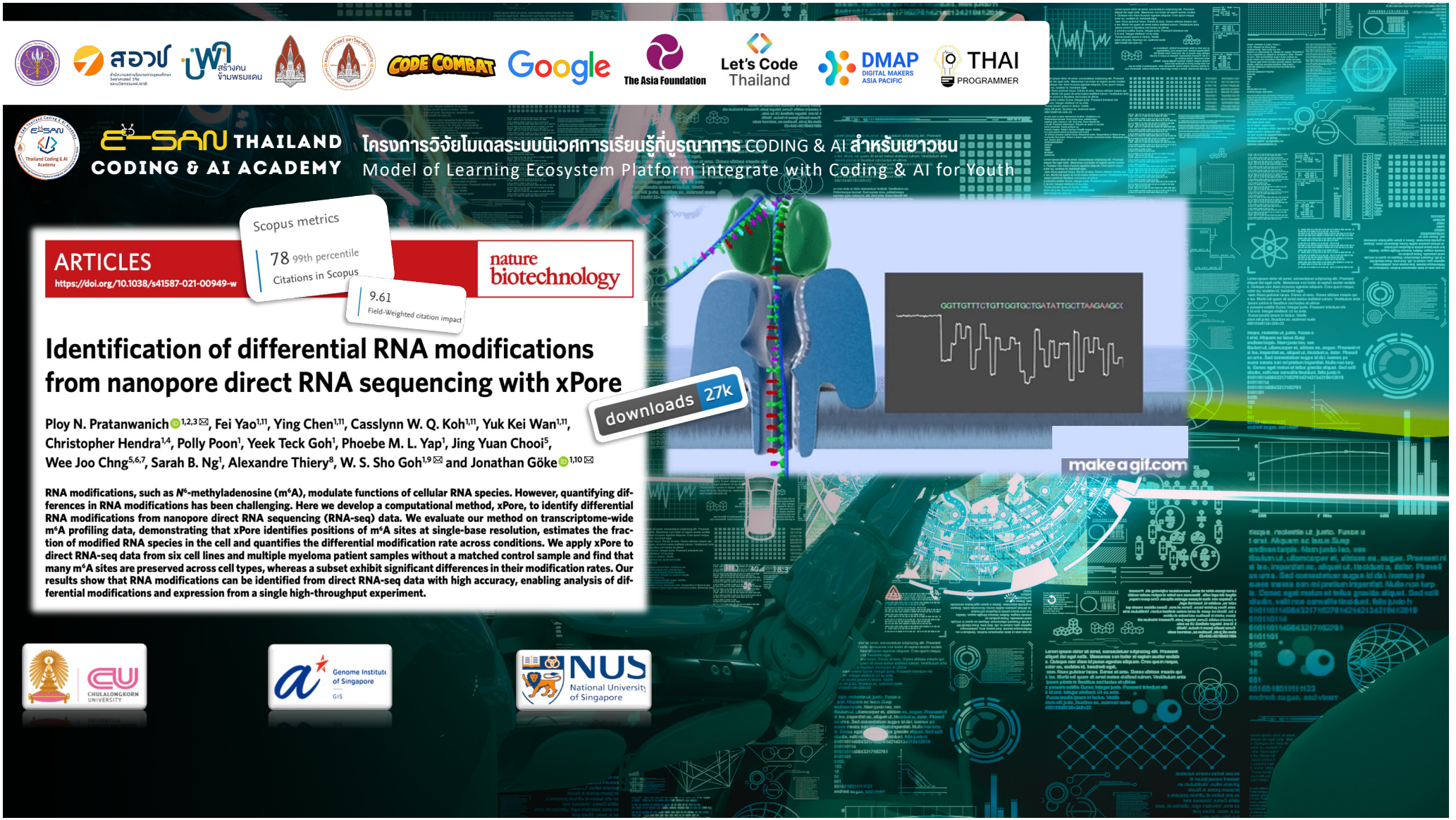

สอวช
สร้างคน ข้ามพรมแดน
CODE COMBAT
Google
The Asia Foundation
Let's Code Thailand
DMAP DIGITAL MAKERS ASIA PACIFIC
THAI PROGRAMMER
Thailand Coding & AI Academy
E-SAN THAILAND
CODING & AI ACADEMY
โครงการวิจัยโมเดลระบบนิเวศการเรียนรู้ที่บูรณาการ CODING & AI สำหรับเยาวชน
Model of Learning Ecosystem Platform 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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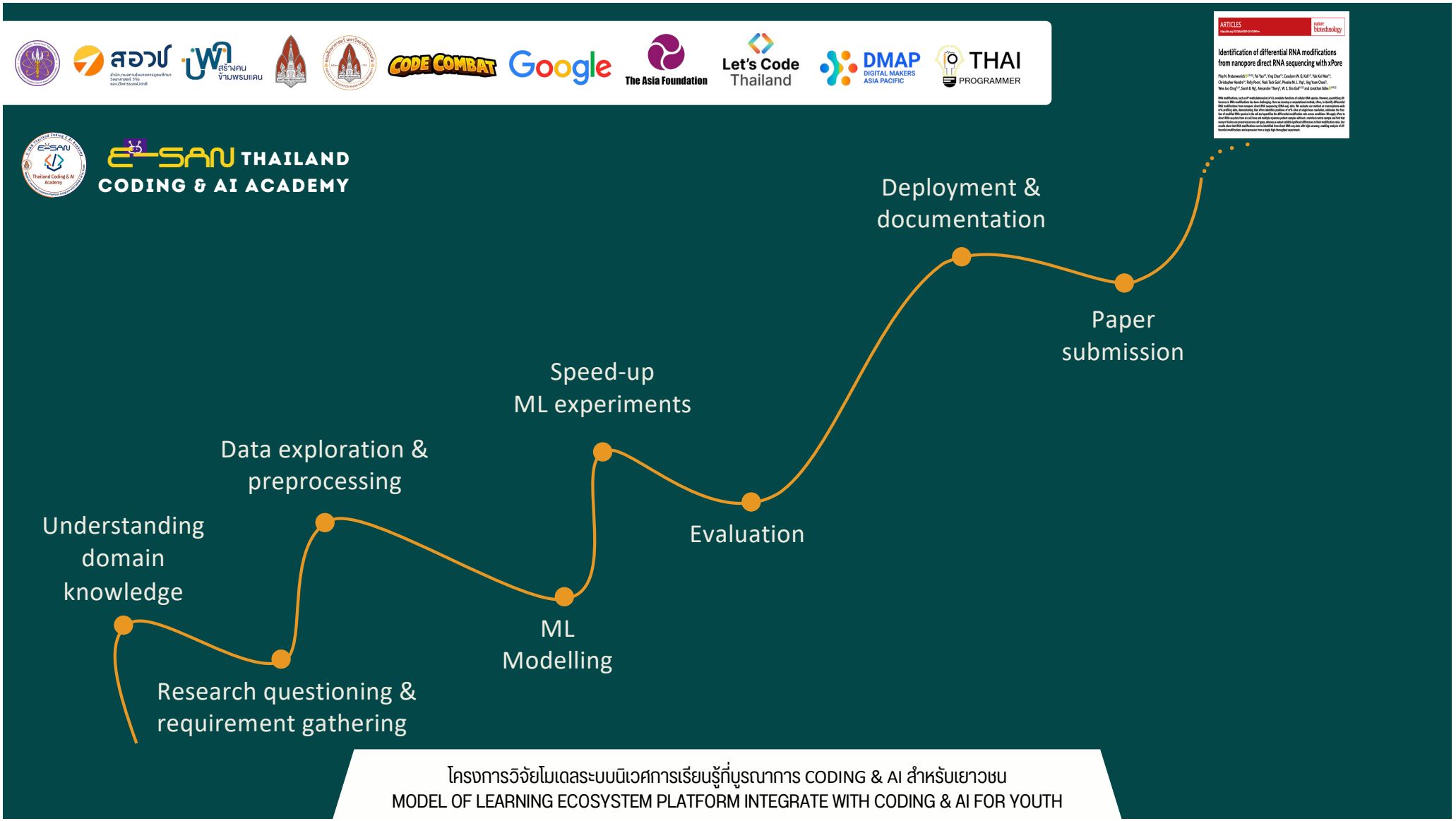
โครงการวิจัยโมเดลระบบนิเวศการเรียนรู้ที่บูรณาการ CODING & AI สำหรับเยาวชน
Model of Learning Ecosystem Platform integrate with Coding & AI for Youth
Scopus metrics
78 99th percentile
Citations in Scopus
9.61
Field-Weighted citation impact
ARTICLES
https://doi.org/10.1038/s41587-021-00949-w
nature biotechnology
Identification of differential RNA modifications from nanopore direct RNA sequencing with xPore
downloads 27k
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 N6-methyladenosine (m6A), 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 m6A profiling data, demonstrating that xPore identifies positions of m6A 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 m6A 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.
GGTTGTTTCTGTTGGTGCTGATATTGCTTAAGAAGC
make a gif.com
CHULALONGKORN UNIVERSITY
Genome Institute of Singapore
NUS National University of Singapore


ESAN 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
nature biotechnology
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


Bioinfomatician
Data Scientist
Biologist
THAILAND CODING & AI ACADEMY
โครงการวิจัยโมเดลระบบนิเวศการเรียนรู้ที่บูรณาการ CODING & AI สำหรับเยาวชน
Model of Learning Ecosystem Platform Integrate with Coding & AI for Youth
xpore 2.1
Oct 9, 2021
xpore is a python package for Nanopore data analysis of differential RNA modifications.
PyPI installation (recommended)
pip install xpore
Installation from our GitHub repository
git clone https://github.com/GoekeLab/xpore.git
cd xpore
python setup.py install
Contributors
Languages
Python 100.0%
Gene
All m6ACE-Seq validated
Partially m6ACE-Seq validated
XPORE
m6ACE-Seq
5' UTR
CDS
3' UTR
True positive rate
False positive rate
AUC = 0.86
Estimated modification rates
Accuracy
Top positions
m6ACE-Seq
DRACH
HEK293T-WT
HEK293T-KO
GGACC
AGACA
XPORE

E-SAN THAILAND CODING & AI ACADEMY

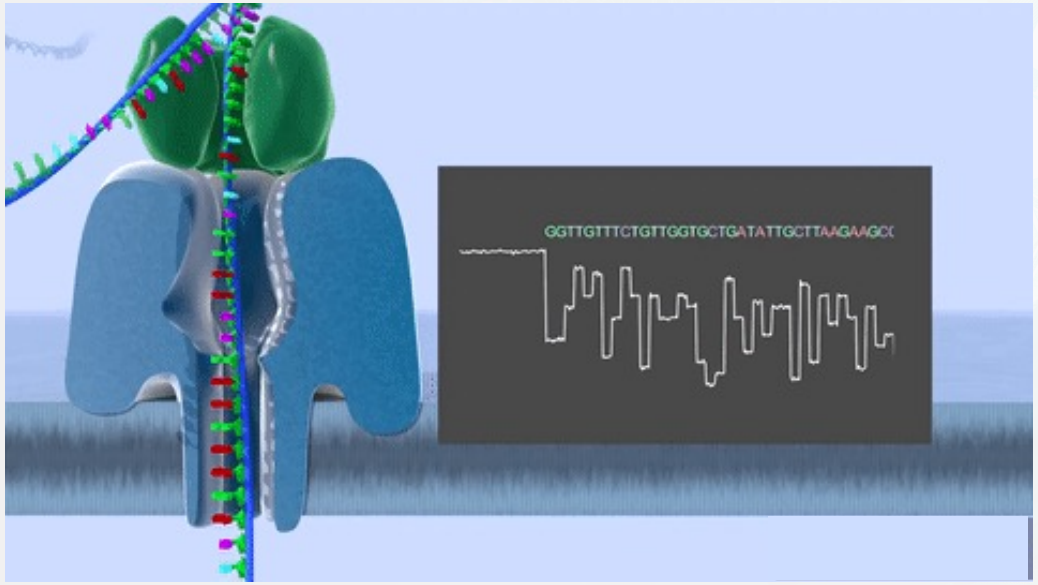
โครงการวิจัยโมเดลระบบนิเวศการเรียนรู้ที่บูรณาการ CODING & AI สำหรับเยาวชน
Model of Learning Ecosystem Platform Integrate with Coding & AI for Youth

Outline

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

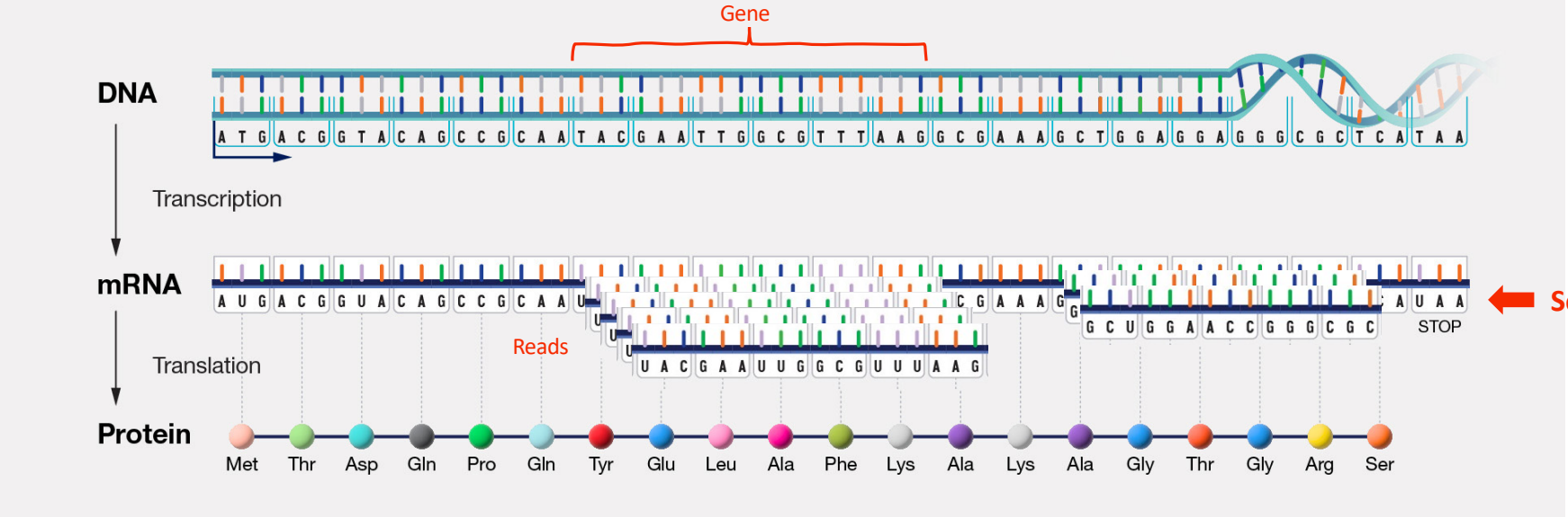
1. Problem Statement
2. Data Collection and Preparation
3. Bayesian [Multi-Sample] Gaussian Mixture Modelling
4. Evaluation
5. Visualization and Presentation
6. Future Work

1. Problem Statement



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

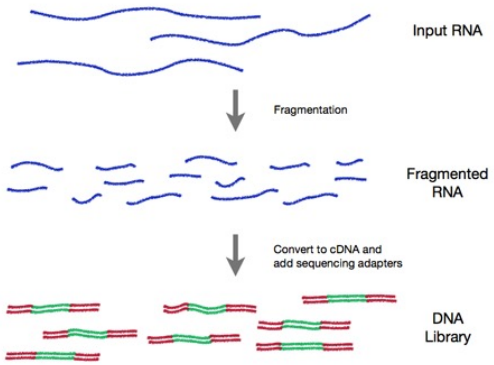
Central Dogma



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

RNA Sequencing

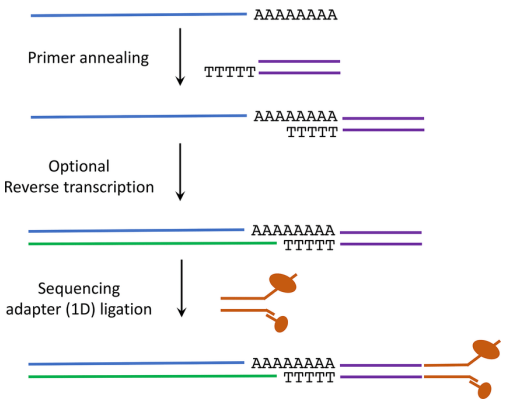
Then

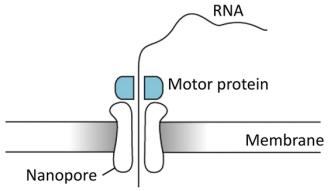


Perform NGS sequencing

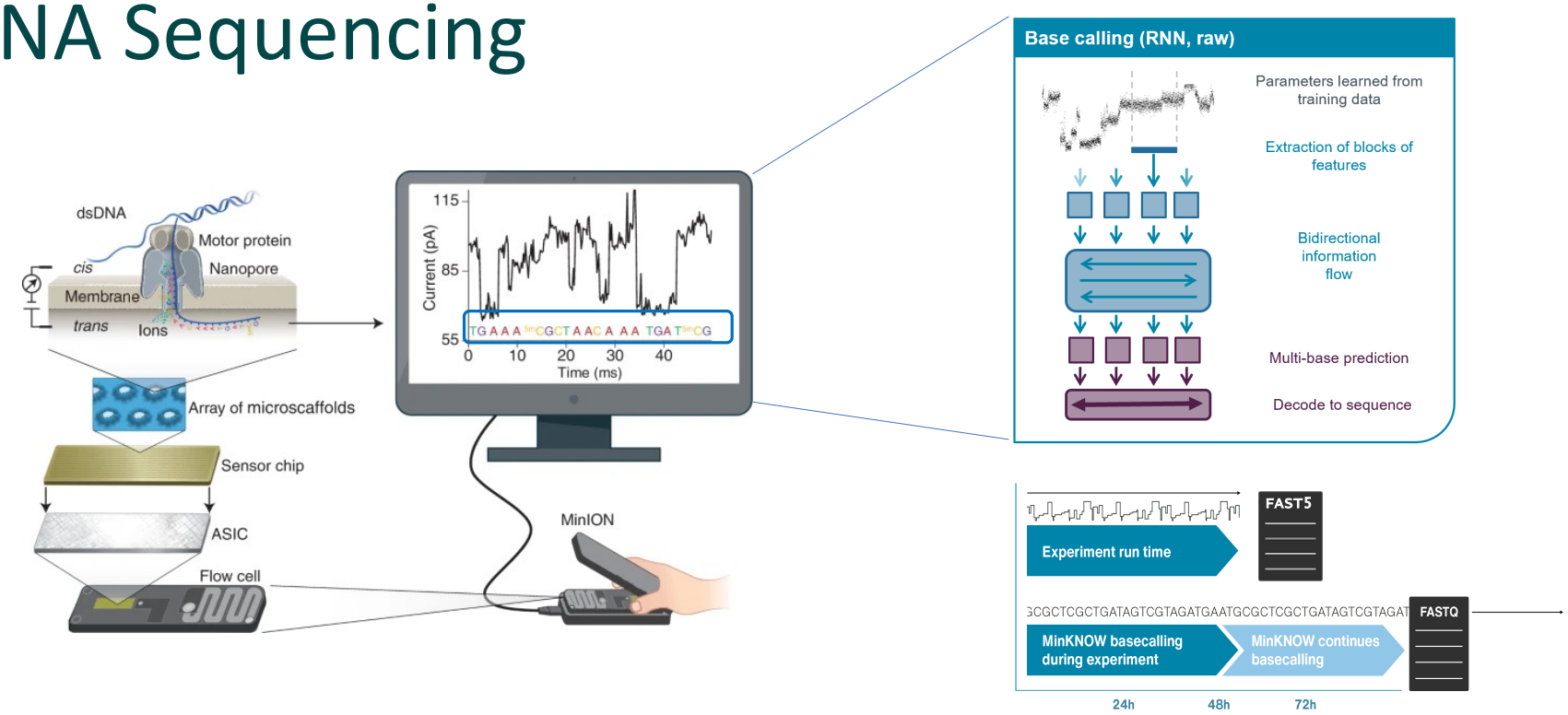
Direct RNA Sequencing

Now



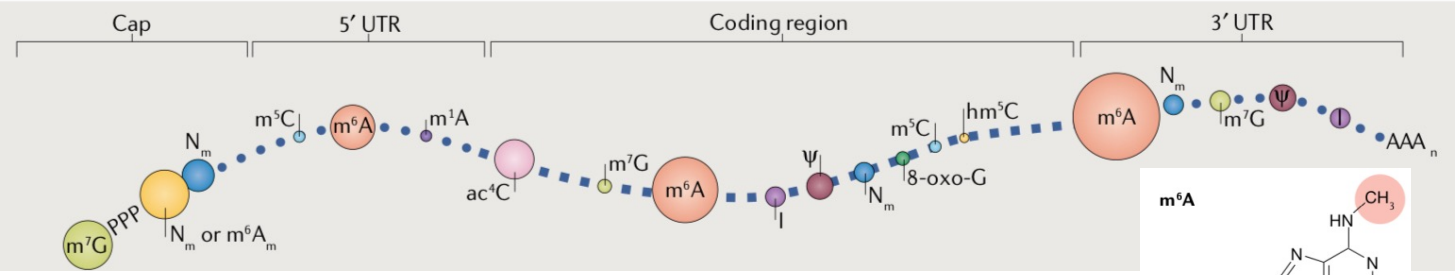


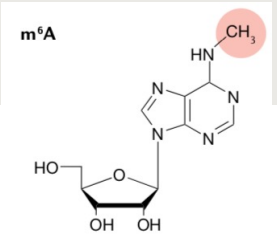
RNA Sequencing



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)

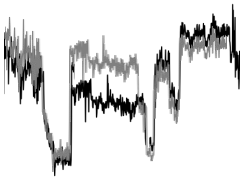
Output Table

Genomic positions	5-mer	Modification rates		Differential modification rates	
		KO	WT	$\bar{W}_{WT} - \bar{W}_{KO}$	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

GGACT
GGm6ACT

Nanopore Sequencing



XPORE

Locate modified positions

Quantify fraction of modified reads -- modification rate

Signal-level modification detection methods

EpiNano

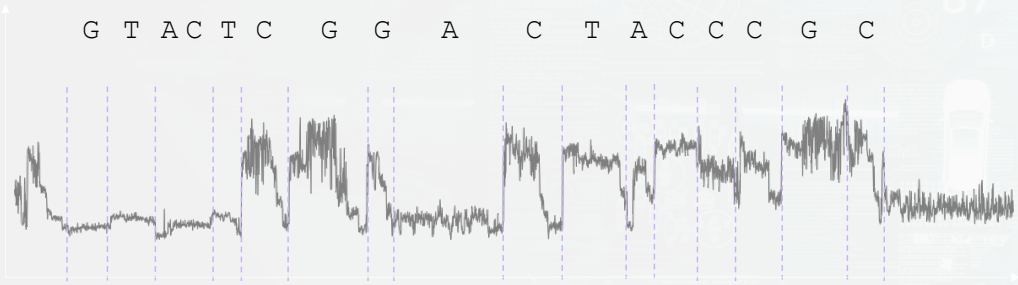
MINES

- m6A
- Training data required.

Tombo

- 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

```
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      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 {
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         }
      }
   }
   GROUP "Raw" {
      GROUP "Reads" {
         GROUP "Read_984" {
            ATTRIBUTE "duration" {
               DATATYPE  H5T_STD_U32LE
               DATA {
               (0): 12639754
               }
            }
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               DATATYPE  H5T_STD_I16LE
               DATASPACE  SIMPLE { ( 76256 ) / ( H5S_UNLIMITED ) }
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               (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
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%&)%&-.),,)**)'',//0-.4-'+),')$(+)()((.+)+'&)())*66.,/--1+*++)+*('*('&&%,+5.*',/,((-+()2/3+)+$
```

FASTA

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

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```

BAM / SAM

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

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D26M2D16M3D10M1D12M1I7M2D8M13S  *       0       0       CAACATCGCGGCCGTGGCGGGATGCTGTGGGAGGGGTCATCCTGCCCAGGCCGGGCCGGCTGCCTACCCTTCCAGACTGTCCGCATCGGCTCCAAGGCTGCTACGCCACCGTCGTGTGAT
GCCATCAGCGGCAGAAGCTGATGGCTGTGACTGAATATCCCCCCGACAGCCATCCTTCCCATCATGCCTGCCACTTCCAGCCCCCACAGGAAGATAGGCCTCATCAGGCTTTTCCGCCAGATAGCAGCAGTTTTCCAGGACAACCGAATGATAGCGTTGCCAGAATGTGGCTTGAG
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
```

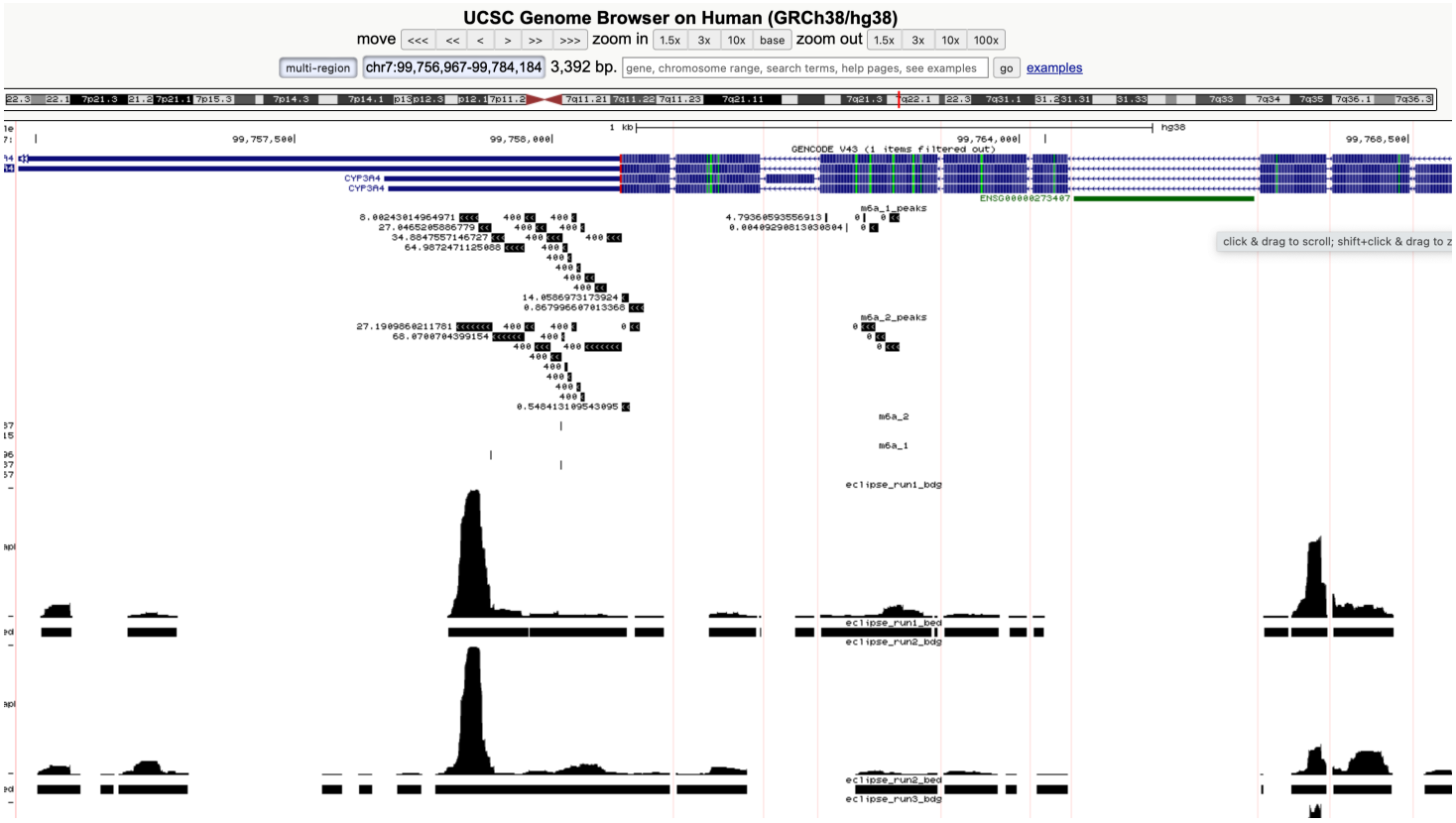

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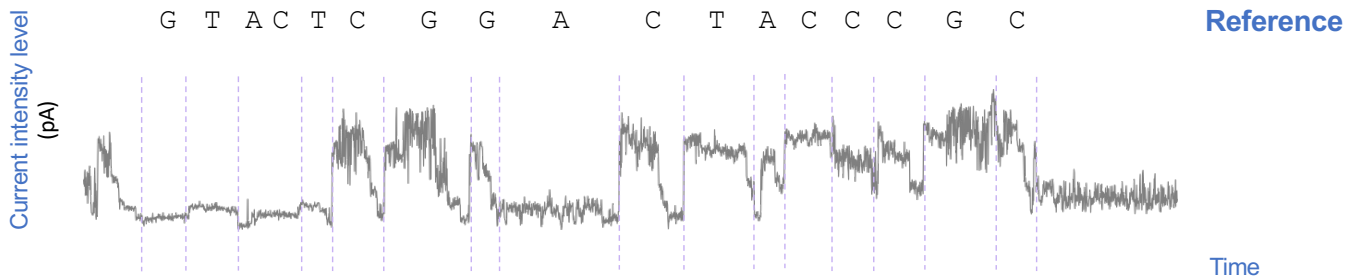
MODEL OF LEARNING ECOSYSTEM PLATFORM INTEGRATE WITH CODING & AI FOR YOUTH

Nanopore pre-processing pipeline for signal-level data analysis



nf-core/nanoseq

Direct RNA sequencing	Basecalling	Sequence aligning	Signal event aligning
Oxford Nanopore	Guppy	Minimap2	Nanopolish
	FAST5 → FASTQ	FASTQ + FASTA → BAM	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
```

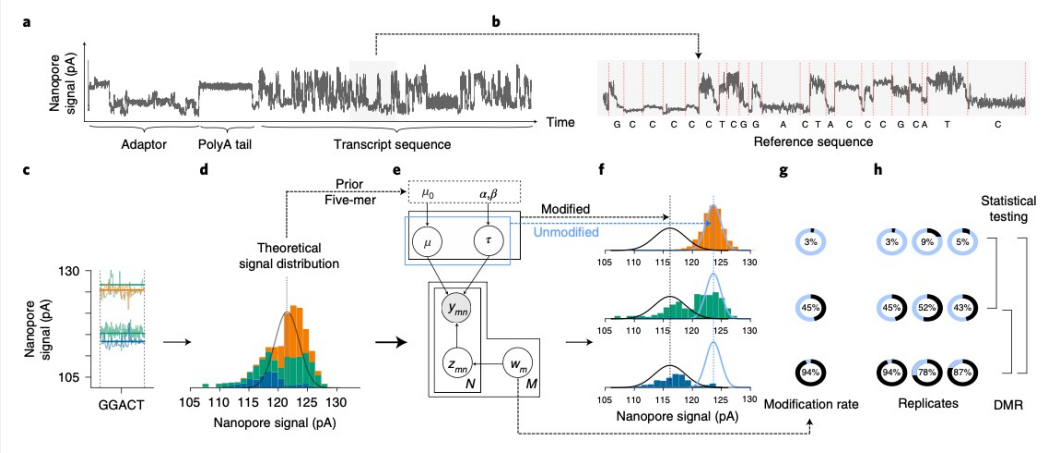
2. Align to transcriptome:

```
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:

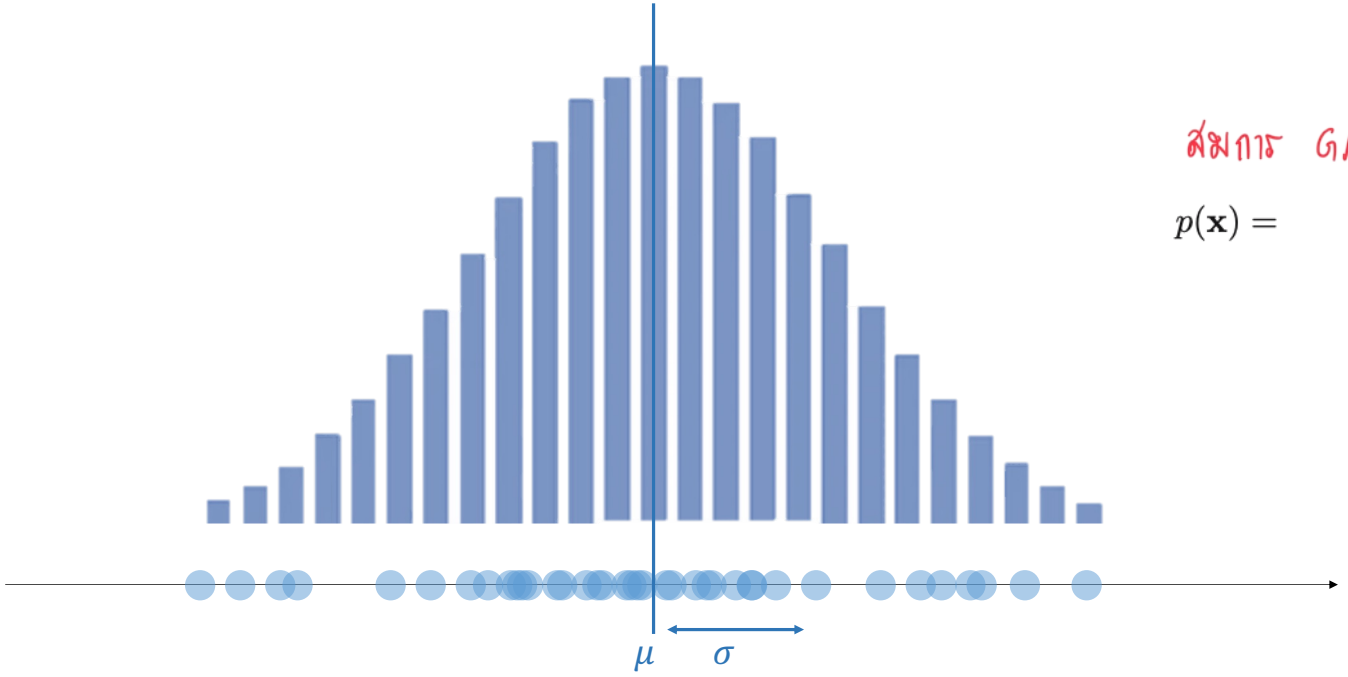
```
nanopolish index -d <PATH/TO/FAST5_DIR> <PATH/TO/FASTQ_FILE>
nanopolish eventalign --reads <PATH/TO/FASTQ_FILE> \
--bam <PATH/TO/BAM_FILE> \
--genome <PATH/TO/FASTA_FILE \
--signal-index \
--scale-events \
--summary <PATH/TO/summary.txt> \
--threads 32 > <PATH/TO/eventalign.txt>
```

3. Bayesian [Multi-Sample] Gaussian Mixture Modelling



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

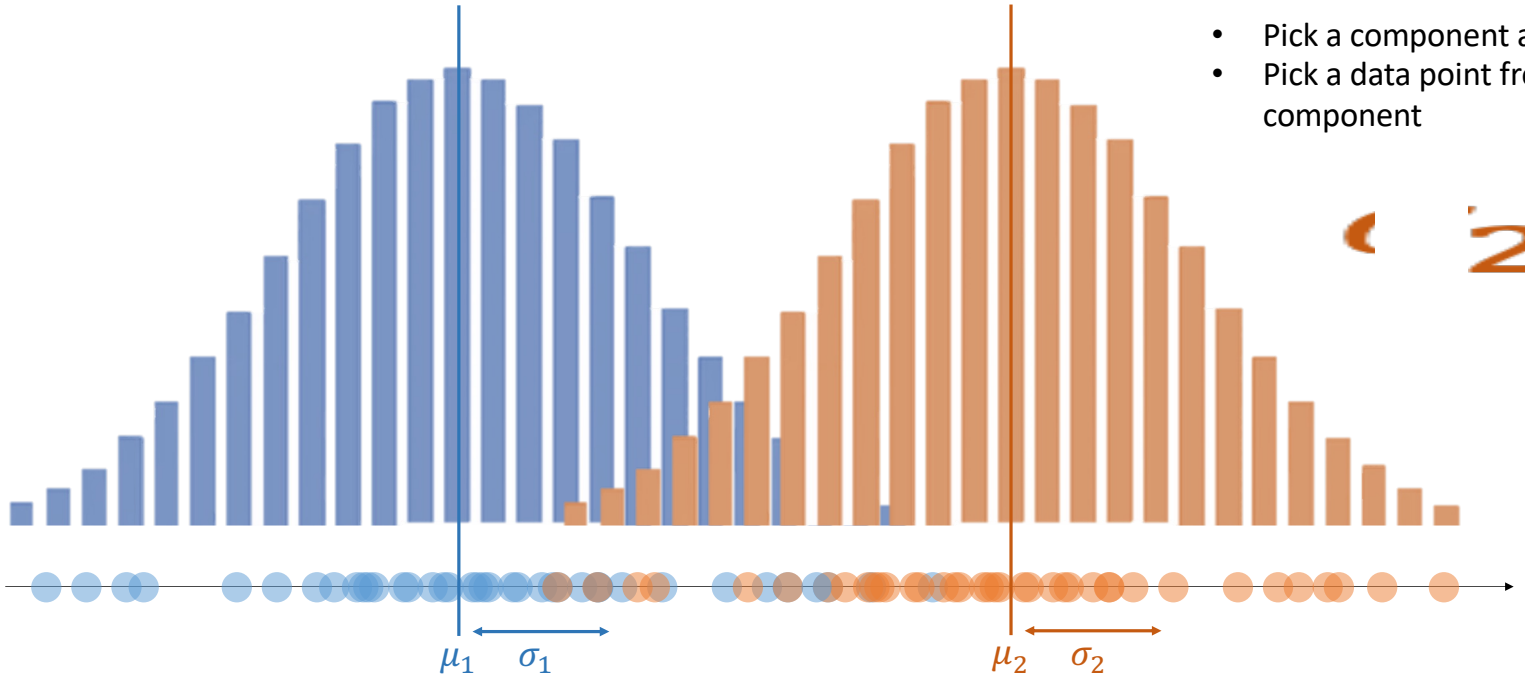
Bayesian Multi-Sample Gaussian Mixture Model



สมการ GMM

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

Bayesian Multi-Sample Gaussian Mixture Model



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

What is GMM?

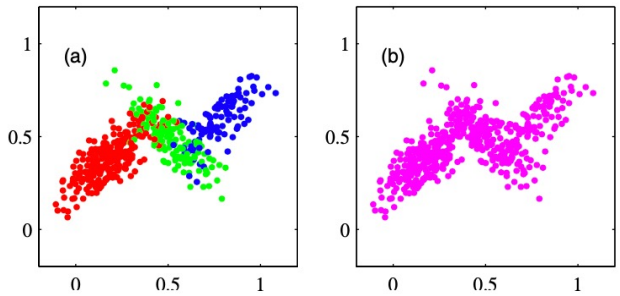
Assumption how data are **generated** as follows

- 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

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

$\boldsymbol{\theta}$

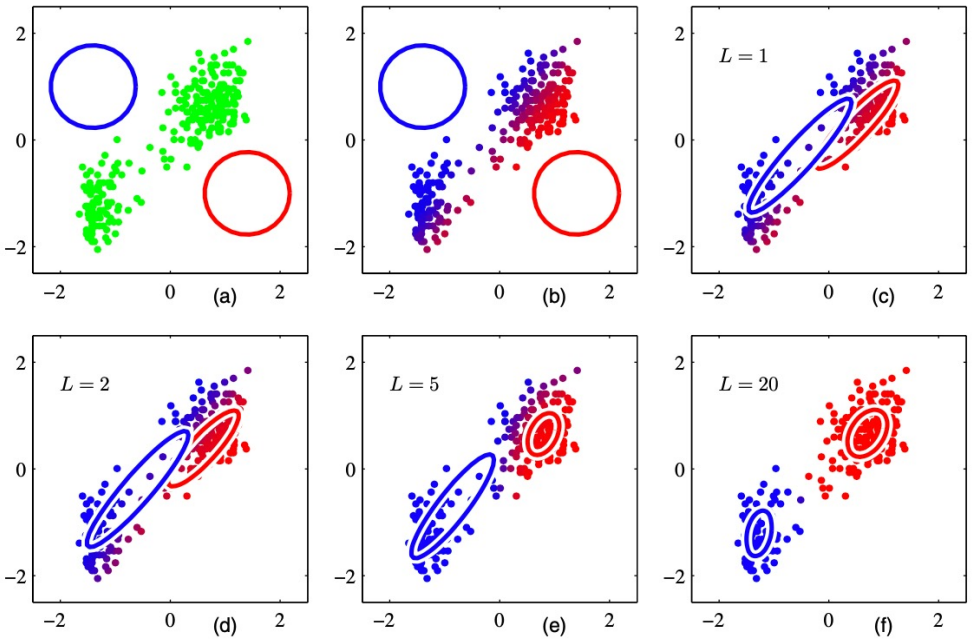
Data



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

GMM Inference

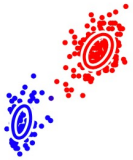
Iterative algorithm



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

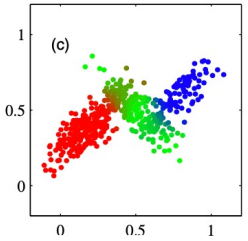
Generative AI

Clustering

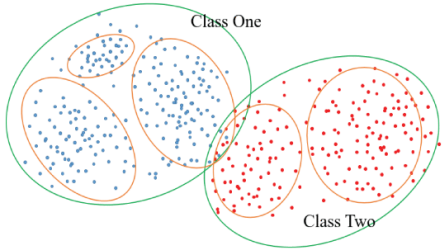


GMM
as a **Density Estimator**

Model
how data are generated



(One-Calss) Classification



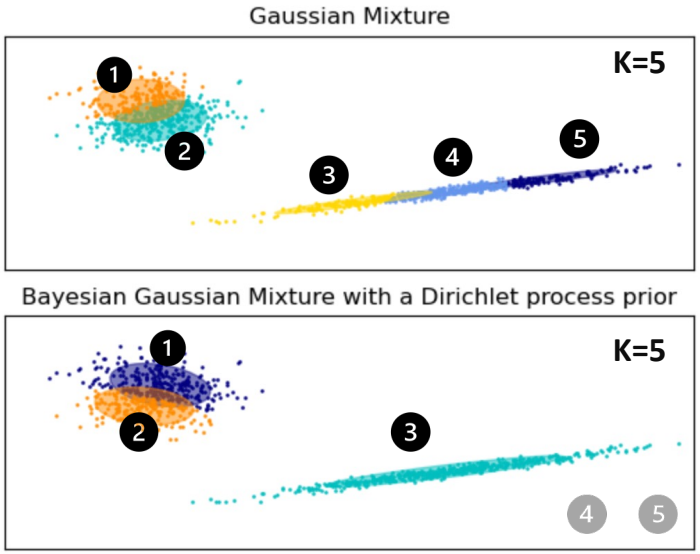
Bayesian Multi-Sample Gaussian Mixture Model

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

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

Θ (over π_k and $\boldsymbol{\mu}_k, \boldsymbol{\Sigma}_k$)

Data



Point estimate = Maximum Likelihood

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

Posterior = Likelihood x Prior

$P(\Theta \mid \text{Data}) = P(\text{Data} \mid \Theta) \times P(\Theta)$

Frequentist vs Bayesian

$P(Data \mid \Theta)$

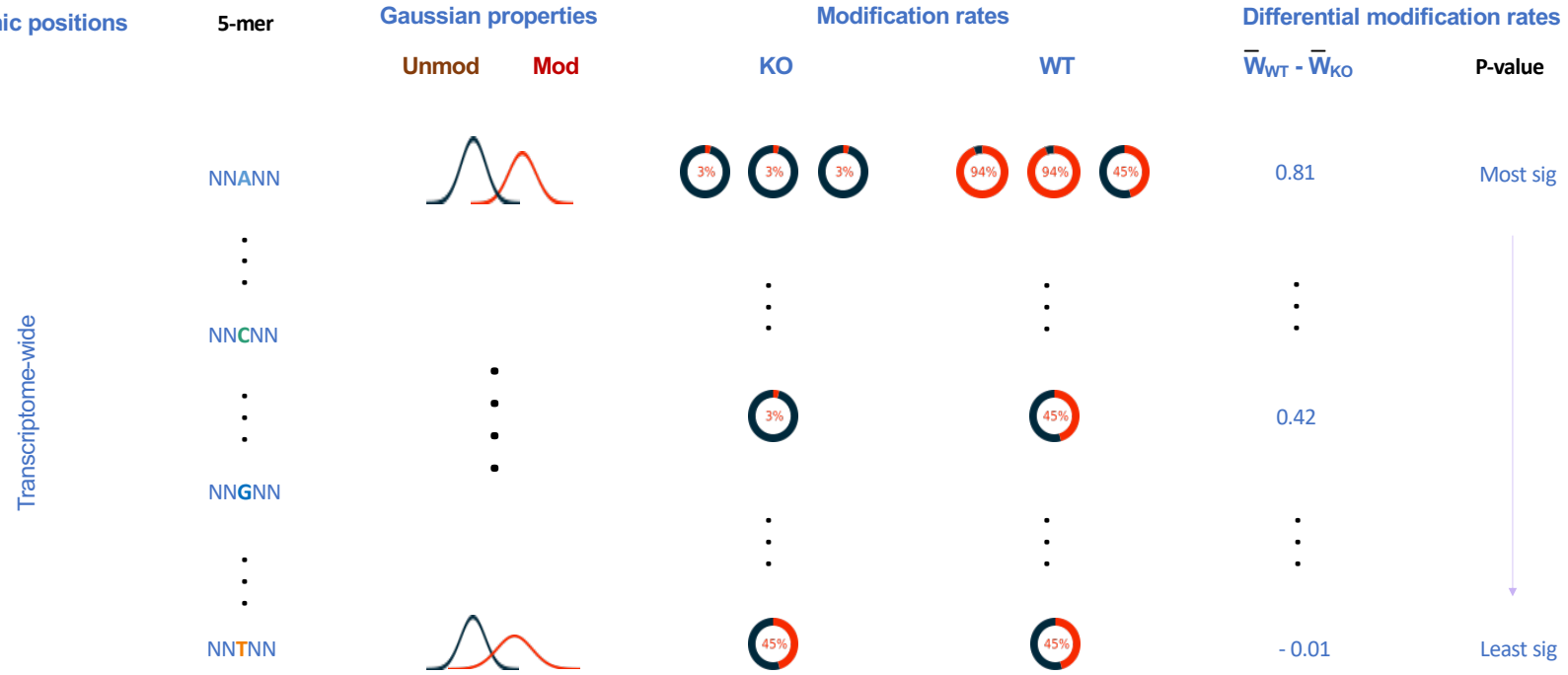
$P(\Theta \mid Data) = P(Data \mid \Theta) \times P(\Theta)$

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



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MODEL OF LEARNING ECOSYSTEM PLATFORM INTEGRATE WITH CODING & AI FOR YOUTH

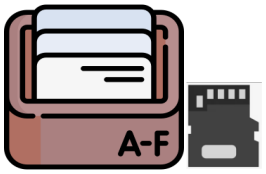
Speed-Up ML Experiments

Automated ML models

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

- Config file
- Python packaging

- 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**

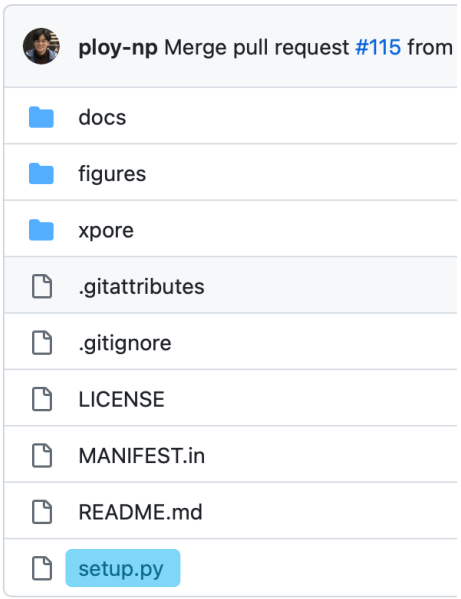
Code | Blame | 78 lines (63 loc) · 2.68 KB | Raw

```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()
```

```
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




```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

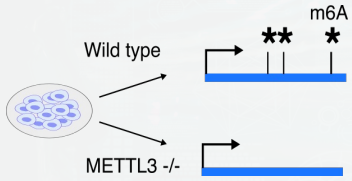
data.index

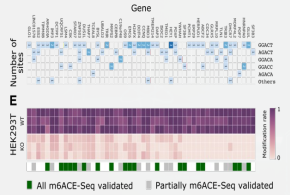
gene_id	start_idx	stop_idx
ENGxx1	0	16856
ENGxx2	16857	29435
…	…	…

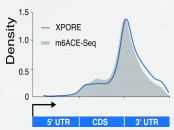
data.json

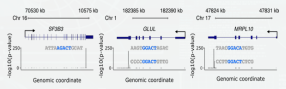
{'ENGxx1': [123,110,…]}, {'ENGxx1': [123,110,…]}, {…}

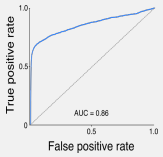
4. Evaluation

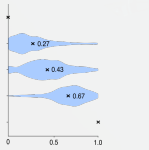


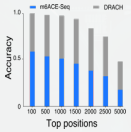


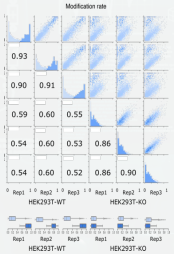


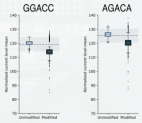






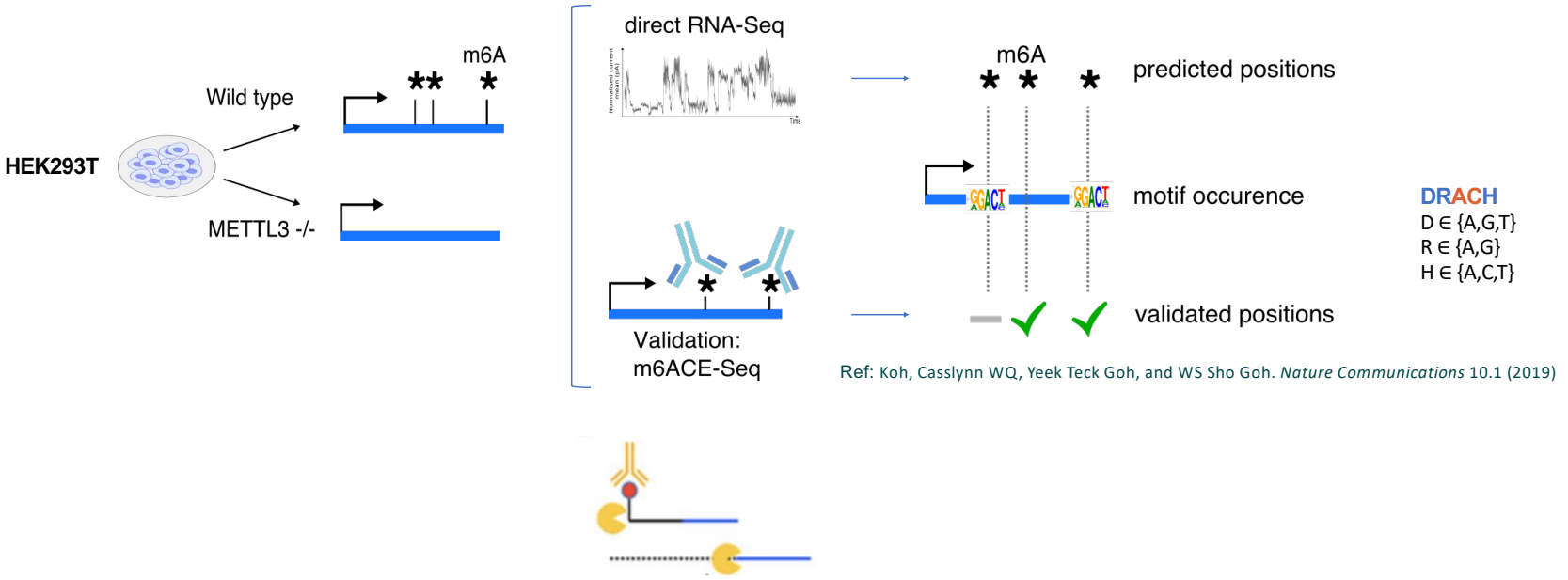






- **Experiment setup**
- **Validation**
- **Applicability**
- **Discovery**

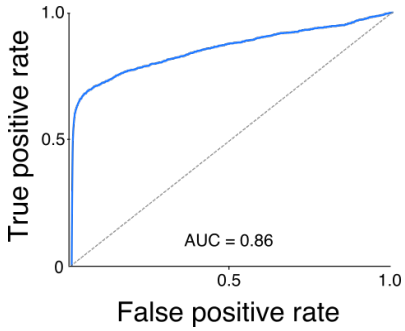
Experiment Setup



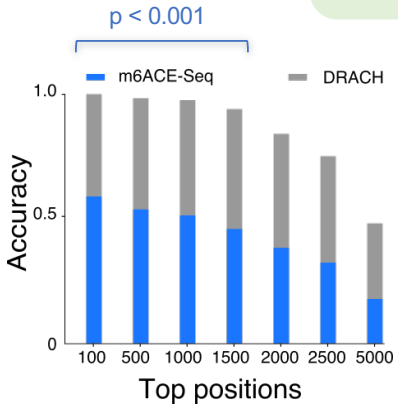
Ref: Koh, Casslynn WQ, Yeek Teck Goh, and WS Sho Goh. *Nature Communications* 10.1 (2019)

Validation: m6A calling

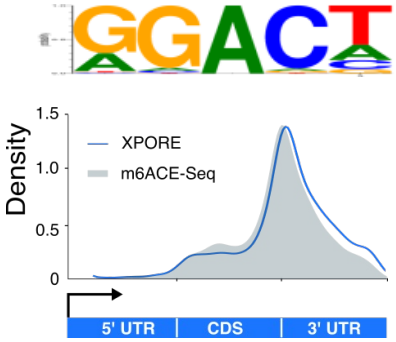
In bioinformatics,
- as the labels are incomplete,
- the false positives may not be wrong.
- Analysis on the predictions is usually required to get more insights.



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

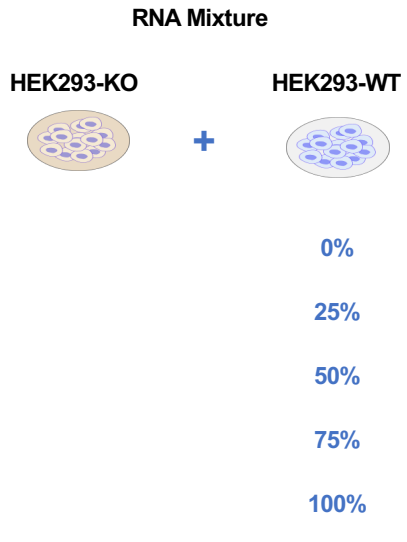


- Around half were identified by m6ACE-Seq.
- With m6ACE-Seq + DRACH, the accuracy is up to >95%.
- dRNA-Seq helps identify a different set of modified sites that had been otherwise missed.

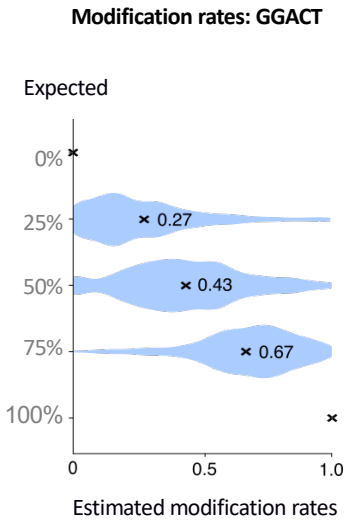


- m6A motifs e.g. GGACT, AGACT are confirmed.
- xPore can facilitate motif discovery in any other pairwise comparison.
- The differentially modified sites are also enriched at stop codons.

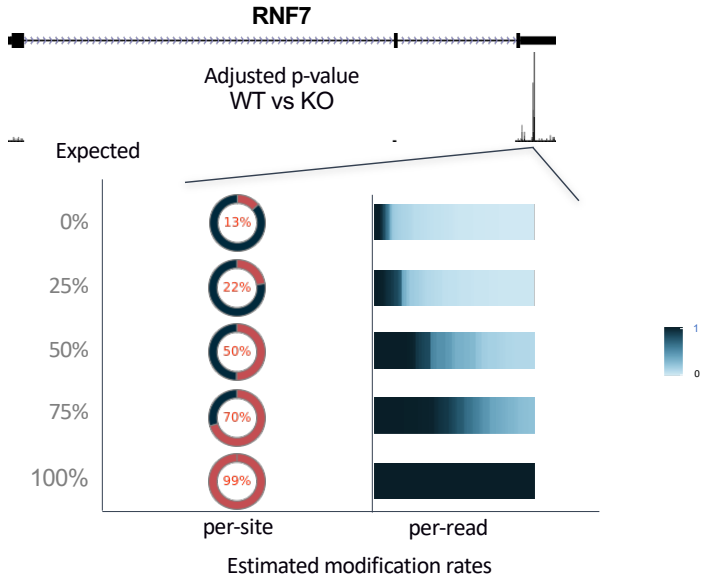
Validation: m6A stoichiometry quantification



- xPore models all RNA mixture samples at once.



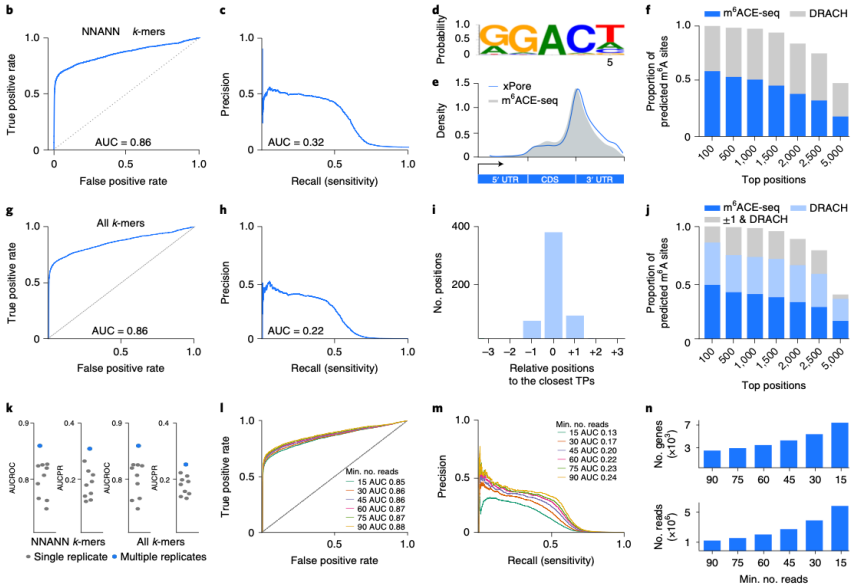
- Estimated modification rates closely match to the expected.



- 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

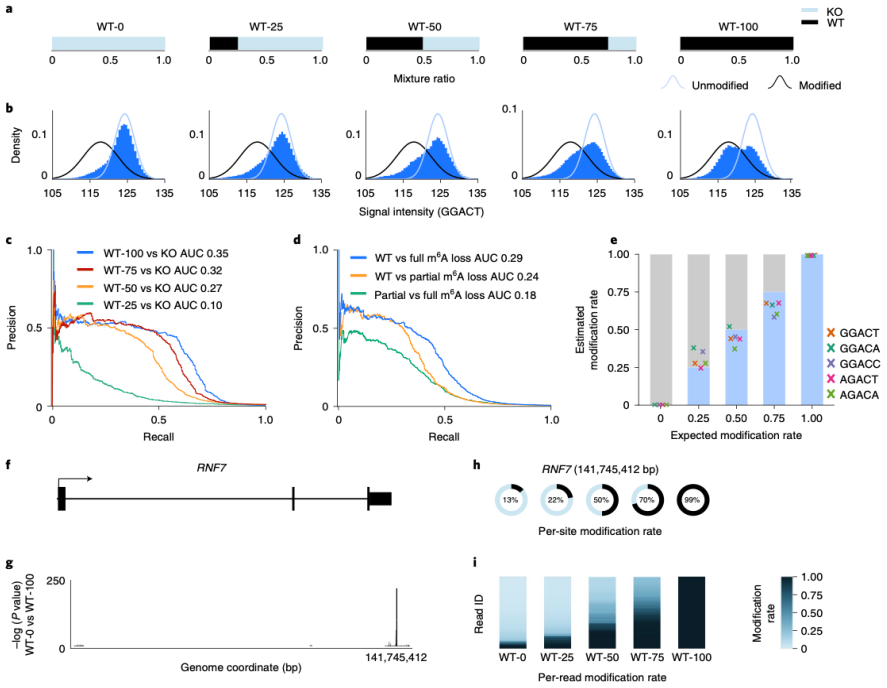
- ROC Curve
- Precision-Recall Curve
- Accuracy

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

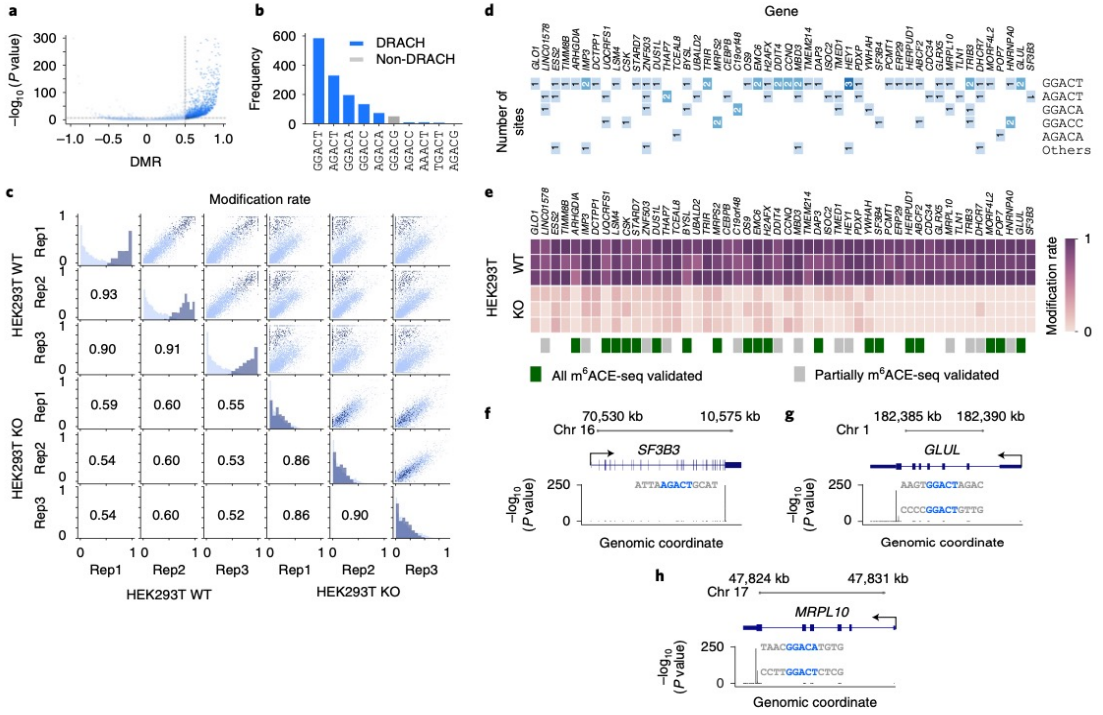
- ROC Curve
- Precision-Recall Curve
- Accuracy

Analysis

- Domain-specific evaluation
- Effects of the data size

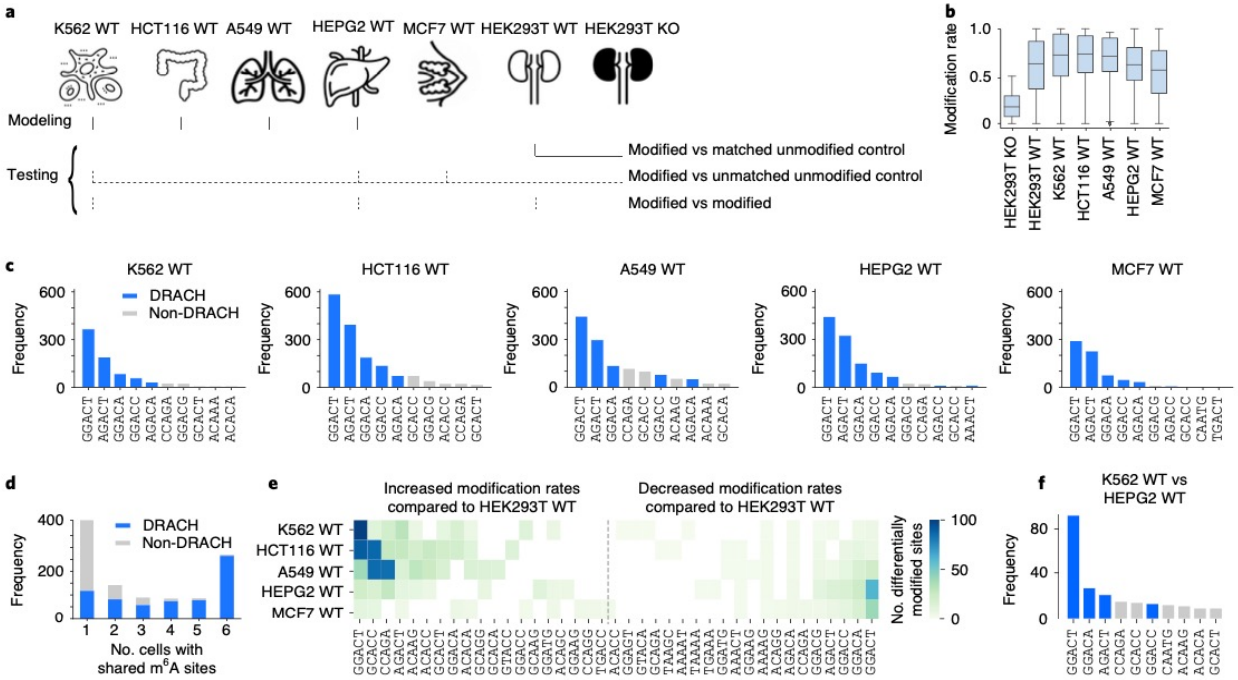
Applicability: Full Dataset

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



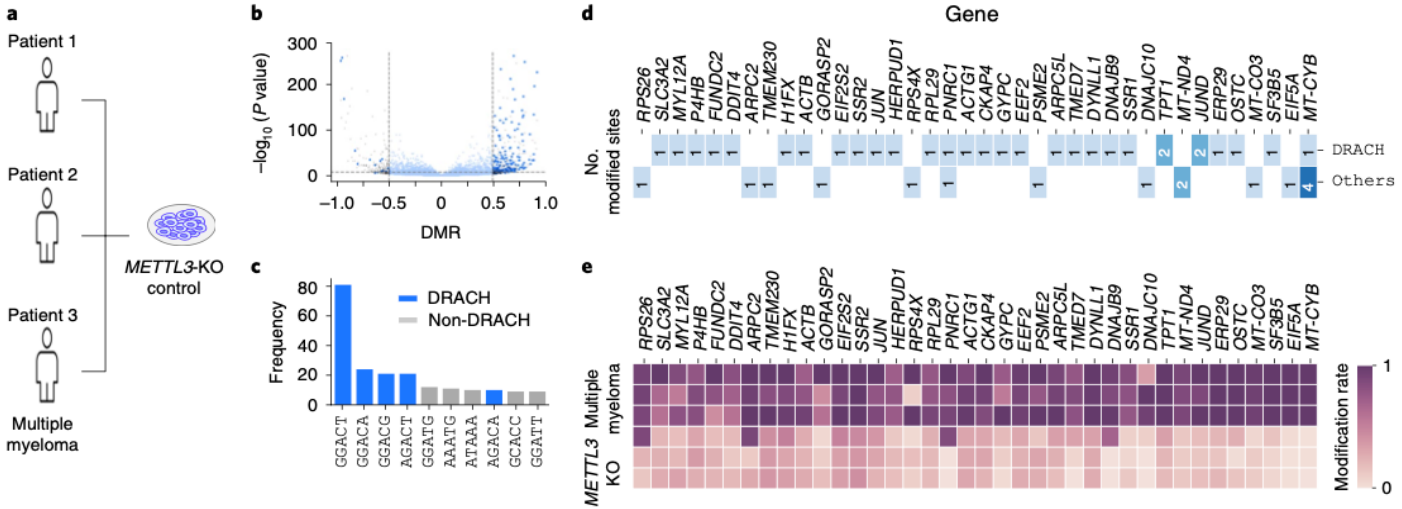
Applicability: Other Datasets

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



Applicability: Clinical Data

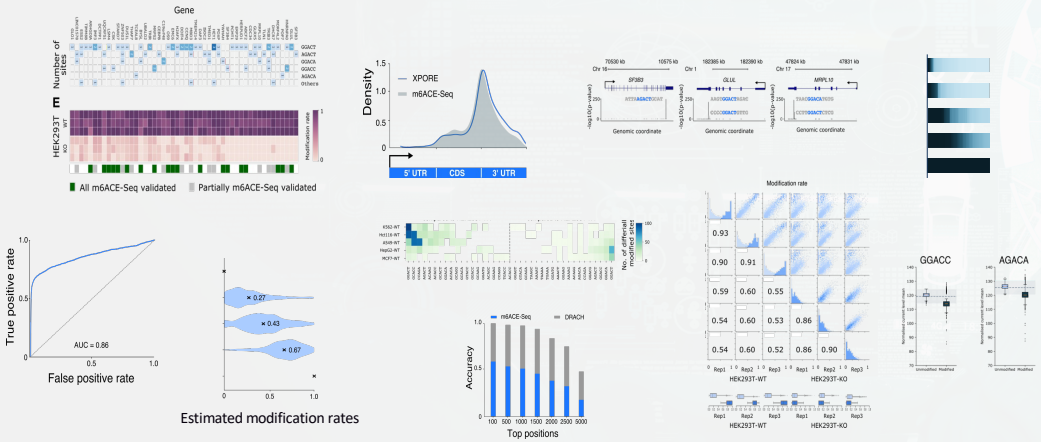
Fig. 6 | Identification of m6A in clinical samples using direct RNA-seq.



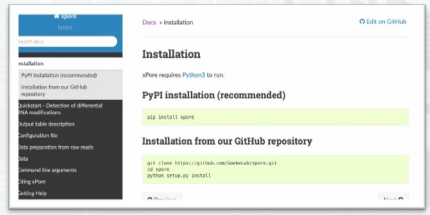
Evaluation: Keys Takeaway

- 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

- xPore: identification of differential RNA modifications.
- xPore identifies m6A sites at single-base resolution.

Validation

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

- 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.

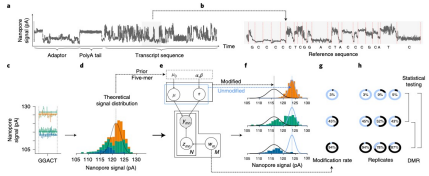
- Identification of m6A across genetically diverse cell lines.
- Variation of m6A across different cell lines.

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



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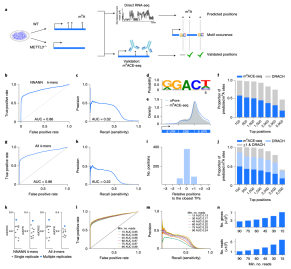

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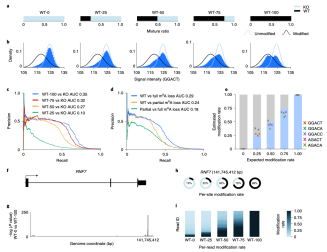

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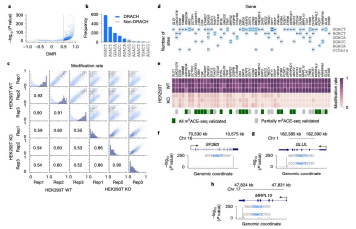

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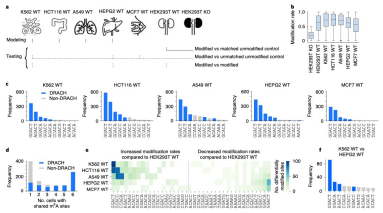

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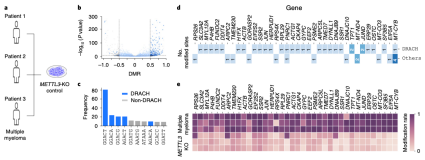


โครงการวิจัยโมเดลระบบนิเวศการเรียนรู้ที่บูรณาการ CODING & AI สำหรับเยาวชน
MODEL OF LEARNING ECOSYSTEM PLATFORM INTEGRATE WITH CODING & AI FOR YOUTH

Choosing the Right Plots

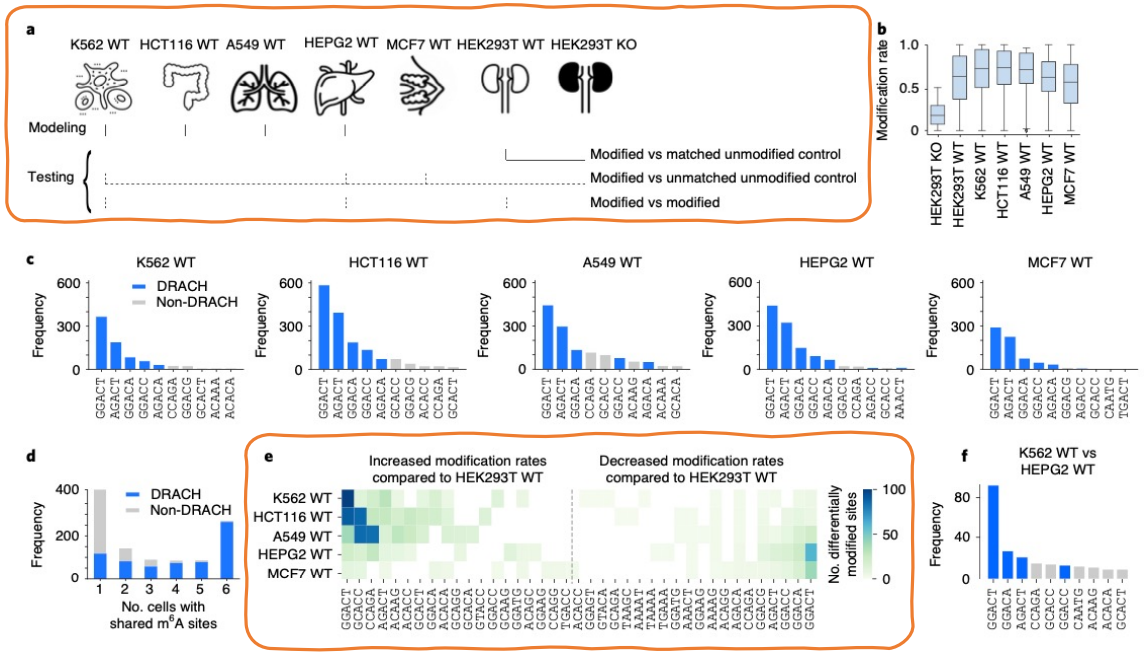
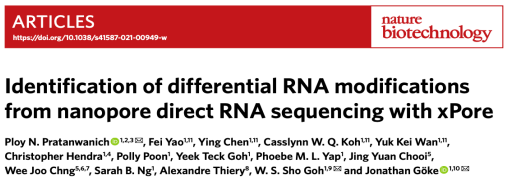


Fig. 5 | Identification of m6A sites across different tissues and cell lines.

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 ✓

https://github.com/GoekeLab/xpore



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

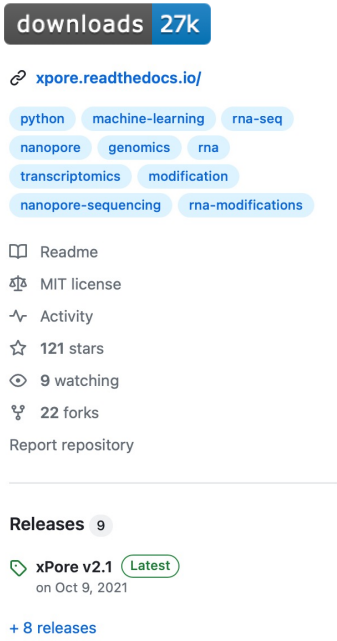
Identification of differential RNA modifications from nanopore direct RNA sequencing with xPore

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✉]

Scopus metrics

78 99th percentile
Citations in Scopus

9.61
Field-Weighted citation impact



downloads 27k

xpore.readthedocs.io/

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

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121 stars
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22 forks
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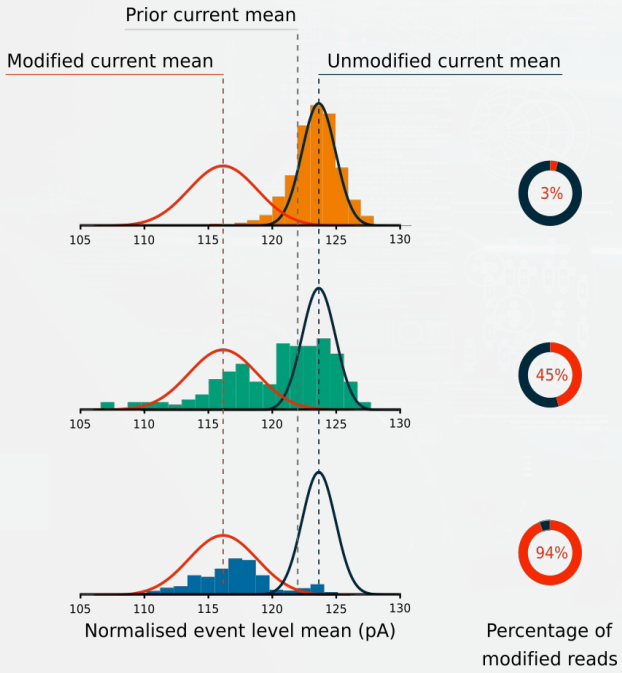
Releases 9

xPore v2.1 Latest
on Oct 9, 2021

+ 8 releases

6. Future Work



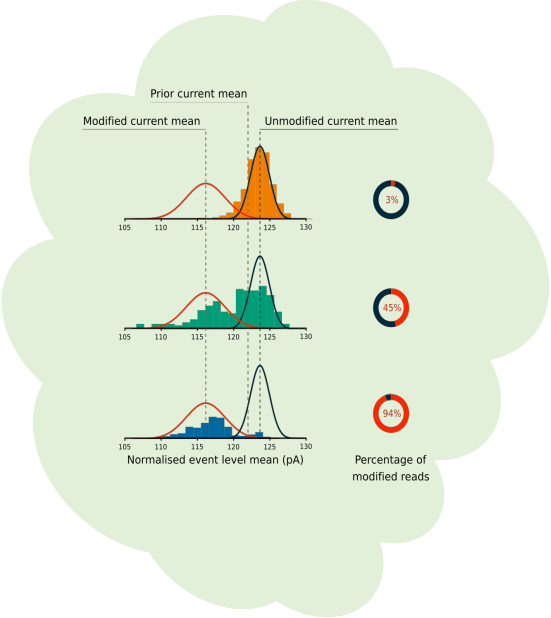
- **Identifying the Limitations**
- **Considering Changes in the Future**

Future Work

Domain-Oriented

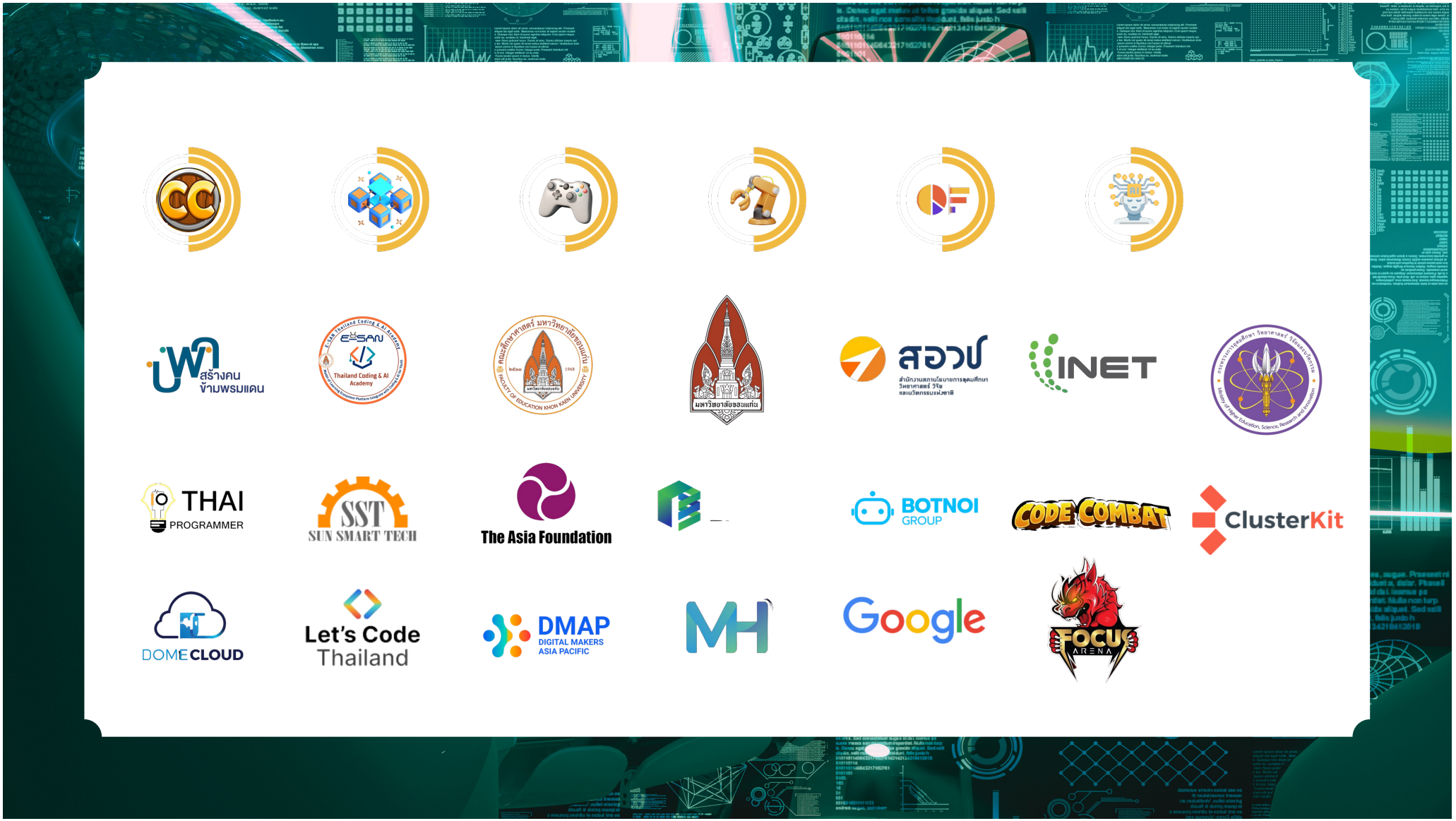
- m6anet nature methods
- **<Gaussian>** mixture model
- Interpretability
  - Modification or basecalled errors
- End-to-end
  - Why?

  → Nanopolish eventalign / Guppy basecaller are subjet to change



Method-Oriented

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