E-SAN THAILAND
CODING & AI ACADEMY

โครงการวิจัยโมเดลระบบนิเวศการเรียนรู้ที่บูรณาการ CODING & AI สำหรับเยาวชน
Model of Learning Ecosystem Platform integrate with Coding & AI for Youth

โครงการย่อยที่ 6
การพัฒนาเยาวชนเพื่อเข้าสู่วิชาชีพขั้นสูงด้าน Coding & AI
ร่วมกับ Coding **Entrepreneur & Partnership: Personal AI**

xPore

AI-Powered App for Bioinformaticians

ผศ. ดร.นฤมล ประทานวณิช
โครงการย่อยที่ 6

E-SAN THAILAND CODING & AI ACADEMY

โครงการวิจัยโมเดลระบบนิเวศการเรียนรู้ที่บูรณาการ CODING & AI สำหรับเยาวชน

Model of Learning Ecosystem Platform integrate with Coding & AI for Youth

ARTICLES

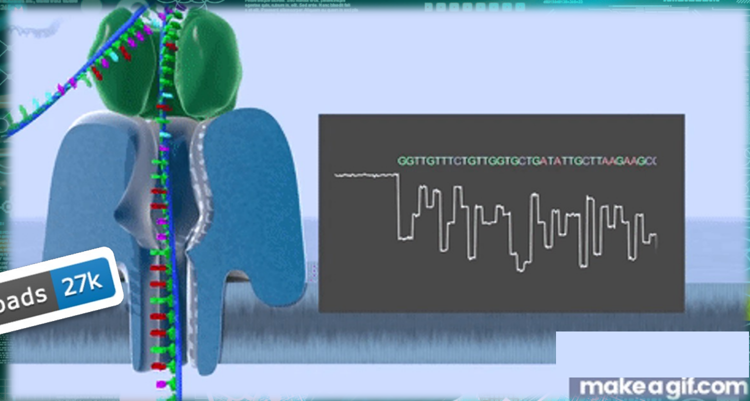
https://doi.org/10.1038/s41587-021-00949-w

nature biotechnology

Identification of differential RNA modifications from nanopore direct RNA sequencing with xPore

Ploy N. Pratanwanich[1,2,3] ✉, Fei Yao[1,11], Ying Chen[1,11], Casslynn W. Q. Koh[1,11], Yuk Kei Wan[1,11], Christopher Hendra[1,4], Polly Poon[1], Yeek Teck Goh[1], Phoebe M. L. Yap[1], Jing Yuan Chooi[5], Wee Joo Chng[5,6,7], Sarah B. Ng[1], Alexandre Thiery[8], W. S. Sho Goh[1,9] ✉ and Jonathan Göke[1,10] ✉

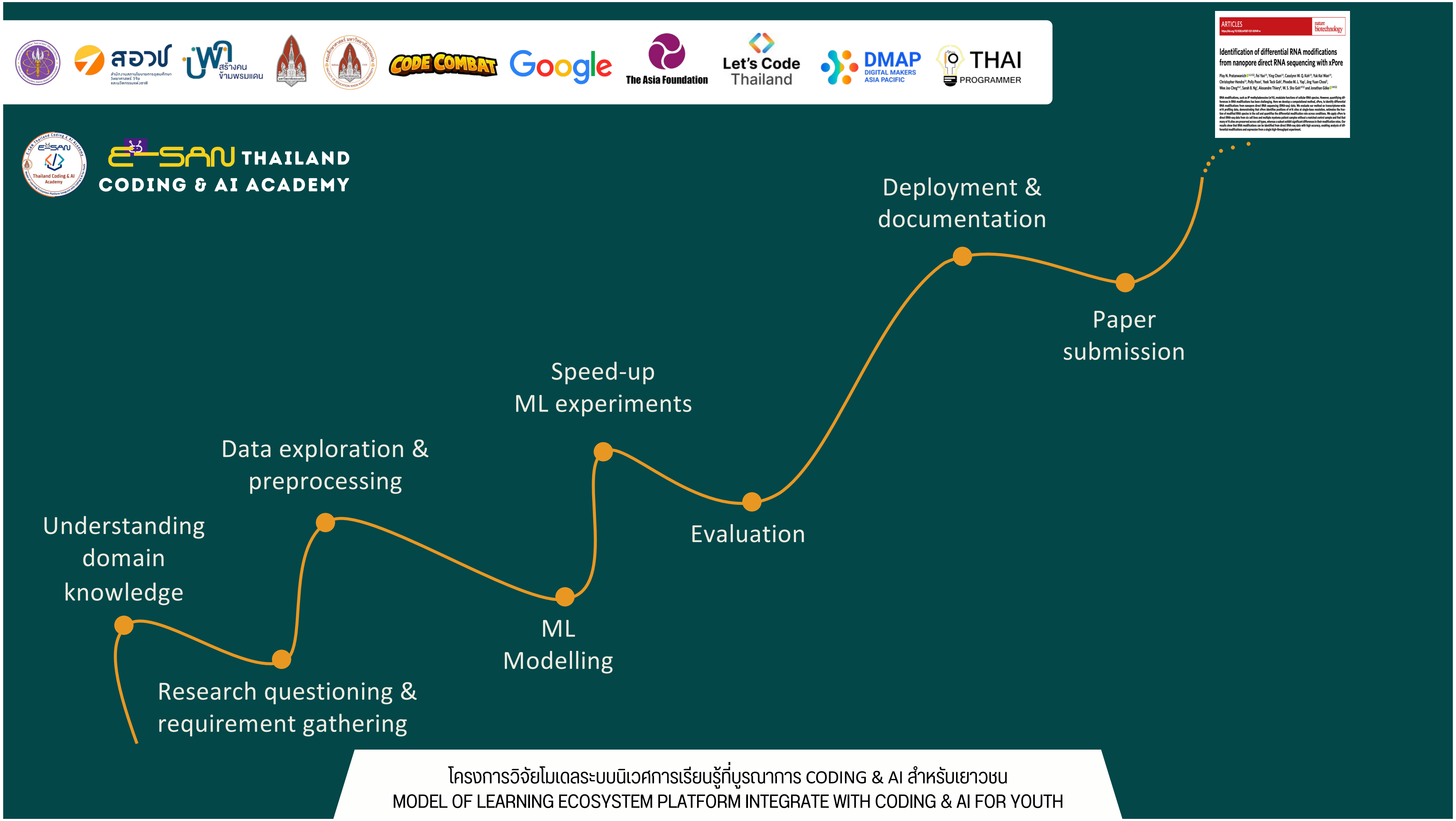
RNA modifications, such as N^6-methyladenosine (m^6A), modulate functions of cellular RNA species. However, quantifying differences in RNA modifications has been challenging. Here we develop a computational method, xPore, to identify differential RNA modifications from nanopore direct RNA sequencing (RNA-seq) data. We evaluate our method on transcriptome-wide m^6A profiling data, demonstrating that xPore identifies positions of m^6A sites at single-base resolution, estimates the fraction of modified RNA species in the cell and quantifies the differential modification rate across conditions. We apply xPore to direct RNA-seq data from six cell lines and multiple myeloma patient samples without a matched control sample and find that many m^6A sites are preserved across cell types, whereas a subset exhibit significant differences in their modification rates. Our results show that RNA modifications can be identified from direct RNA-seq data with high accuracy, enabling analysis of differential modifications and expression from a single high-throughput experiment.



CHULALONGKORN UNIVERSITY

Genome Institute of Singapore GIS

NUS National University of Singapore

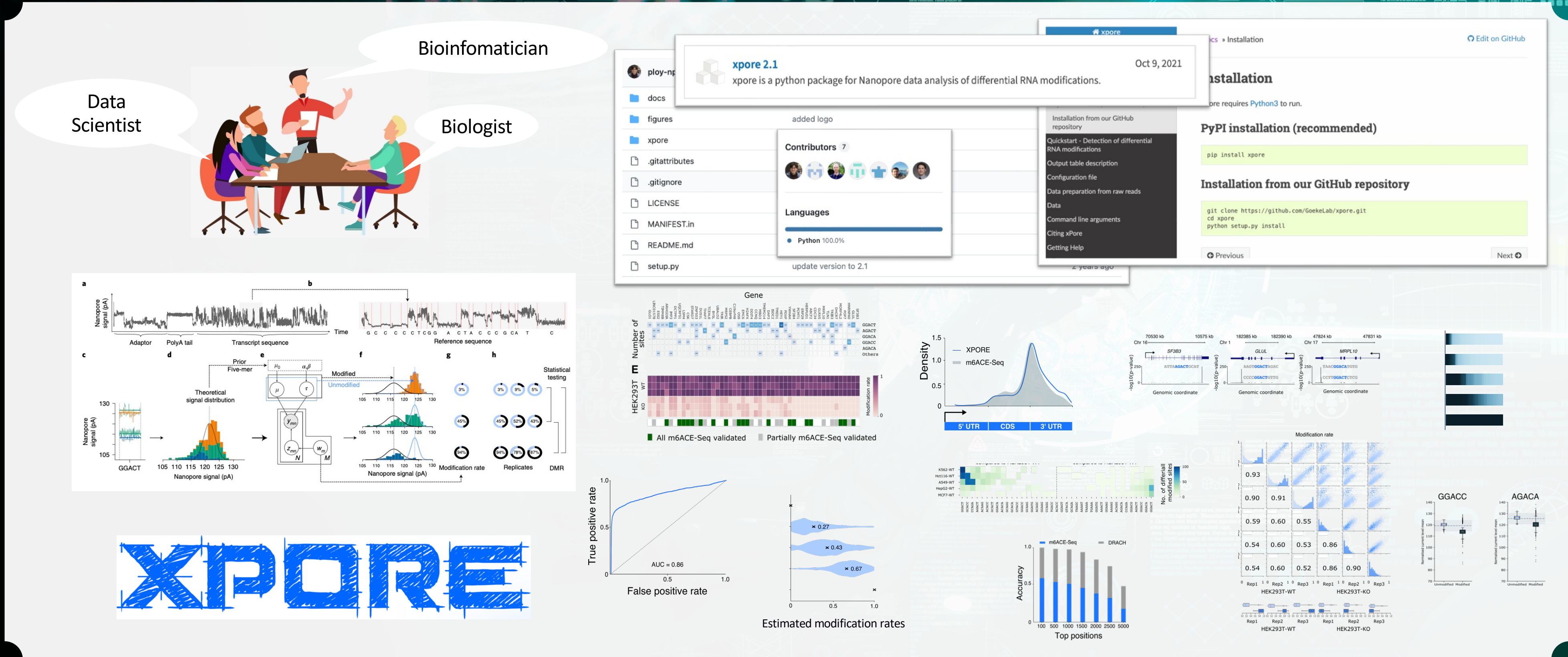


E-SAN THAILAND
CODING & AI ACADEMY
Understanding domain knowledge
Research questioning & requirement gathering
Data exploration & preprocessing
ML Modelling
Speed-up ML experiments
Evaluation
Deployment & documentation
Paper submission
ARTICLES
Identification of differential RNA modifications from nanopore direct RNA sequencing with xPore
โครงการวิจัยโมเดลระบบนิเวศการเรียนรู้ที่บูรณาการ CODING & AI สำหรับเยาวชน
MODEL OF LEARNING ECOSYSTEM PLATFORM INTEGRATE WITH CODING & AI FOR YOUTH

E-SAN THAILAND
CODING & AI ACADEMY

โครงการวิจัยโมเดลระบบนิเวศการเรียนรู้ที่บูรณาการ CODING & AI สำหรับเยาวชน

Model of Learning Ecosystem Platform integrate with Coding & AI for Youth



E-SAN THAILAND CODING & AI ACADEMY

โครงการวิจัยโมเดลระบบนิเวศการเรียนรู้ที่บูรณาการ CODING & AI สำหรับเยาวชน

Model of Learning Ecosystem Platform integrate with Coding & AI for Youth

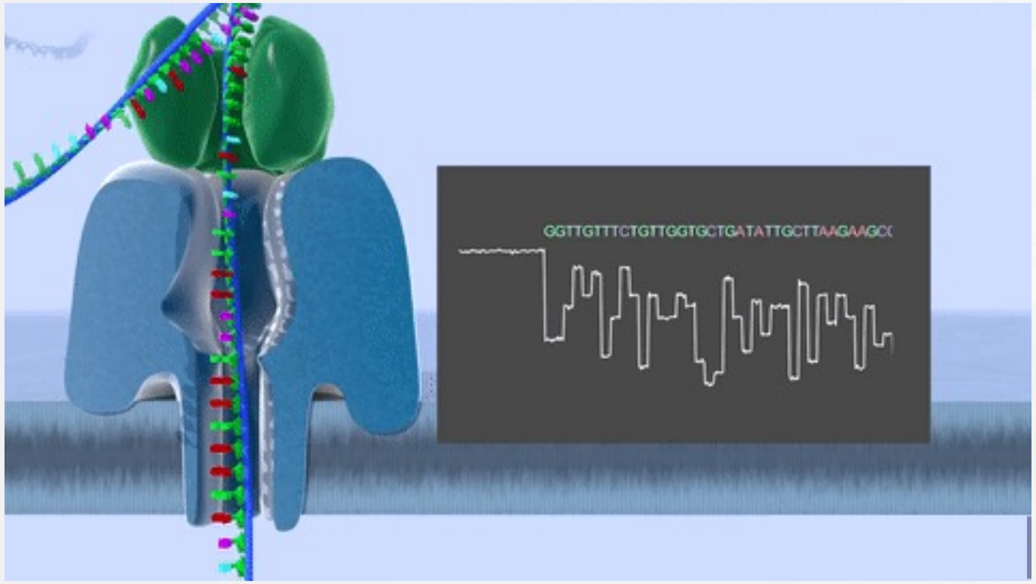
Outline

การพัฒนาเยาวชนเพื่อเข้าสู่วิชาชีพขั้นสูงด้าน Coding & AI ร่วมกับ Coding Entrepreneur & Partnership: Personal AI

1. Problem Statement
2. Data Collection and Preparation
3. Bayesian [Multi-Sample] Gaussian Mixture Modelling
4. Evaluation
5. Visualization and Presentation
6. Future Work

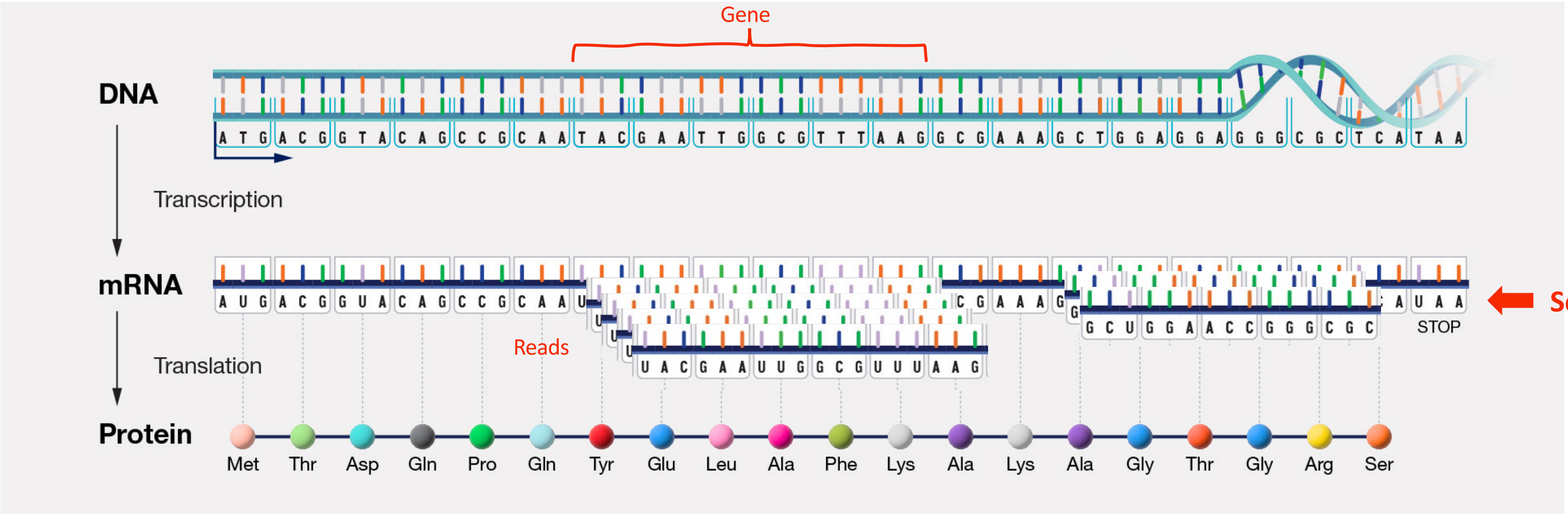
Try Coding

1. Problem Statement



- **Nanopore Sequencing**
- **RNA Modification**
- **Inputs & Outputs**
- **Research Objectives**

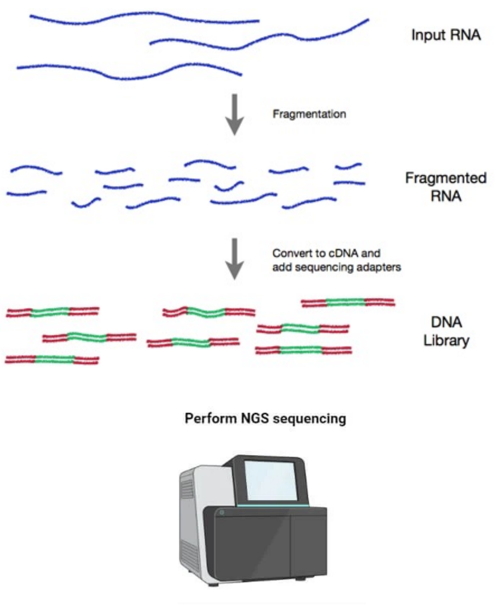
Central Dogma



Source: https://www.genome.gov/genetics-glossary/Central-Dogma

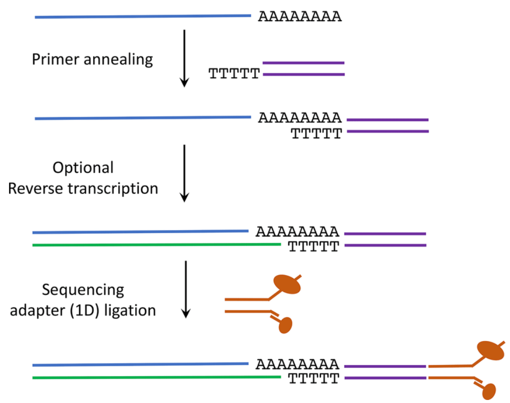
RNA Sequencing

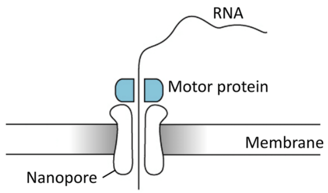
Then



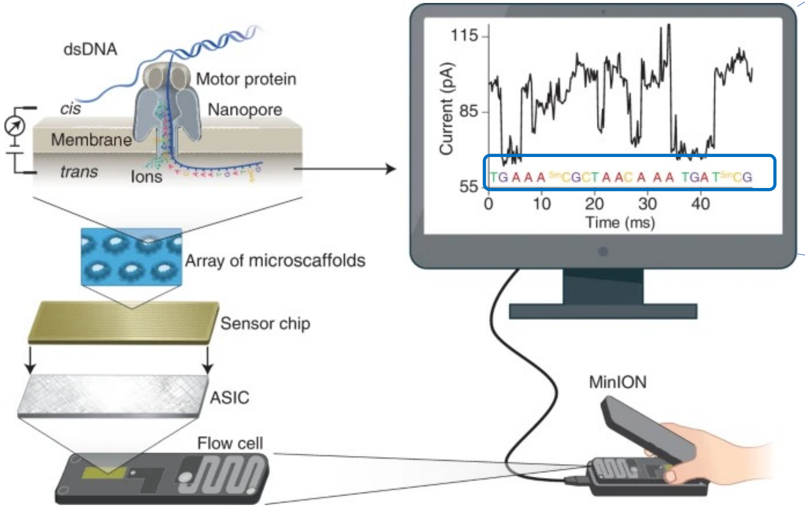
Direct RNA Sequencing

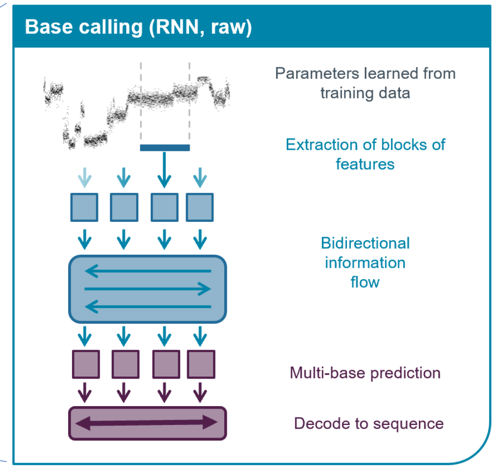
Now

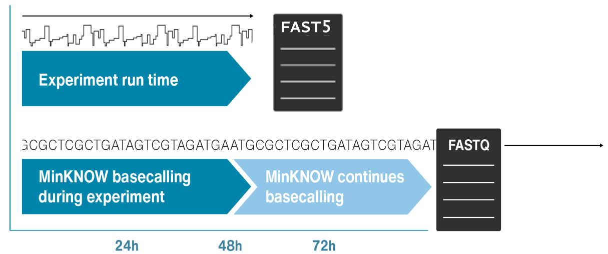




RNA Sequencing

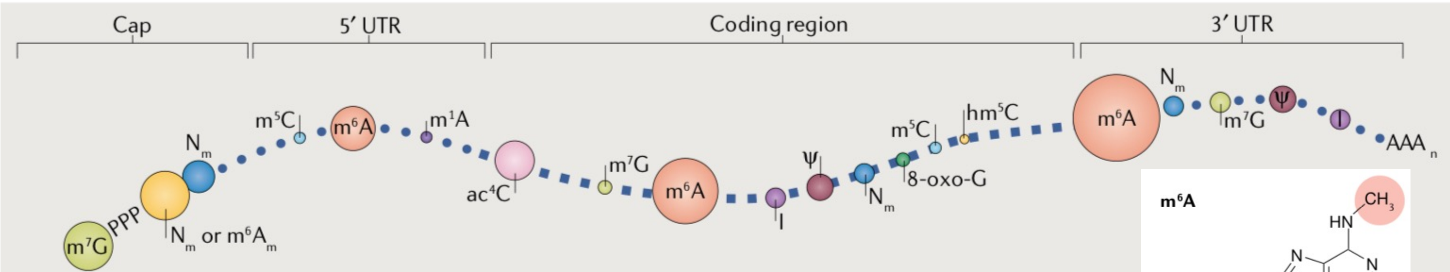


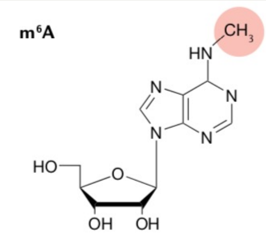




Ref: Yunhao Wang, *et al.*, "Nanopore sequencing technology, bioinformatics and applications", *Nature Biotechnology* (2021)

RNA modifications



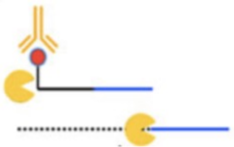


Ref: Zaccara, Sara, Ryan J. Ries, and Samie R. Jaffrey. *Nature Reviews Molecular Cell Biology* (2019)

Splicing | RNA Instability | Translation | Disease-related

Single-base-resolution CLIP-based detection methods

*Use **antibodies** to induce **truncations** or mutations at m6A sites during **reverse transcription**.*



m6ACE-Seq

Ref: Koh, Casslynn WQ, Yeek Teck Goh, and WS Sho Goh. *Nature Communications* 10.1 (2019)

Output Table

Genomic positions	5-mer	Modification rates: KO	Modification rates: WT	Differential modification rates: $\bar{W}_{WT} - \bar{W}_{KO}$	Differential modification rates: P-value
Transcriptome-wide	NNANN	3% 3% 3%	94% 94% 45%	0.81	Most sig
	⋮	⋮	⋮	⋮	↓
	NNCNN				
	⋮	3%	45%	0.42	
	NNGNN				
	⋮	⋮	⋮	⋮	
	NNTNN	45%	45%	- 0.01	Least sig

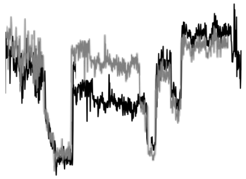
Research Objectives

XPORE

GGACT

GGm6ACT

Nanopore Sequencing



- Locate modified positions
- *Quantify fraction of modified reads -- modification rate*

Signal-level modification detection methods

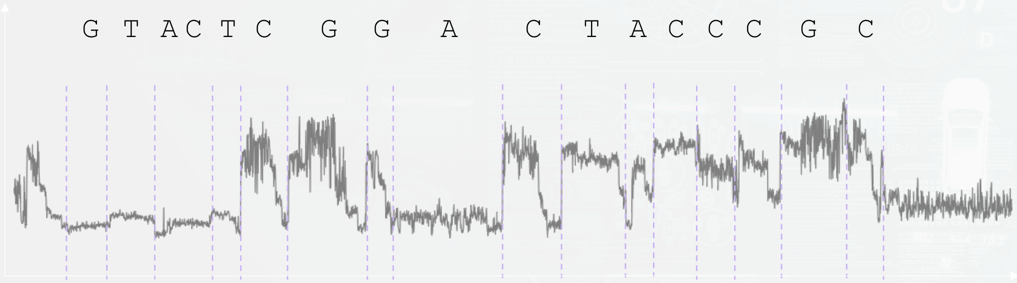
EpiNano MINES

- m6A
- Training data required.

Tombo nanocompore

- All modification types.
- No training data required.

2. Data Collection and Preparation



- **Nanopore Raw Signal Data**
- **Sequencing Data**
- **Genome Browser**
- **Nanopore Data Pipeline**

FAST5

- Raw signal - Sequencing output
- Intensity level (pA)
- HDF5 format (binary), storing large and complex data

```
HDF5 "GISPC936_20181120_FAK27249_MN18749_sequencing_run_SHO_20112018_Empty
GROUP "/" {
   ATTRIBUTE "file_version" {
      DATATYPE  H5T_IEEE_F64LE
      DATASPACE  SCALAR
      DATA {
      (0): 0.6
      }
   }
   GROUP "PreviousReadInfo" {
      ATTRIBUTE "previous_read_id" {
         DATATYPE  H5T_STRING {
            STRSIZE 38;
            STRPAD H5T_STR_NULLTERM;
            CSET H5T_CSET_ASCII;
            CTYPE H5T_C_S1;
         }
         DATASPACE  SCALAR
         DATA {
         (0): "ac7312ce-d058-4382-a6c6-8471302869b9"
         }
      }
      ATTRIBUTE "previous_read_number" {
         DATATYPE  H5T_STD_U32LE
         DATASPACE  SCALAR
         DATA {
         (0): 976
         }
      }
   }
   GROUP "Raw" {
      GROUP "Reads" {
         GROUP "Read_984" {
            ATTRIBUTE "duration" {
               DATATYPE  H5T_STD_U32LE
```

```
   DATA {
   (0): 12639754
   }
}
DATASET "Signal" {
   DATATYPE  H5T_STD_I16LE
   DATASPACE  SIMPLE { ( 76256 ) / ( H5S_UNLIMITED ) }
   DATA {
   (0): 595, 492, 497, 502, 500, 499, 514, 495, 515, 512, 531,
   (11): 529, 515, 483, 497, 529, 510, 521, 524, 525, 523, 514,
   (22): 519, 517, 512, 520, 522, 519, 521, 517, 535, 514, 505,
   (33): 537, 527, 512, 521, 528, 523, 530, 530, 529, 529, 521,
   (44): 527, 515, 537, 522, 512, 485, 480, 481, 478, 465, 467,
   (55): 472, 476, 463, 469, 476, 454, 450, 446, 468, 471, 470,
   (66): 466, 468, 467, 466, 460, 458, 466, 467, 464, 465, 467,
   (77): 465, 459, 476, 470, 477, 460, 486, 470, 485, 486, 468,
   (88): 475, 470, 472, 472, 468, 456, 457, 452, 448, 440, 440,
   (99): 473, 470, 454, 442, 448, 449, 455, 461, 443, 455, 448,
   (110): 449, 444, 462, 456, 461, 459, 467, 459, 461, 458, 472,
   (121): 461, 463, 467, 456, 471, 468, 471, 475, 467, 466, 471,
   (132): 477, 459, 473, 482, 466, 477, 470, 461, 464, 452, 454,
   (143): 457, 468, 457, 466, 472, 474, 441, 456, 470, 467, 444,
   (154): 442, 455, 451, 456, 470, 469, 473, 479, 478, 468, 472,
   (165): 462, 466, 458, 435, 436, 464, 467, 455, 462, 463, 471,
   (176): 455, 459, 446, 460, 442, 453, 465, 465, 480, 465, 478,
   (187): 467, 475, 483, 512, 502, 539, 521, 506, 521, 523, 516,
   (198): 518, 511, 514, 518, 530, 516, 528, 503, 503, 510, 524,
   (209): 529, 526, 513, 504, 469, 476, 472, 470, 468, 476, 476,
   (220): 476, 471, 459, 457, 432, 443, 472, 466, 477, 467, 471,
   (231): 470, 474, 449, 468, 456, 457, 460, 459, 459, 456, 469,
   (242): 457, 469, 475, 468, 465, 465, 463, 446, 455, 458, 461,
   (253): 456. 448. 446. 462. 444. 464. 462. 469. 479. 471. 502.
```

FASTQ

- Basecalled sequence
- Text format:
  - Name/ID, starting with "@"
  - Sequence
  - Optional info, starting with "+"
  - Quality of the sequence, encoding the probability error

```
@2fdda8fc-621a-415e-baaf-1b088a91302d runid=385c19b6dda90bee8f8b01b21643004d7cb1d235 sampleid=SHO_20112018_EmptyE2-9 read=65062 ch=111 start_time=2018-11-20T22:13:23Z
CAACAUCGCGGCCGUGGCGGGAUGCUGUGGGAGGGGUCAUCCUGCCCAGGCCGGGCCGGCUGCCUACCCUUCCAGACUGUCCGCAUCGGCUCCAAGGCUGCUACGCCACCGUCGUGUGAUGCCAUCAGCGGCAGAAGCUGAUGGCUGUGACUGAAUAUCCCCCGACAGCCAUCCU
UCCCAUCAUGCCUGCCACUUCCAGCCCCCACAGGAAGAUAGGCCUCAUCAGGCUUUUCCGCCAGAUAGCAGCAGUUUUCCAGGACAACCGAAUGAUAGCGUUGCCAGAAUGUGGCUUGAGUGCAGAGGGACAAGCUUUUUCGUGCGACACCAGCUUGCGGAAACACAAGAUCCUGA
UGAAGGUUUCCCCAACCAGGUCCUGAAGCCCUUCCUGAGGAUUCCAAGUACCAAAAUCUGCUGCCCCUUUUGUGGGGCACUAUCGCUGCUGGUCAAAUGAAGAGCCCGGGUCAAAGAUGGUCACGGAUCUUUAAGGACUGUGCCAUCACCCUGCCGCUGCAUGGGUGGGCGGCAUU
GAUGACACCAUCCAGCAGGCGGCUUUAUCAACUACUCCUUGACCCAGCCUGCCCUGGUGCAGGAGCUUGUAGGAGGCCUCACCUGCCACAGCCCAGACCCACUCCCUGCUCCAGCACCAGCCCCACCGUUGACCACCCCUGUUGGACCAGUACAUCAGAGAGCAACGCGCGAGAAG
GAUUUGUCAUGCGGCCAAUGGGUCAGAUCCUGACACUGGGUUCCGGACUUGCAGCAGCCUGUUCGGCCAGCCCCUGCGCAUAAAUACAUUUGUGUUAUUGGCUGUGCUCUCCUUGCCGAGCUCGUGGAAGAACAUUGUCGAGGAGUGUUUGUCACUUGGUUCACUAGAGGUAAUGA
UAUUGUCGUGUAGGUAAUGGGCACUUGGAGAUGCAGAGGAUUCCAUUUCAGAUGUCAGUCACCGAAUCUCGUCCUUAGUUUCCCUAUUGGACGUGAUAUGGGAGCAAAGACUUCCAUUACCCAGGUCCAAGGCAGAGAGAGAGAUCUGAAAAGAUAGGGCUAUUGUCCCCUGGCC
CUUGGUCACUGCCUUUUUGCUGGCACGACCUGAGCCUCCCUCCUUGGGGCACAACCUAUCCCAUUGCCACAGUAGCUCAUCAAGCAGUUGAGUGCUGAGAAUGGCACACCUGGUGAGAGCCUGUUGUGUGCCAGGCUUUGUGUGUGAGUGCUGUCAUGUAUUAGUUCCUUACUGCU
GACCACUGUACCCAUAUCACAGAGAAGGAGCAGAGAAAUUAAGUGGGCUUGCUCCACAAGGUCAUGCAGUUAGUAAGUGGCAGACUACGGACUUGAACCAAGCCUCUGCUUUGAGUCGCGUCCUGAAUUUUUCACUAGAGCUCACCACAUUUCAGGUUACCCAGAAGUGGGUCCA
UCCAUCCAGGUGUGCUUGGAUGUUAGUUCCACCCUCGAGGUGUCUGUGAAAAGUUGGGAGCAUUGUUUUUAUAAAAAUGAAUAUUUAAAAAACC
+
%$$$$$%%&(,)++),-(-7.+/0)))&..,%*,0/&$$&),*(/6.)')%''5--.+,)&((-(*)2++)-.+-.354688.+'')*1)(*0-,0.,'($)',+*-*/3--)))+(+-.23(&+&&'*0'%%'(/'((+-,-2++,./,++,,2231.:42($&''(+/4.+-+'
)'-)**),,.07110-'%&()+..4783-++&(*&*(+*+./0.1*,,,)(.''/.**1(+-(((&*+)*,++-)*.***').&&),+)*2-.01.,))'&())7,,-.,+)+1)&&%%&%)++*)*0*)+.*''),*)''''))**,-.2,+*,)&&&,(3/-,,-/-.-/1)+,
+,.++-).(197/.+++)3)*-+*-0)*+2-'',(,+)*0)+,+/-4,))*3)42,+'.++-0-22'+,)-,02172()*&(&%&&&))+,2***&$&+.,,,+/0)&+/*+,5.**+1.*'(*,1,.%+)+&(-0,-..-/51.&$$&-.&*+,.1/.-()*/.&'+)),.'')-
((%')(,.0(())&**)1.*)+(*,),-0/-(**)'(*$&'(,8-(*9-909://420/,).*)*.1+*&$')%*+..))(-/002/))(&(/*//-.4,)*'/51-.,.611/0+/..,922(#''%$)''0,*/61*,,4,7-,1..2/...*-,*)***-84-,*)++,+1)5
++23,+)))((%(*1*1,+,,+&)+/333,+++,))(()-'**3+6(&'(((*+,03:5-,)-$(,.706-792,-,*))(25/)**+,+((&).--2.240/132)('&,+-0.--..**)(&'(.+.-2)%$()0)+**+,%'%&'*0(+*)*0-0+*-'((1+)')++52/0*
)',,0.+'&(%$&)&**),,,,((204.,+,,+*(&&,(*7261//3*,./**++,25-+*,,))-&%+,,22/13-0)3)(2.#((()--'(),-,,(')++')'/..(%%$%'+'()%&12()0.,*-,'/&%''''&&(()-.))*341,)('(01)*+-0.*,66.'%().
*0-.+''&%$()''('('))(&/,()('&)(()'),&',+$'+(+*0.,,*--&*+,''0-&&(,./4/+**+-1-.*,%,+-,,/0+'%&(()'('%,.//-2-+*(//.9431/.++'-%()*(*()**/++-'*/.)-,)()&%($(*,'$%%(*+*+*&/2,),),'()))*
*-0)'&)''%'/.(''&&')*&'&*+/-,)(''&'12,/.3+)((**-/.,()'*$'+,*.*+575645810.,-))'*'&(*)'&&%)4,+.5./0-..#%)+++'+.(((*'-,()'&)*0.+-10)/+)'%$''(*++*)&$(($%&'*(%'))))%(0-.01,+()+)'*/(
%&)%&-.),,)**)'',//0-.4-'+),')$(+)()((.+)+'&)())*66.,/--1+*+*)+*('*('&&%,+5.*',/,((-+()2/3+)+$
```

FASTA

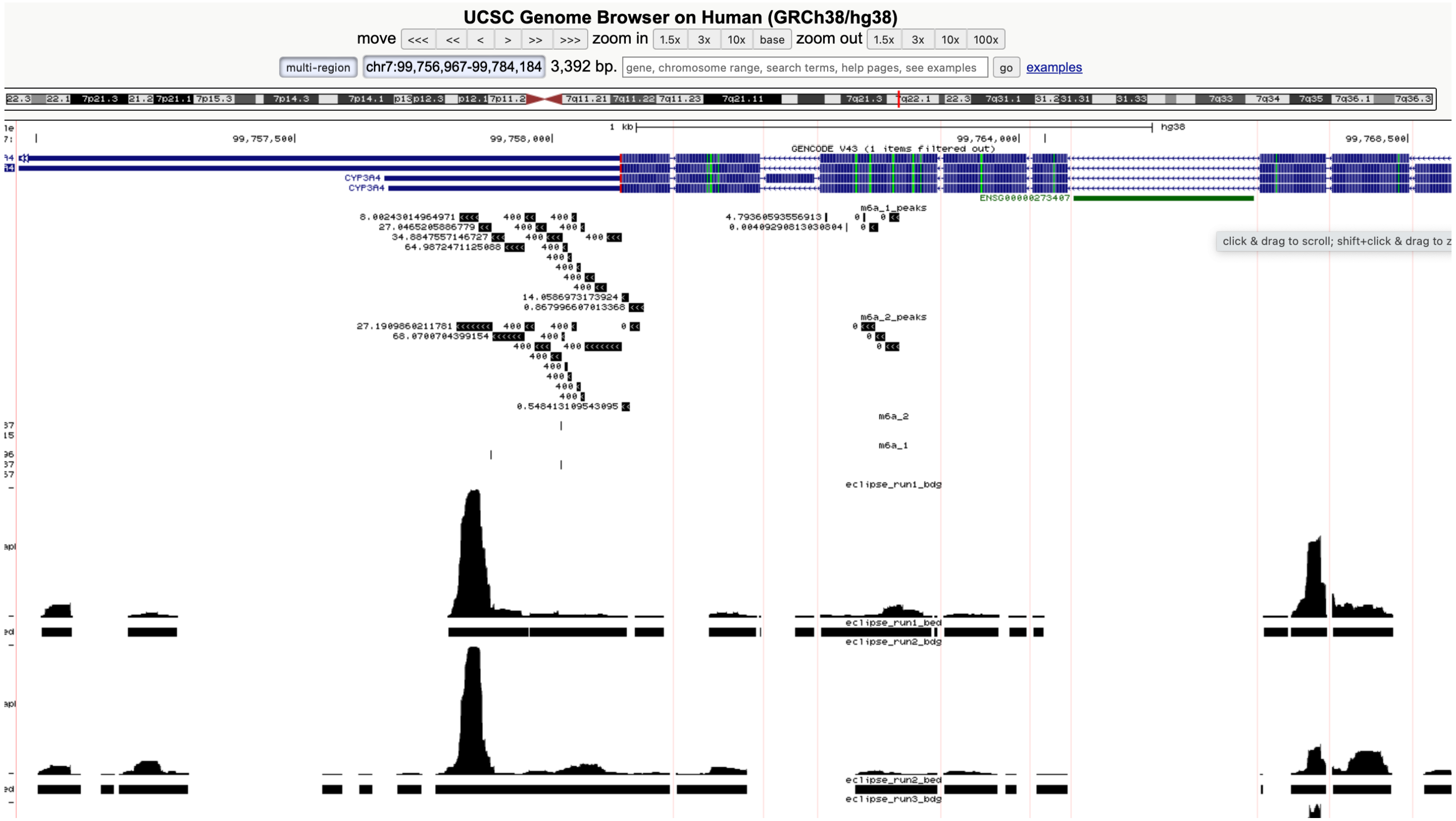
- Reference sequence
- Text format:
  - Sequence ID, starting with ">", optionally followed by other attributes
  - Sequence

```
>ENST00000480901.1 cdna chromosome:GRCh38:17:47828308:47831525:-1 gene:ENSG00000159111.12 gene_biotype:protein_coding transcript_biotype:retained_intron gene_symbol:MRPL10 desc
ription:mitochondrial ribosomal protein L10 [Source:HGNC Symbol;Acc:HGNC:14055]
TTCTTCCGGTGGAGATGGCTGCGGCCGTGGCGGGGATGCTGCGAGGGGGTCTCCTGCCCC
AGGCGGGTAAGGAGTGGCCCAGGTCCTCACGGCGTGTCTTGCGGCCGCTCTCTAGTCCTC
ATCTGCCCTCCTCTACTACTGATTCTTCCCATAATCTCTGACCCCAGCTAGATCGCTGGC
CTCCTTACCCCGTCCAGTTCCTTGTGACTCGACTGGCCGGCTGCCTACCCTCCAGACTGT
CCGCTATGGCTCCAAGGCTGTTACCCGCCACCGTCGTGTGATGCACTTTCAGCGGCAGAA
GCTGATGGCTGTGACTGAATATATCCCCCCGAAACCAGCCATCCACCCATCATGCCTGCC
ATCTCCTCCCAGCCCCCCACAGGAGGTAAGGAGGAATTTGGGTACATGTCACTTGGTGGT
GGGATGGTGGATTAAAGTAATCTTGTCTCTGGCCATAGTGAAGTAGGACACTCAGCCATT
GTCATGCACGTCATTATTTCAGTTTGACTGCCTGATCCAGATATTTTAAGATGAAATCCG
CACTTGATTCTGTATTGGCTTTTGGGCTCTGGATTGGG
```

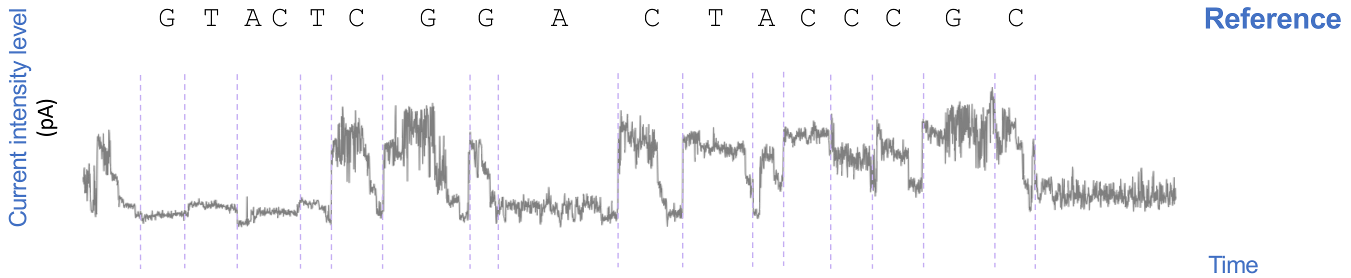
BAM / SAM

- Alignment results (FASTQ aligned with FASTA)
- BAM – Binary / SAM – Text

```
2fdda8fc-621a-415e-baaf-1b088a91302d    0       ENST00000351111.6       13      13      7S11M1D13M1I6M1I5M1D6M1I18M1I14M1D2M1I16M2D19M1D1M1D31M2D12M3D9M1I17M4D7M1D11M2D27M3D36M
1D3M1D15M1D10M1I13M1I13M1I28M1D29M1D31M1D13M1I3M2D10M1I12M1D7M2D7M1I7M1I16M2I12M1I5M1I21M2D8M2D21M2D10M1D10M3D25M2D41M1D8M1I29M2I13M1D7M1D10M3D16M2I15M1D16M1I17M1D25M3D2M1I3M1I
13M1I2M3D8M2D15M2D7M3I14M4I5M1D51M1I10M1D8M1D13M2I12M1D21M6I6M1D29M1D14M1I7M1I5M3D29M2I20M1D11M2I13M2I36M1I11M1D14M1D14M2D38M1I8M3I28M2I2M1D15M1D11M1D2M1D15M1D12M1I6M2I21M1D4M3
D26M2D16M3D10M1D12M1I7M2D8M13S  *       0       0       CAACATCGCGGCCGTGGCGGATGCTGTGGGAGGGGTCATCCTGCCCAGGCCGGGCCGGCTGCCTACCCTTCCAGACTGTCCGCATCGGCTCCAAGGCTGCTACGCCACCGTCGTGTGAT
GCCATCAGCGGCAGAAGCTGATGGCTGTGACTGAATATCCCCCGACAGCCATCCTTCCCATCATGCCTGCCACTTCCAGCCCCACAGGAAGATAGGCCTCATCAGGCTTTTCCGCCAGATAGCAGCAGTTTTCCAGGACAACCGAATGATAGCGTTGCCAGAATGTGGCTTGAG
TGCAGAGGGACAAGCTTTTTCGTGCGACACCAGCTTGCGGAAACACAAGATCCTGATGAAGGTTTCCCCAACCAGGTCCTGAAGCCCTTCCTGAGGATTCCAAGTACCAAAATCTGCTGCCCCTTTTGTGGGGCACTATCGCTGCTGGTCAAATGAAGAGCCCGGGTCAAAGATGG
TCACGGATCTTTAAGGACTGTGCCATCACCCTGCCGCTGCATGGGTGGGCGGCATTGATGACACCATCCAGCAGGCGGCTTTATCAACTACTCCTTGACCCAGCCTGCCCTGGTGCAGGAGCTTGTAGGAGGCCTCACCTGCCACAGCCCAGACCCACTCCCTGCTCCAGCACCAG
CCCCACCGTTGACCACCCCTGTTGGACCAGTACATCAGAGAGCAACGCGCGAGAAGGATTTGTCATGCGGCCAATGGGTCAGATCCTGACACTGGGTTCCGGACTTGCAGCAGCCTGTTCGGCCAGCCCCTGCGCATAAATACATTTGTGTTATTGGCTGTGCTCTCCTTGCCGAG
CTCGTGGAAGAACATTGTCGAGGAGTGTTTGTCACTTGGTTCACTAGAGGTAATGATATTGTCGTGTAGGTAATGGGCACTTGGAGATGCAGAGGATTCCATTTCAGATGTCAGTCACCGAATCTCGTCCTTAGTTTCCCTATTGGGACGTGATATGGGAGCAAAGACTTCCATTA
CCCAGGTCCAAGGCAGAGAGAGAGATCTGAAAAGATAGGGCTATTGTCCCCTGGCCCTTGGTCACTGCCTTTTTGCTGGCACGACCTGAGCCTCCCTCCTTGGGGCACAACCTATCCCATTGCCACAGTAGCTCATCAAGCAGTTGAGTGCTGAGAATGGCACACCTGGTGAGAGC
CTGTTGTGTGCCAGGCTTTGTGTGTGAGTGCTGTCATGTATTAGTTCCTTACTGCTGACCACTGTACCCATATCACAGAGAAGGAGCAGAGAAATTAAGTGGGCTTGCTCCACAAGGTCATGCAGTTAGTAAGTGGCAGACTACGGGACTTGAACCAAGCCTCTGCTTTGAGTCGC
GTCCTGAATTTTTCACTAGAGCTCACCACATTTCAGGTTACCCAGAAGTGGGTCCATCCATCCAGGTGTGCTTGGATGTTAGTTCCACCCTCGAGGTGTCTGTGAAAAGTTGGGAGCATTGTTTTTATAAAAATGAATATTTAAAAAACC  %$$$$$%%&(,)++),-(-7.+/0
)))&..,%*,0/&$$&),*(/6.)')%''5--.+,)&((-(*)2++)-.+-.354688.+'')*1)(*0-,0.,'($)',+*-*/3--)))+(+-.23(&+&&'*0'%%'(/'((+-,-2++,./,++,,2231.:42($&''(+/4.+-+')'-)**),,.07110-'%&()+..
4783-++&(*&*(+*+./0.1*,,,)(.''/.**1(+-(((&*+)*,++-)*.***').&&),+)*2-.01.,))'&())7,,-.,+)+1)&&%%&%)++*)*0*)+.*''),*)''''))**,-.2,+*,)&&&,(3/-,,-/-.-/1)+,+,.++-).(197/.+++)3)*-+*
-0)*+2-'',(,+)*0)+,+/-4,))*3)42,+'.++-0-22'+,)-,02172()*&(&%&&&))+,2***&$&+.,,,+/0)&+/*+,5.**+1.*'(*,1,.%+)+&(-0,-..-/51.&$$&-.&*+,.1/.-()*/.&'+)),.'')-((%')(,.0(())&**)1.*)+(*
,),-0/-(**)'(*$&'(,8-(*9-909://420/,).*)*.1+*&$')%*+..))(-/002/))(&(/*//-.4,)*'/51-.,.611/0+/..,922(#''%$)''0,*/61*,,4,7-,1..2/...*-,*)***-84-,*)++,+1)5++23,+)))((%(*1*1,+,,+&)
+/333,+++,))(()-'**3+6(&'(((*+,03:5-,)-$(,.706-792,-,*))(25/)**+,+((&).--2.240/132)('&,+-0.--..**)(&'(.+.-2)%$()0)+**+,%'%&'*0(+*)*0-0+*-'((1+)')++52/0*)',,0.+'&(%$&)&**),,,,((
204.,+,,+*(&&,(*7261//3*,./**++,25-+*,,))-&%+,,22/13-0)3)(2.#((()--'(),-,,(')++')'/..(%%$%'+'()%&12()0.,*-,'/&%''''&&(()-.))*341,)('(01)*+-0.*,66.'%().*0-.+''&%$()''('('))(&/,
()('&)(()'),&',+$'+(+*0.,,*--&*+,''0-&&(,./4/+**+-1-.*,%,+-,,/0+'%&(()'('%,.//-2-+*(//.9431/.++'-%()*(*()**/++-'*/.)-,)()&%($(*,'$%%(*+*+*&/2,),),'()))**-0)'&)''%'/.(''&&')*&'&
*+/-,)(''&'12,/.3+)((**-/.,()'*$'+,*.*+575645810.,-))'*'&(*)'&&%)4,+.5./0-..#%)+++'+.(((*'-,()'&)*0.+-10)/+)'%$''(*+++*)&$(($%&'*(%'))))%(0-.01,+()+)'*/(%&)%&-.),,)**)'',//0-.4-
'+),')$(+)()((.+)+'&)())*66.,/--1+*+*)+*('*('&&%,+5.*',/,((-+()2/3+)+$  NM:i:212        ms:i:1794       AS:i:1794       nn:i:0  ts:A:+  tp:A:P  cm:i:50 s1:i:490        s2:i:47d
e:f:0.1026
```


UCSC Genome Browser on Human (GRCh38/hg38)
move <<< << < > >> >>> zoom in 1.5x 3x 10x base zoom out 1.5x 3x 10x 100x
multi-region chr7:99,756,967-99,784,184 3,392 bp. gene, chromosome range, search terms, help pages, see examples go examples
GENCODE V43 (1 items filtered out)
CYP3A4
m6a_1_peaks
m6a_2_peaks
m6a_2
m6a_1
eclipse_run1_bdg
eclipse_run1_bed
eclipse_run2_bdg
eclipse_run2_bed
eclipse_run3_bdg
click & drag to scroll; shift+click & drag to z

Nanopore pre-processing pipeline for signal-level data analysis



nf-core/ nanoseq

Direct RNA sequencing — **Basecalling** — **Sequence aligning** — **Signal event aligning**

Oxford Nanopore

Guppy

FAST5 → FASTQ

Minimap2

FASTQ + FASTA → BAM

Nanopolish

FAST5 + FASTA + BAM → Event Align Output https://xpore.readthedocs.io/en/latest/

Data preparation from raw reads

1. After obtaining fast5 files, the first step is to basecall them. Below is an example script to run Guppy basecaller. You can find more detail about basecalling at Oxford nanopore Technologies:

```
guppy_basecaller -i </PATH/TO/FAST5> -s </PATH/TO/FASTQ> --flowcell <FLOWCELL_ID> --kit <KI
```

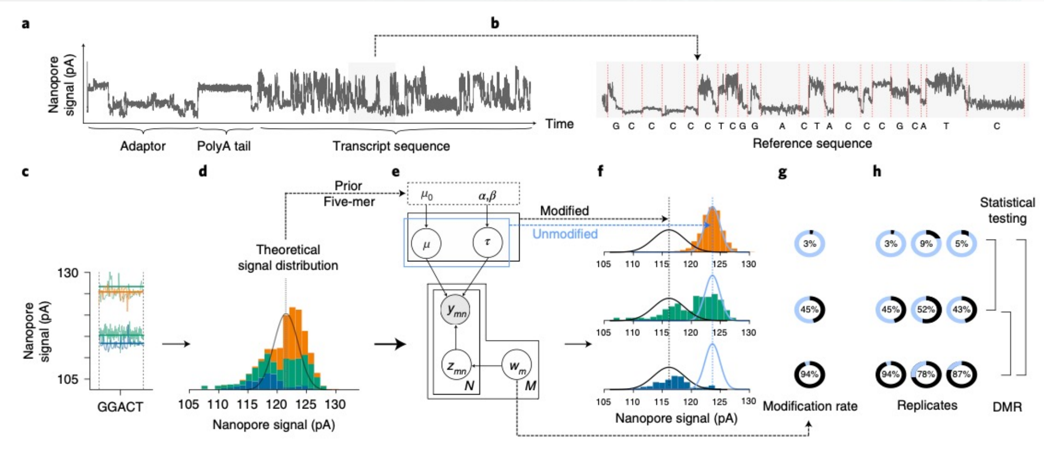
2. Align to transcriptome:

```
minimap2 -ax map-ont -uf -t 3 --secondary=no <MMI> <PATH/TO/FASTQ.GZ> > <PATH/TO/SAM> 2>> <
samtools view -Sb <PATH/TO/SAM> | samtools sort -o <PATH/TO/BAM> - &>> <PATH/TO/BAM_LOG>
samtools index <PATH/TO/BAM> &>> <PATH/TO/BAM_INDEX_LOG>
```

3. Resquiggle using nanopolish eventalign:

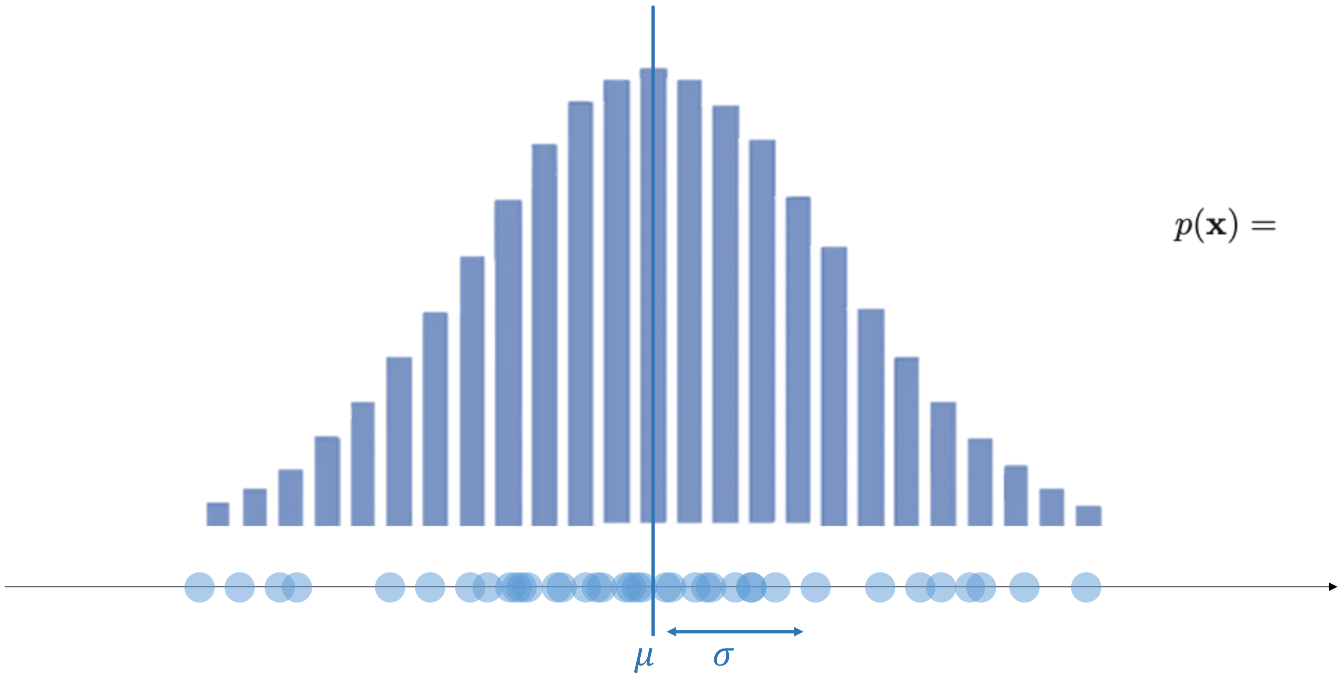
```
nanopolish index -d <PATH/TO/FAST5_DIR> <PATH/TO/FASTQ_FILE>
nanopolish eventalign --reads <PATH/TO/FASTQ_FILE> \
--bam <PATH/TO/BAM_FILE> \
--genome <PATH/TO/FASTA_FILE \
--signal-index \
--scale-events \
--summary <PATH/TO/summary.txt> \
--threads 32 > <PATH/TO/eventalign.txt>
```

3. Bayesian [Multi-Sample] Gaussian Mixture Modelling



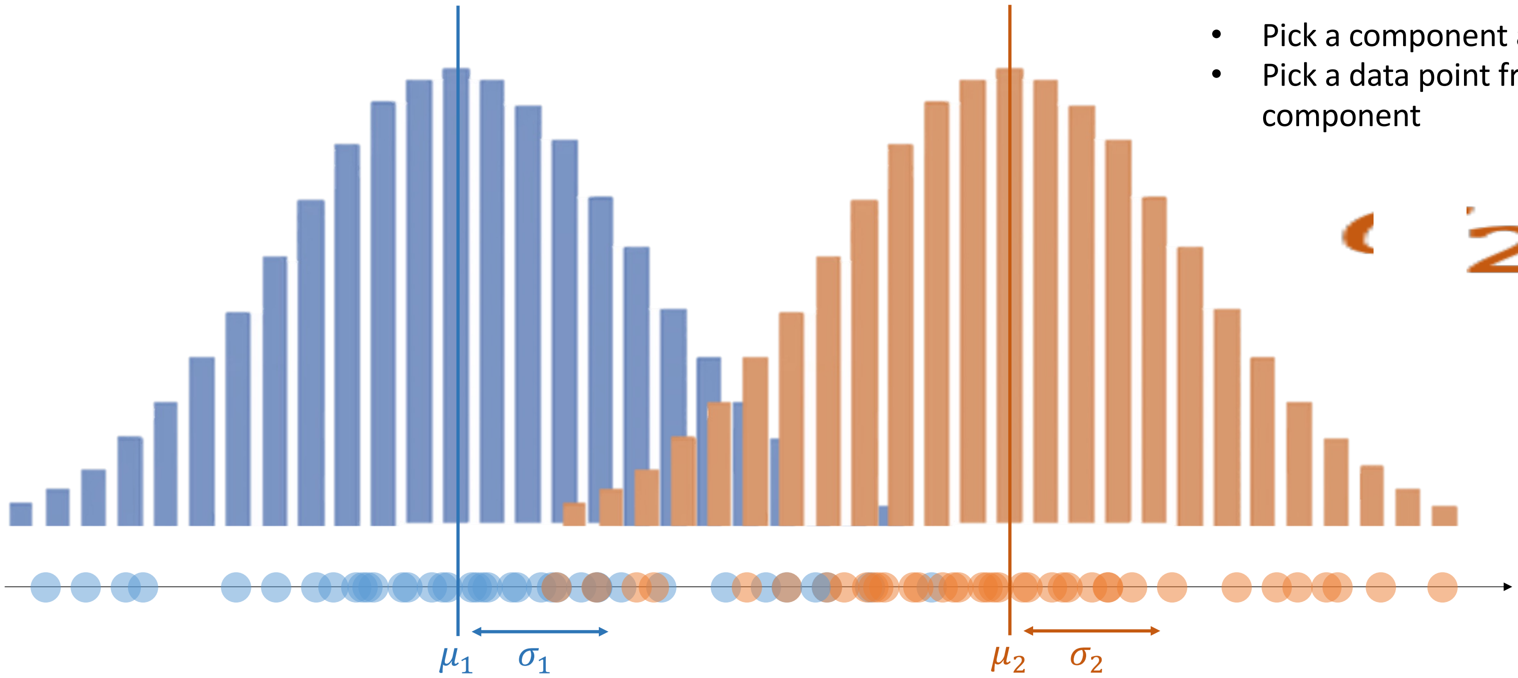
- [Bayesian] GMM
- Where did the idea come from?
- How Multi-Sample?
- Why Bayesian?
- Speed-Up ML Experiments

Bayesian Multi-Sample Gaussian Mixture Model



$$p(\mathbf{x}) = \quad \mathcal{N}(\mathbf{x}|\boldsymbol{\mu}\ ,\boldsymbol{\Sigma}\ ).$$

Bayesian Multi-Sample Gaussian Mixture Model



- Pick a component at random
- Pick a data point from the chosen component

What is GMM?

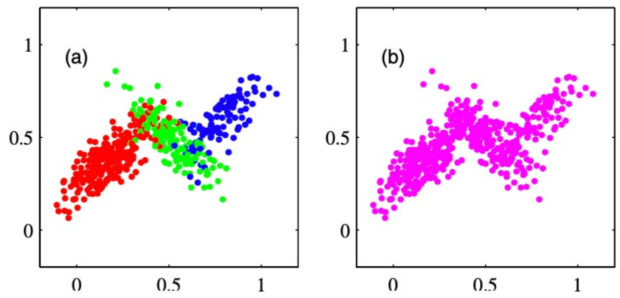
Assumption how data are **generated** as follows

- There are K components
- Each component is defined as a Gaussian distribution
- Pick a component at random
- Pick a data point from the chosen component

$\boldsymbol{\Theta}$

$$p(\mathbf{x}) = \sum_{k=1}^{K} \boxed{\pi_k} \mathcal{N}(\mathbf{x}|\boxed{\boldsymbol{\mu}_k, \boldsymbol{\Sigma}_k}).$$

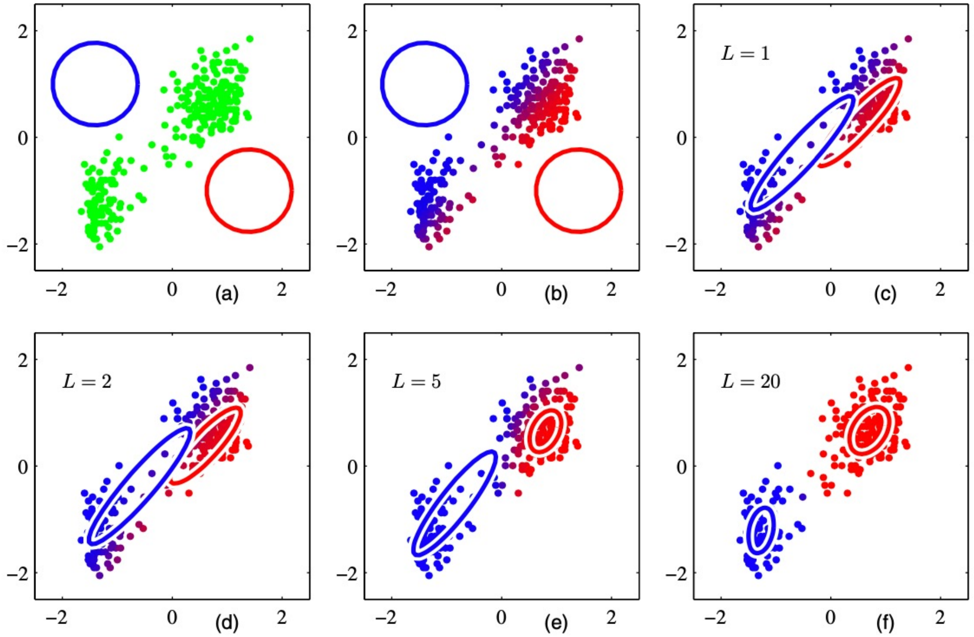
Data



Source: Christopher M. Bishop, “Pattern Recognition and Machine Learning”, 2006

GMM Inference

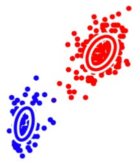
Iterative algorithm



Source: Christopher M. Bishop, "Pattern Recognition and Machine Learning", 2006

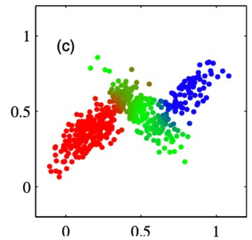
Generative AI

Clustering

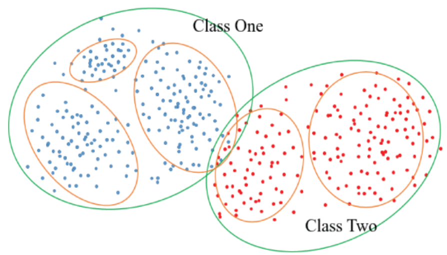


GMM
as a **Density Estimator**

Model
how data are generated



(One-Calss) Classification



Bayesian Multi-Sample Gaussian Mixture Model

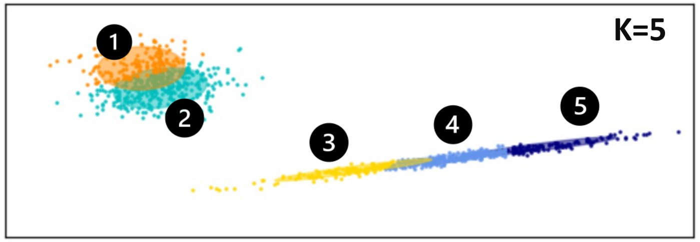
Learning algorithm for making inference on the **latent** variables

Θ

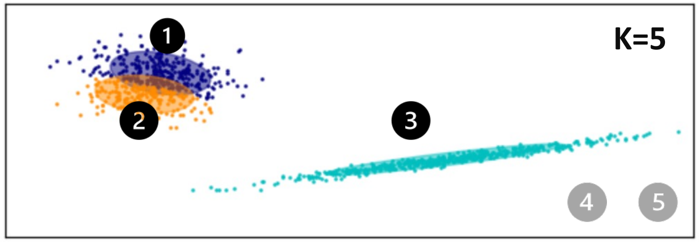
$$p(\mathbf{x}) = \sum_{k=1}^{K} \boxed{\pi_k} \mathcal{N}(\mathbf{x}|\boxed{\boldsymbol{\mu}_k, \boldsymbol{\Sigma}_k}).$$

Data

Gaussian Mixture



Bayesian Gaussian Mixture with a Dirichlet process prior



Point estimate = Maximum Likelihood

Θ = $\underset{\Theta}{\text{argmax}}$ P(Data | Θ)

Posterior = Likelihood x Prior

P(Θ | Data) = P(Data | Θ) x P(Θ)

Frequentist vs Bayesian

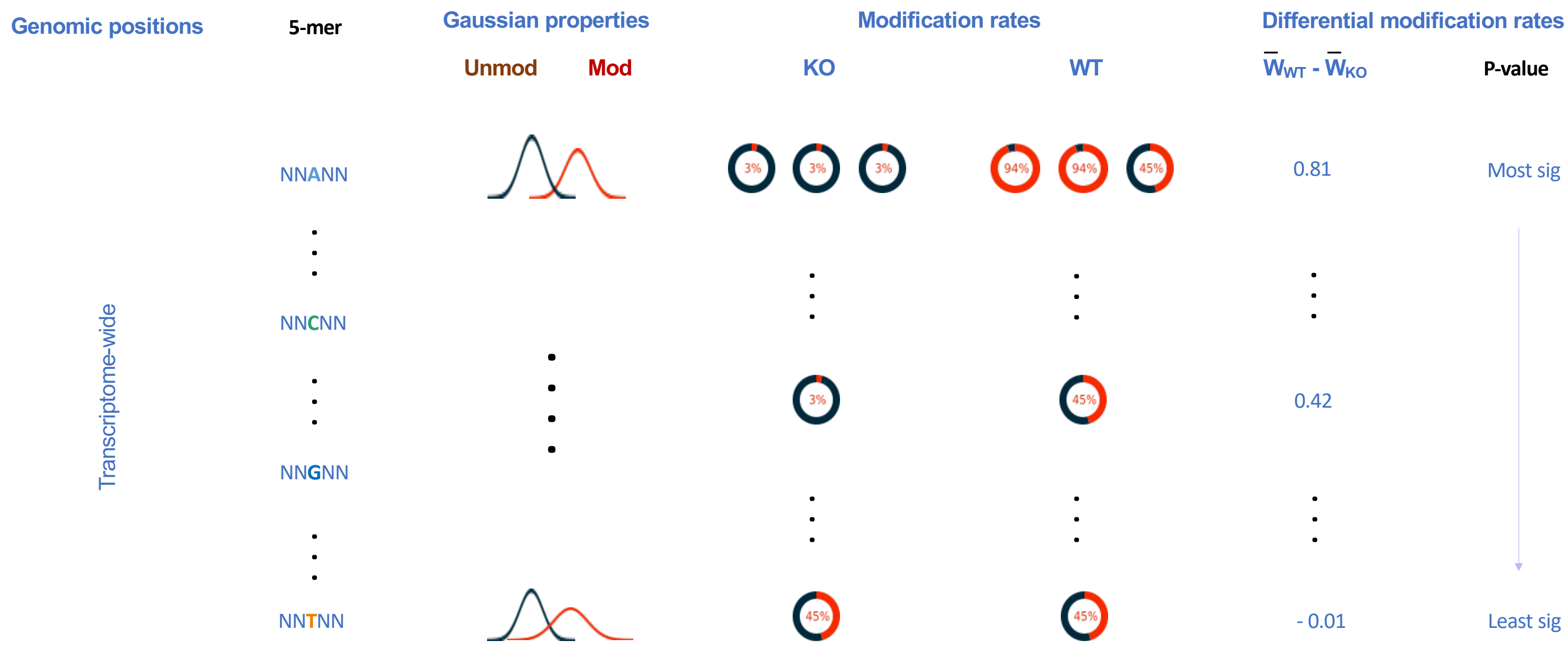
$P(Data \mid \Theta)$ $P(\Theta \mid Data) = P(Data \mid \Theta) \times P(\Theta)$

Aspect	Frequentist	Bayesian
Probability interpation	Long term frequency	Posterior
Treatment of parameters	Fixed / Point estimates	Random / Probability distributions
Prior information	No	Yes
Sample size requirement	Larger	Smaller
Interpretation of results	Focused on the observed data	In the context of prior beliefs and their updates based on the observed data
Computational complexity	Simpler	More complex

Bayesian Multi-Sample Gaussian Mixture Model

- Each site has 2 distributions at maximum
  - Unmodified
  - Modified by only one modification type
- To accommodate comparison **across many samples**
- Nanopolish Eventalign assume a Gaussian distribution for each signal event (kmer)
- Mean and variance of unmodified k-mer is estimated by the eventalign algorithm *in priori*
- Fast → **Parallelisation**

Output Table



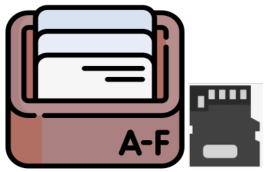
Speed-Up ML Experiments

- Config file
- Python packaging

Automated ML models

- Hyper-parameter settings
- Multiple datasets
- Different models / methods

- Parallelization



- File indexing

Why config files?

- Automating tasks
- Centralised configuration
- Documentation
- Portability

YAML, JSON, TOML, and INI are the popular and standardised formats of configuration files

```
xpore diffmod --config Hek293T_config.yml
```

Configuration file

xpore / xpore / diffmod / configurator.py

Code | Blame | 78 lines (63 loc) · 2.68 KB | Raw

```python
import yaml
import os
from collections import defaultdict

from ..utils import misc

def get_condition_run_name(condition_name,run_name):
    return '-'.join([condition_name,run_name])

class Configurator(object):
    def __init__(self, config_filepath):
        self.filepath = os.path.abspath(config_filepath)
        self.filename = self.filepath.split('/')[-1]
        self.yaml = yaml.safe_load(open(self.filepath, 'r'))

    def get_paths(self):
        paths = {}

        if 'prior' in self.yaml:
            paths['model_kmer'] = os.path.abspath(self.yaml['prior'])
        else:
            paths['model_kmer'] = os.path.join(os.path.dirname(__file__),'model_kmer.csv')

        paths['out_dir'] = os.path.join(os.path.abspath(self.yaml['out']))
        paths.update(misc.makedirs(paths['out_dir'],sub_dirs=['models']))
        paths['model_filepath'] = os.path.join(paths['out_dir'], 'models', '%s.model')
        return paths
```

```python
config = Configurator(config_filepath)
paths = config.get_paths()
data_info = config.get_data_info()
method = config.get_method()
criteria = config.get_criteria()
prior_params = config.get_priors()
```

```yaml
data:
    <CONDITION_NAME_1>:
        <REP1>: <DIR_PATH_TO_DATA_JSON>
        ...

    <CONDITION_NAME_2>:
        <REP1>: <DIR_PATH_TO_DATA_JSON>
        ...

    ...

out: <DIR_PATH_FOR_OUTPUTS>

criteria:
    readcount_min: <15>
    readcount_max: <1000>

method:
    # To speed up xpore-diffmod, you can use a statistical test (currently only t-test is imple
    # to remove positions that are unlikely to be differentially modified. So, xpore-diffmod wi
    # those significant positions by the statistical test -- usually the P_VALUE_THRESHOLD very
    # If you want xPore to test every genomic/transcriptomic position, please remove this pref
    prefiltering:
        method: t-test
        threshold: <P_VALUE_THRESHOLD>

    # Here are the parameters for Bayesian inference. The default values shown in <> are used,
    max_iters: <500>
    stopping_criteria: <0.00001>
```

Python Packaging

ploy-np Merge pull request #115 from

- docs
- figures
- xpore
- .gitattributes
- .gitignore
- LICENSE
- MANIFEST.in
- README.md
- setup.py

```python
"""Setup for the xpore package."""

from setuptools import setup,find_packages

__pkg_name__ = 'xpore'


with open('README.md') as f:
    README = f.read()

setup(
    author="Ploy N. Pratanwanich",
    maintainer_email="naruemon.p@chula.ac.th",
    name=__pkg_name__,
    license="MIT",
    description='xpore is a python package for Nanopore data analysis of differential RNA modifications.',
    version='v2.1',
    long_description=README,
    long_description_content_type='text/markdown',
    url='https://github.com/GoekeLab/xpore',
    packages=find_packages(),
    include_package_data=True,
    install_requires=[
            'numpy>=1.18.0',
            'pandas>=0.25.3',
            'scipy>=1.4.1',
            'PyYAML',
            'h5py>=2.10.0',
            'pyensembl>=1.8.5',
            'ujson>=4.0.1'
            ],
    python_requires=">=3.8",
    entry_points={'console_scripts': ["xpore={}.scripts.xpore:main".format(__pkg_name__)]},
    classifiers=[
        # Trove classifiers
        # (https://pypi.python.org/pypi?%3Aaction=list_classifiers)
        'Development Status :: 1 - Planning',
        'License :: OSI Approved :: MIT License',
        'Programming Language :: Python',
        'Programming Language :: Python :: 3.8',
        'Topic :: Software Development :: Libraries',
        'Topic :: Scientific/Engineering :: Bio-Informatics',
        'Intended Audience :: Science/Research',
    ],
)
```

Parallelization / Multiprocessing

```
import multiprocessing
```

When Data are Too Big to Fit in the Memory

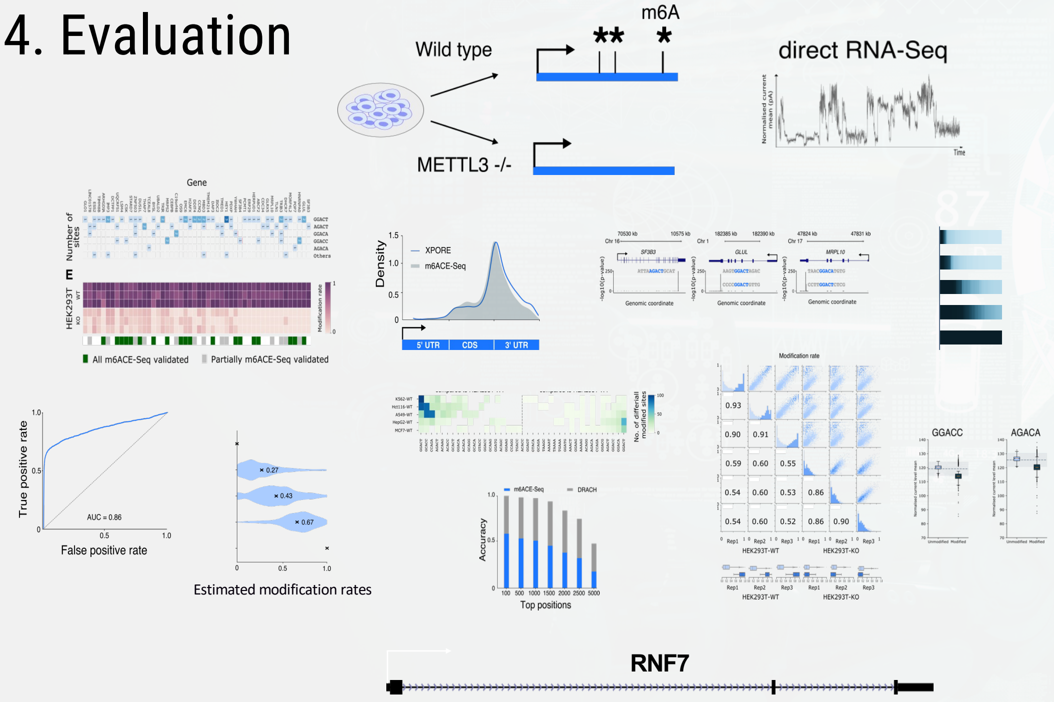
data.index

gene_id	start_idx	stop_idx
ENGxx1	0	16856
ENGxx2	16857	29435
…	…	…

data.json

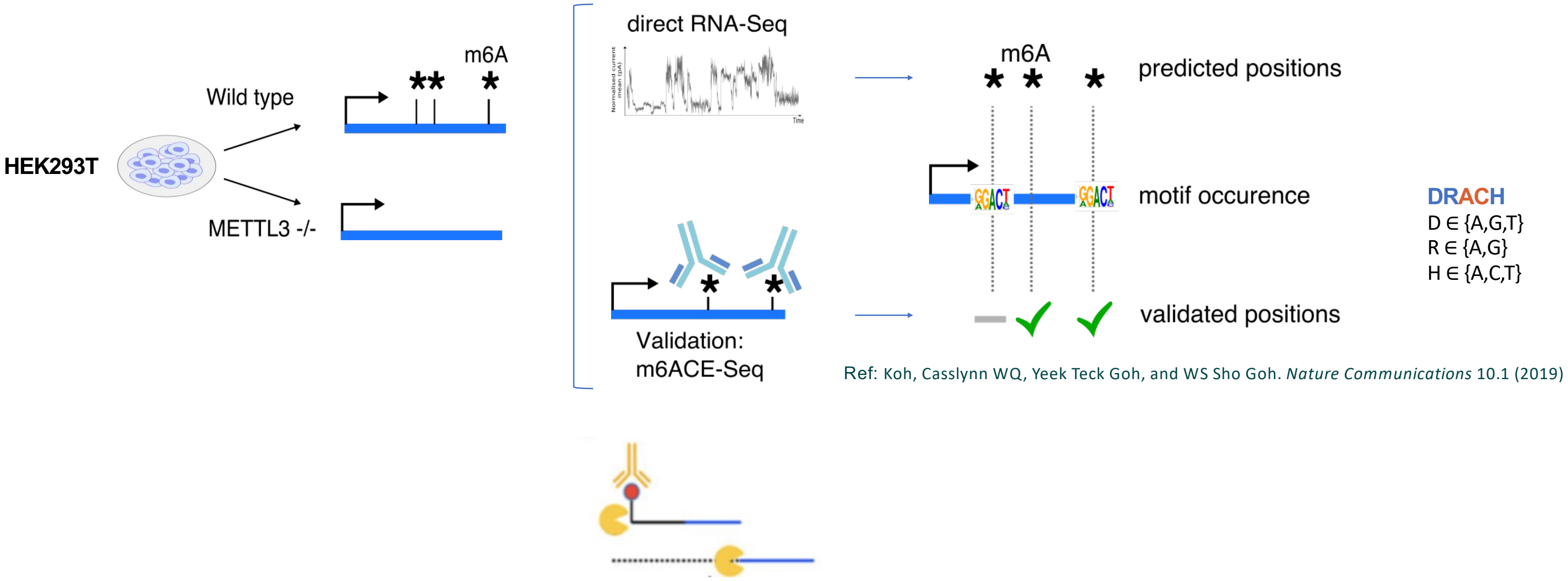
{'ENGxx1': [123,110,…]}, {'ENGxx1': [123,110,…]}, {…}

4. Evaluation



- **Experiment setup**
- **Validation**
- **Applicability**
- **Discovery**

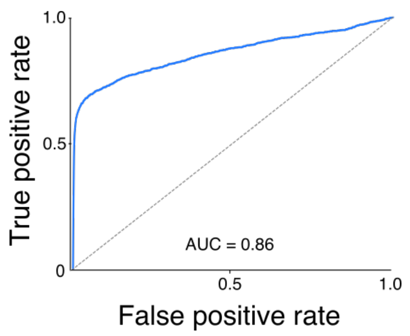
Experiment Setup

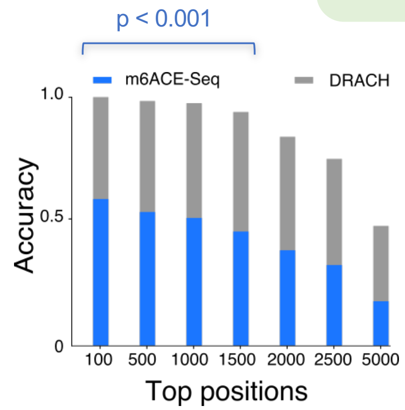


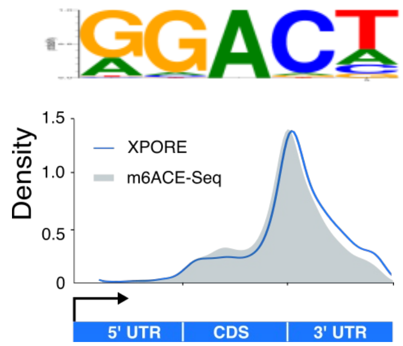
Ref: Koh, Casslynn WQ, Yeek Teck Goh, and WS Sho Goh. *Nature Communications* 10.1 (2019)

Validation: m6A calling

In bioinformatics,
- as the labels are incomplete,
- the false positives may not be wrong.
- Analysis on the predictions is usually required to get more insights.





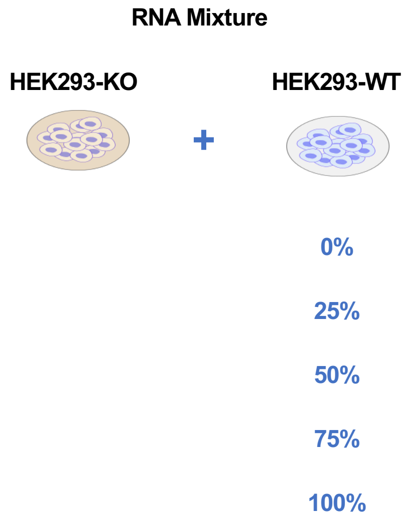


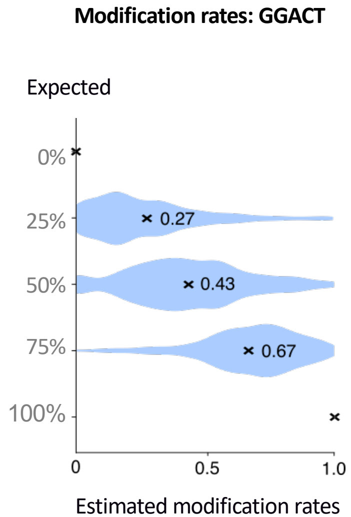
- ~1 million sites were tested.
- xPore achieves AUCROC of 86% to call differentially m6A sites.

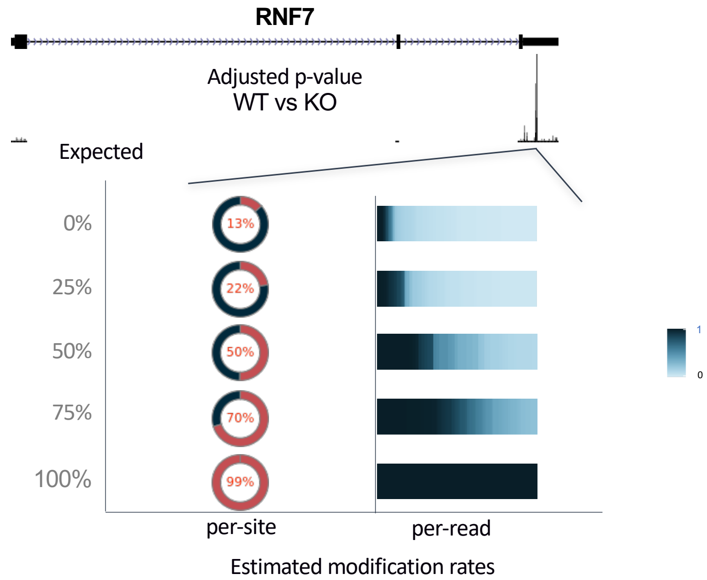
- Around half were identified by m6ACE-Seq.
- With m6ACE-Seq + DRACH, the accuracy is up to >95%.
- dRNA-Seq helps identify a different set of modified sites that had been otherwise missed.

- m6A motifs e.g. GGACT, AGACT are confirmed.
- xPore can facilitate motif discovery in any other pairwise comparison.
- The differentially modified sites are also enriched at stop codons.

Validation: m6A stoichiometry quantification







- xPore models all RNA mixture samples at once.

- Estimated modification rates closely match to the expected.

- Modification rates estimated by xPore can be interpretable as fractions of modified reads in a cell.
- This allows the analysis of differential modifications.

Validation: ML Metrics & Result Analysis

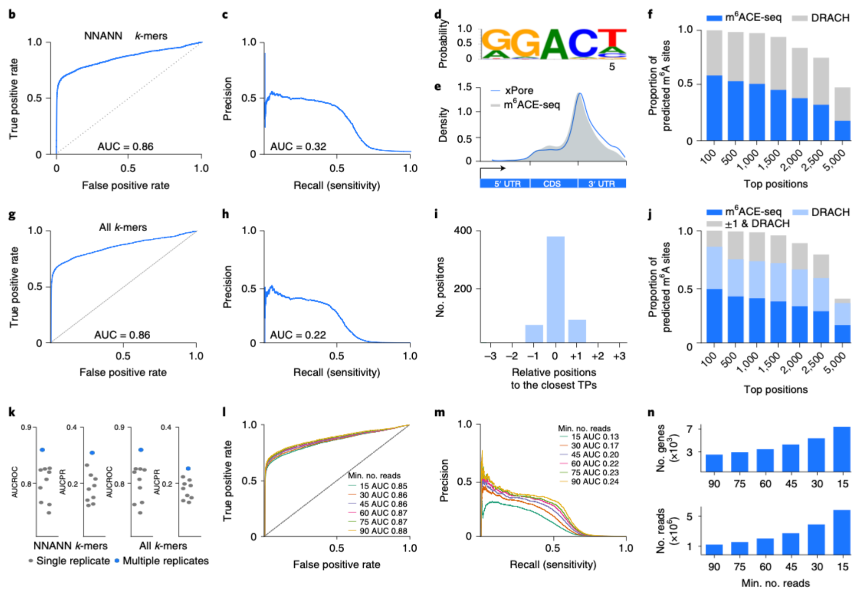
Fig. 2 | Detection of m6A sites in the human transcriptome.

ML Metrics

- ROC Curve
- Precision-Recall Curve
- Accuracy

Analysis

- Domain-specific evaluation
- Effects of the data size



Validation: ML Metrics & Result Analysis

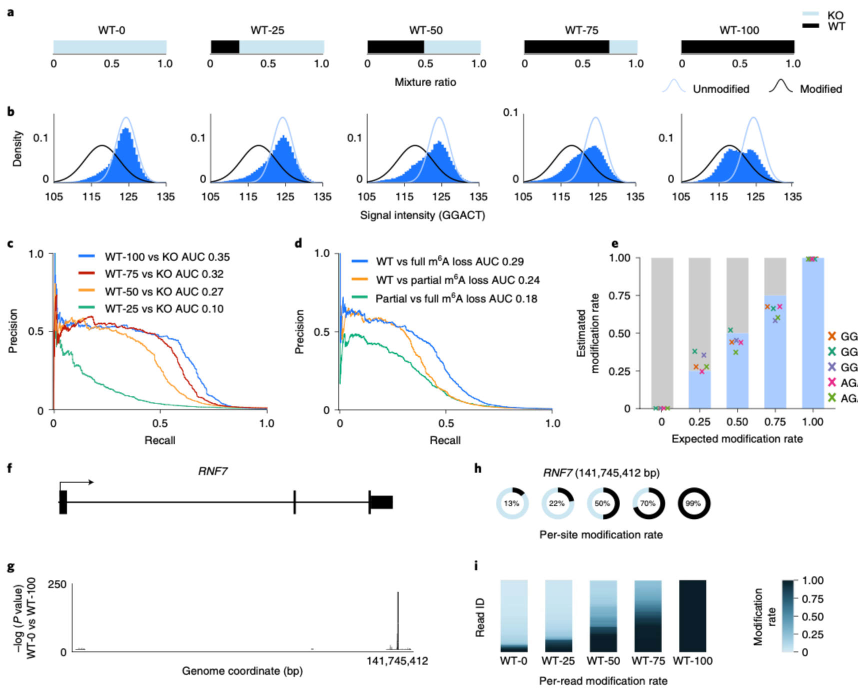
Fig. 3 | xPore modification-rate estimates correspond to the fraction of modified RNA species in the cell

ML Metrics

- ROC Curve
- Precision-Recall Curve
- Accuracy

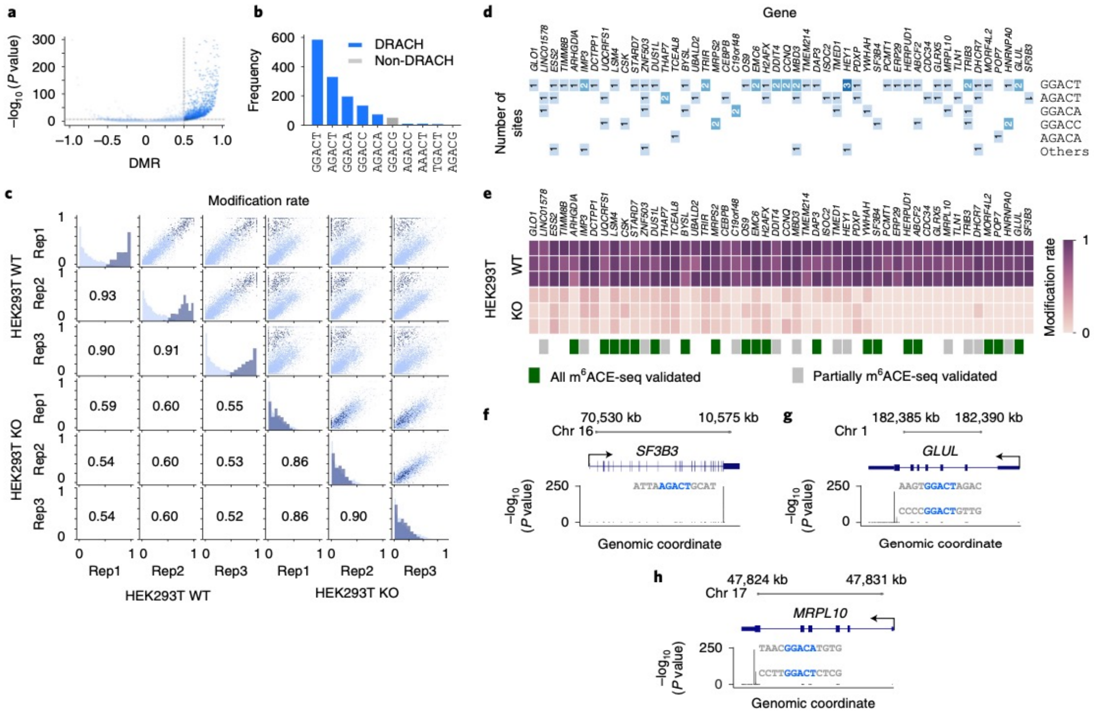
Analysis

- Domain-specific evaluation
- Effects of the data size



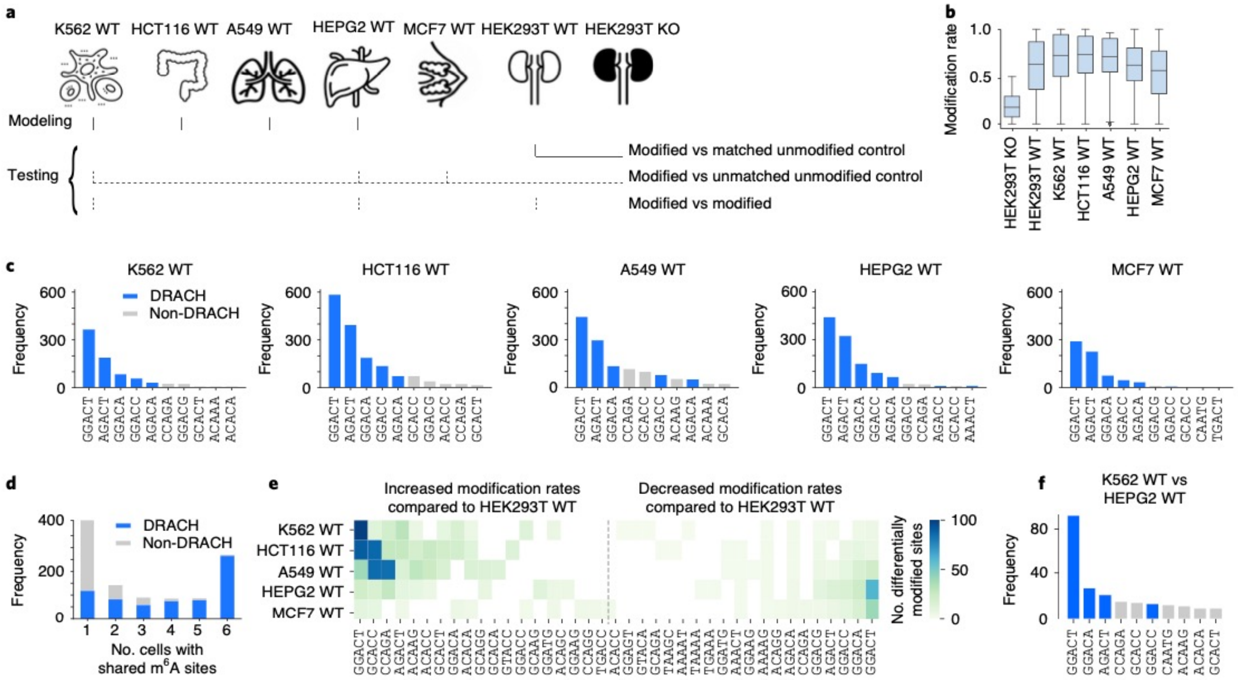
Applicability: Full Dataset

Fig. 4 | Transcriptome-wide identification of differentially modified positions.



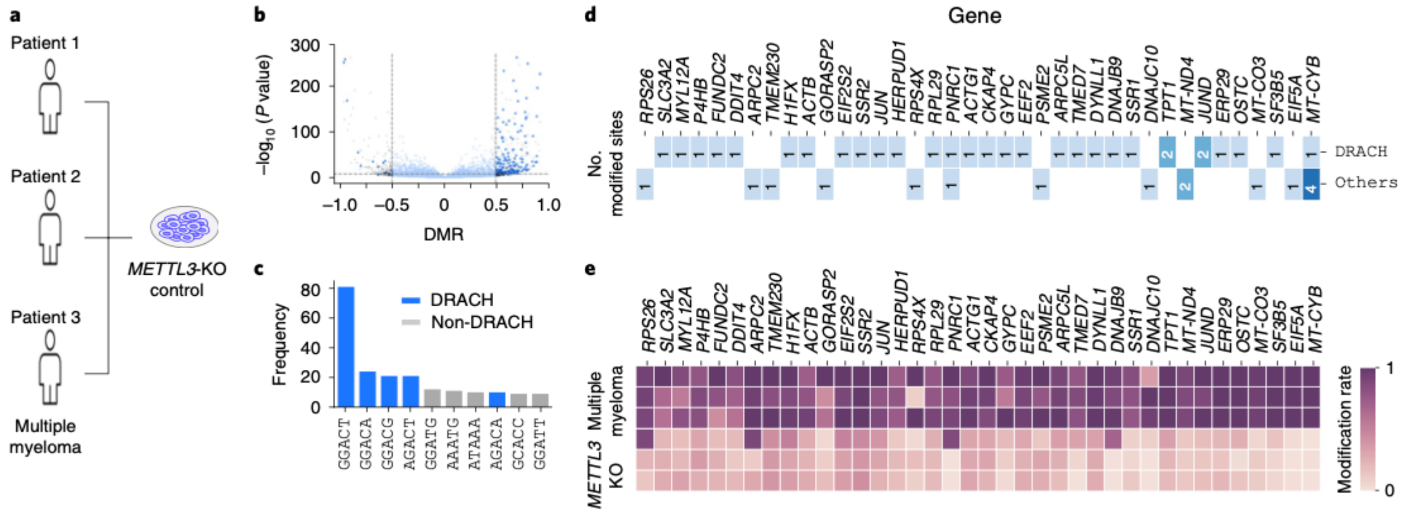
Applicability: Other Datasets

Fig. 5 | Identification of m6A sites across different tissues and cell lines.



Applicability: Clinical Data

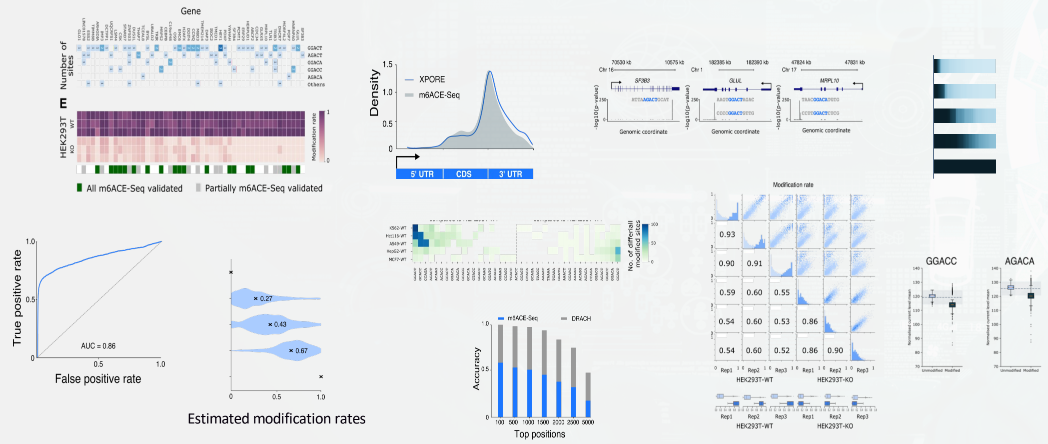
Fig. 6 | Identification of m6A in clinical samples using direct RNA-seq.



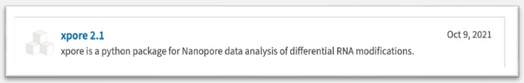
Evaluation: Keys Takeaway

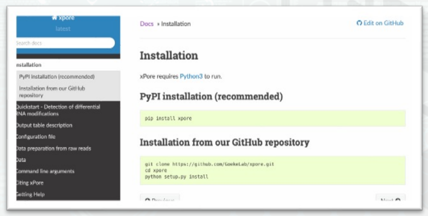
- Validation
  - Using appropriate ML metrics
  - Analyzing the results to get more insights
- Comparison with other state-of-the-art methods
- Applicability
  - External / Other data
  - Human evaluation
  - Discovery

5. Visualization and Presentation



- **Storylining**
- **Choosing the Right Plots**
- **Source Code**
- **Online Documentation**





Storylining

Method overview

- Fig. 1 | Schematic workflow: quantification of RNA modifications from direct RNA-seq data using xPore

Validation

- Fig. 2 | Detection of m6A sites in the human transcriptome.
  - xPore: identification of differential RNA modifications.
  - xPore identifies m6A sites at single-base resolution.
  - Replicates increase precision.
  - Pooling data increases sensitivity.
- Fig. 3 | xPore modification-rate estimates correspond to the fraction of modified RNA species in the cell
  - xPore identifies modified positions with low stoichiometry.
  - Quantitative estimation of RNA-modification rates.

Applicability & Discovery

- Fig. 4 | Transcriptome-wide identification of differentially modified positions.
  - DMRs as estimates of effect size.
- Fig. 5 | Identification of m6A sites across different tissues and cell lines.
  - Identification of m6A across genetically diverse cell lines.
  - Variation of m6A across different cell lines.
- Fig. 6 | Identification of m6A in clinical samples using direct RNA-seq.
  - Identification of m6A in clinical cancer samples.

Choosing the Right Plots

Fig. 1 | Schematic workflow: quantification of RNA modifications from direct RNA-seq data using xPore

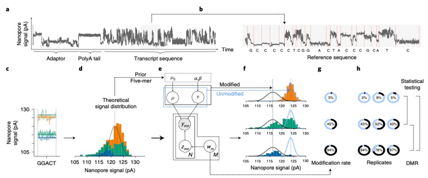


Fig. 2 | Detection of m6A sites in the human transcriptome.

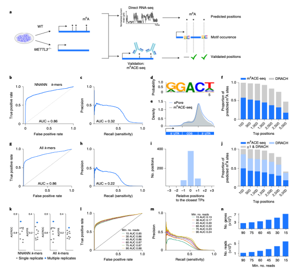


Fig. 3 | xPore modification-rate estimates correspond to the fraction of modified RNA species in the cell

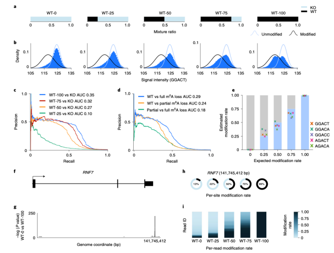


Fig. 4 | Transcriptome-wide identification of differentially modified positions.

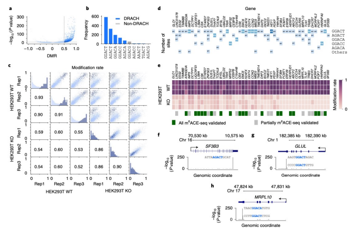


Fig. 5 | Identification of m6A sites across different tissues and cell lines.

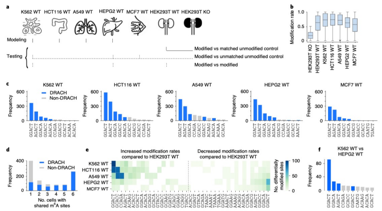
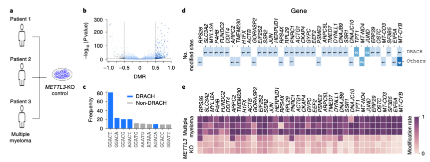


Fig. 6 | Identification of m6A in clinical samples using direct RNA-seq.



Choosing the Right Plots

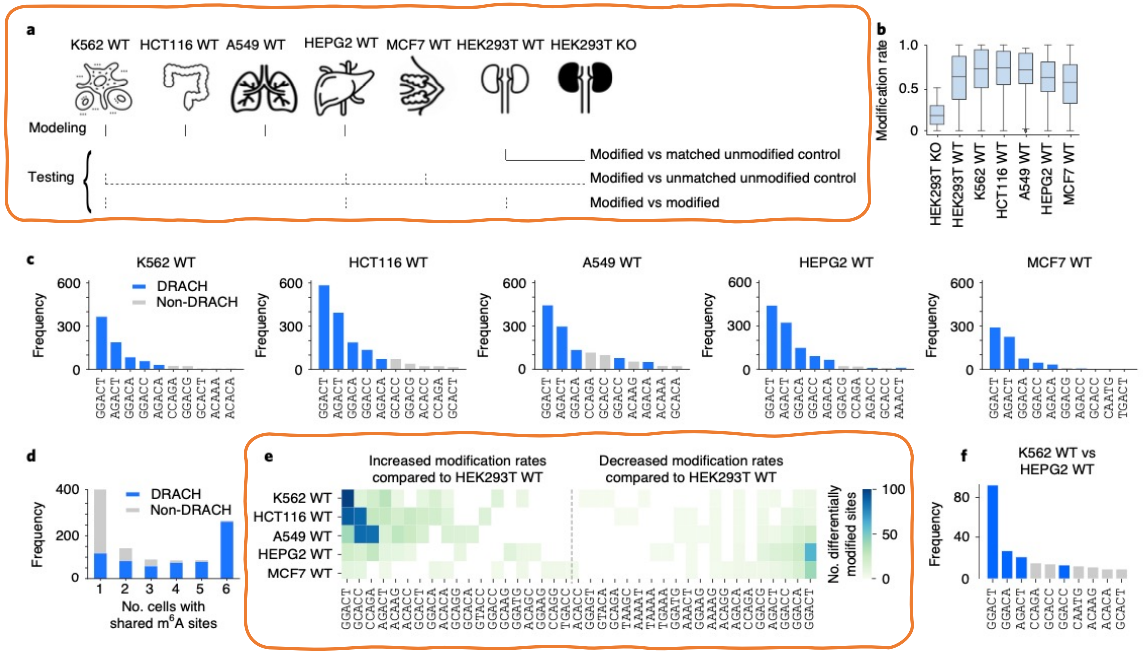


Fig. 5 | Identification of m6A sites across different tissues and cell lines.

3 Key Success to Develop AI-Powered Apps

1. Alignment with the actual needs
2. Sufficient generalization and evaluation
3. Simple deployment and serving
    - Online documentation
    - Easy installation
    - Source code
    - Data availability
    - Lightweight
    - Fast

downloads 27k

xpore.readthedocs.io/

python · machine-learning · rna-seq · nanopore · genomics · rna · transcriptomics · modification · nanopore-sequencing · rna-modifications

Readme
MIT license
Activity
121 stars
9 watching
22 forks
Report repository

Releases 9
xPore v2.1 Latest
on Oct 9, 2021
+ 8 releases

https://github.com/GoekeLab/xpore

ARTICLES nature biotechnology
https://doi.org/10.1038/s41587-021-00949-w

Identification of differential RNA modifications from nanopore direct RNA sequencing with xPore

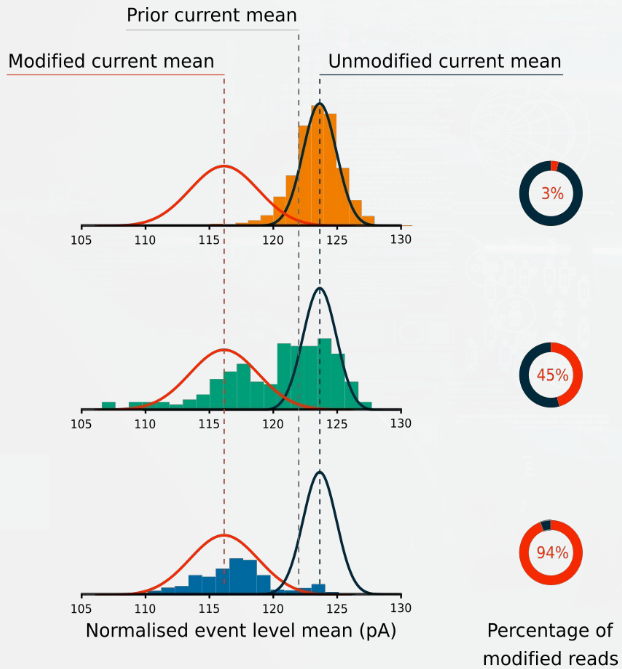
Ploy N. Pratanwanich[1,2,3], Fei Yao[1,11], Ying Chen[1,11], Casslynn W. Q. Koh[1,11], Yuk Kei Wan[1,11], Christopher Hendra[1,4], Polly Poon[1], Yeek Teck Goh[1], Phoebe M. L. Yap[1], Jing Yuan Chooi[5], Wee Joo Chng[5,6,7], Sarah B. Ng[1], Alexandre Thiery[8], W. S. Sho Goh[1,9] and Jonathan Göke[1,10]

Scopus metrics

78 99th percentile
Citations in Scopus

9.61
Field-Weighted citation impact

6. Future Work



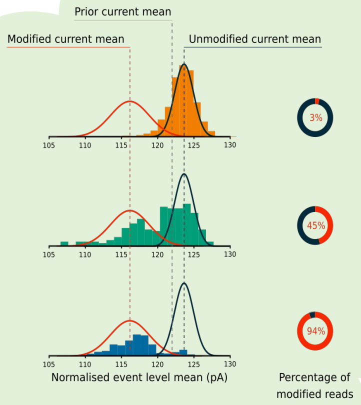
- **Identifying the Limitations**
- **Considering Changes in the Future**

Future Work

Domain-Oriented

- m6anet
- <Gaussian> mixture model
- Interpretability
  - Modification or basecalled errors
- End-to-end
  - Why?

  → Nanopolish eventalign / Guppy basecaller are subjet to change



Method-Oriented

- Deep autoencoder + GMM
- CNN + GMM
- **Other models + GMM**

สร้างคน
ข้ามพรมแดน

E-SAN Thailand Coding & AI Academy

Thailand Coding & AI Academy

FACULTY OF EDUCATION KHON KAEN UNIVERSITY

มหาวิทยาลัยขอนแก่น

สอวน

สำนักงานสภานโยบายการอุดมศึกษา วิทยาศาสตร์ วิจัยและนวัตกรรมแห่งชาติ

INET

Ministry of Higher Education, Science, Research and Innovation

THAI PROGRAMMER

SST
SUN SMART TECH

The Asia Foundation

BOTNOI GROUP

CODE COMBAT

ClusterKit

DOME CLOUD

Let's Code Thailand

DMAP
DIGITAL MAKERS ASIA PACIFIC

MH

Google

FOCUS ARENA