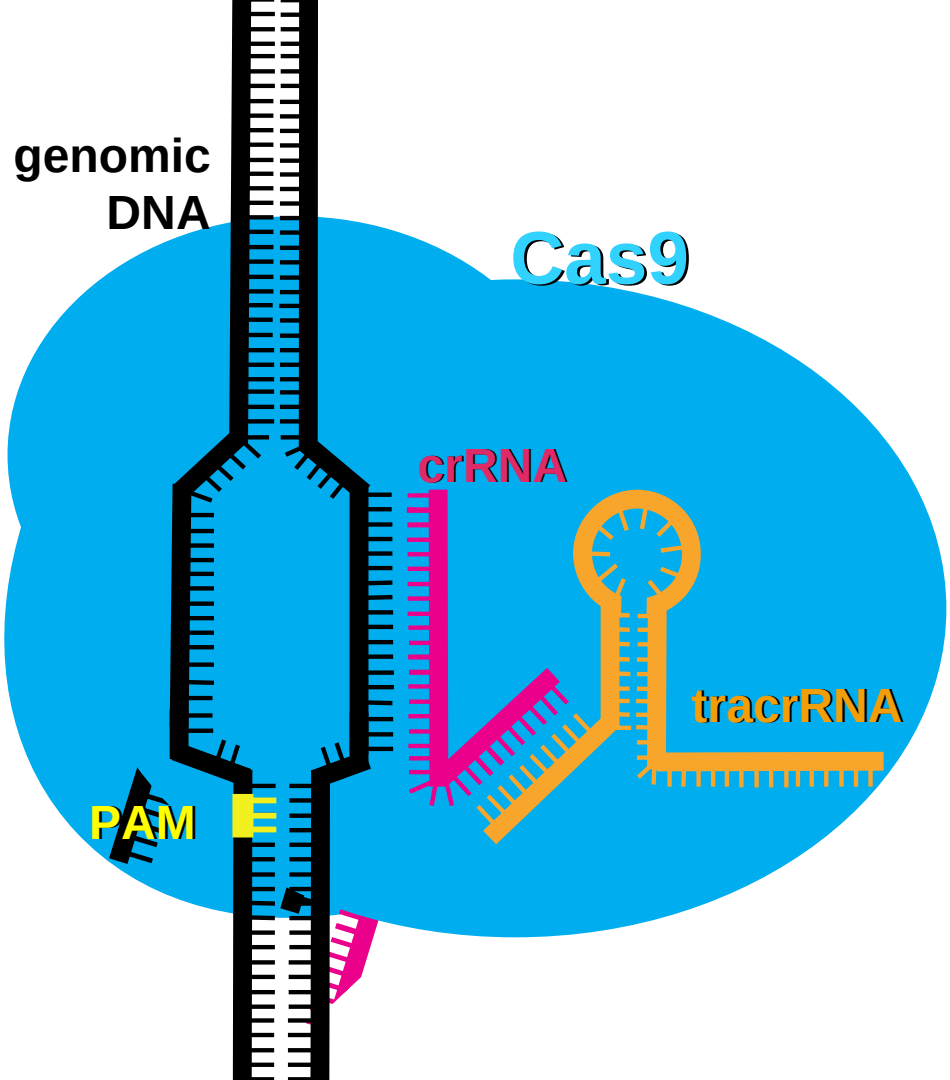
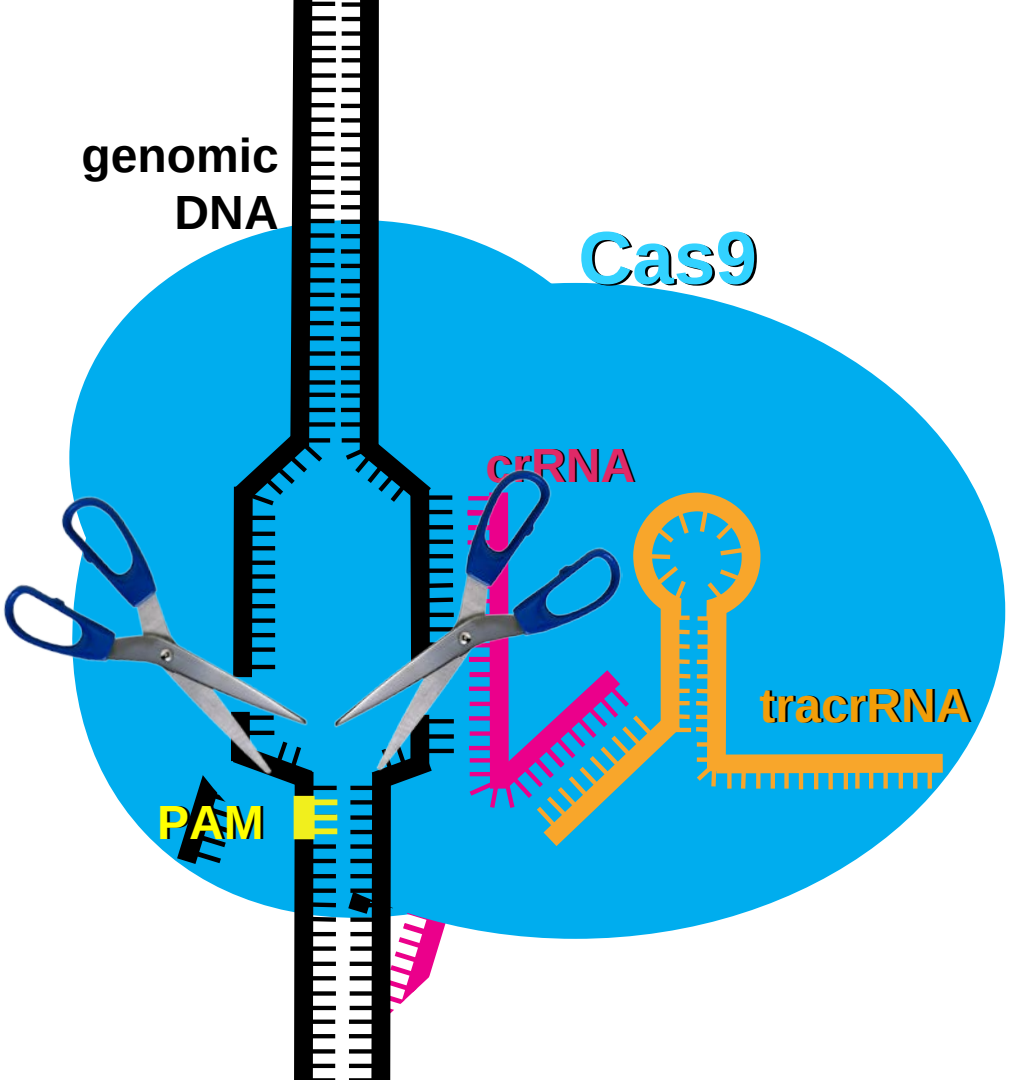
Approach



Oxford NANOPORE Technologies




genomic
DNA
Cas9
crRNA
tracrRNA
PAM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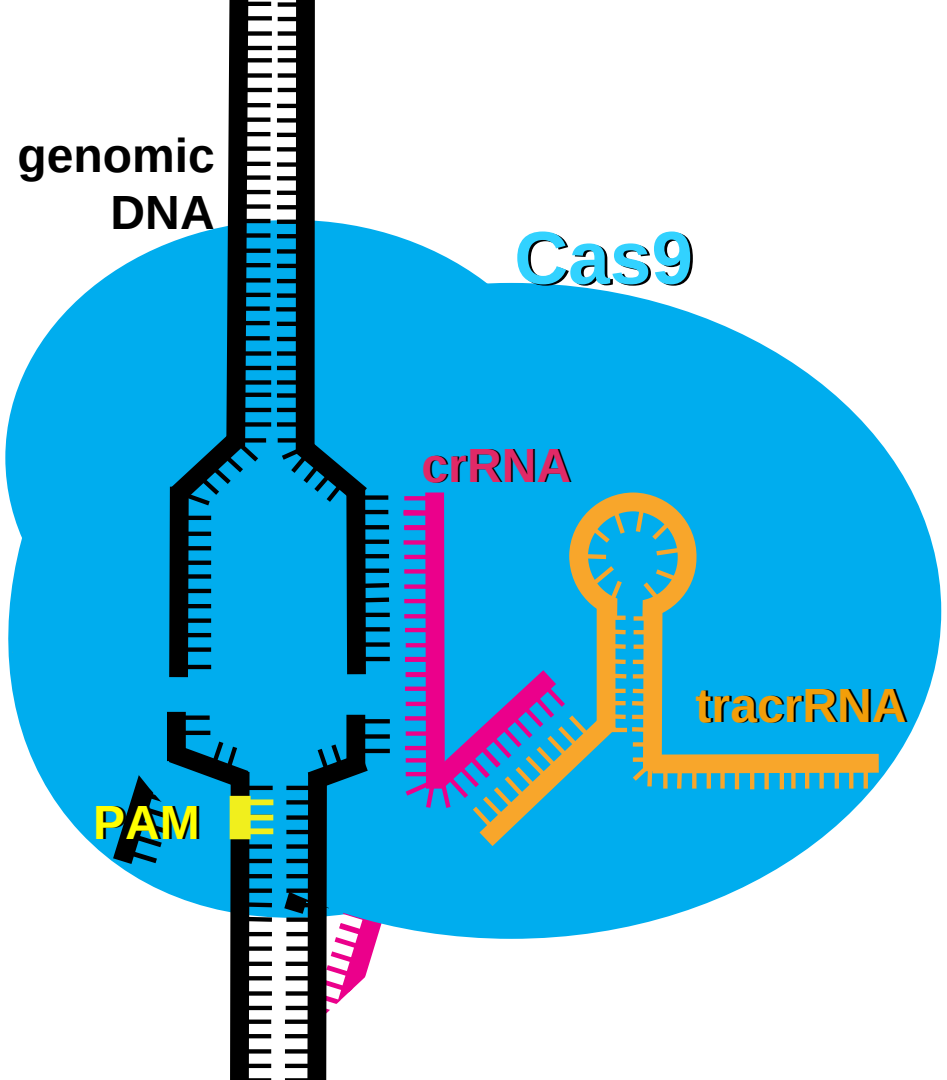
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DNA
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crRNA
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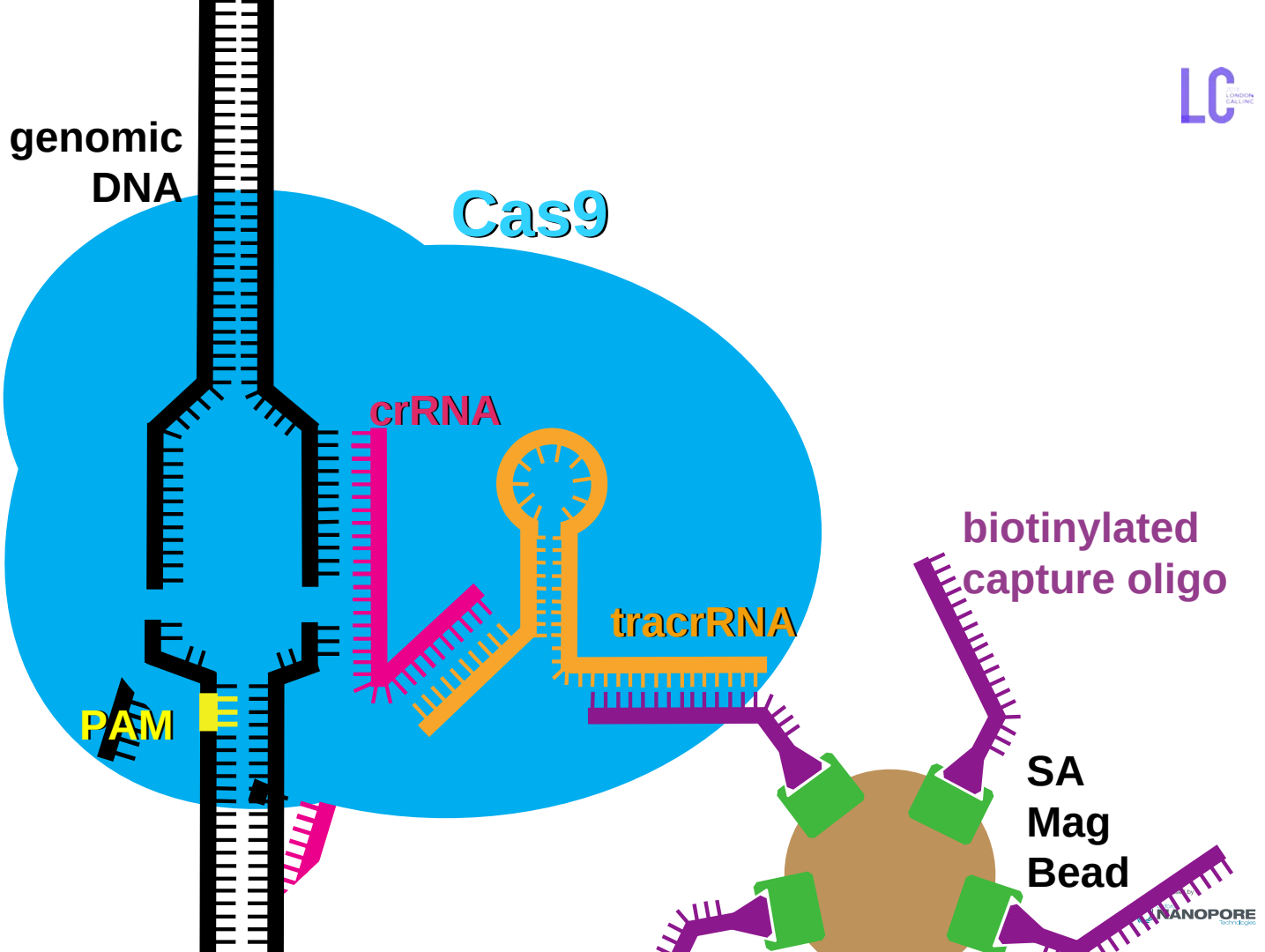
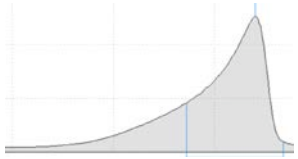
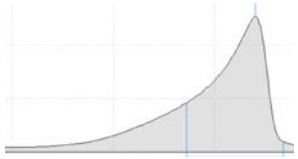

genomic
DNA
Cas9
crRNA
tracrRNA
PAM
biotinylated
capture oligo
SA
Mag
Bead

Workflow



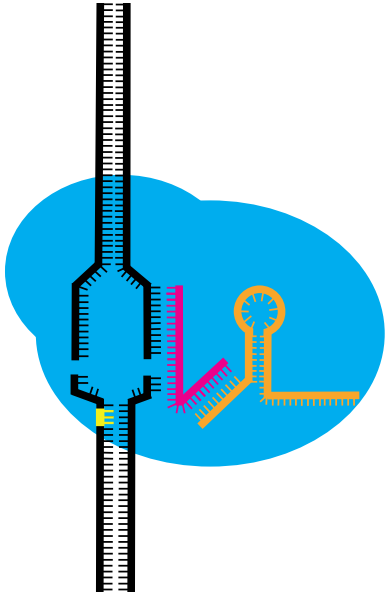
Shear and End-Prep DNA

Workflow

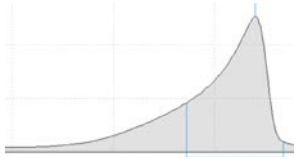


Shear and End-Prep DNA

Combine With Cas9/RNA complex



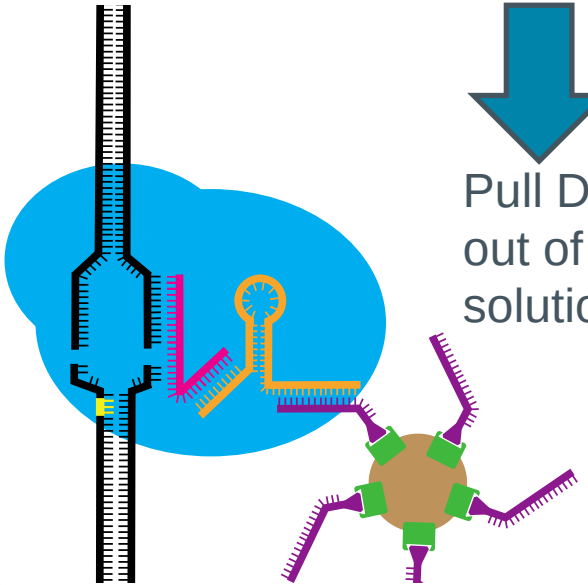
Workflow



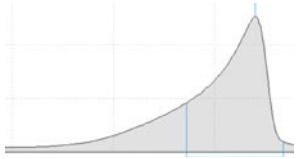
Shear and End-Prep DNA

Combine With Cas9/RNA complex

Pull DNA out of solution



Workflow

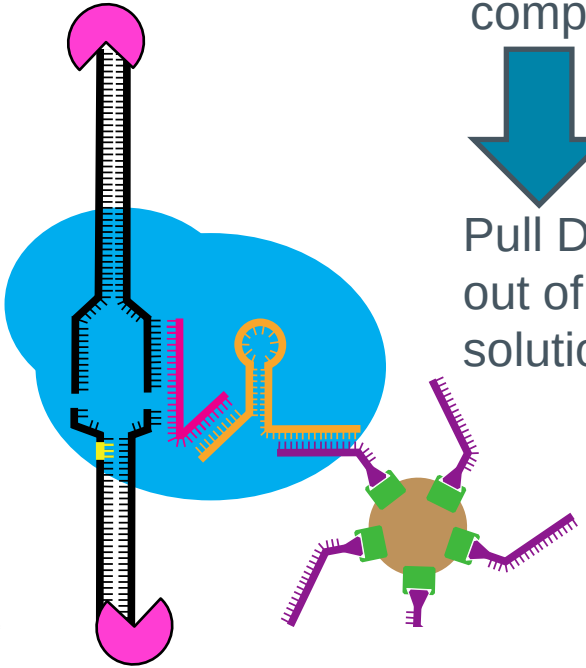


Shear and End-Prep DNA

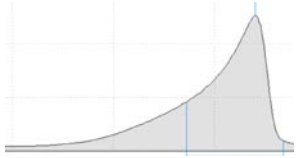
Combine With Cas9/RNA complex

Pull DNA out of solution

Ligate Adaptor while on Mag beads



Workflow



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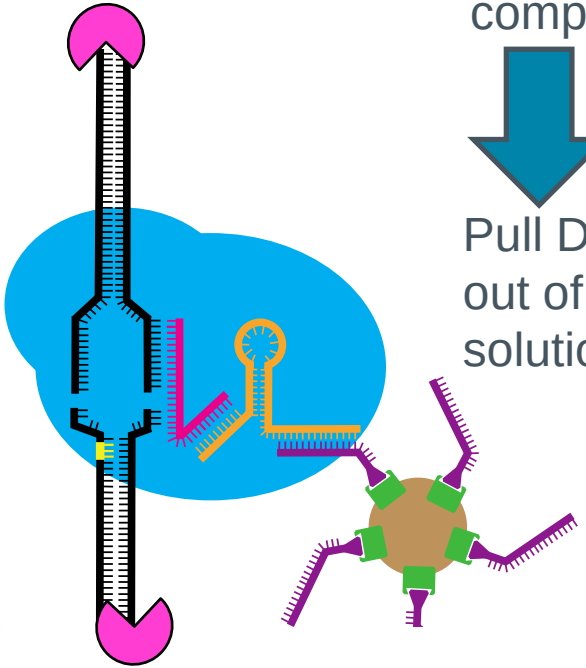
Combine With Cas9/RNA complex

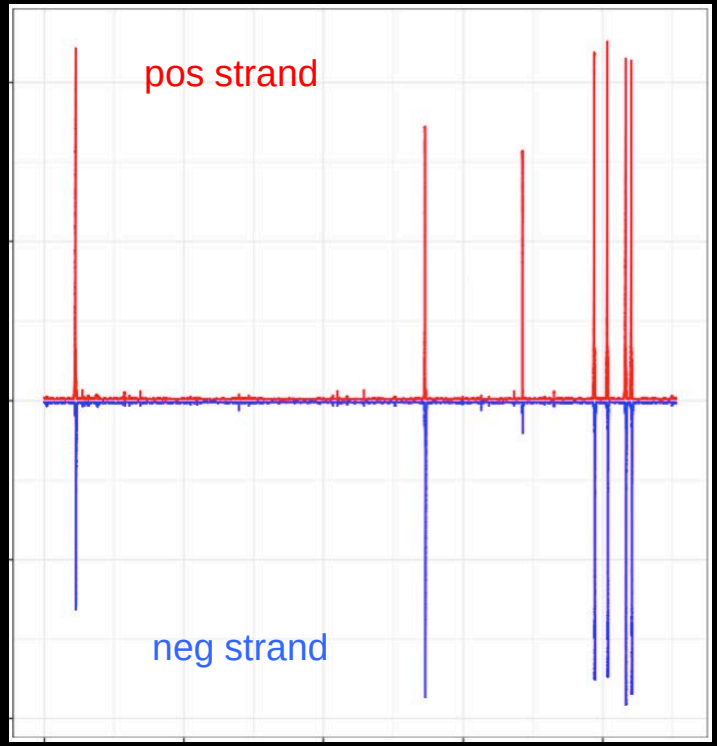


Pull DNA out of solution

Ligate Adaptor while on Mag beads

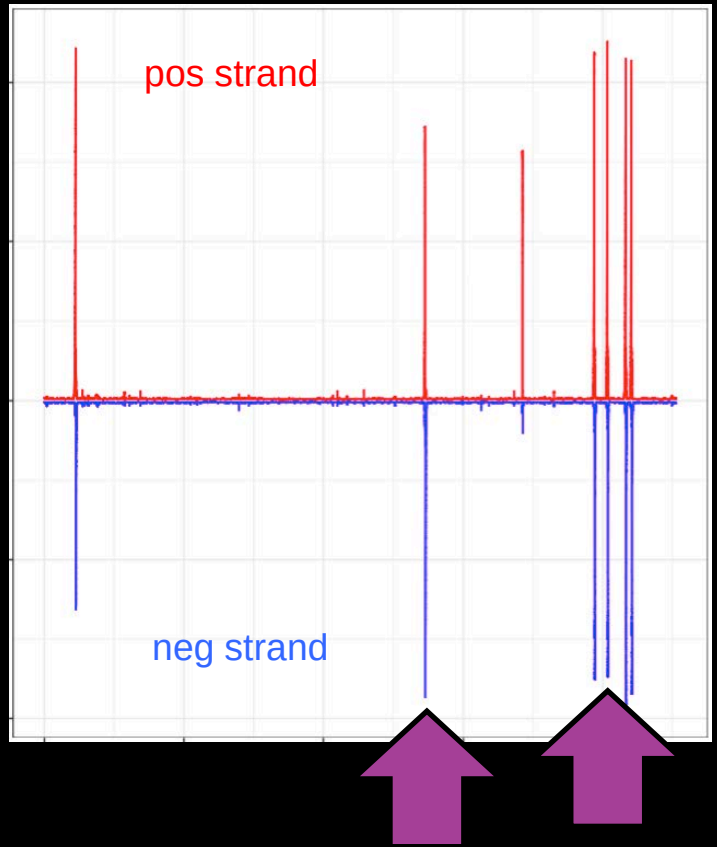
Run on MinION Flow Cell

Enrichment Yield

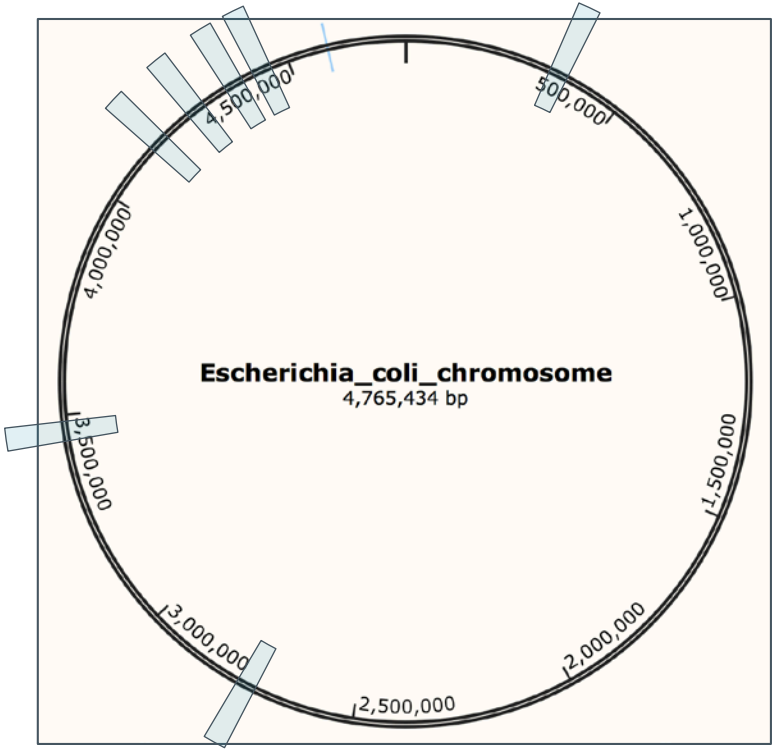


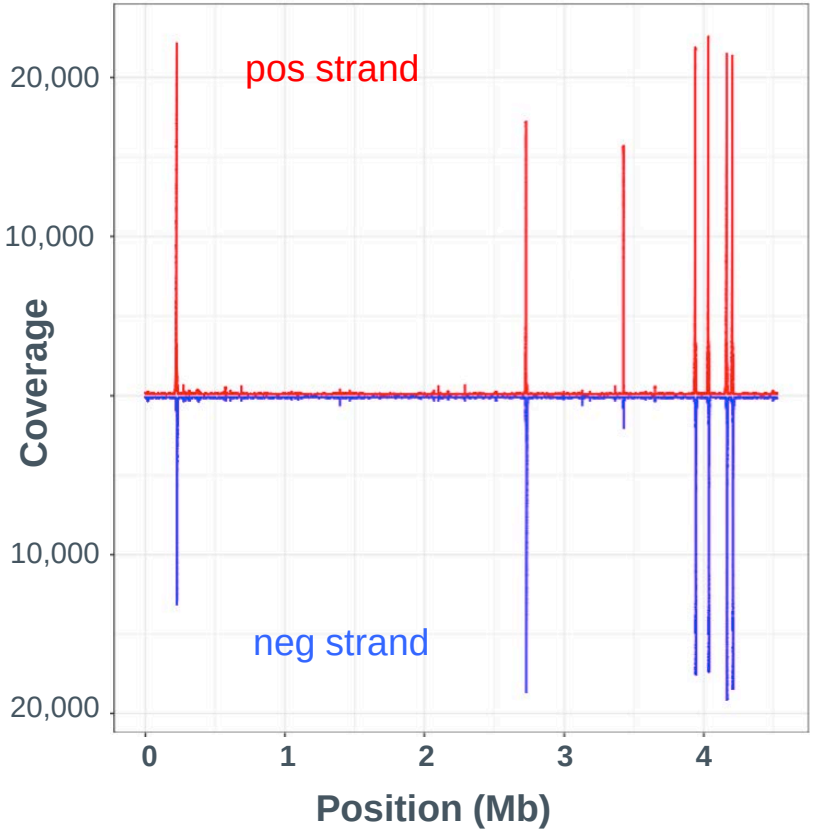
Enrichment Yield

16s rRNA gene is slow to evolve and used in characterizing microbial identification



Enrichment from E. Coli DNA





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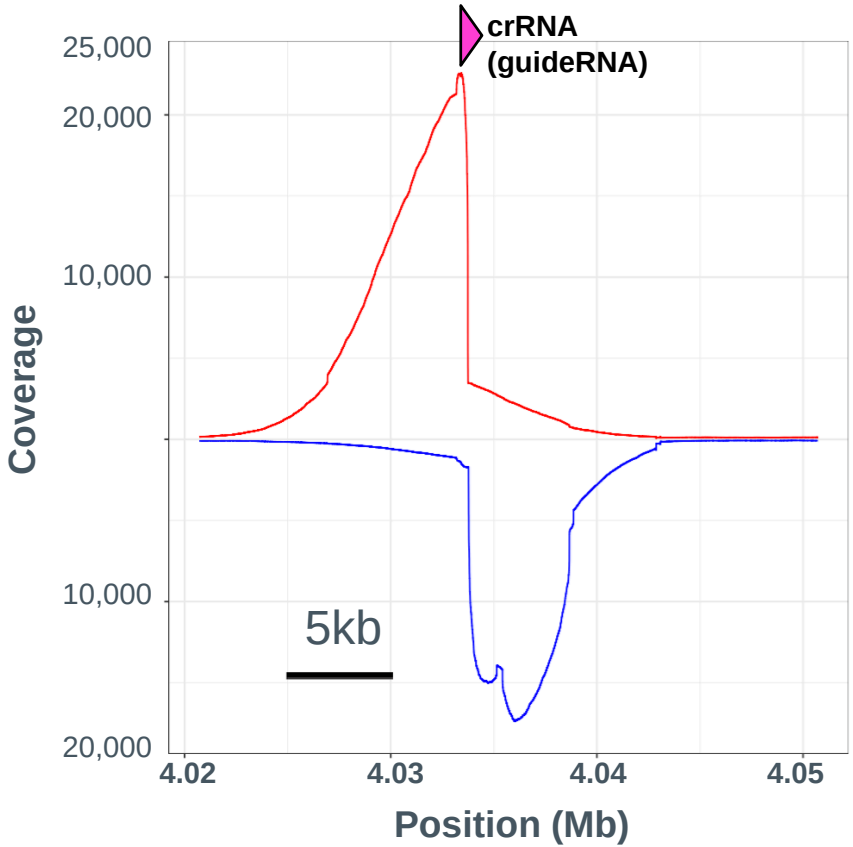

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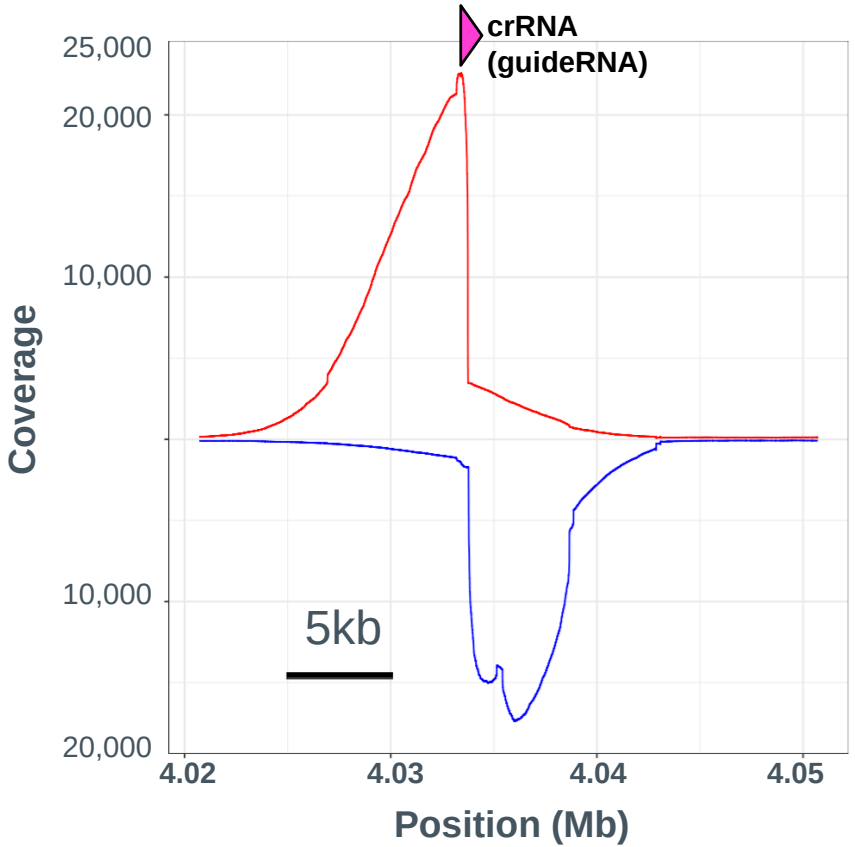

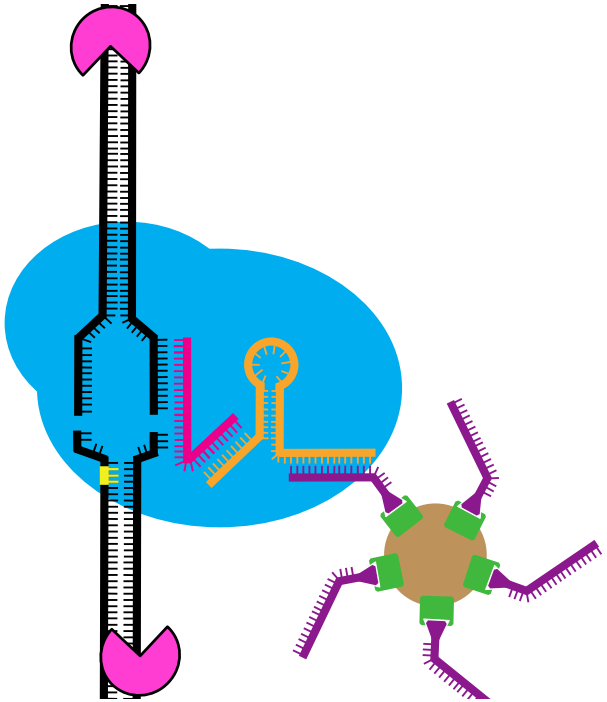

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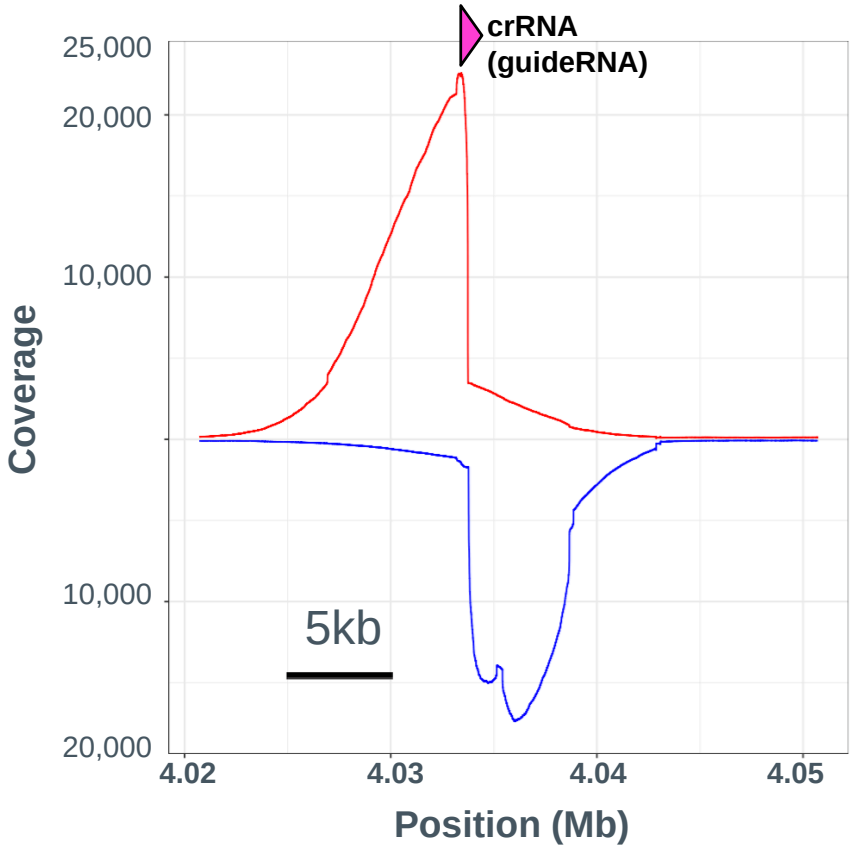

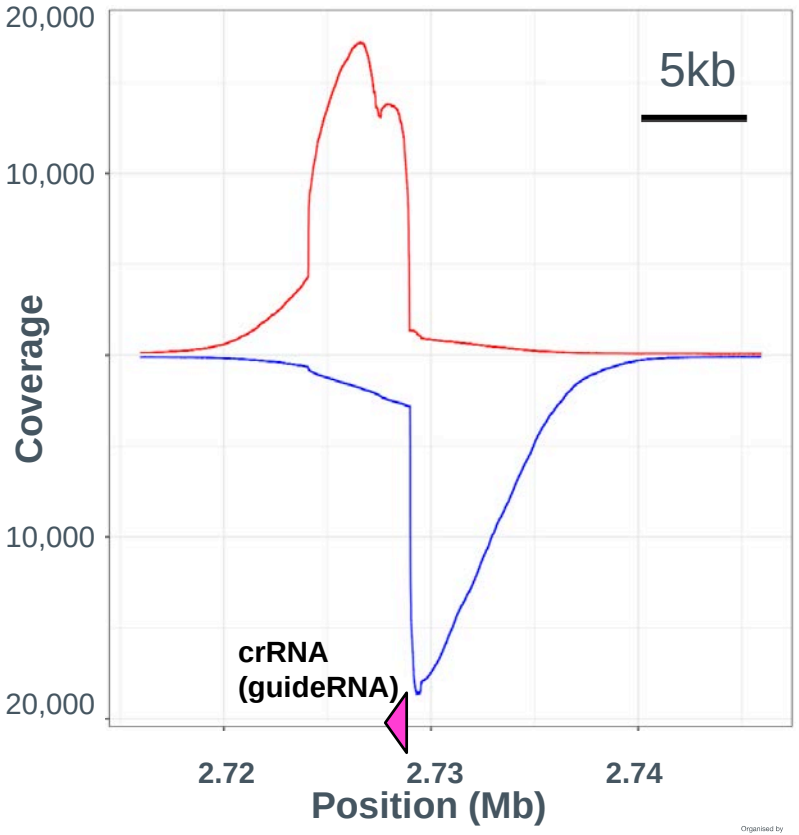

Enrichment in Mixed Microbial Population

Species	Genomic DNA fraction	Predicted crRNA binding sites
Escherichia coli	12	7
Salmonella enterica	12	7
Bacillus subtilis	12	1
Lactobacillus fermentum	12	2
Enterococcus faecalis	12	0
Staphylococcus aureus	12	0
Listeria monocytogenes	12	0
Pseudomonas aeruginosa	12	0
Cryptococcus neoformans	2	5
Saccharomyces cerevisiae	2	2

Tested performance of this crRNA on a more diverse population

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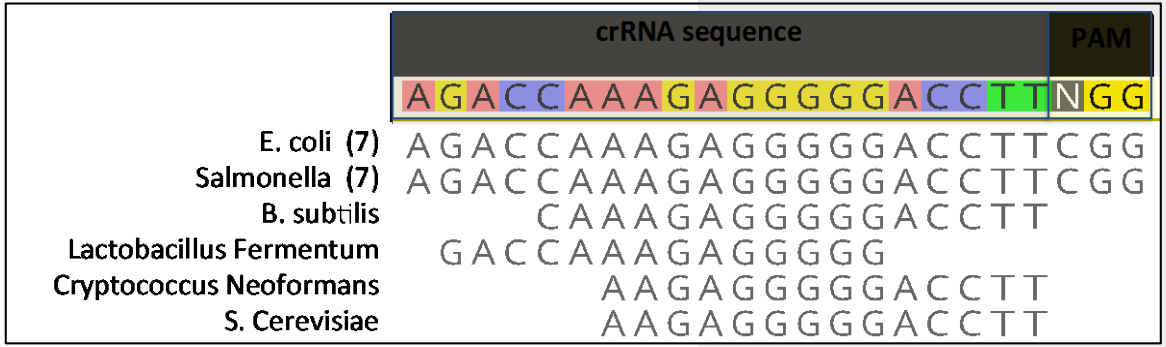

Enrichment in Mixed Microbial Population
Input: 1ug combined microbial genomic DNA

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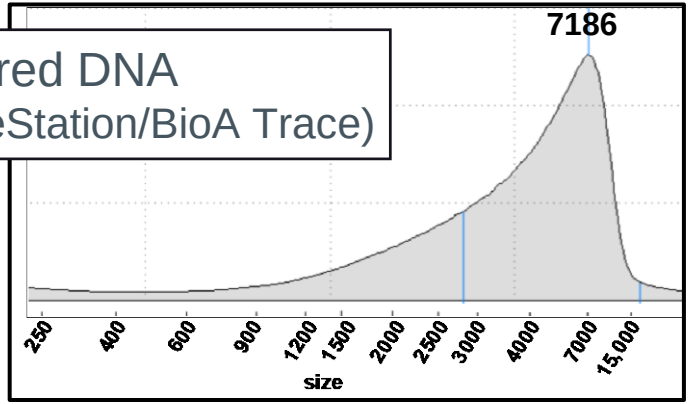
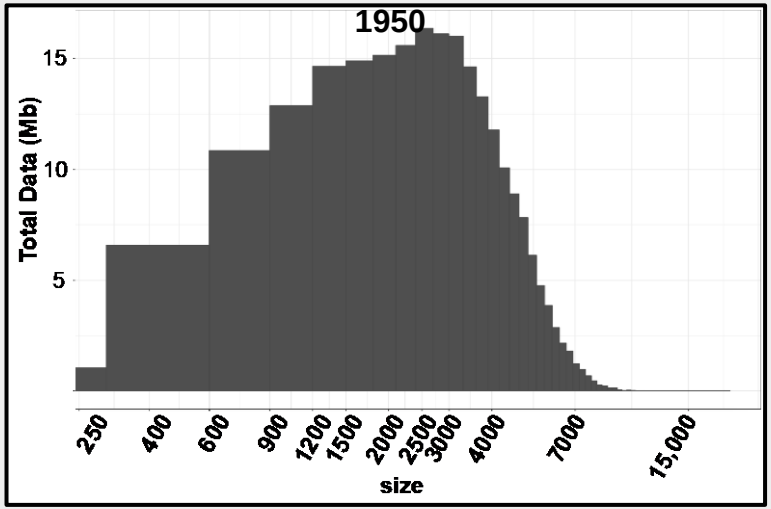
Sheared DNA
(TapeStation/BioA Trace)



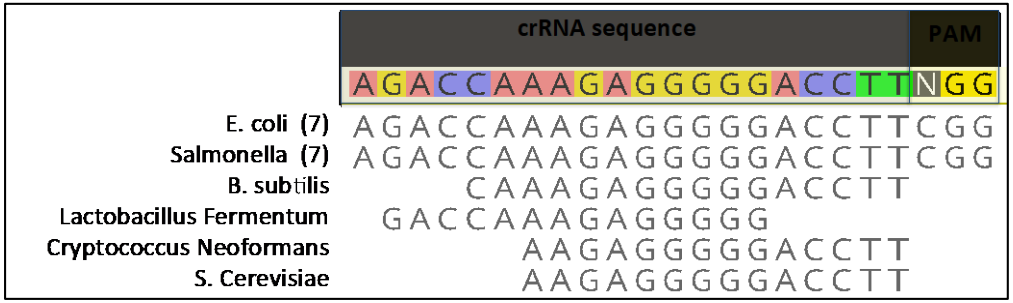
Total Data	230 Mb
Mean Read size	1950 bp

Size distribution of Data



Enrichment in Mixed Microbial Population

Significant coverage in species with crRNA/PAM site from mixed population



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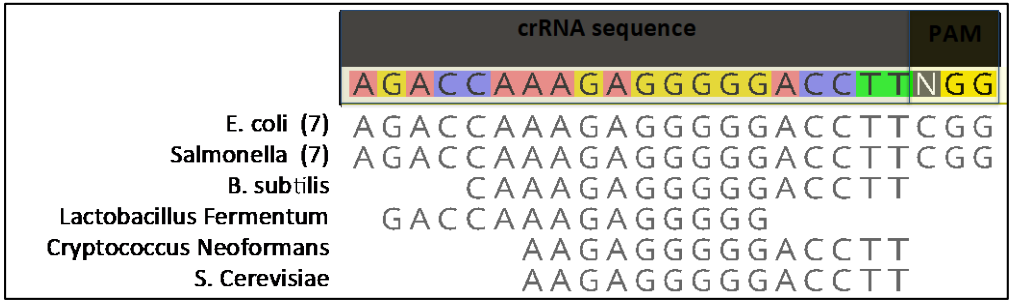
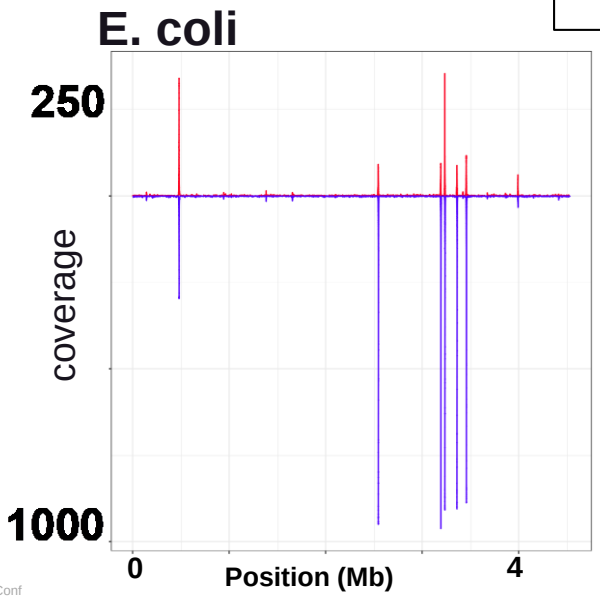
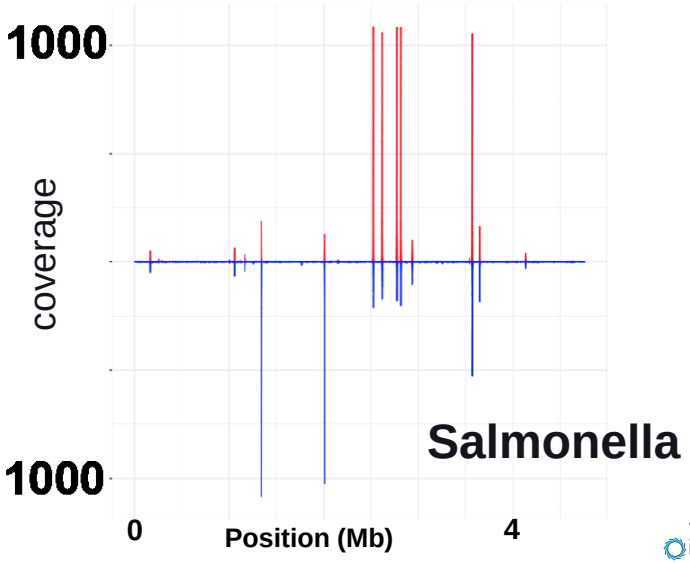
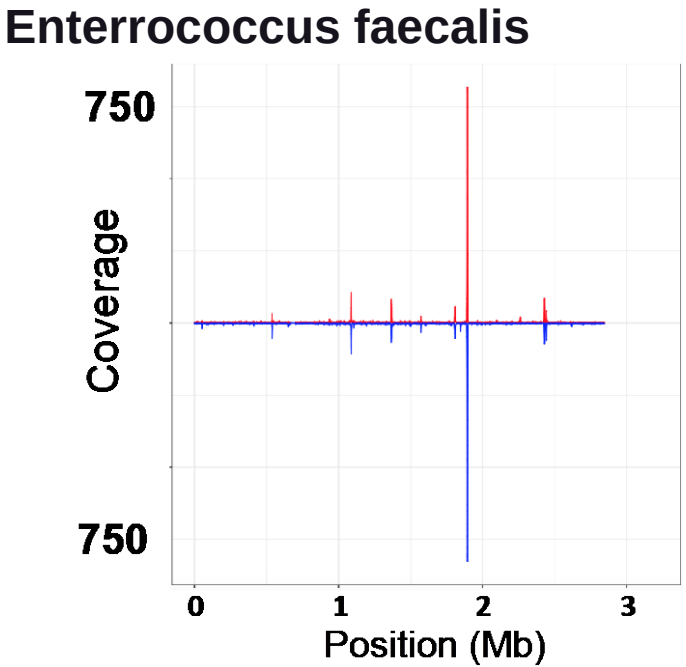






Enrichment in Mixed Microbial Population

Enrichment peaks observed in species without BLAST-identified crRNA target sequence

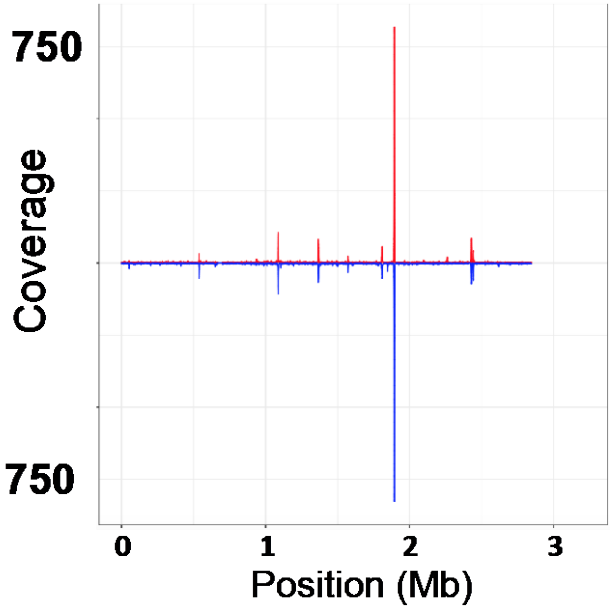


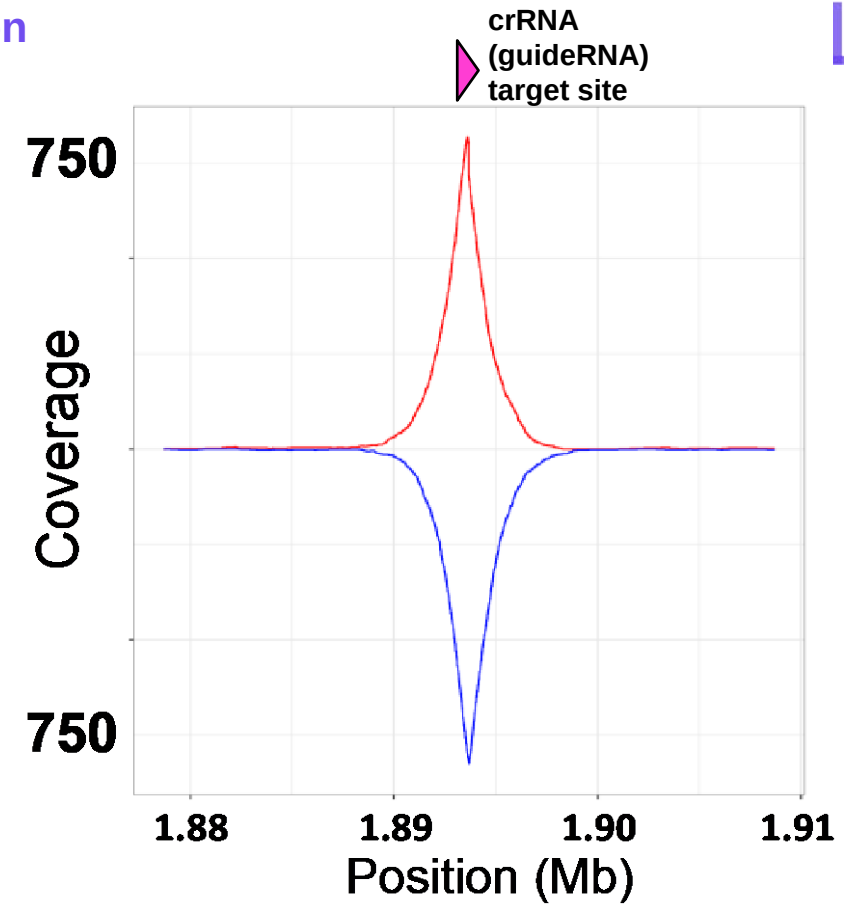
	crRNA sequence																	PAM	
	A	G	A	C	C	A	A	A	G	A	G	G	G	G	G	A	C	C	T	T	N	G	G
E. coli (7)	A	G	A	C	C	A	A	A	G	A	G	G	G	G	G	A	C	C	T	T	C	G	G
Salmonella (7)	A	G	A	C	C	A	A	A	G	A	G	G	G	G	G	A	C	C	T	T	C	G	G
B. subtilis				C	A	A	A	G	A	G	G	G	G	G	A	C	C	T	T				
Lactobacillus Fermentum			G	A	C	C	A	A	A	G	A	G	G	G	G	G							
Cryptococcus Neoformans							A	A	G	A	G	G	G	G	G	A	C	C	T	T			
S. Cerevisiae							A	A	G	A	G	G	G	G	G	A	C	C	T	T			

Enrichment in Mixed Microbial Population

Enrichment peaks observed in species without BLAST-identified crRNA target sequence

Enterrococcus faecalis





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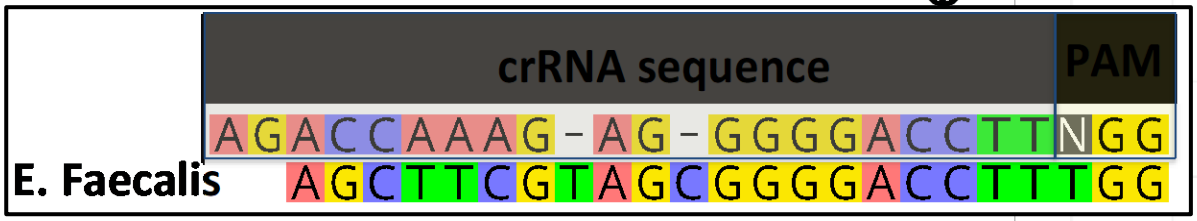
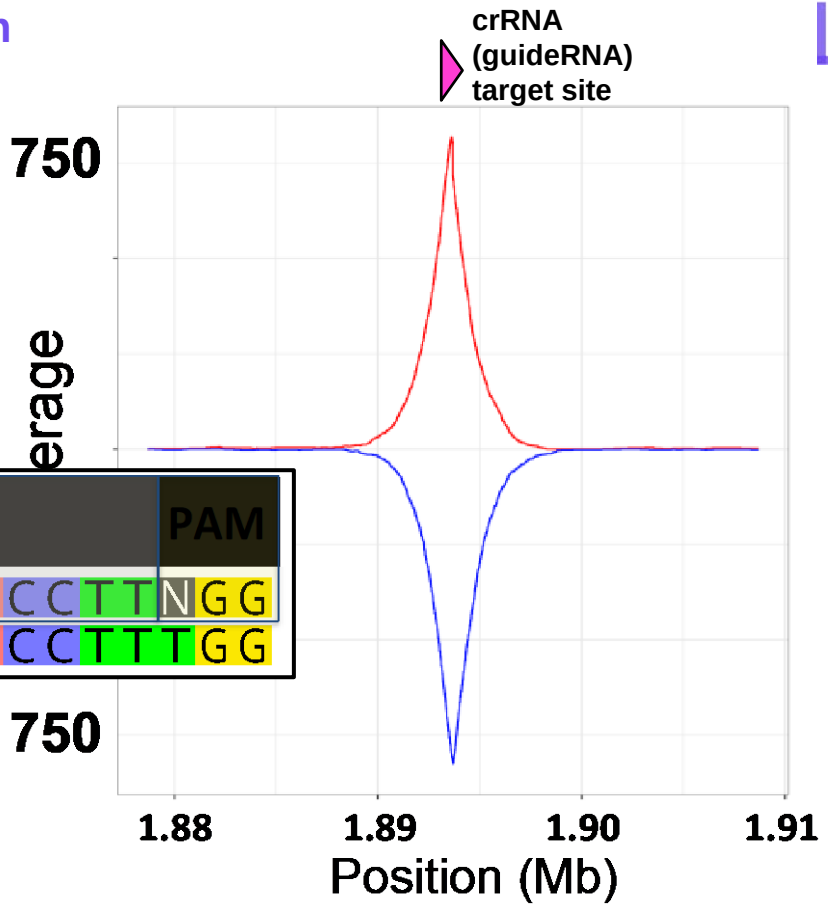




Human Enrichment: hTERT gene

- hTERT gene encodes a core protein protein component of Telomerase
- hTERT activity is widespread in human cancers
- Methylation frequently disrupted at hTERT promoter in human cancers
- Repetitive and high GC nature of this region make it difficult to query with PCR amplicons

Liu et al. Genes. 2016 Jul; 7(7): 38.

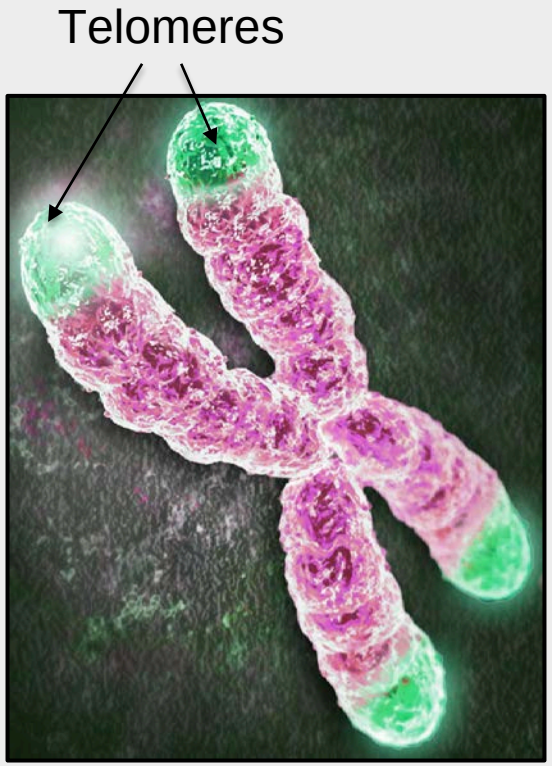
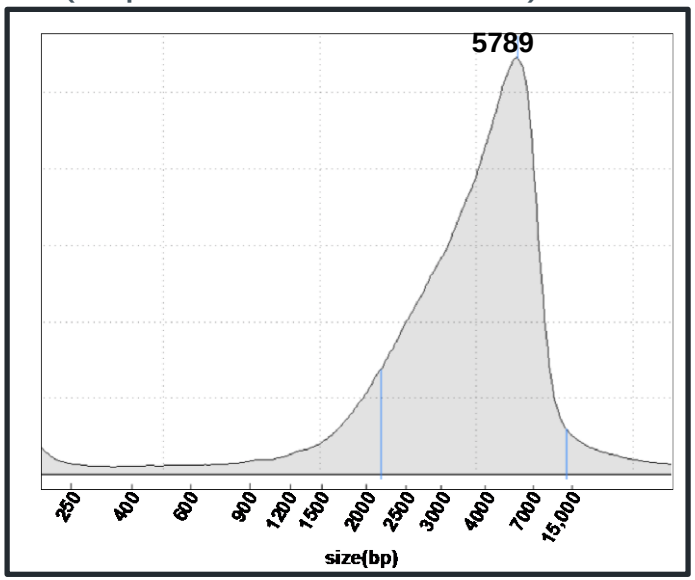


Image source: Stanford Medicine

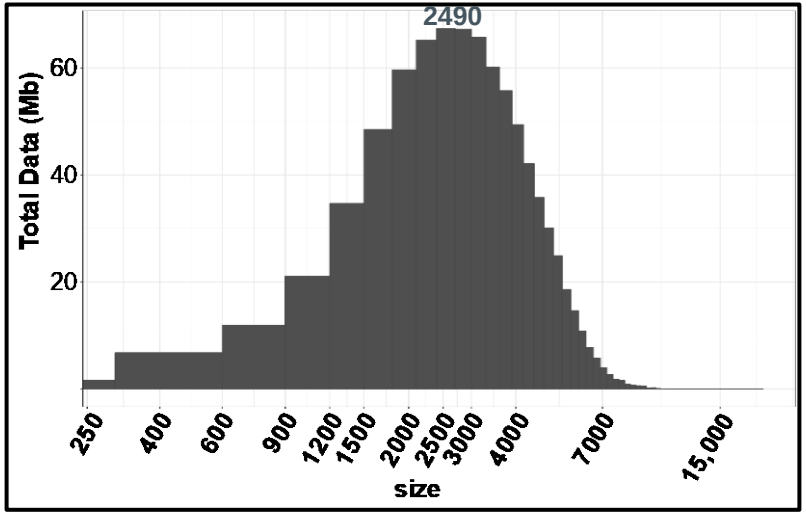
Cas9 Enrichment in human Samples

Input DNA: 2ug thyroid cancer cell line genomic DNA

Sheared DNA
(TapeStation/BioA Trace)

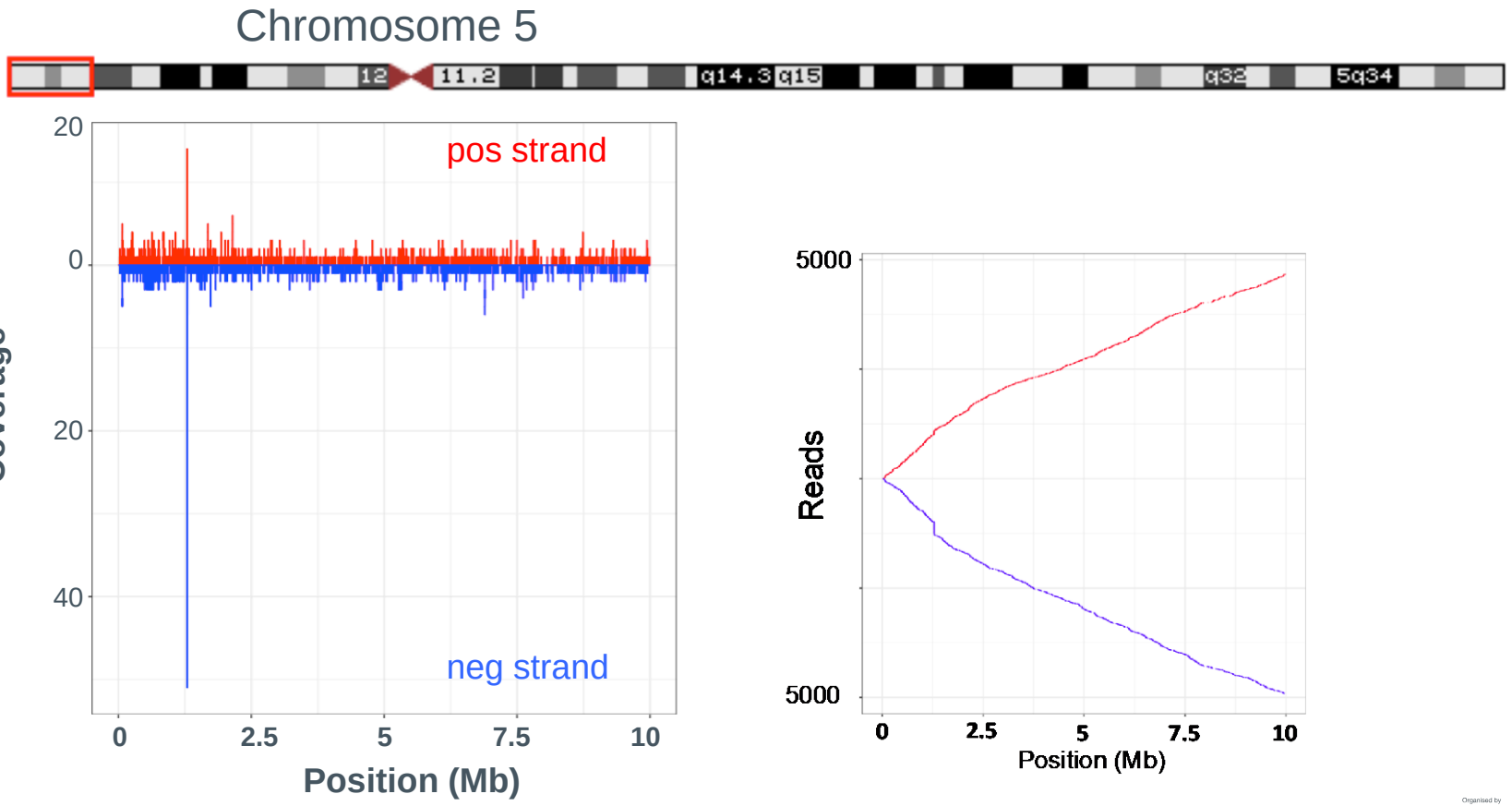


Size distribution of Data

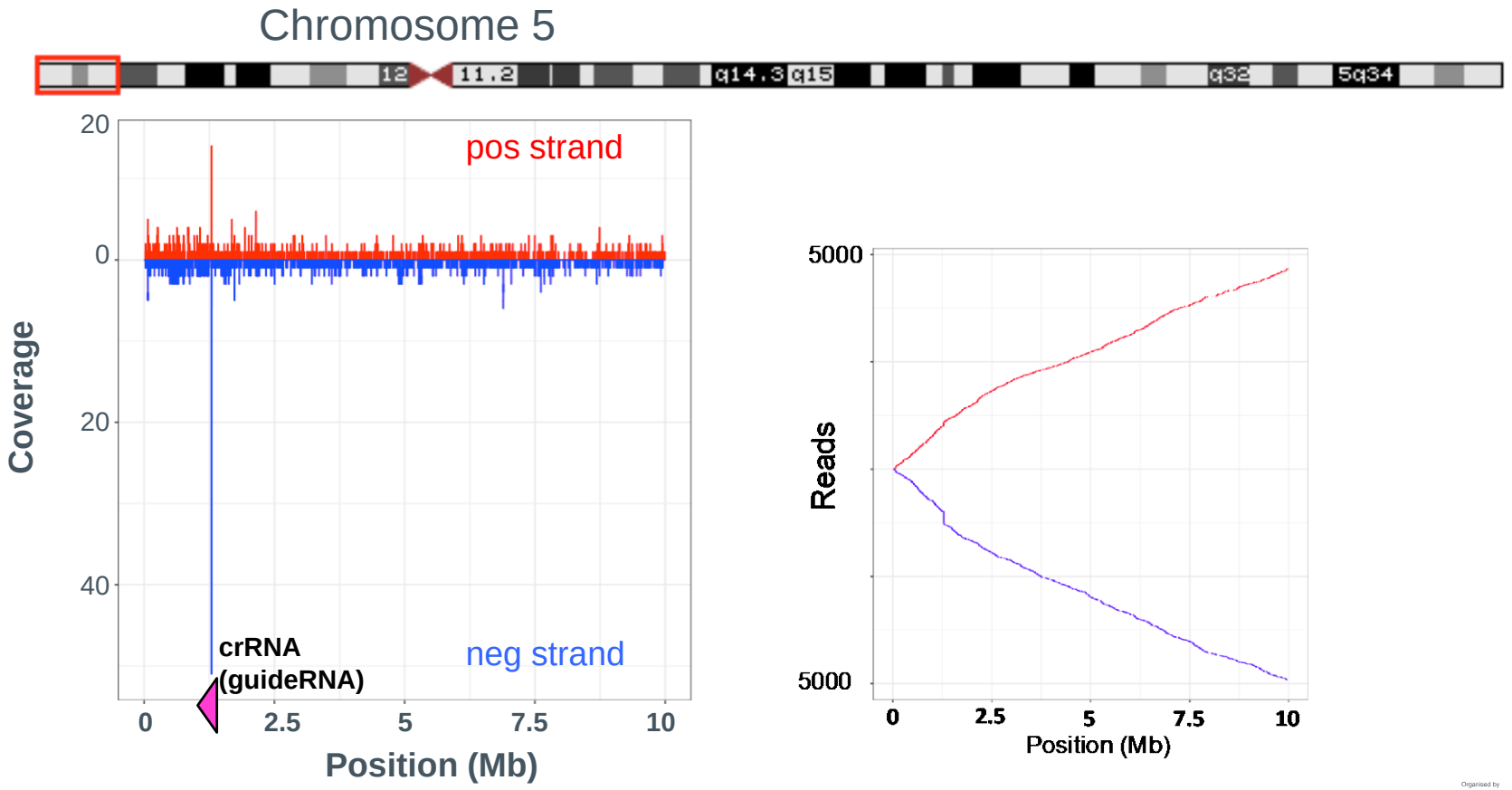


Total Data	820Mb
Mean Read Size	2490bp
Mean On-Target Read Size **On-target = (+/-)30kb**	2400bp

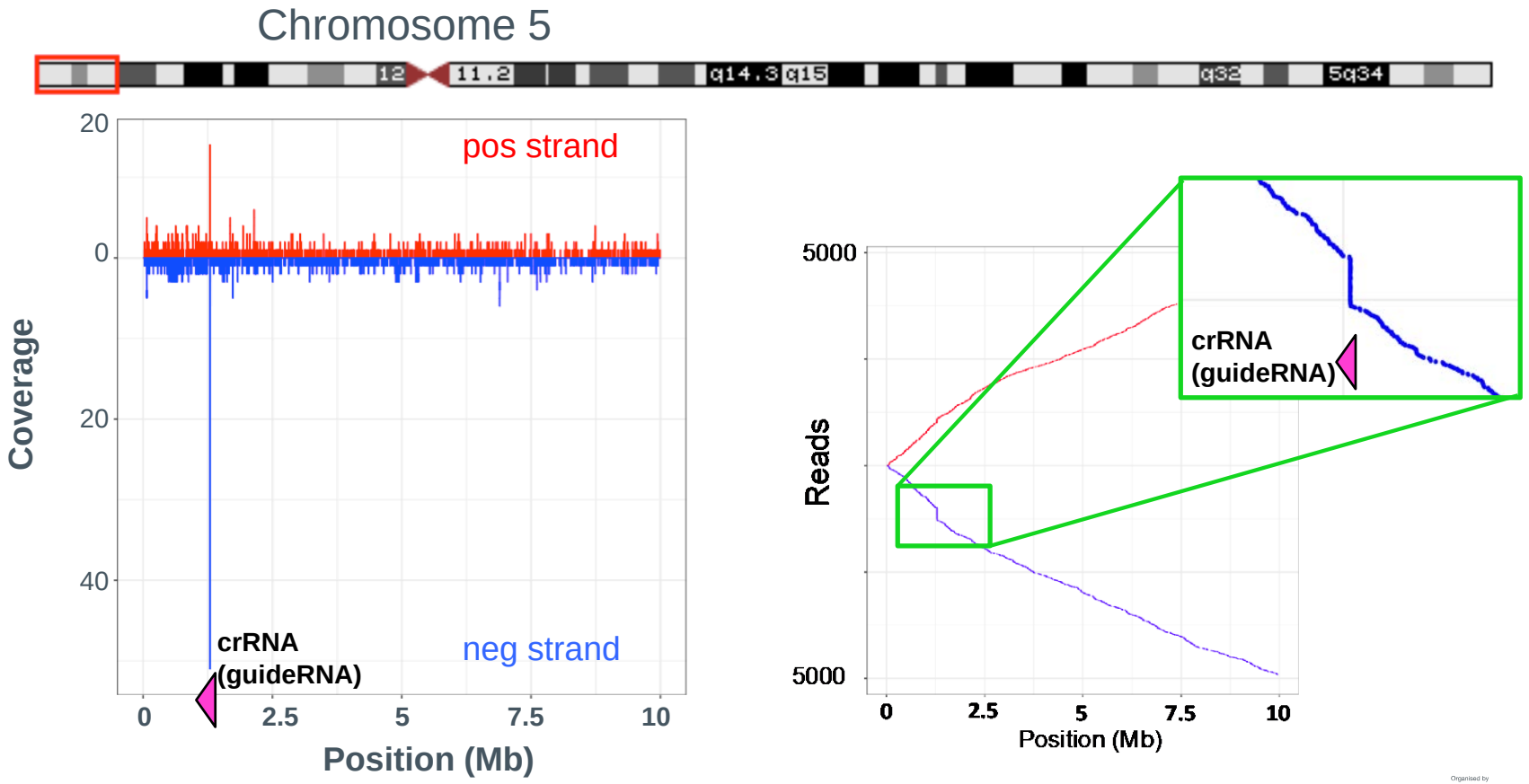
Cas9 Enrichment in human Samples



Cas9 Enrichment in human Samples

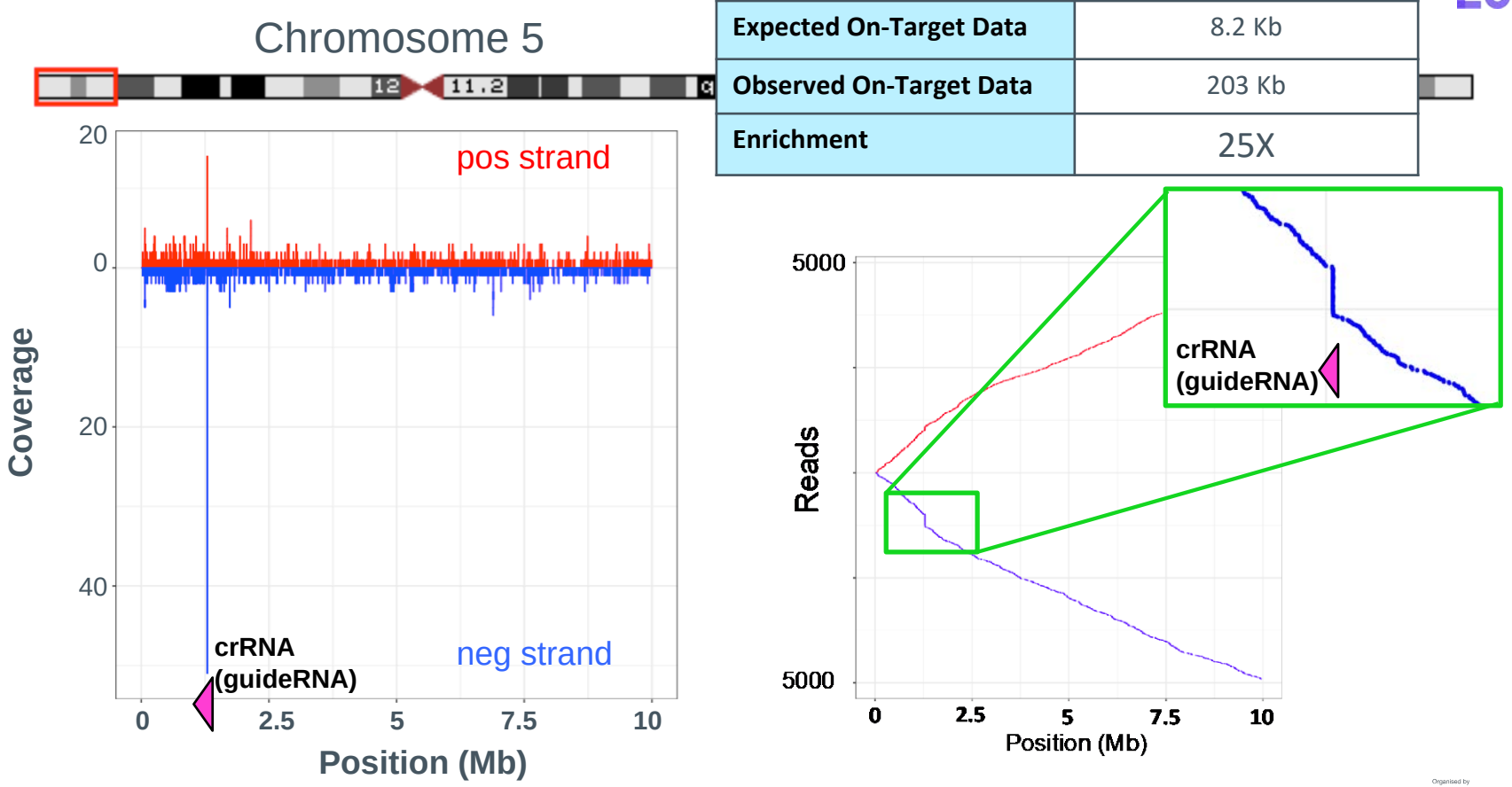


Cas9 Enrichment in human Samples



Cas9 Enrichment in human Samples

Total Data	820 Mb
Expected On-Target Data	8.2 Kb
Observed On-Target Data	203 Kb
Enrichment	25X



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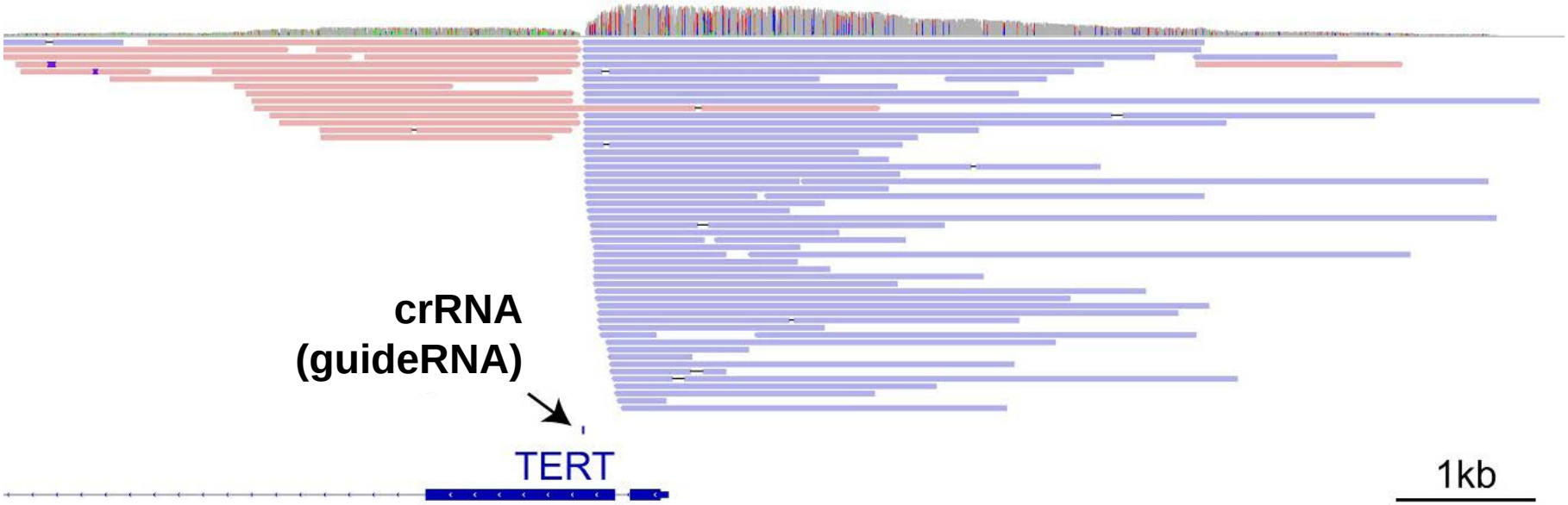
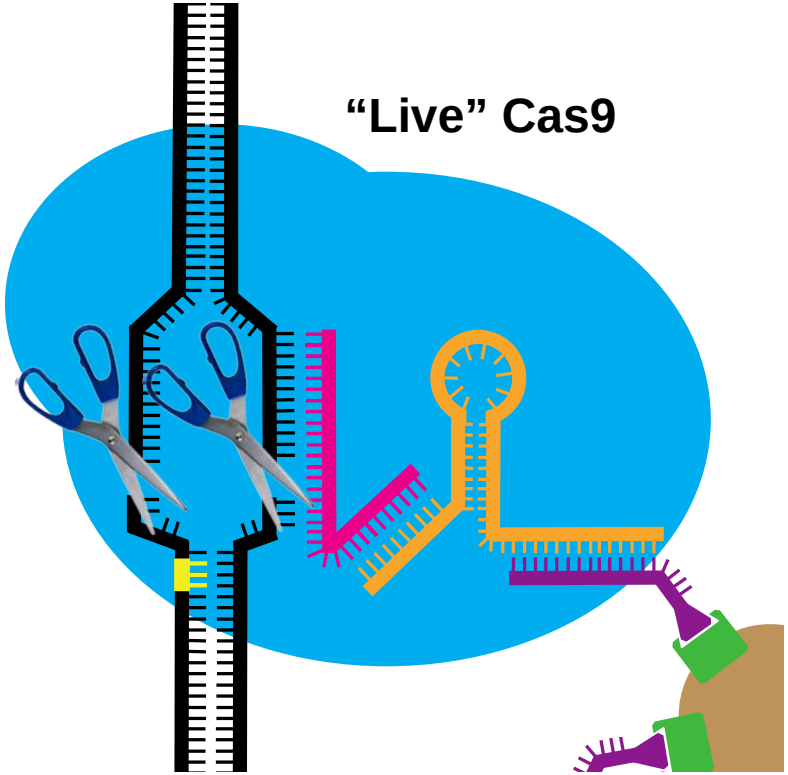
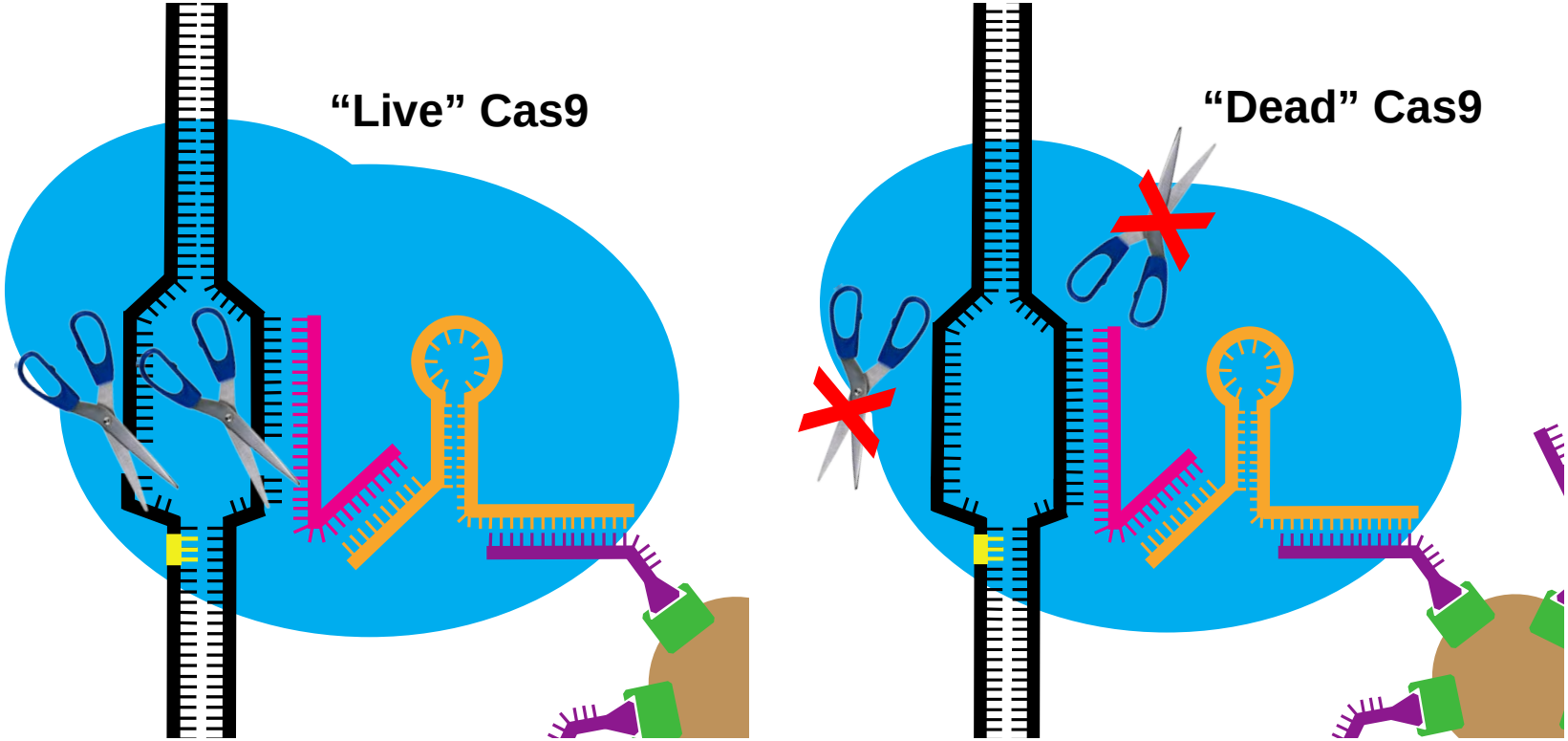


Catalytically Inactive Cas9



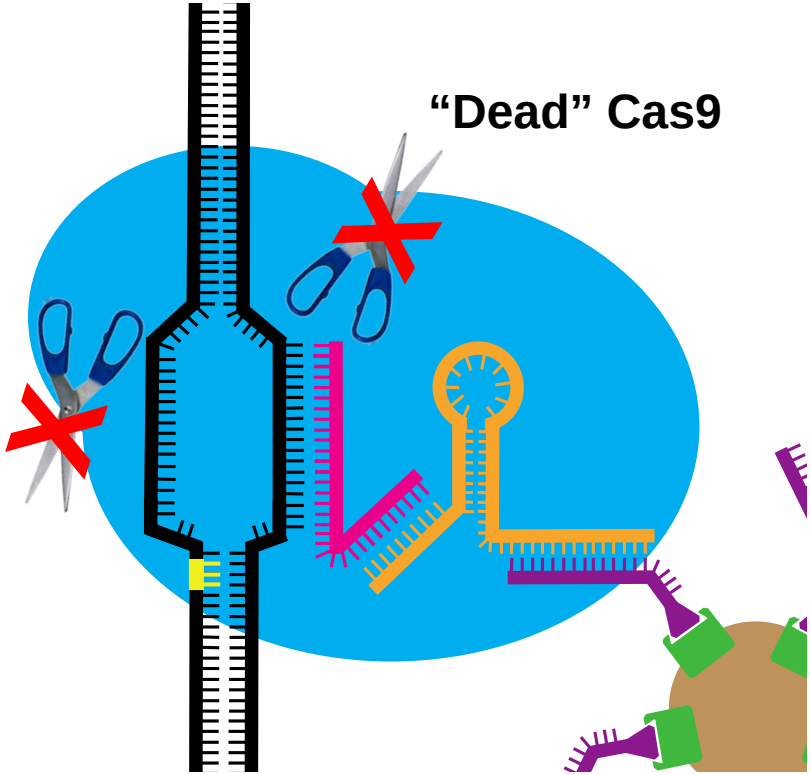
Catalytically Inactive Cas9



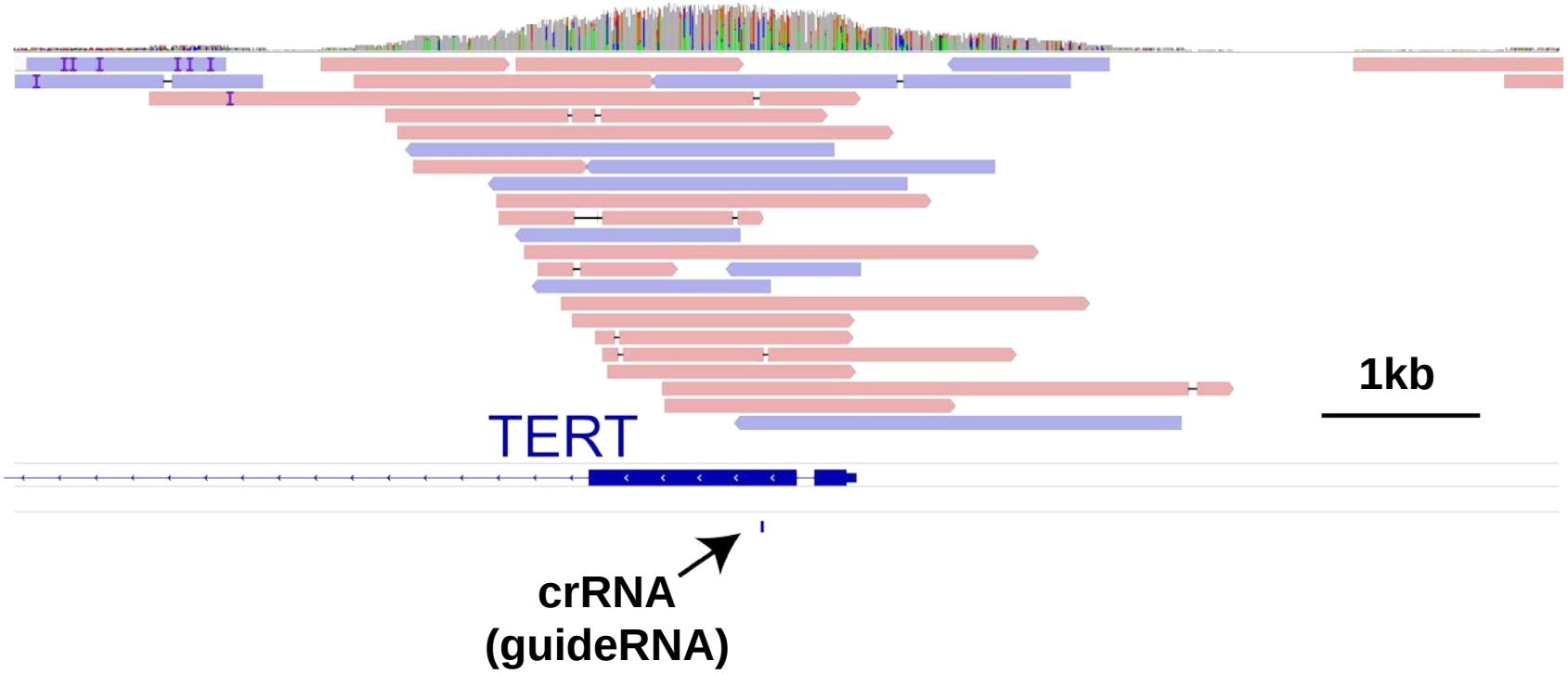
Catalytically Inactive Cas9

Reads centered at location of crRNA

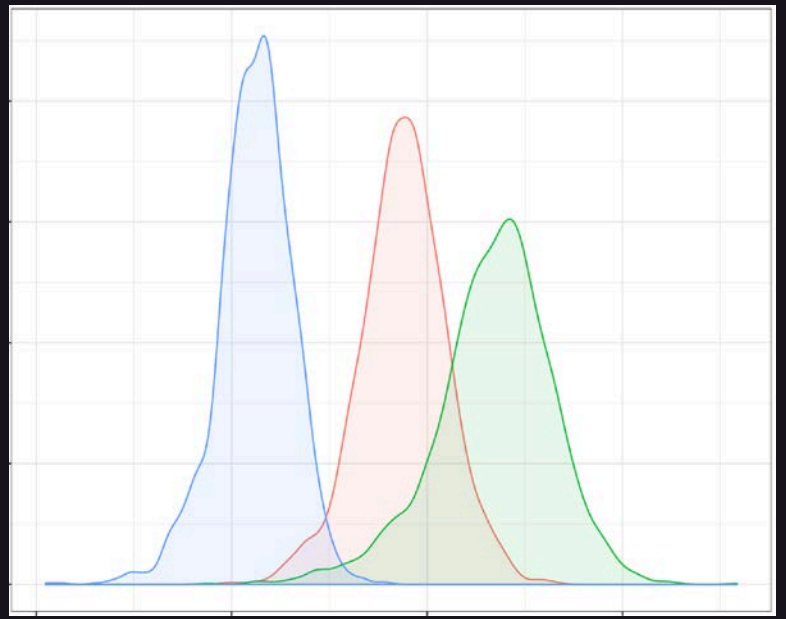
Cas9 complex evicted by ONT motor protein



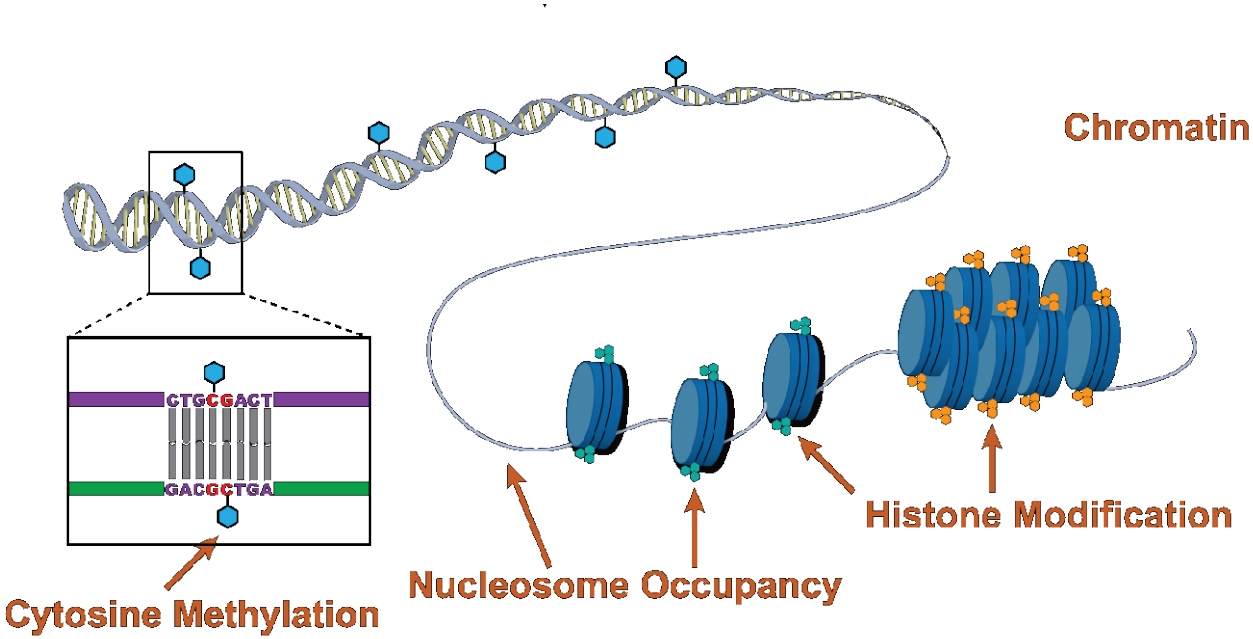
Enrichment with DeadCas9

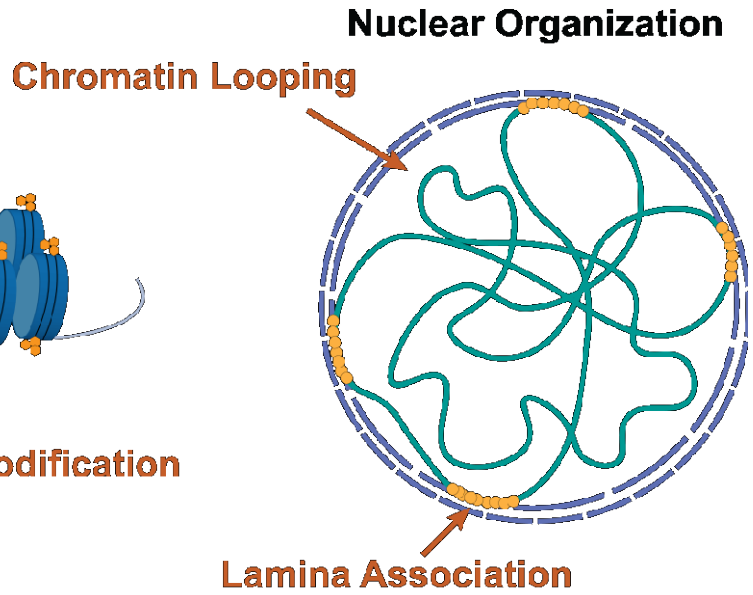


Methylation Analysis



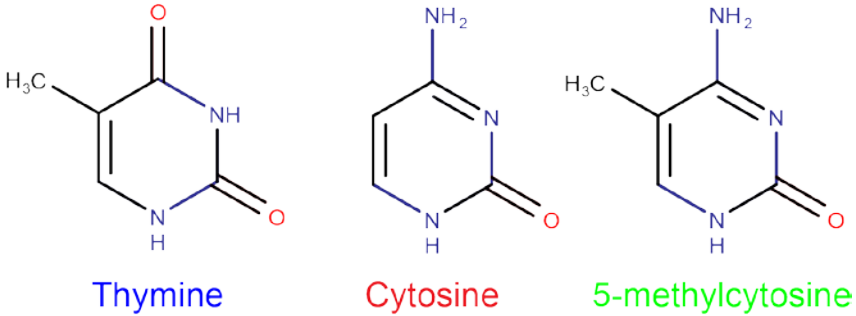
Epigenetics – 3,000 ft view

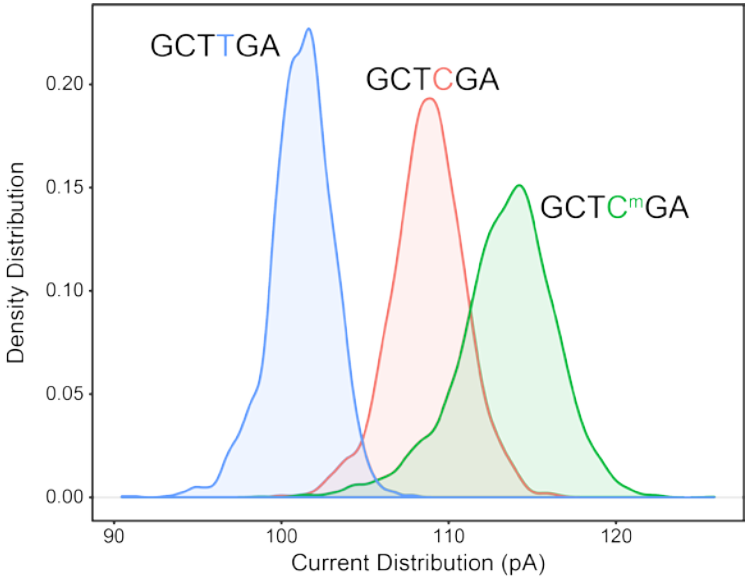




Cytosine methylation part a complex organizational system that regulates transcriptional activity

5-mC Detection with Nanopore Sequencing





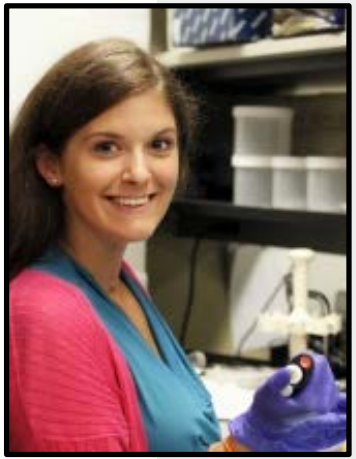
Training sets generated by treating PCR amplicons with methyltransferase (M. SssI)

Able to distinguish between (TG / CG / C^mG) in context of different k-mers

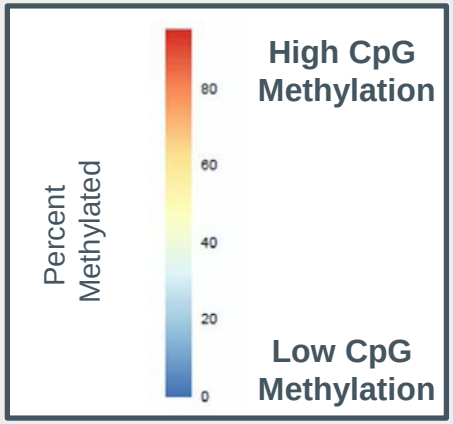
Simpson JT. et al. Nature Methods. 14, p.407–410 (2017)

Methylation at the hTERT promoter

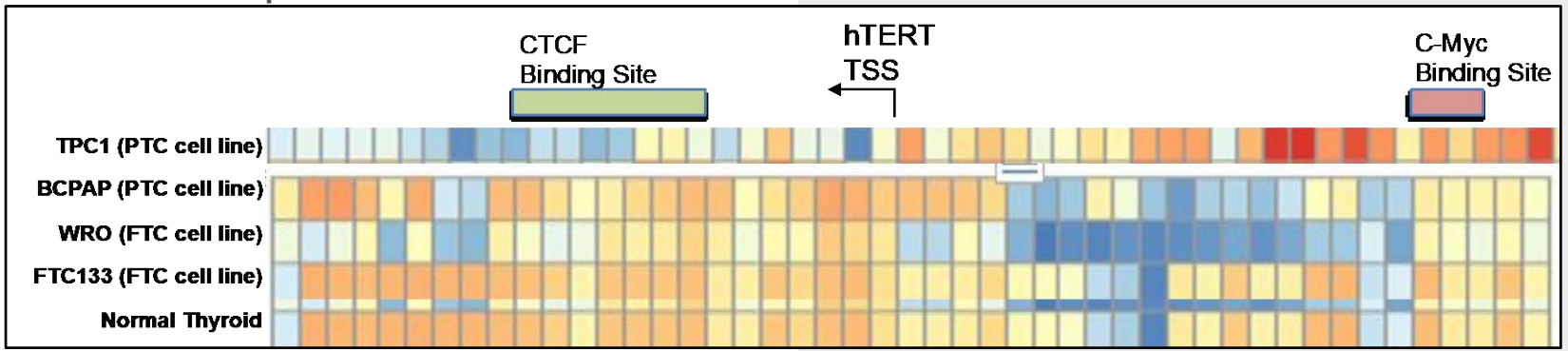
Thyroid carcinomas cell lines show distinct pattern of hypermethylation at the hTERT TSS



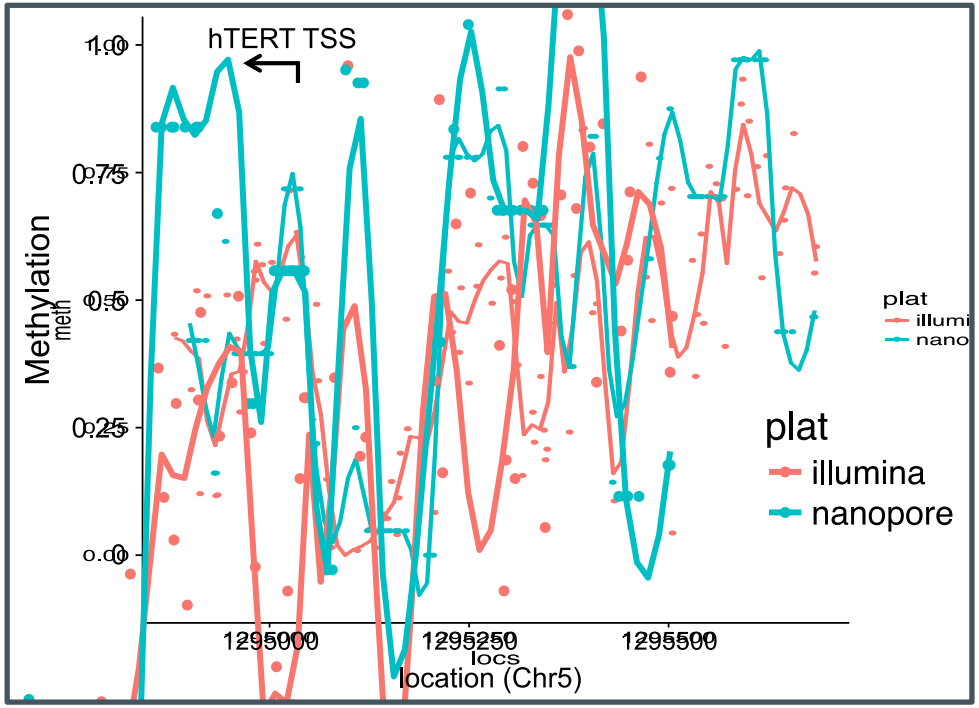
Brittany Avin



illumina BS-amplicon data

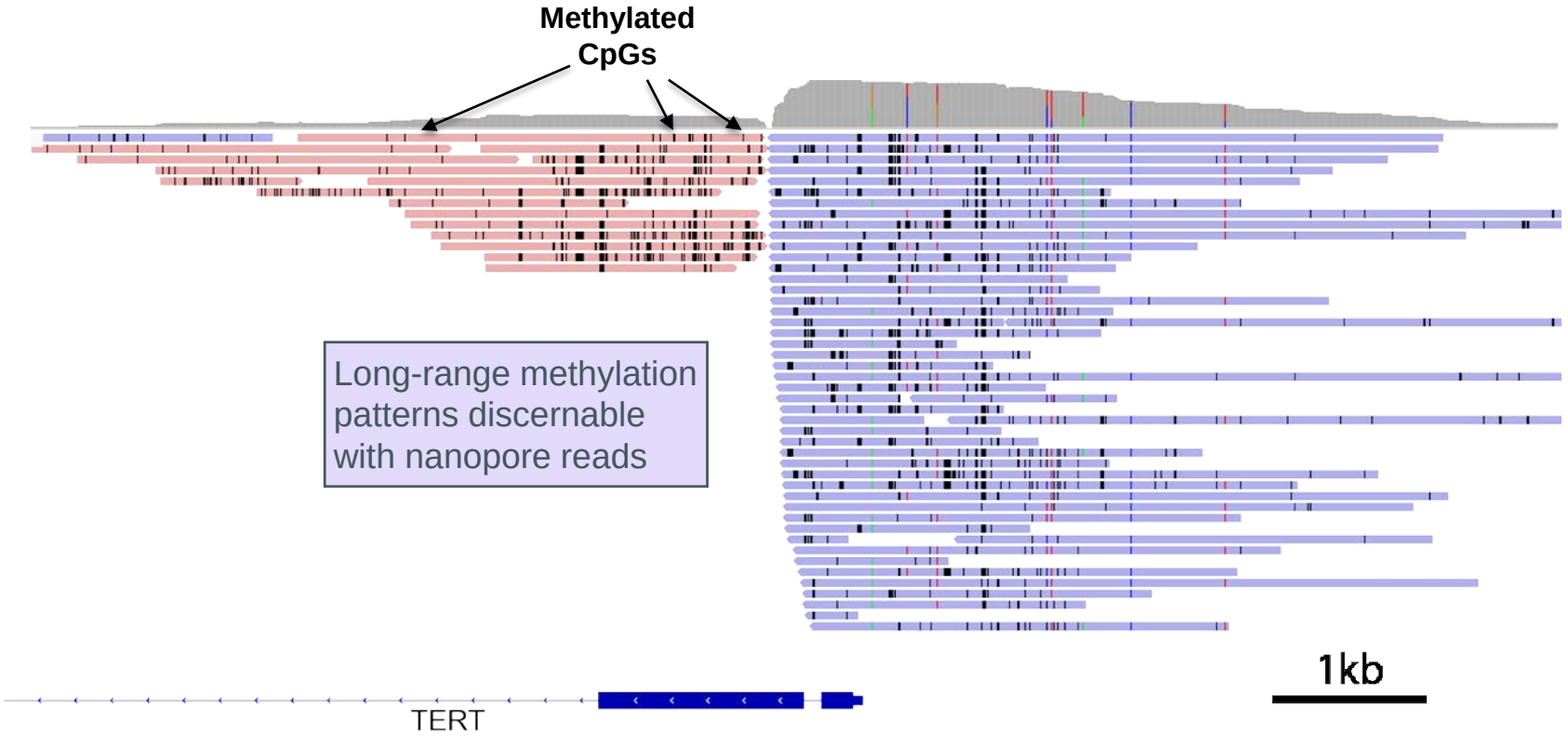


illumina and nanopore methylation calls at the hTERT promoter in BCPAP thyroid cancer cell line

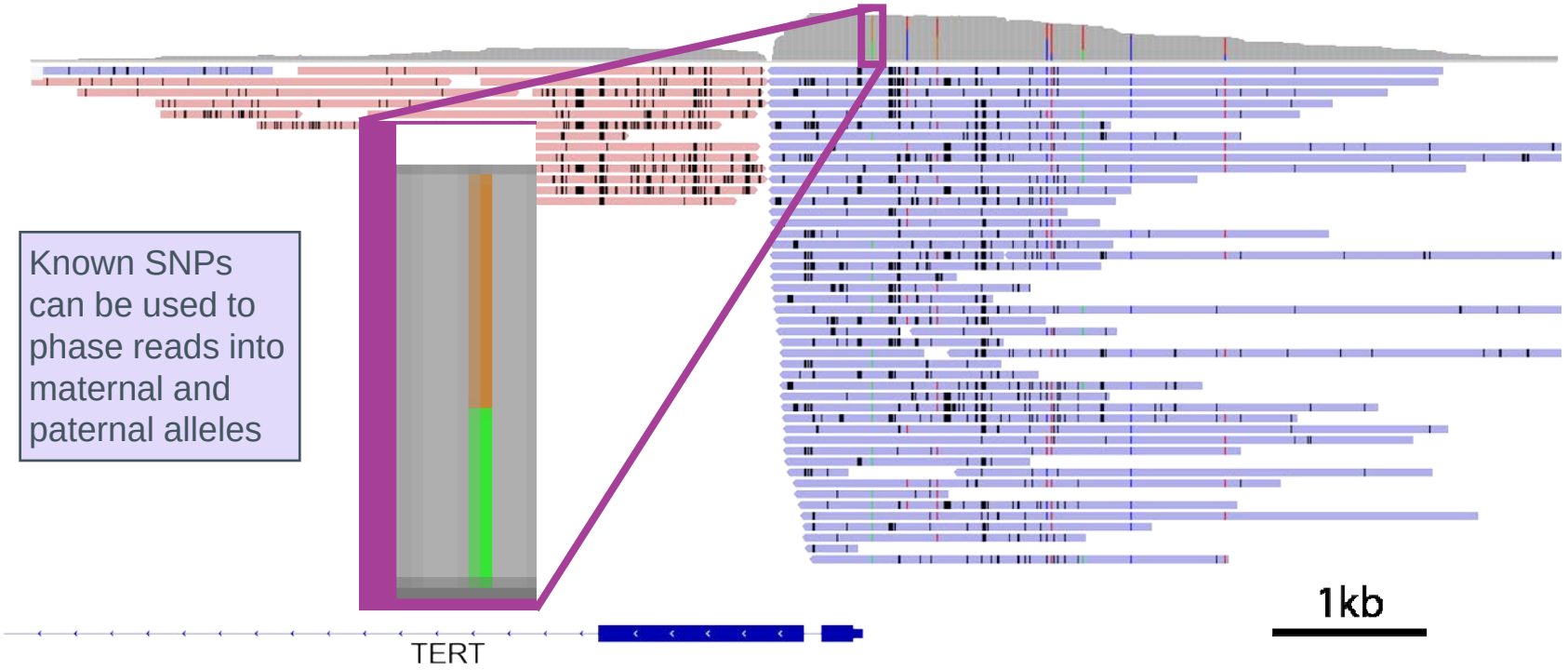


Methylation calls in nanopore enrichment data demonstrates overlap with pattern observed in illumina data (BS-amplicons)

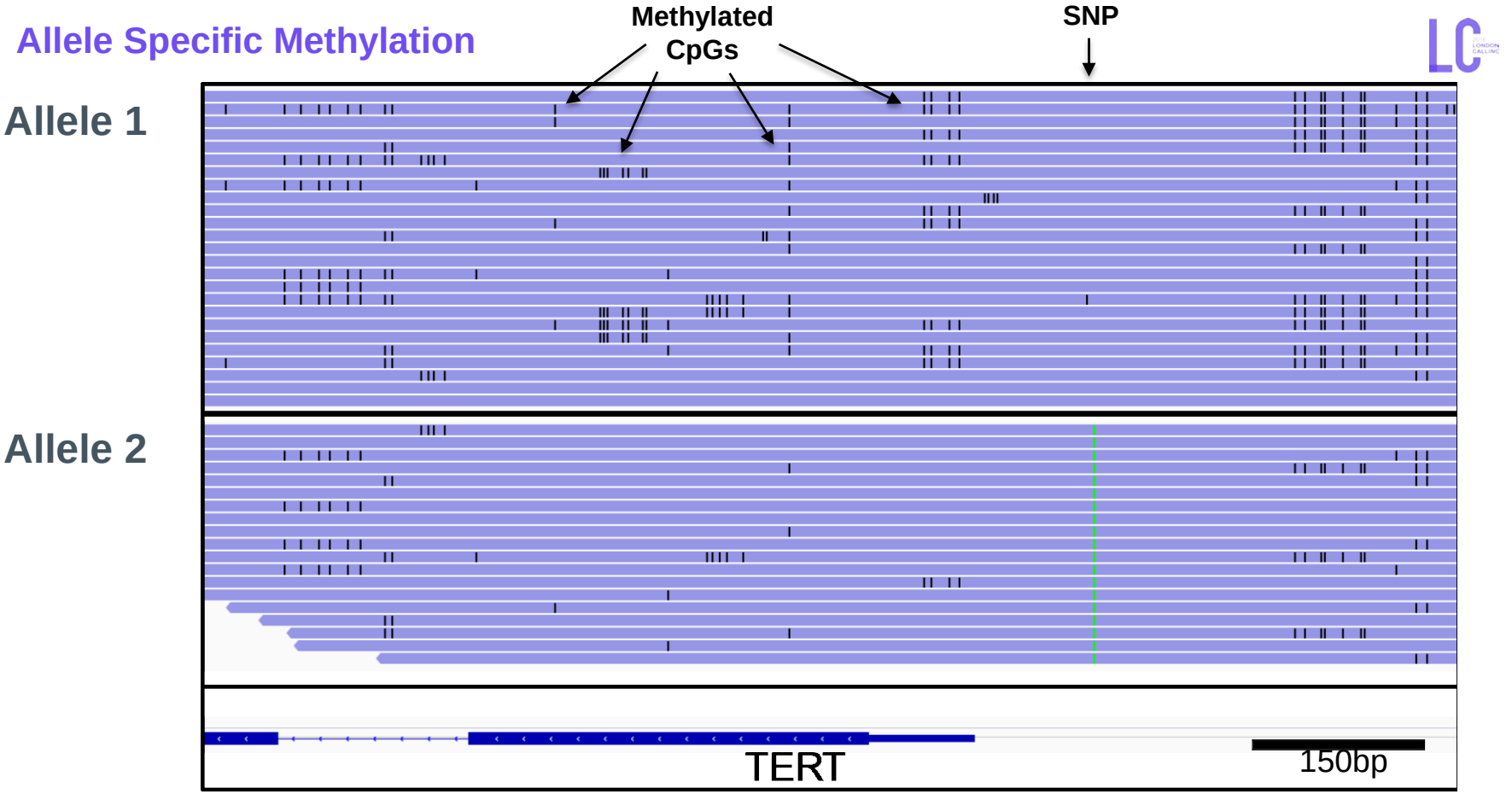
Methylation Calls Visualized



Using SNPs to Phase Reads



Allele Specific Methylation



Clinical Applications

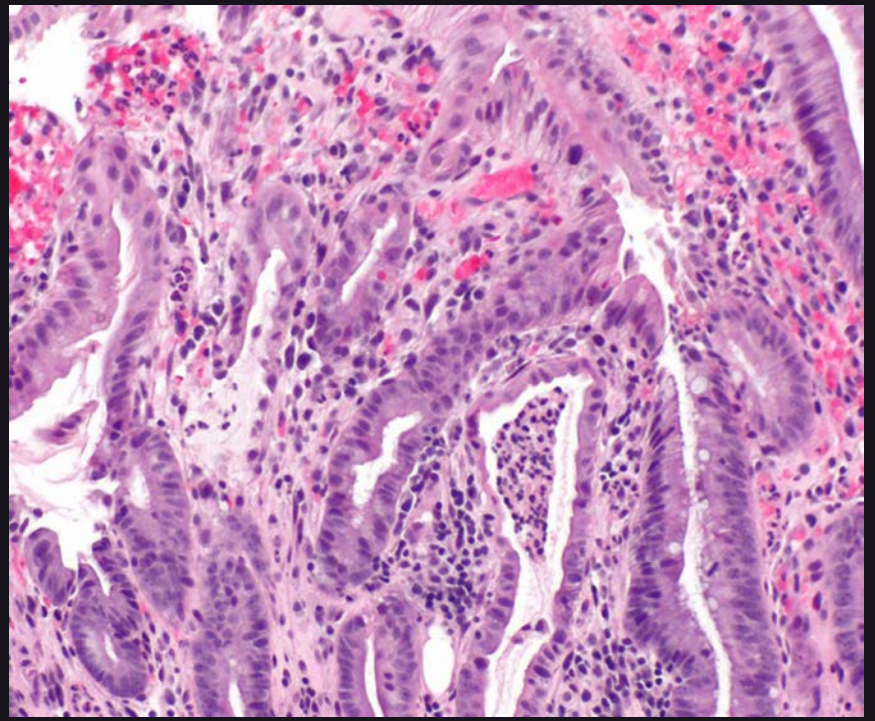
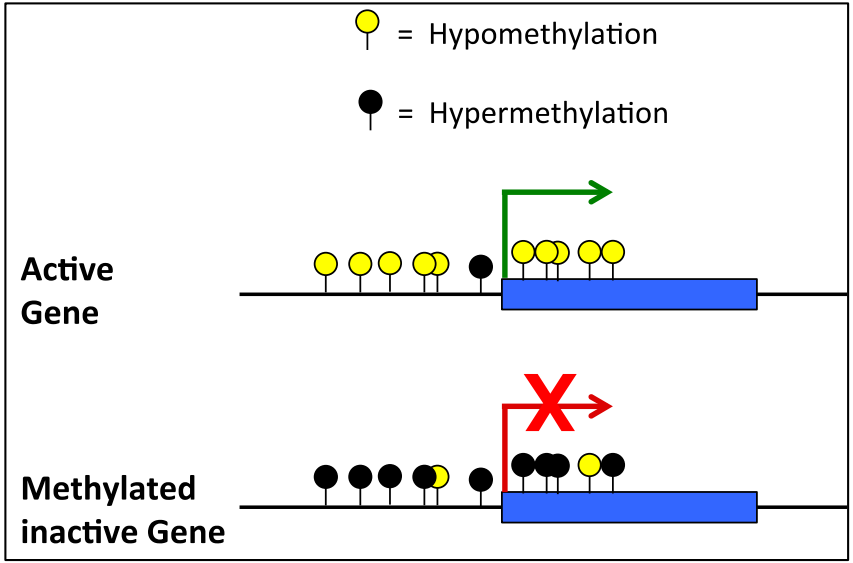


Image source: wikimedia commons By Nephron, CC BY-SA 4.0,

Clinical Applications : Cancer Screening

Profiling simultaneous of numerous cancer genes facilitates evaluating the cancer methylome

Diagnostic and prognostic potential



Clinical Applications : FSHD

FSHD (Facioscapulohumeral muscular dystrophy) caused by shortening or hypomethylation of 3.2kb tandem repeat on chr4

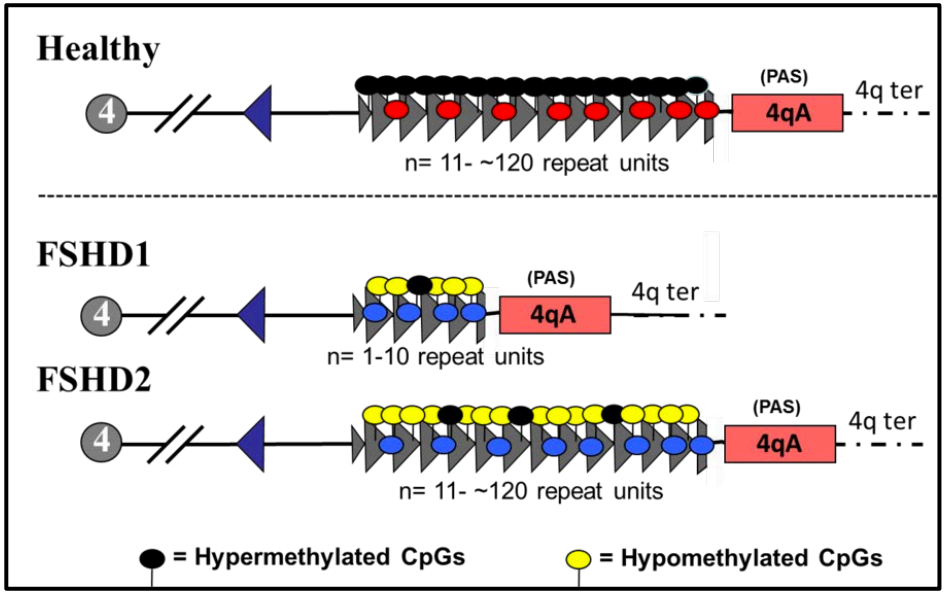


Image source: wikimedia commons
Image author: Peter Jones, PhD

Oxford NANOPORE Technologies

- James Graham
- Andy Heron

- Winston Timp, PhD
- Rachael Workman, MS
- Brittany Avin
- Norah Sadowski
- Yunfan Fan
- Isac Lee
- Evi Mercken
- Roham Razaghi

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