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Computational Approaches to RNA Structure and Function
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mRNA design with deep generative models

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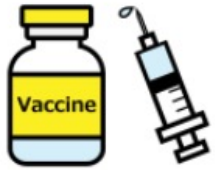
mRNA sequence design

- **Applications of mRNA are attracting attention.**
  - Great expectations for medical applications due to the success of mRNA vaccines for COVID-19
  - Production of useful biomaterials by bacteria
    - New functions unique to biotechnology, reduction of environmental stress
    - Control and optimization of mRNA transcription and translation for metabolic pathway optimization



Biopharmaceuticals

Bioplastics

Biofuels

Alternative Proteins

High-performance fibers

Cosmetic materials

mRNA sequence design

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  - Great expectations for medical applications due to the success of mRNA vaccines for COVID-19
  - Production of useful biomaterials by bacteria
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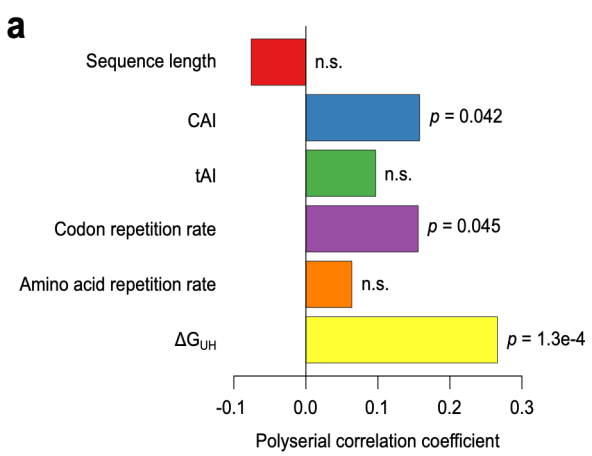
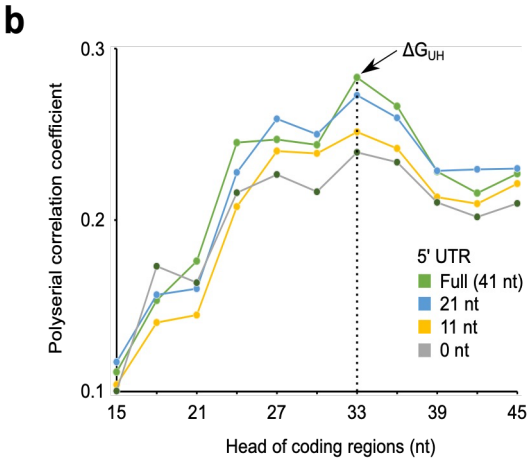
- **mRNA transcription, translation efficiency, and stability** are affected by codon adaptation as well as primary sequences and secondary structures of promoters, UTRs, terminators, etc.

- Existing mRNA sequence design techniques based on codon optimization are insufficiently optimized.
  - Not fully utilizing the high degree of design flexibility





[Saito et al., 2019]

mRNA sequence design with deep generative models

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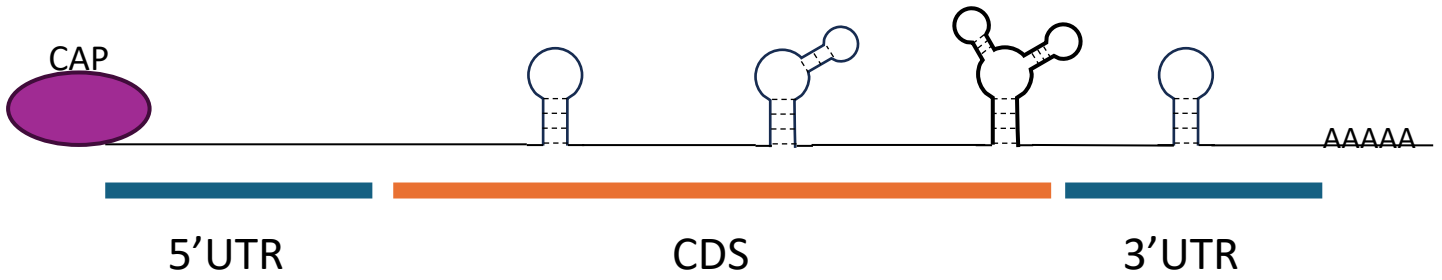
- We reasonably design highly efficient and high-performance mRNA using deep generative models such as **diffusion models**.



- Learning mRNA features from large-scale data enables designing sequences that maximize protein production with:
  - Weak secondary structures around the start codons.
  - High stability in the CDS and 3′ UTR regions.
  - An appropriate translation speed in the host.

Maximize the production of the target protein

Diffusion models

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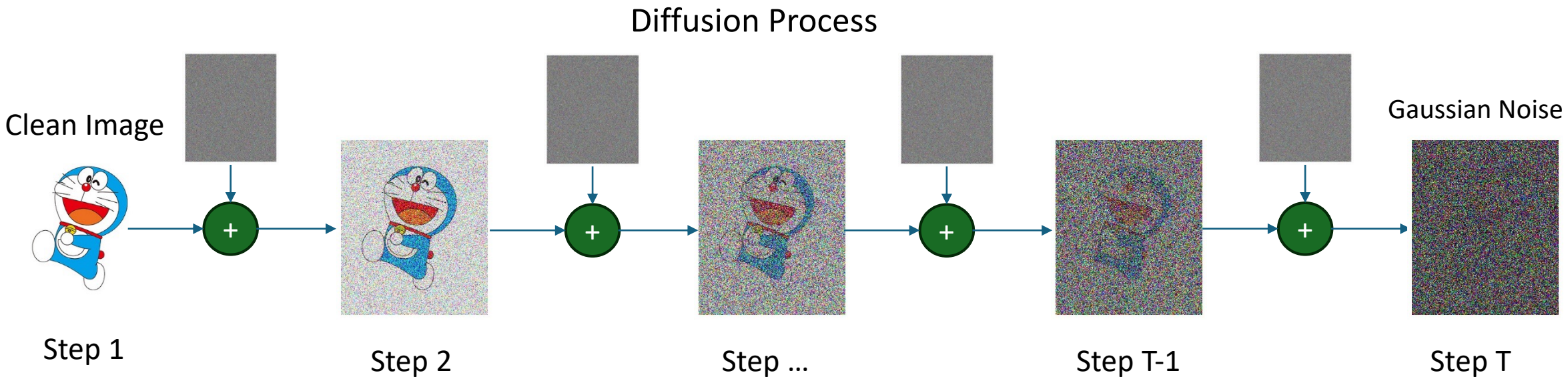
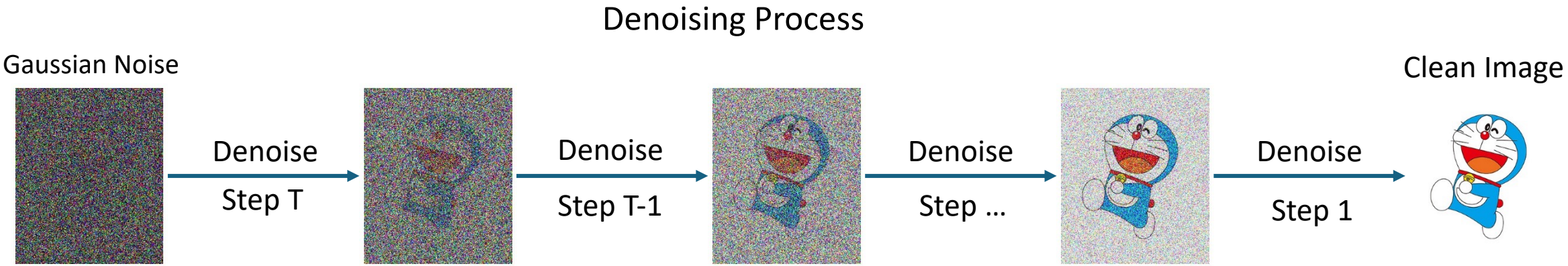
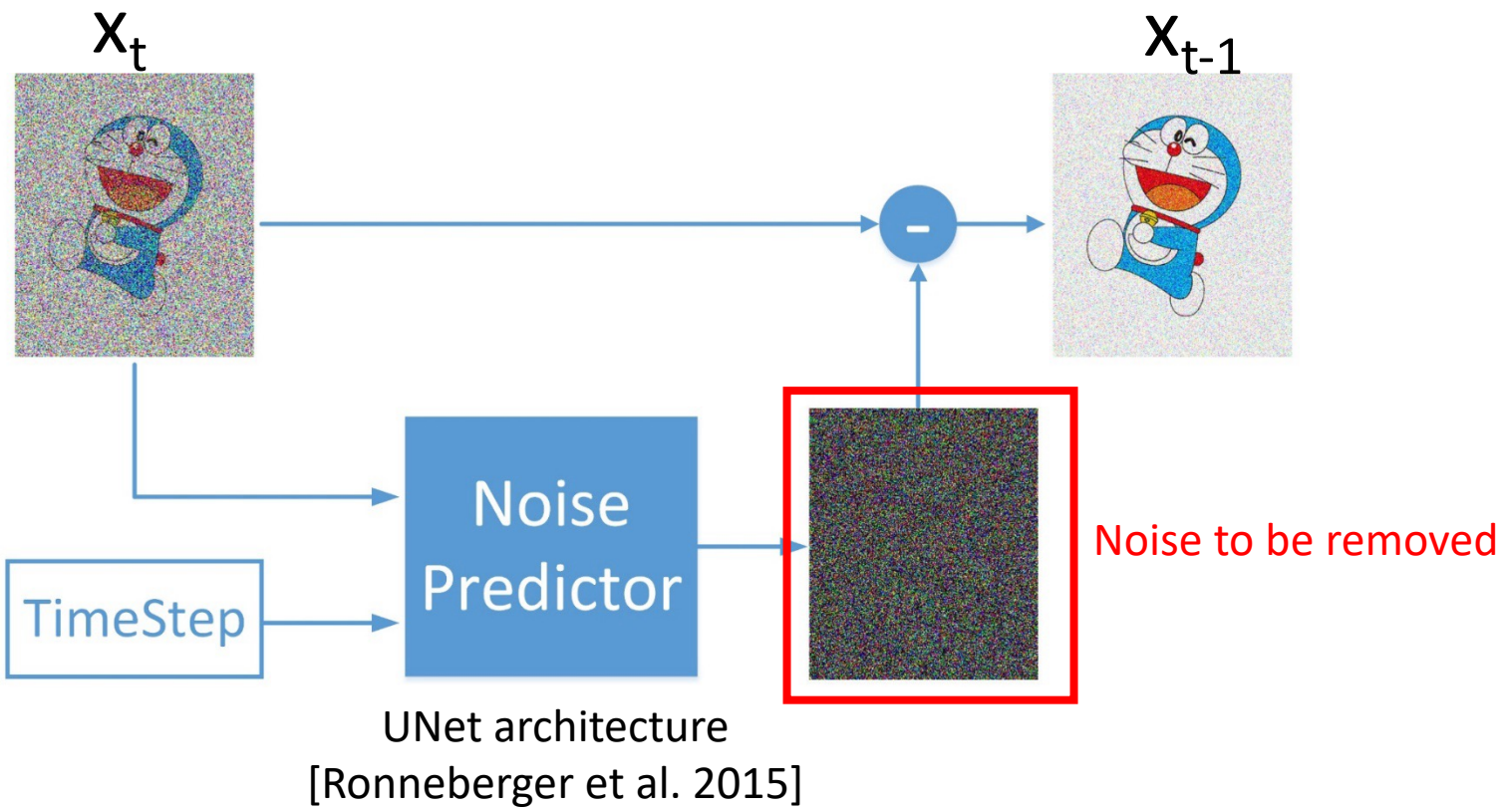




Diffusion models

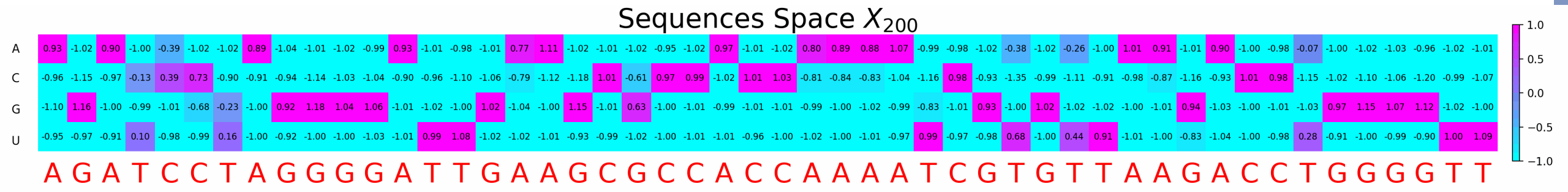
- The deep neural network predicts the noise to be removed at each time step.



UNet architecture
[Ronneberger et al. 2015]

Diffusion models

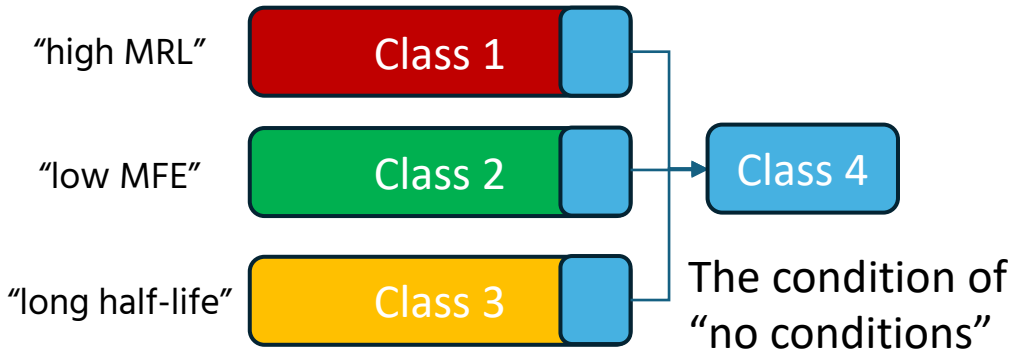
- Apply to RNA sequences



Guiding the model toward the target condition

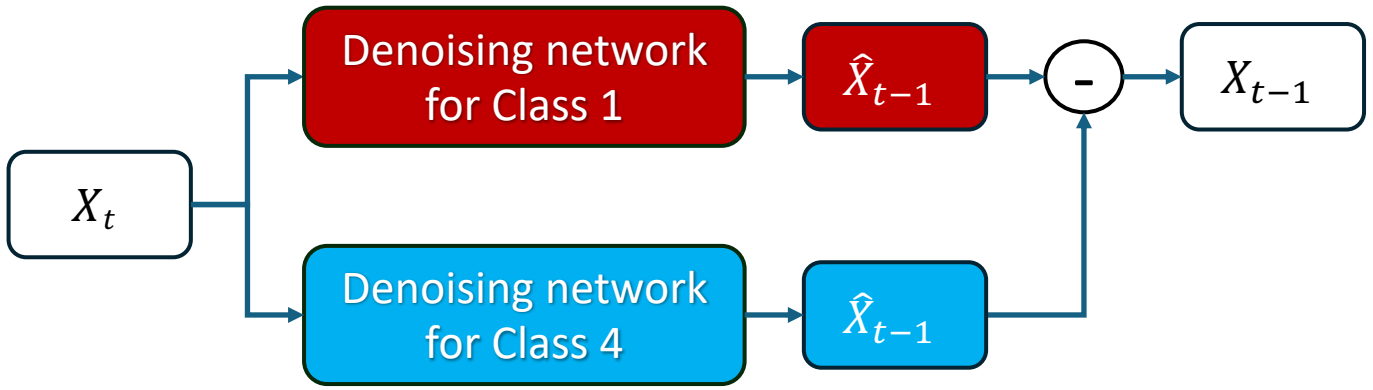
- **Classifier-free guidance** [1] generates RNA sequences that match target conditions such as “high expression”, “low energy”, or “long stability”.

Training



Train the denoising networks for each class

Conditional generation



Move predictions **toward the target condition**, and **away from the unconditional direction**

[1] Nichol et al. "Improved denoising diffusion probabilistic models." *International conference on machine learning*. PMLR, 2021.

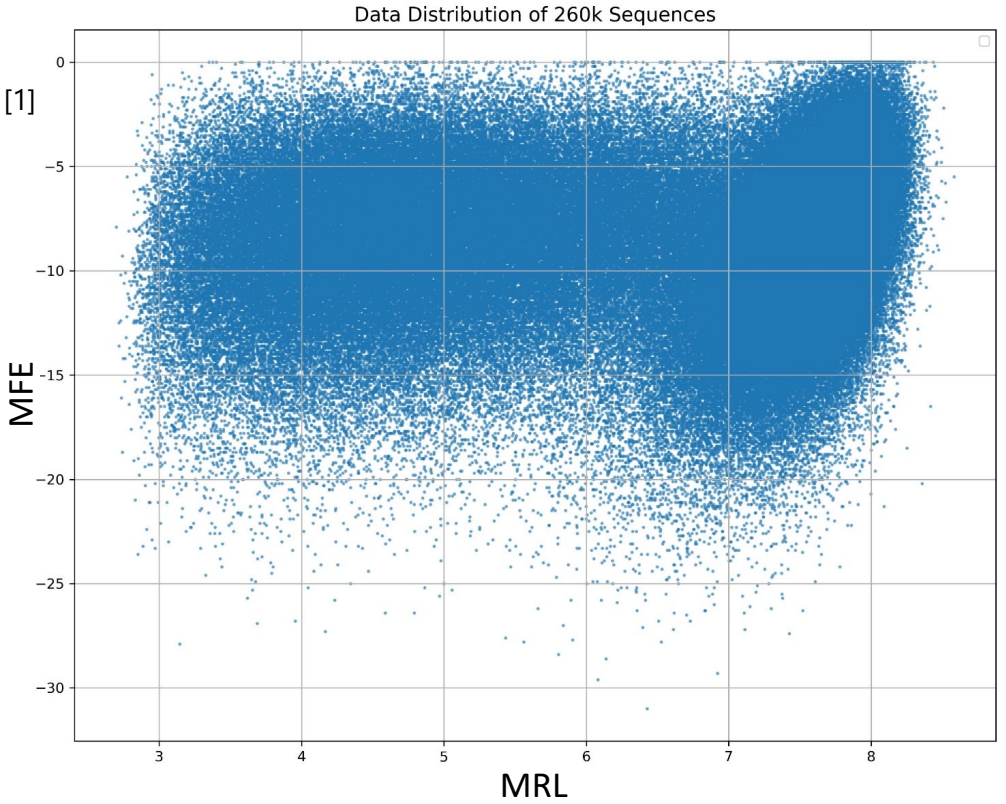
Controlling RNA sequence generation by two target conditions

- We trained a diffusion model to generate 5′ UTR RNA sequences conditioned on two target conditions, **translation efficiency (Mean Ribosome Load; MRL)** and **minimum free energy (MFE)**, and evaluated whether the generated sequences reflect the specified target conditions.

- Dataset:
  - Randomly-generated 260k eGFP 5′ UTR sequences (GSM3130435) [1]
  - Length: 50bp
  - MRL predicted by UTR-LM [2]
  - MFE predicted by RNAfold [3]
- Labels: combinations of two conditions (3x3=9 groups)

Label	MRL
low	4
medium	6
high	8

Label	MFE
low	-20
medium	-10
high	-2



[1] Sample, P. J. *et al.* Human 5′ UTR design and variant effect prediction from a massively parallel translation assay. *Nat. Biotechnol.* **37**, 803–809 (2019).
[2] Chu, Y. *et al.* A 5′ UTR language model for decoding untranslated regions of mRNA and function predictions. *Nat. Mach. Intell.* **6**, 449–460 (2024).
[3] Lorenz, R. *et al.* "ViennaRNA Package 2.0." *Algorithms Mol. Biol.* **6**, 26 (2011).

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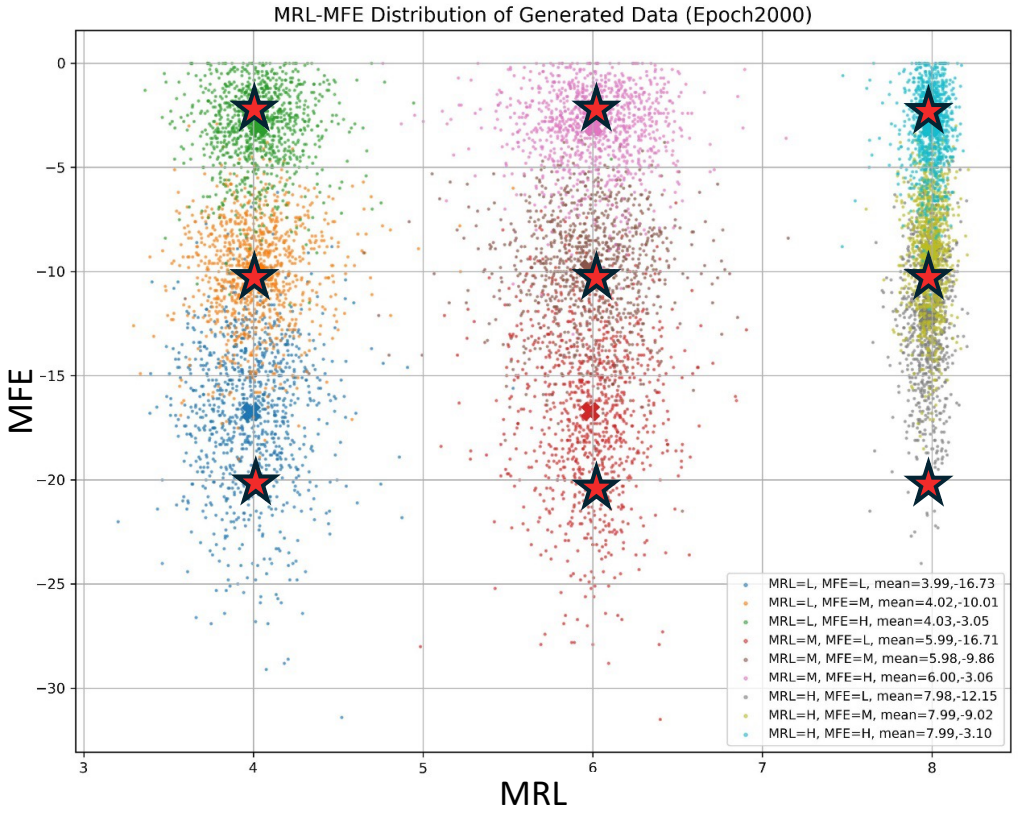
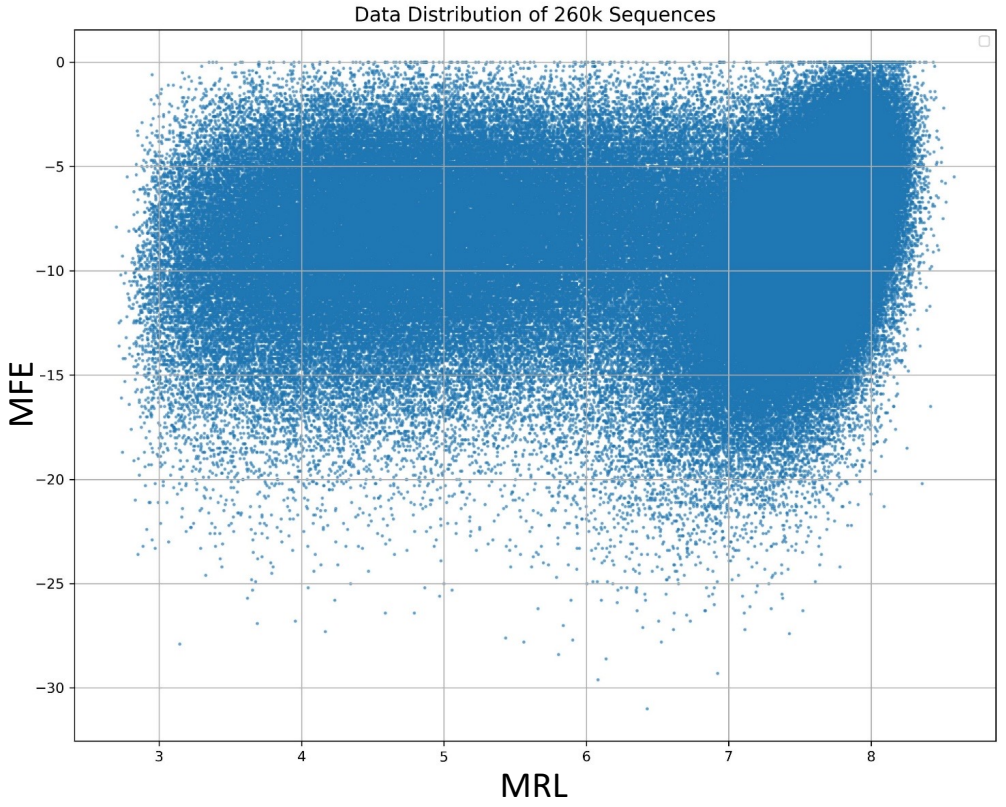
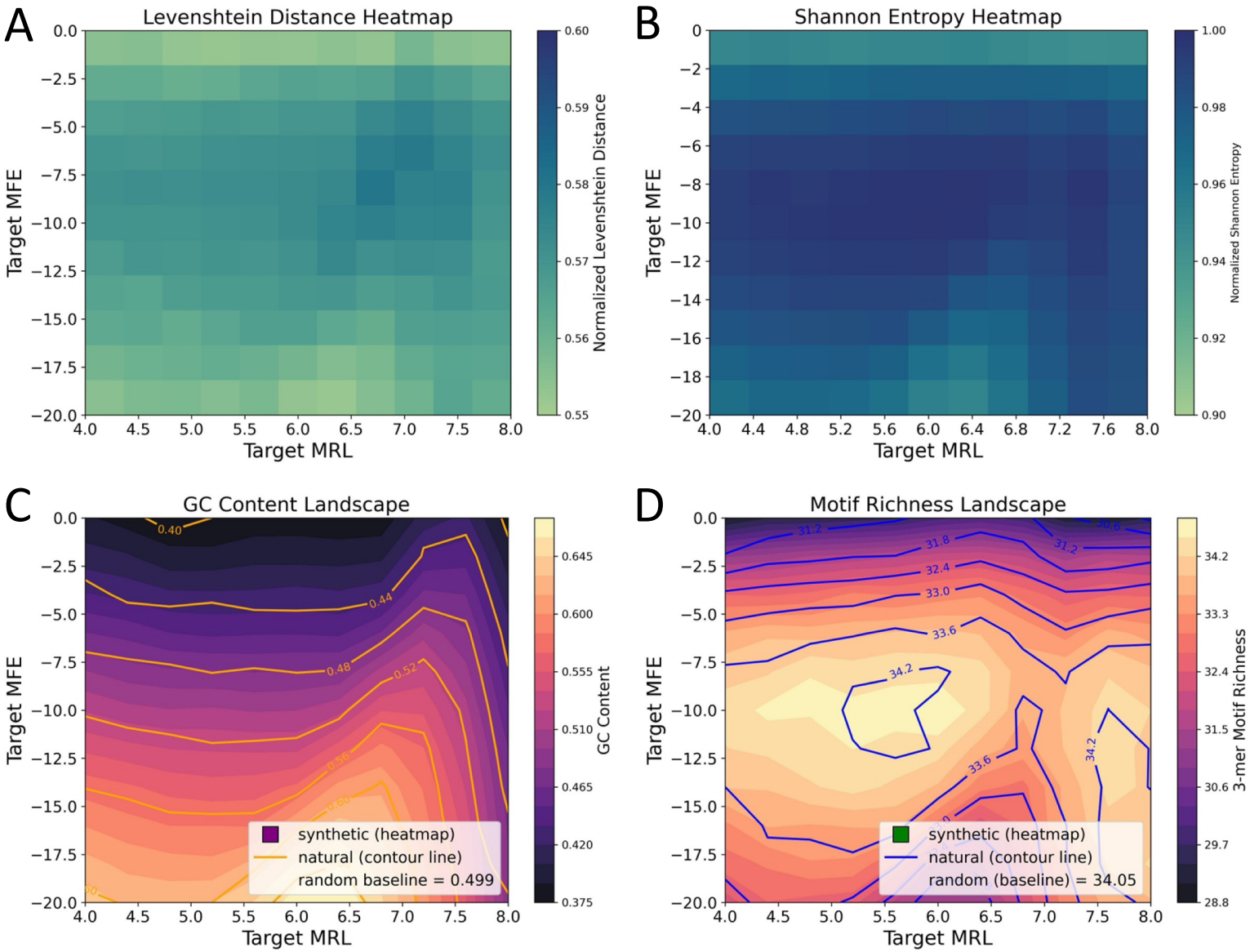


Distribution of generated data (1k samples)



Distribution of training data

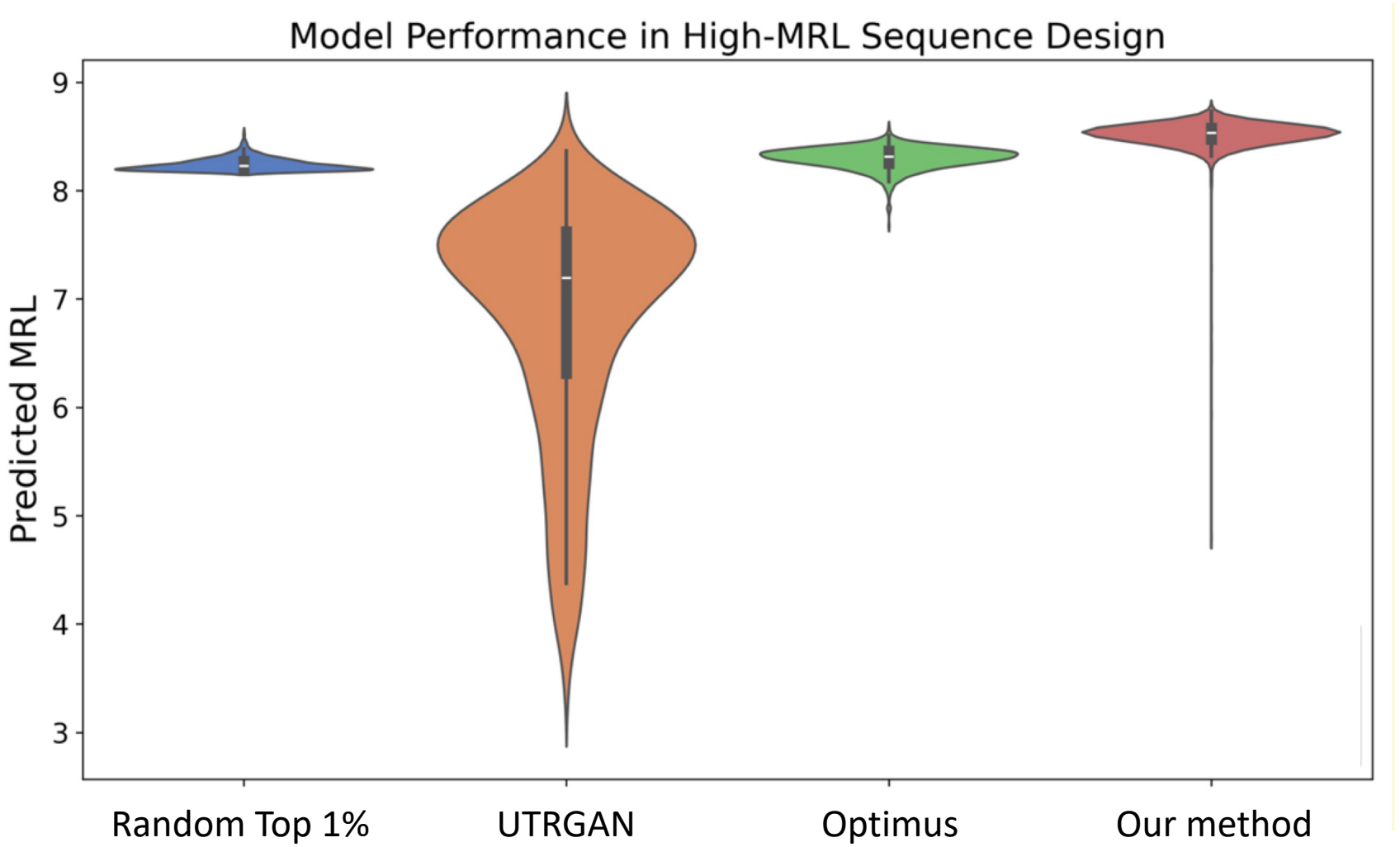
Quality of generated sequences



- Panel A: The generated sequences are not mere copies of the training data.
- Panel B: They are highly diverse.
- Panels C and D: Their distributions closely resemble the training data.

Our method outperforms other methods

- We compare our method with UTRGAN [Barazandeh et al. 2025] and Optimus 5-Prime [Sample et al. 2019].



Localized conditional generation via Repaint technique[1]

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Masked input | Denoising process | Completed images

[1]: Lugmayr, Andreas, et al. "Repaint: Inpainting using denoising diffusion probabilistic models." *Proceedings of the IEEE/CVF conference on computer vision and pattern recognition*. 2022.

RNA design with fixed nucleotides (Repaint extension)

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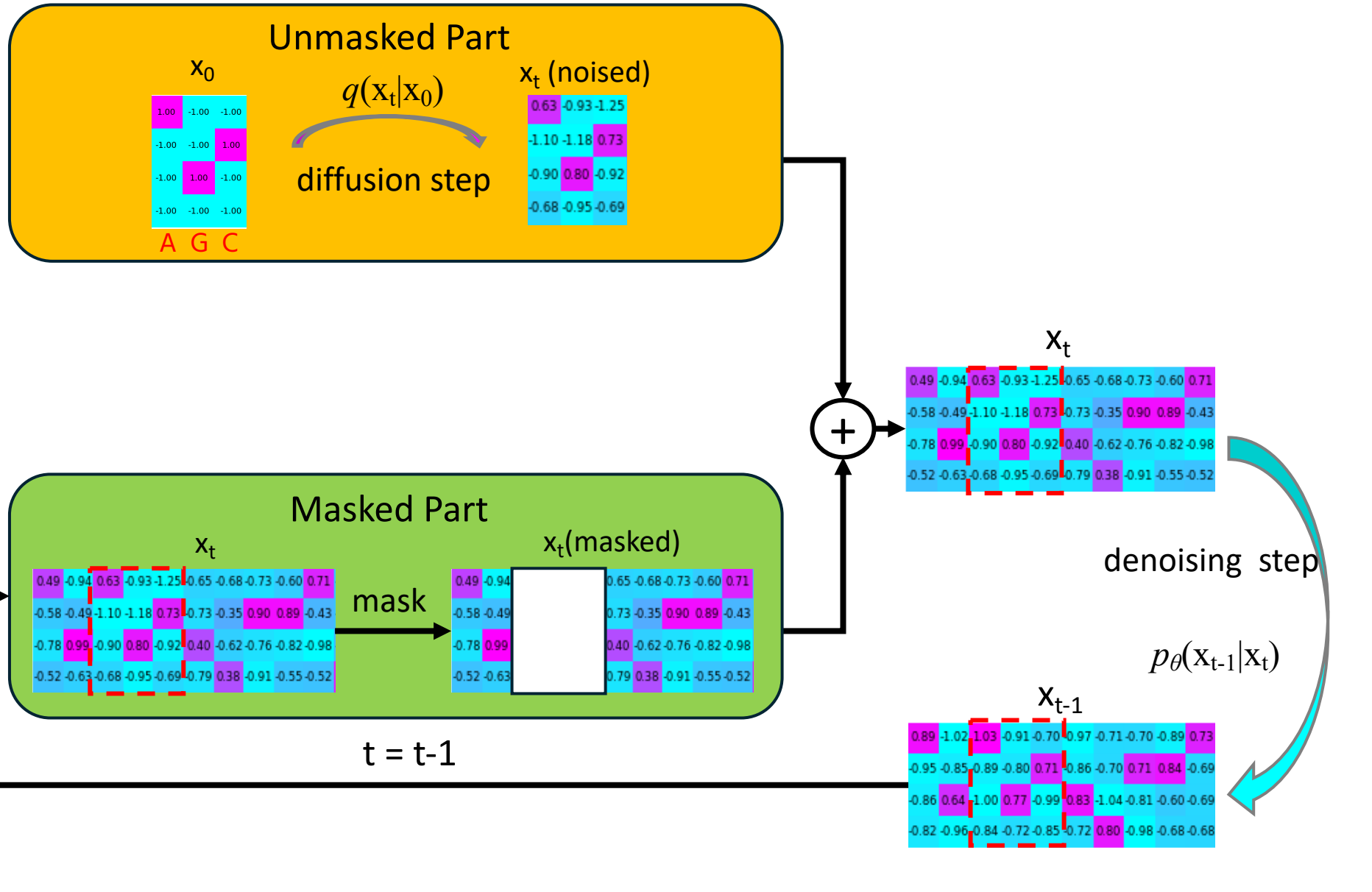
Unmasked part
fixed nucleotides
(given condition)

Masked part
are generated from
Gaussian noise



RNA design with fixed amino acids (Repaint extension)

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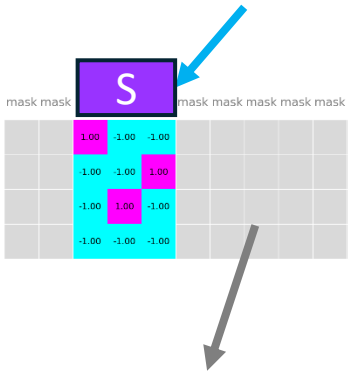
Unmasked part
fixed amino acids
(given condition)



Masked part
are generated from
Gaussian noise

Unmasked Part

x_0

$q(x_t|x_0)$

diffusion step

x_t (noised)

S

UCU
UCC
UCA
UCG
AGU
AGC

select the most probable codon

Masked Part

x_t

mask

x_t(masked)

x_t

denoising step

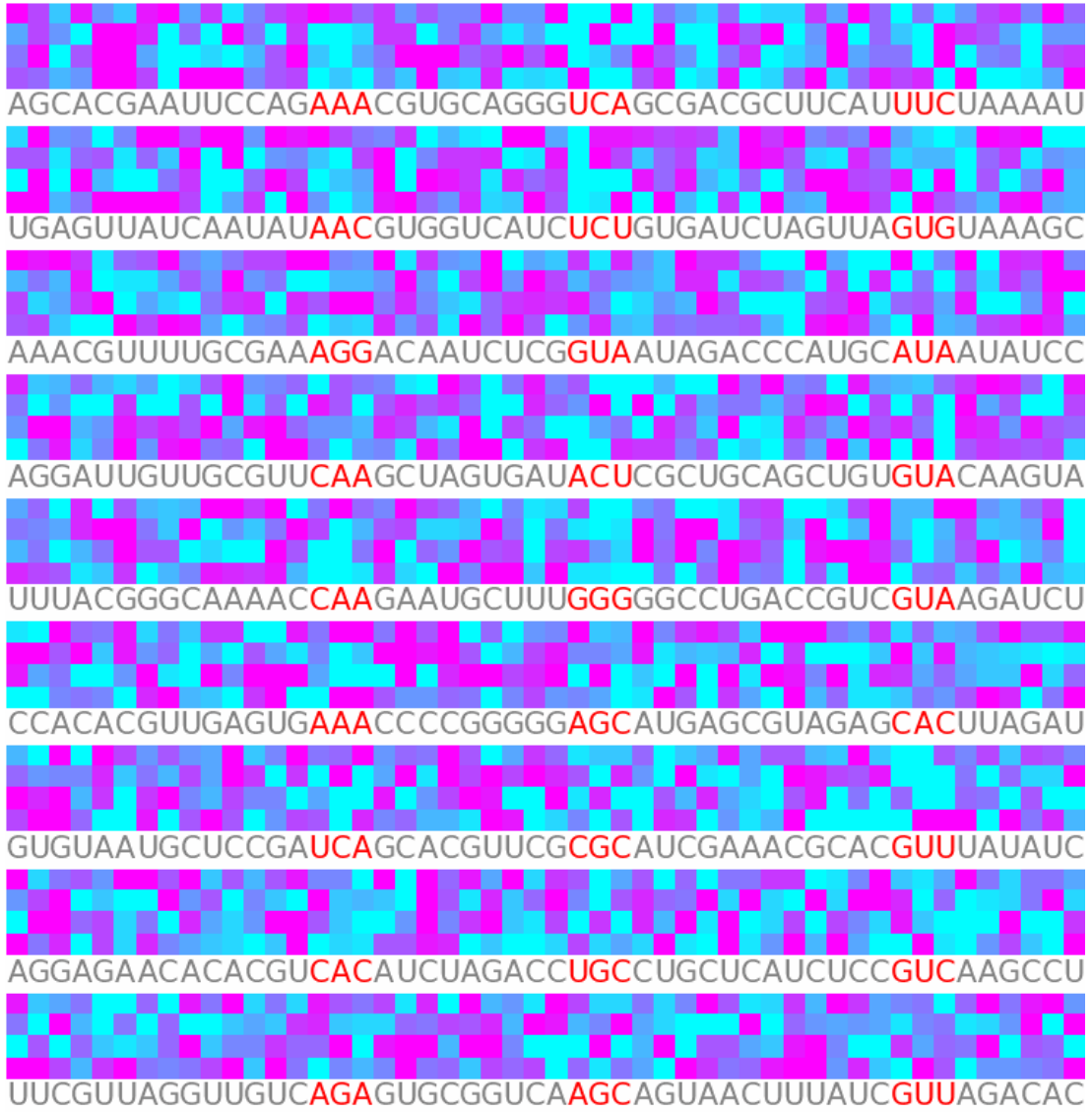
$p_\theta(x_{t-1}|x_t)$

x_{t-1}

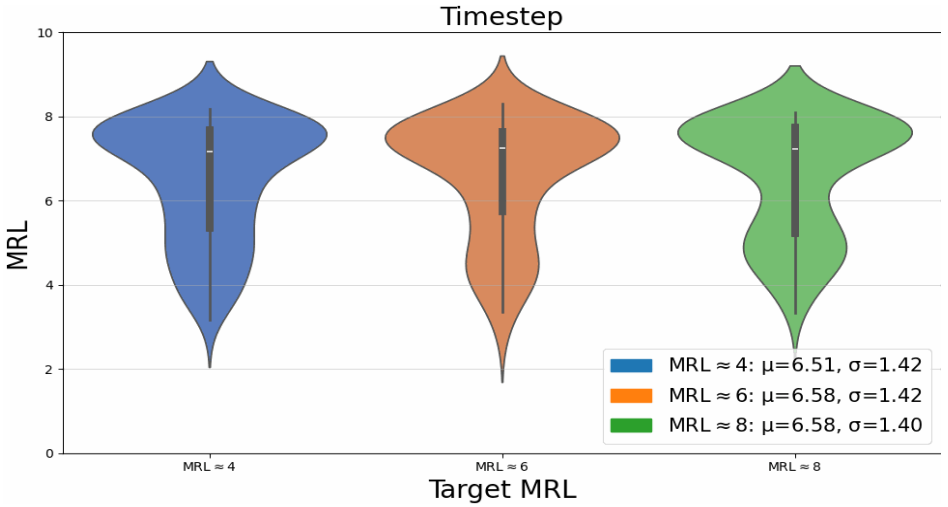
t = t-1

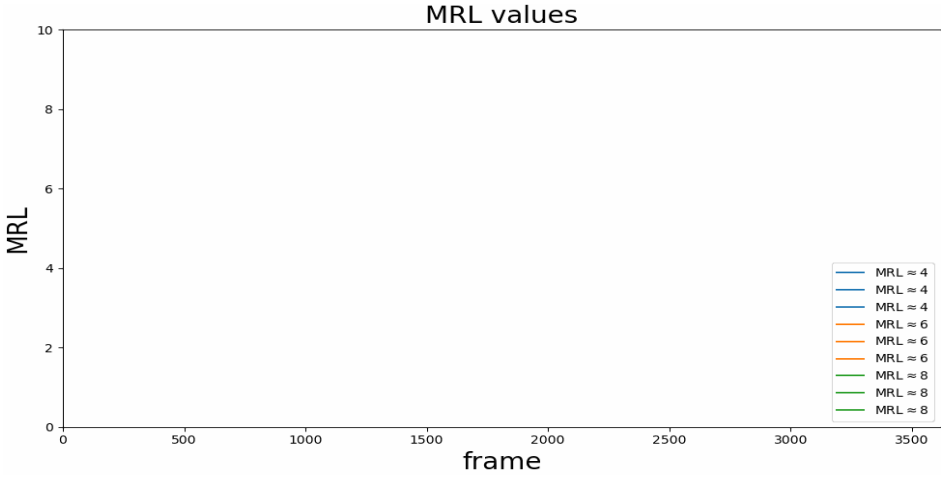
Conditional RNA generation with fixed amino acids (by target MRL)

- Target MRL: 4, 6, 8



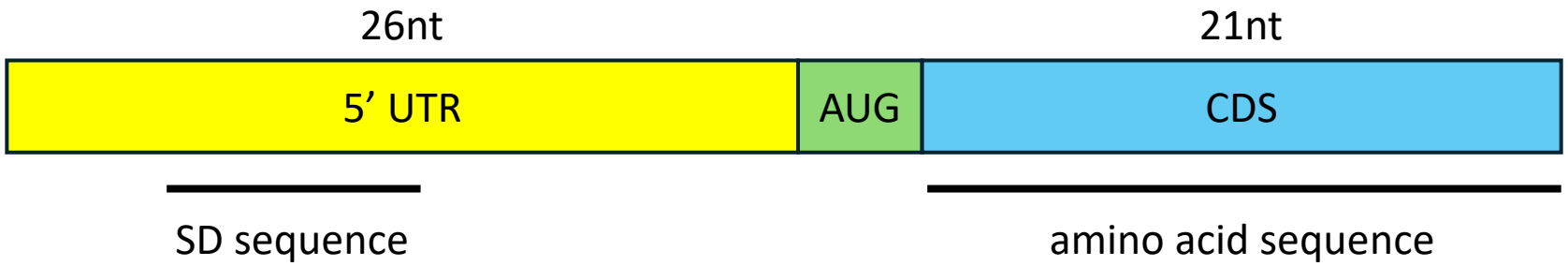

```
'K': ['AAA', 'AAG']
'S': ['UCU', 'UCC', 'UCA', 'UCG', 'AGU', 'AGC']
'V': ['GUU', 'GUC', 'GUA', 'GUG']
```





UTR-Diffusion: Designing mRNAs near 5′ UTR

- Enables the design of mRNA sequences near the 5′ UTR with high translational efficiency (high MRL and MFE)
- Designs can also specify the SD sequence and the amino acid sequence around the start codon



- An integrated pipeline with LinearDesign [Zhang et al., 2023] has been established for full-length mRNA design

UTR-Diffusion: Designing the NanoLuc 5′ UTR–CDS Junction

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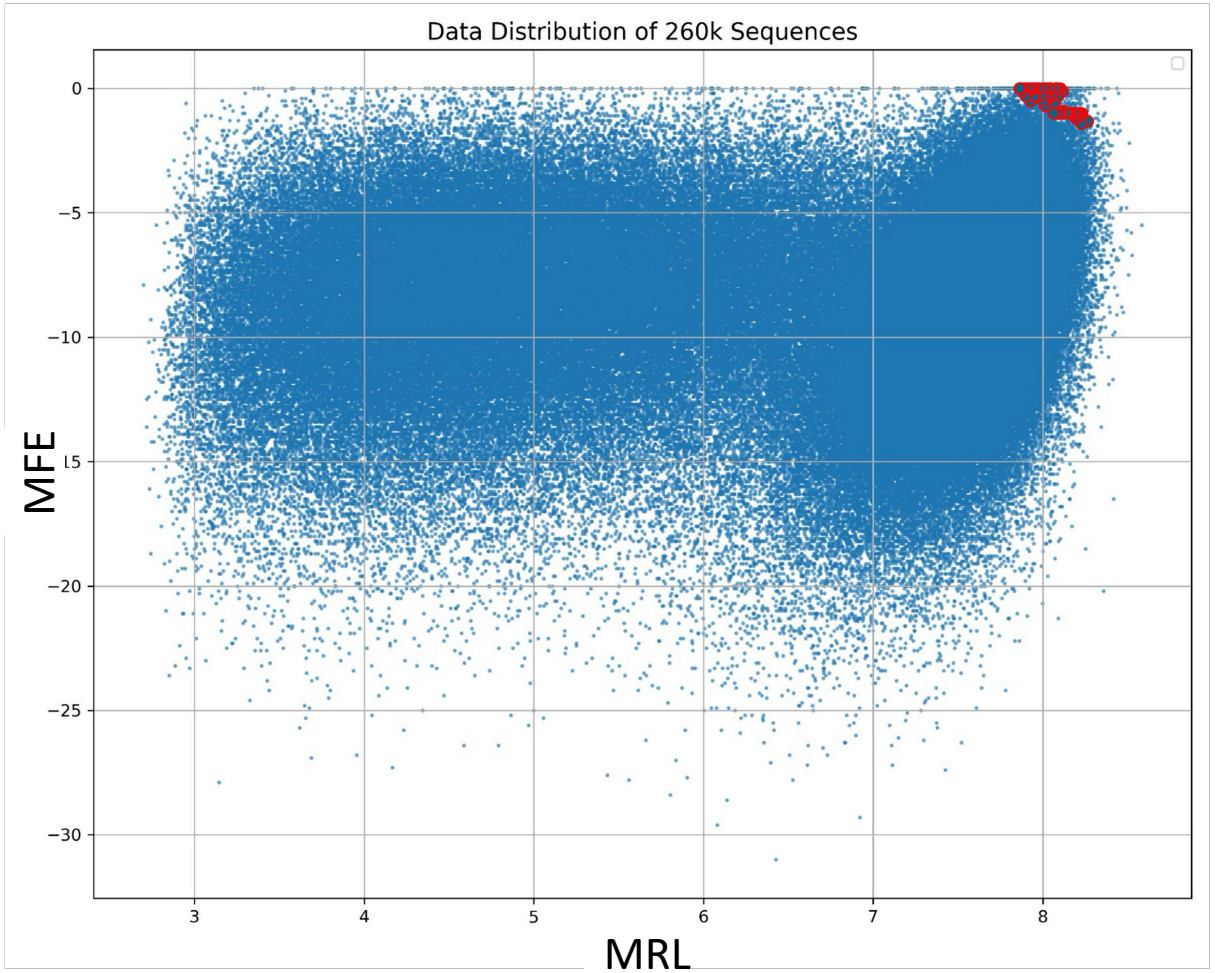
- We jointly designed the NanoLuc 5′ UTR and the first eight CDS codons (encoding MVFTLEDF), targeting high MRL and high MFE.



Current limitations and next steps

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- **Fixed-length generation**
  - 50-nt windows do not directly scale to full-length mRNA.
  - Next: use of transformer-based discrete diffusion models and integration with CDS-design tools.
- **Data and domain dependence**
  - Control relies on a large HEK293-derived, predictor-labeled dataset.
  - Next: broader datasets and more data-efficient guidance.
- **Experimental validation**
  - MRL and MFE are evaluated in silico.
  - Next: collaborative wet-lab validation and closed-loop refinement.

Summary

- **Diffusion models make mRNA design controllable.**
- **Goal-directed design:** Diffusion models can generate mRNA sequences for specific design goals.
- **Conditional generation:** The model can control predicted MRL and MFE together.
- **Biological constraints:** Repaint keeps required nucleotides, SD sequences, or amino acids while redesigning the other positions.
- **Computational proof of concept:** NanoLuc candidates show high predicted MRL and MFE while preserving the required amino-acid sequence.

- **Next step:** Test selected generated sequences in wet-lab experiments.

Acknowledgements

- Sato Lab (Institute of Science Tokyo)
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  - Dr. Bian Bian
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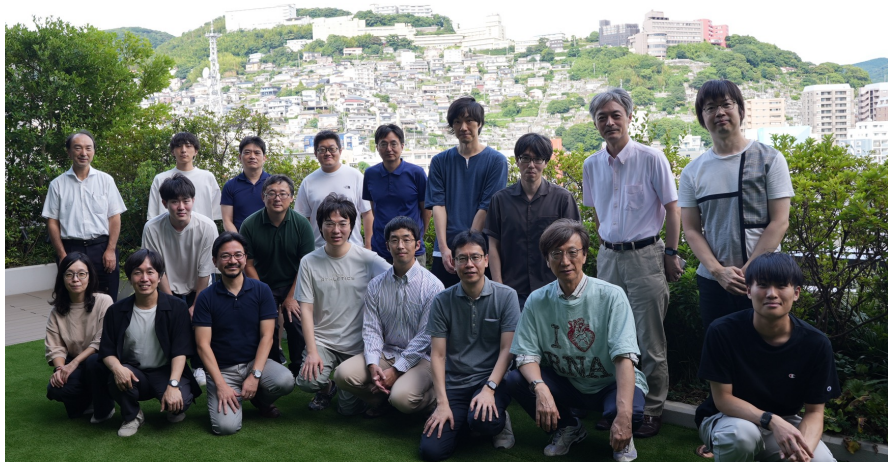
  - Prof. Hiroshi Abe
  - Dr. Fumitaka Hashiya

Sato Lab Party
(2026 Feb)



RNA Dojo
(2025 Aug)