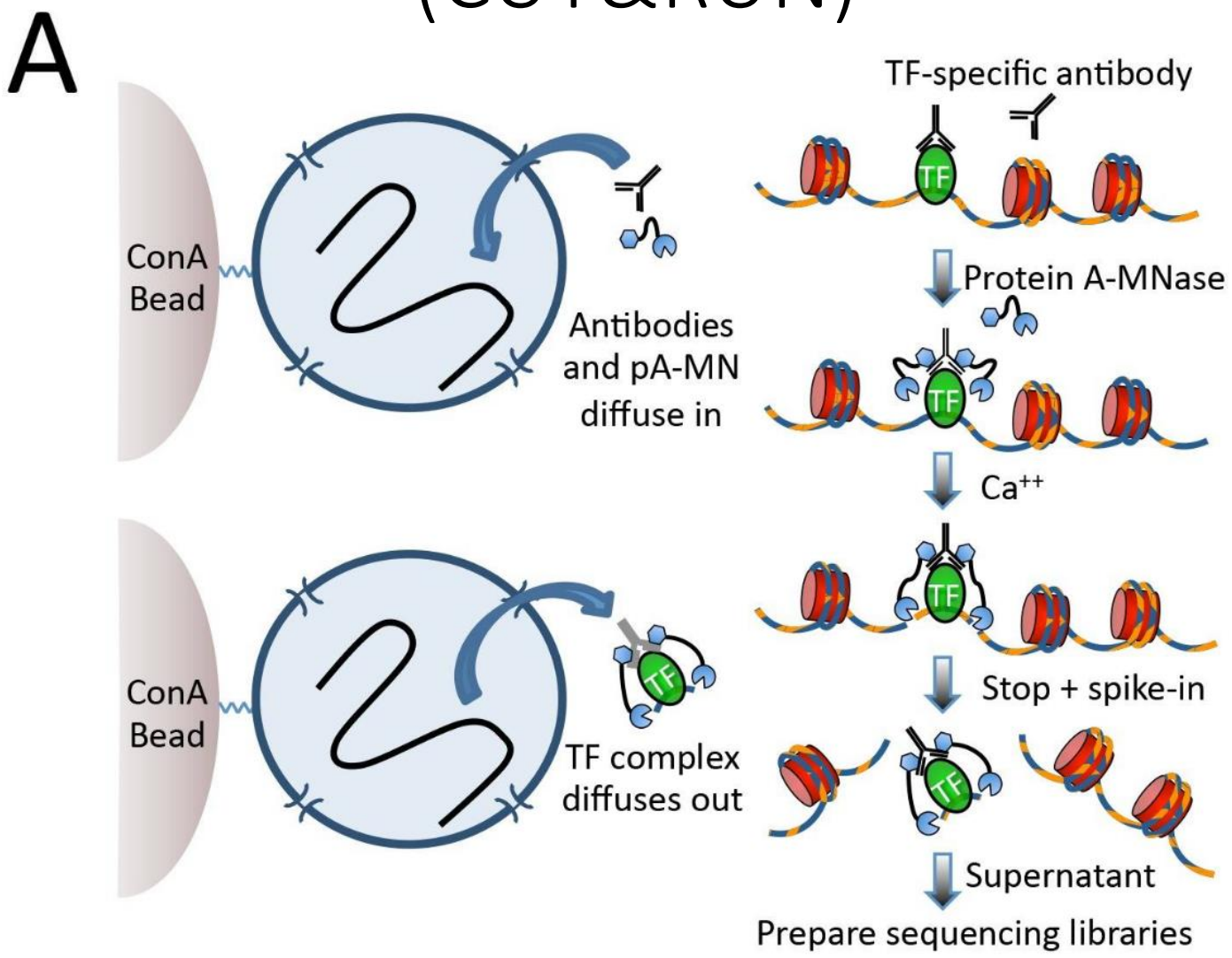
CUT&RUN

The stronger version of ChIP-seq

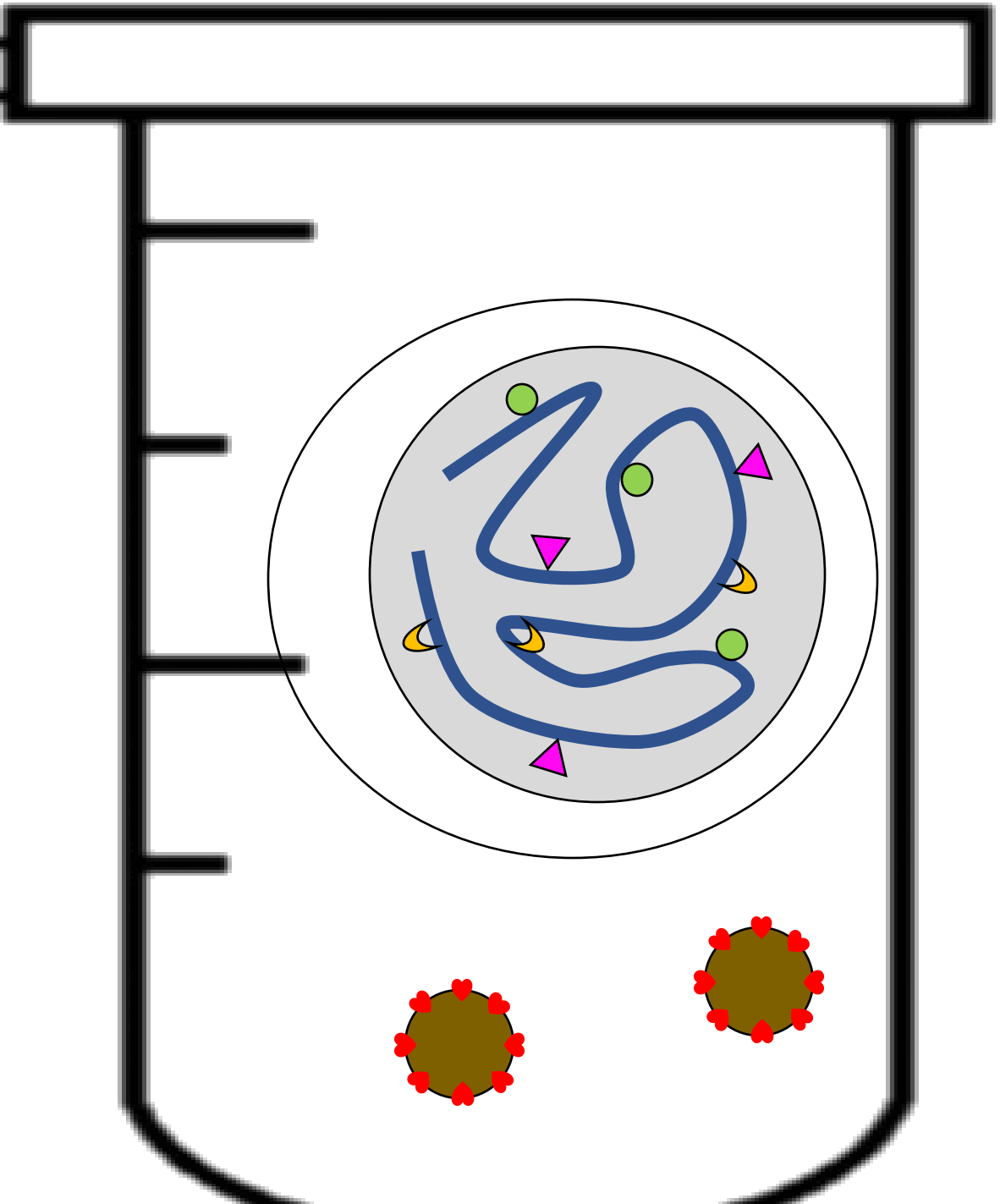
Laureano Tomás-Daza
SubGM3
28/04/2021

Cleavage **U**nder **T**argets **and** **R**elease **U**sing **N**uclease (CUT&RUN)



Skene, P. J., & Henikoff, S. (2017). An efficient targeted nuclease strategy for high-resolution mapping of DNA binding sites. *eLife*

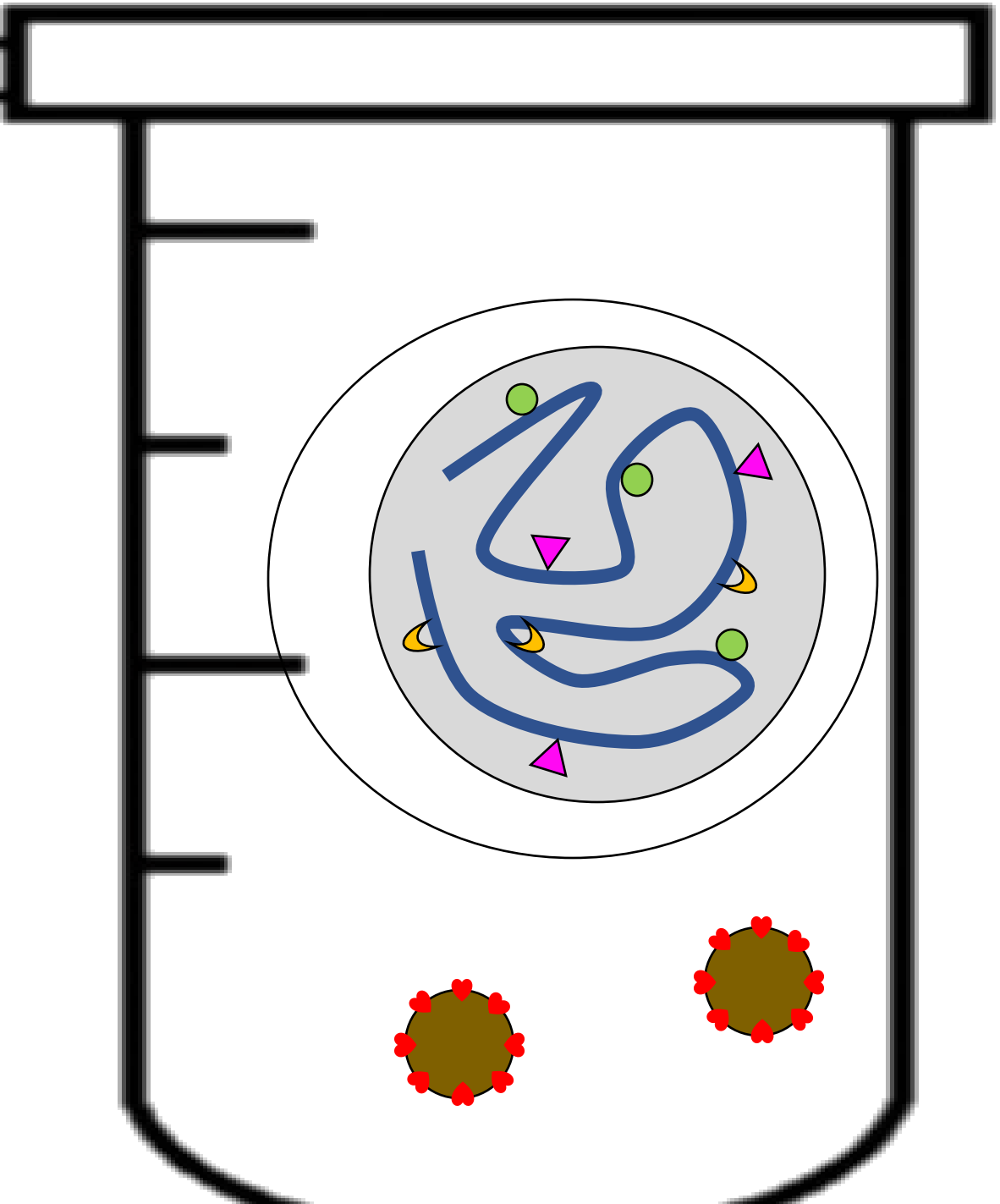
CUT&RUN



- Add ConA-coated microscopic magnetic beads

Slide from: Llorenç Rovirosa

CUT&RUN



- Add ConA-coated microscopic magnetic beads
- They stick to the cell surface

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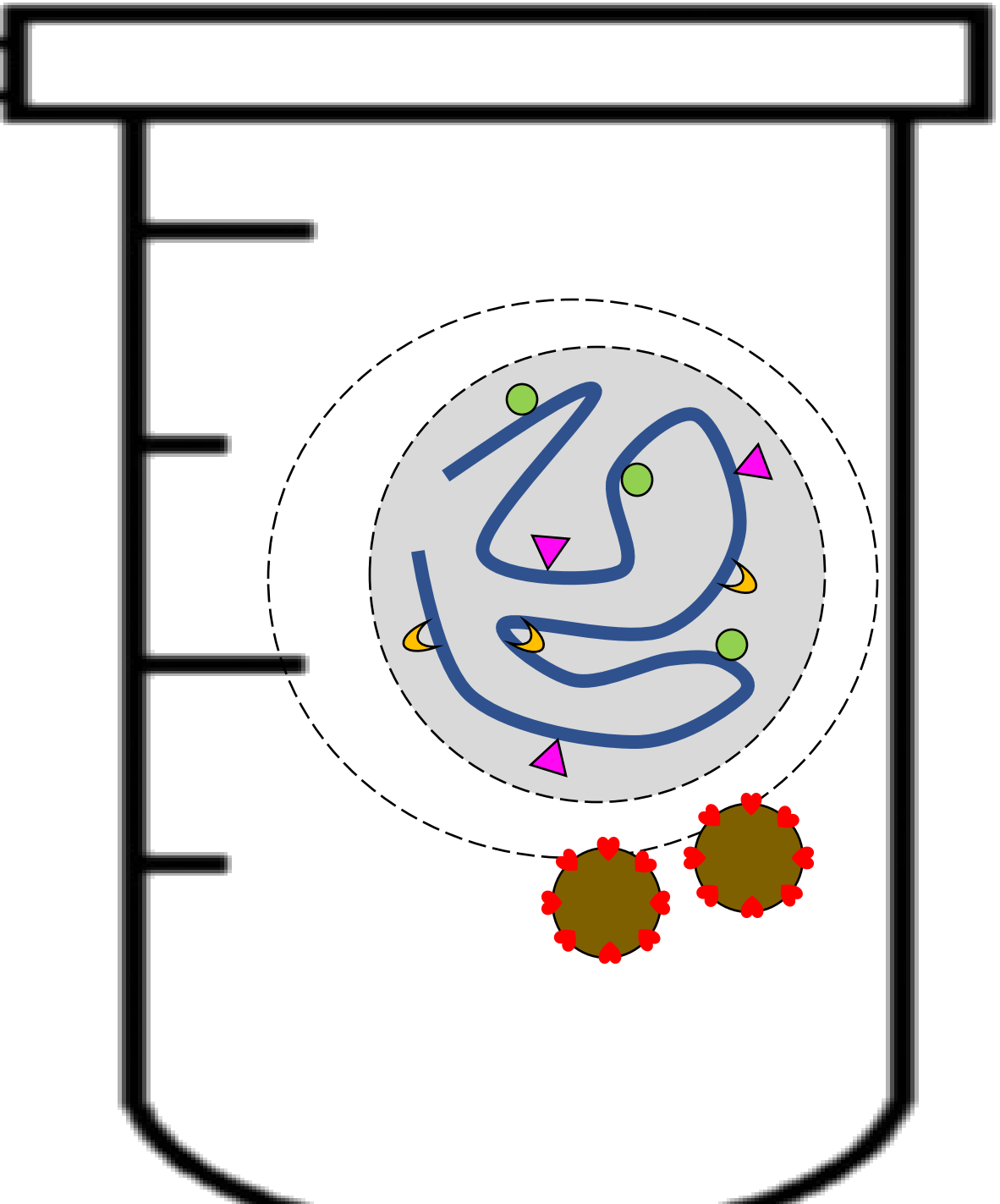

- Permeabilize plasmatic and nuclear membrane
- Add antibody, which is able to permeate inside the nucleus and bind

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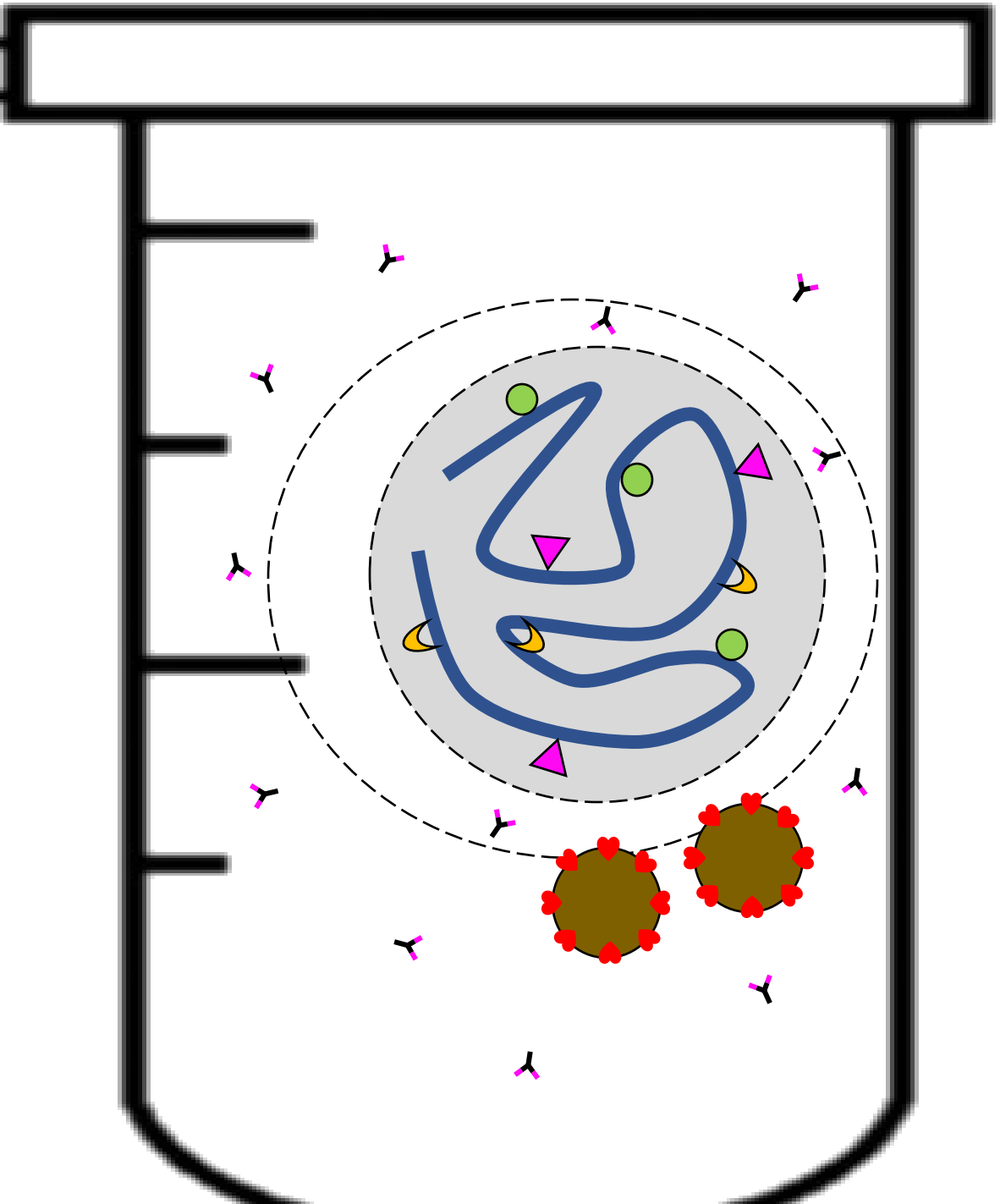

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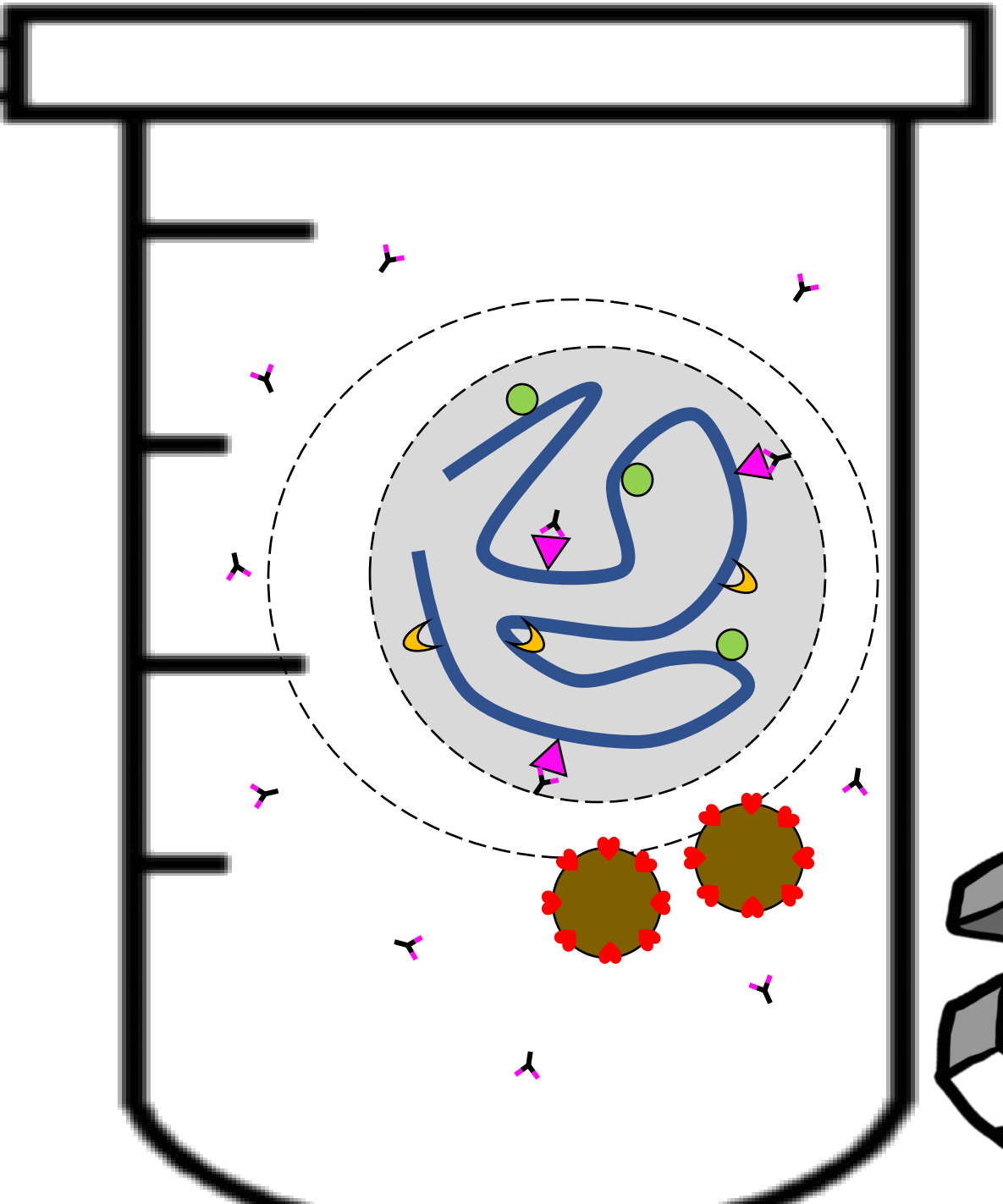
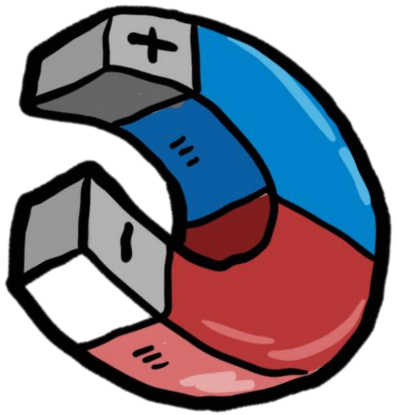


- Place on magnet and excess wash antibody



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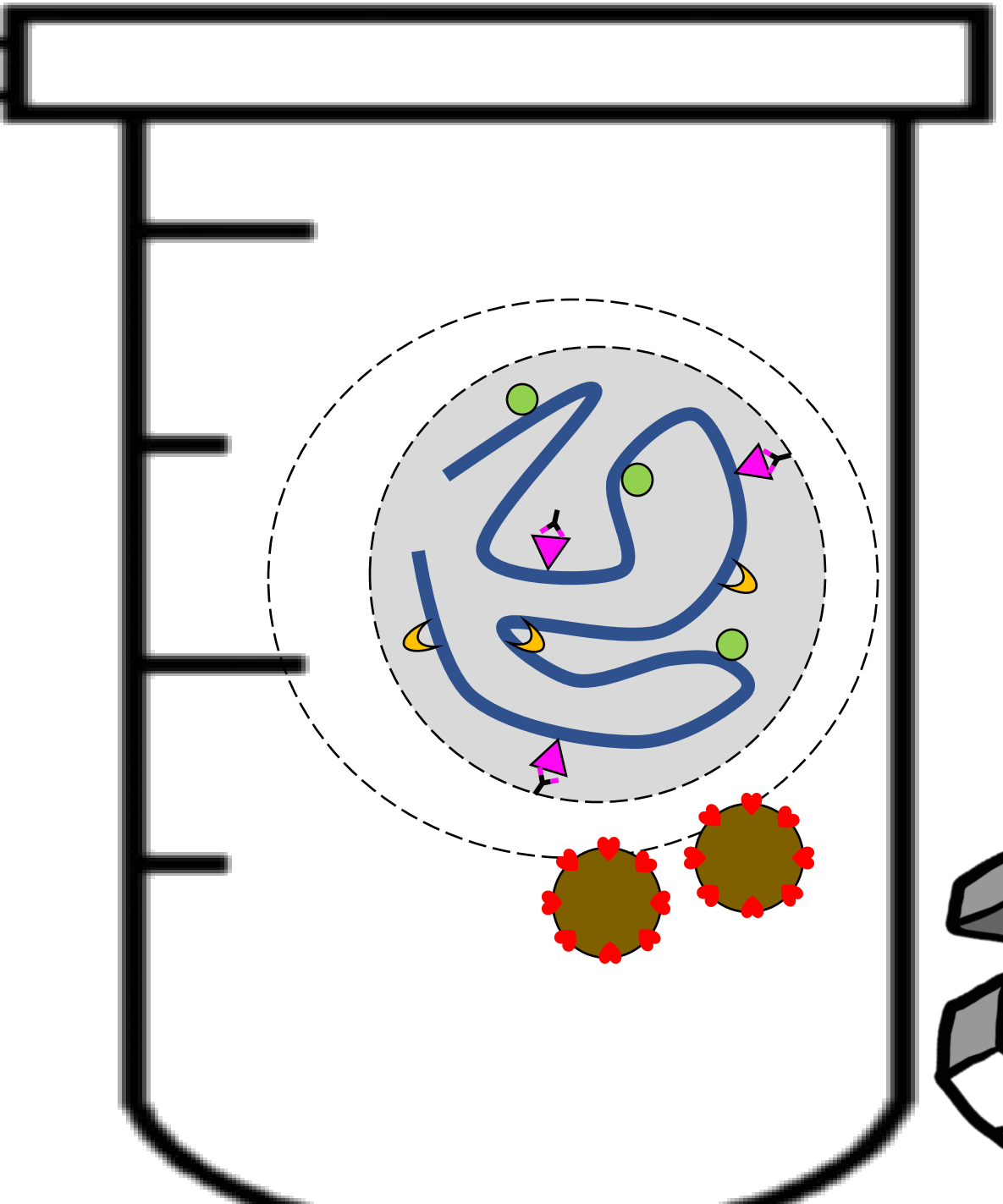
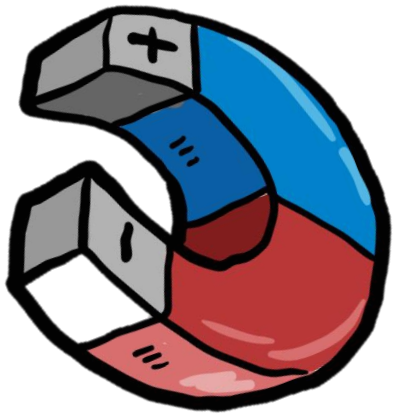
CUT&RUN





- Remove from magnet
- We have intact permeabilized cells with the antibody bound to our protein of interest in native chromatin

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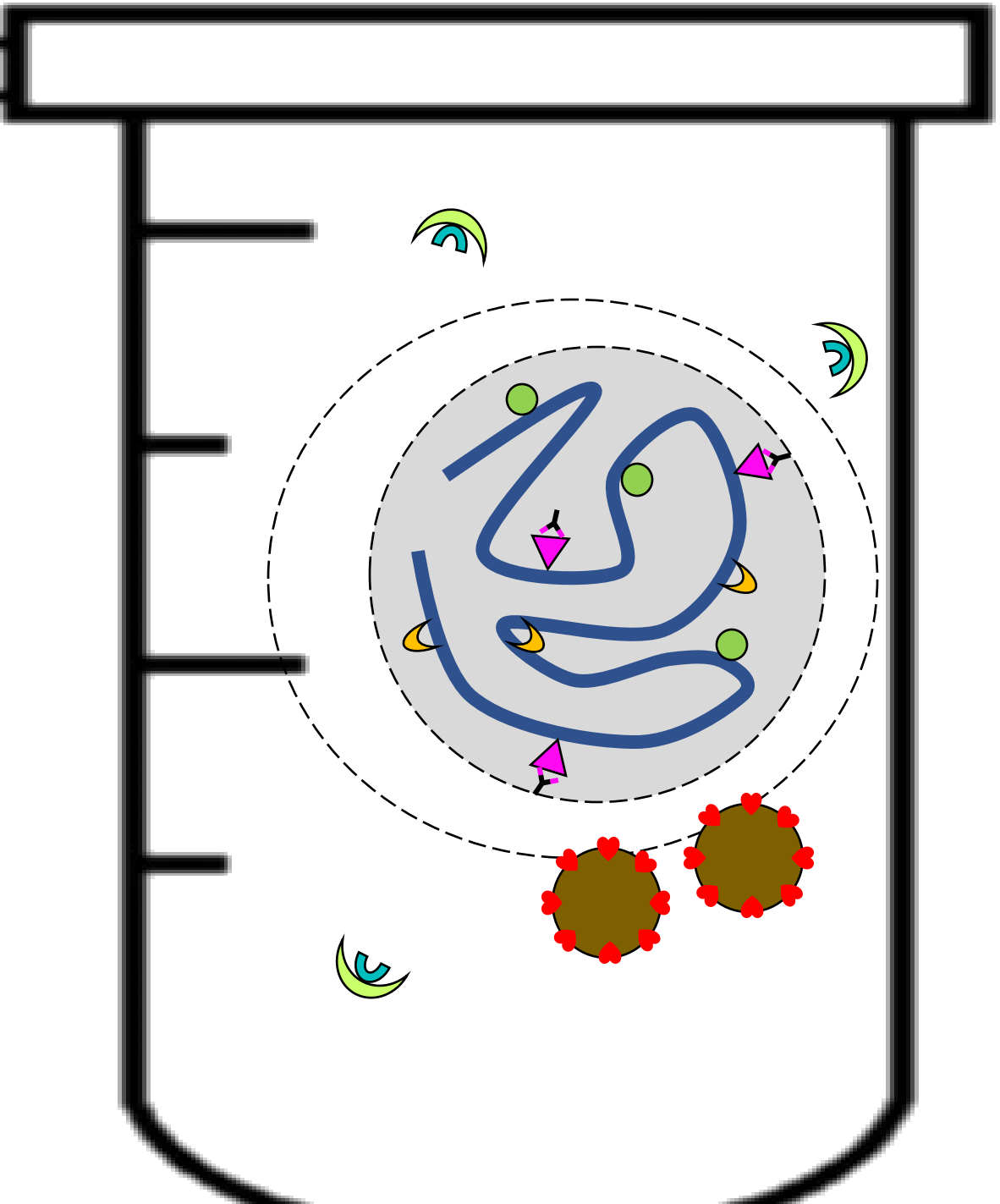

- Add pA/G-MNase fusion protein to the sample.
- p/AG binds ( ) to the constant fraction of antibodies
- MNase ( ) is an inespecific nuclease which cuts wherever it finds accessible

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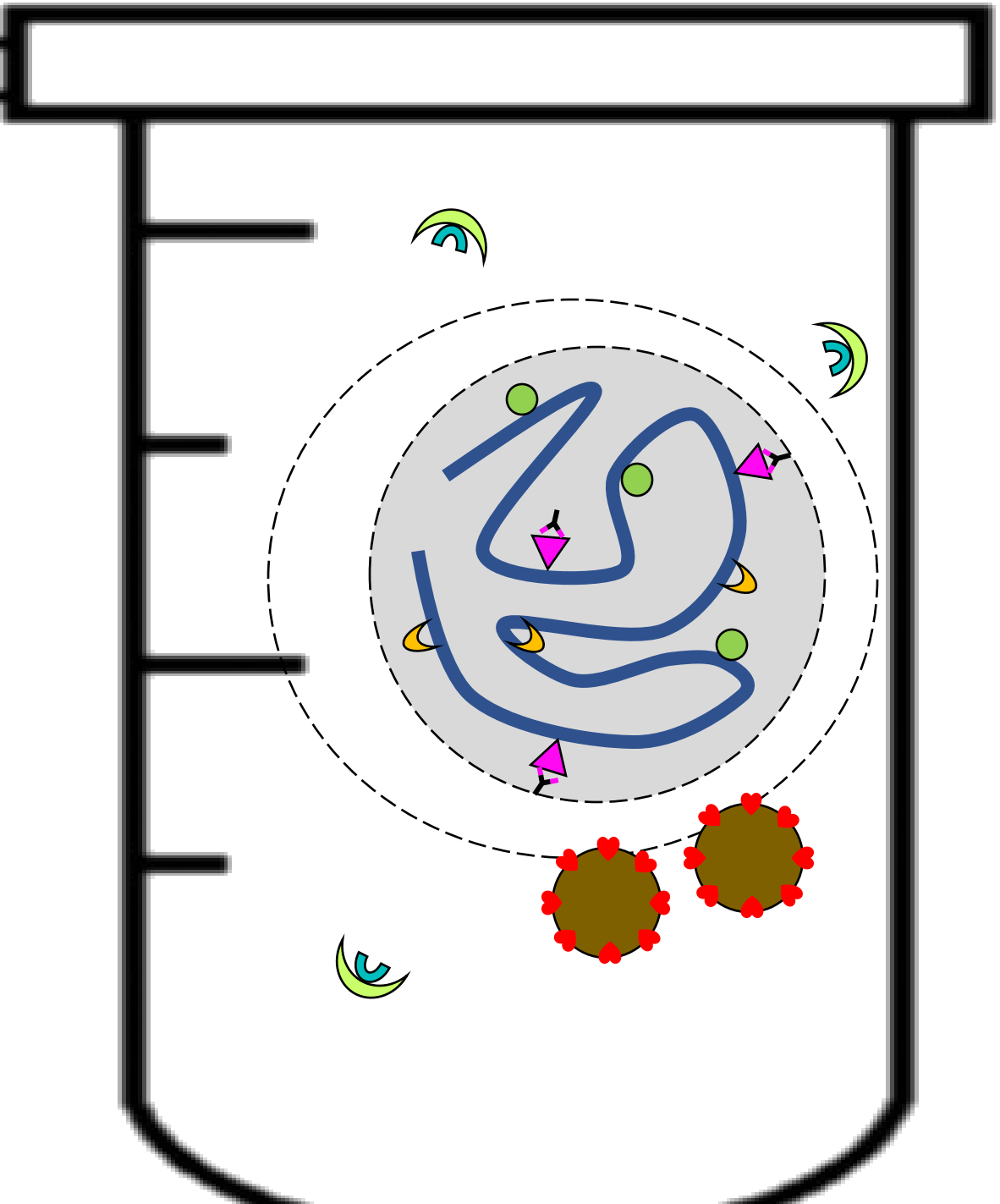
CUT&RUN



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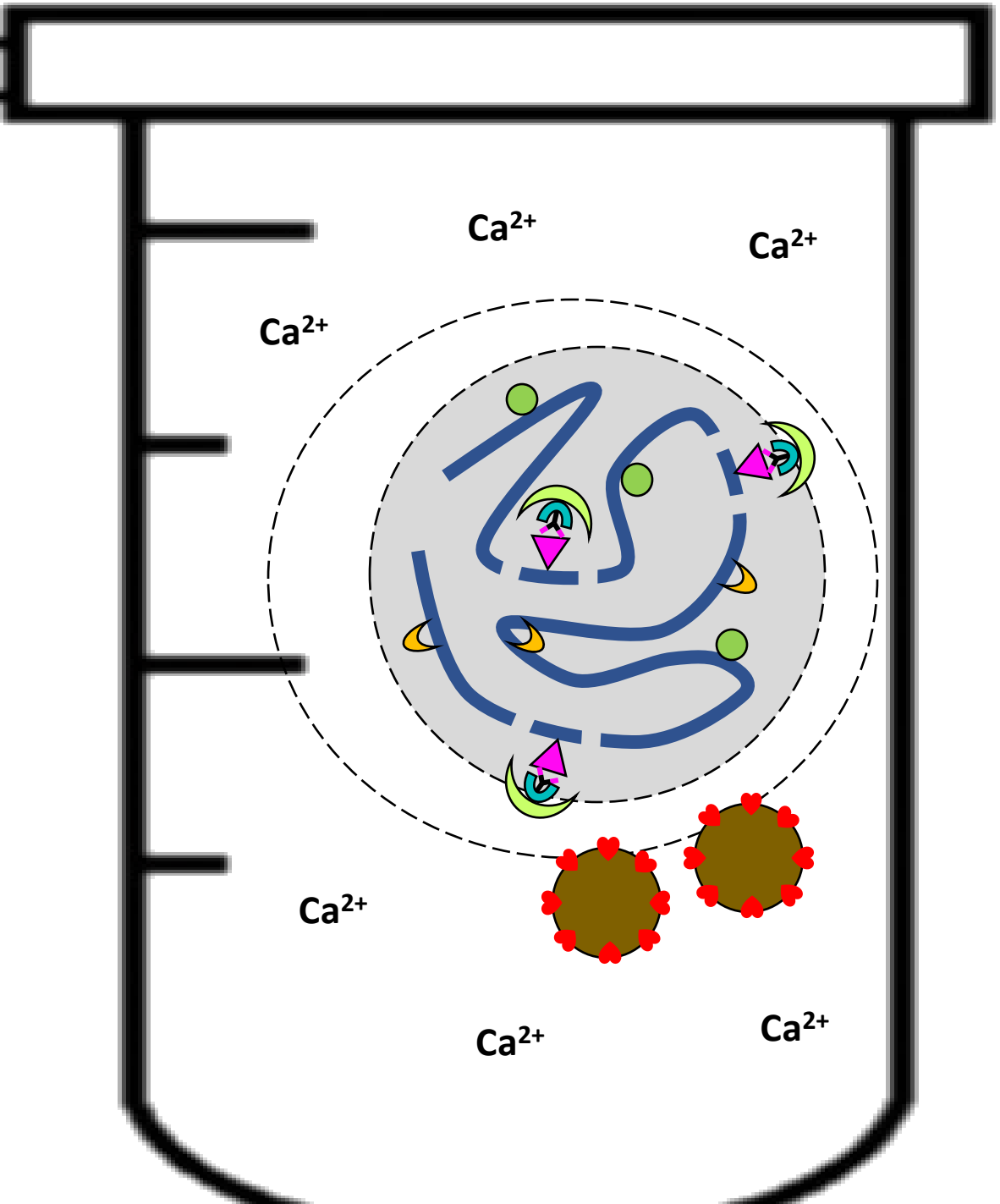

- Activation of Nuclease
- Nuclease cuts only in the regions where it has bound

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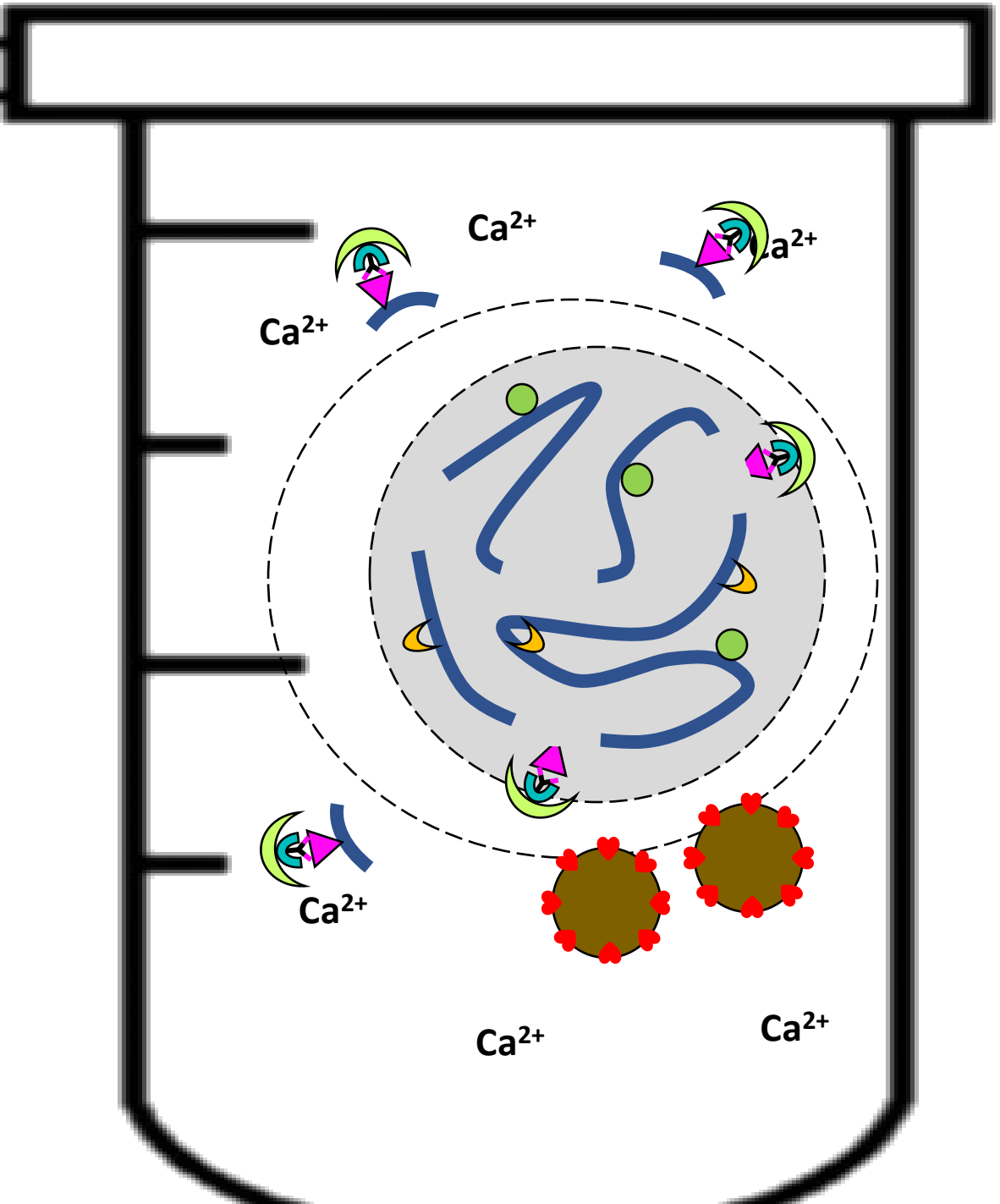

- Cut fragments are able to solubilize and permeate through the membrane; while genomic DNA does not

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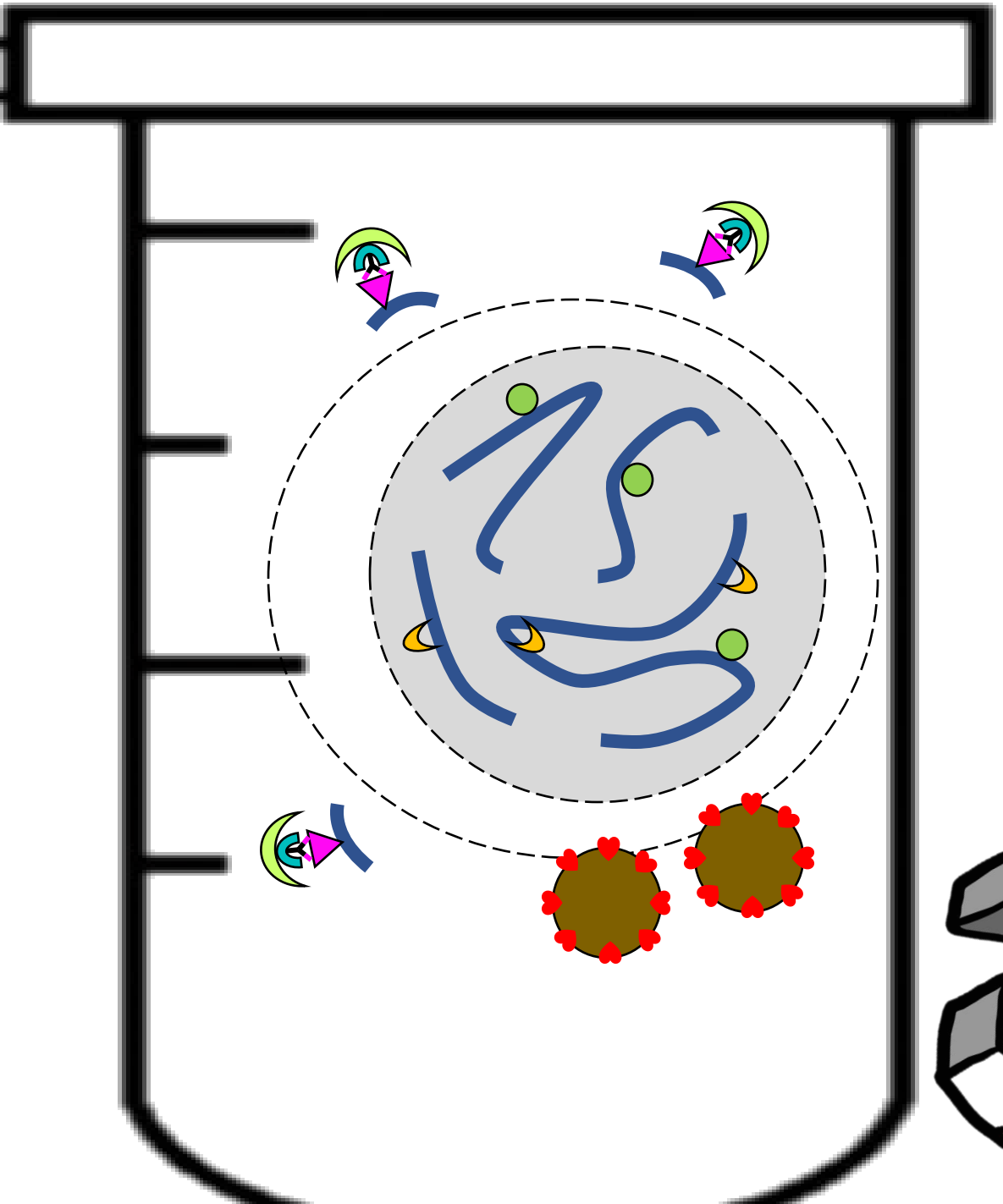
CUT&RUN



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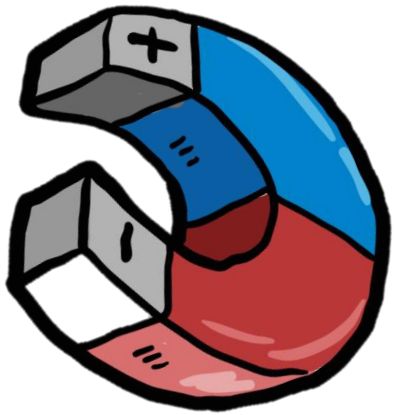

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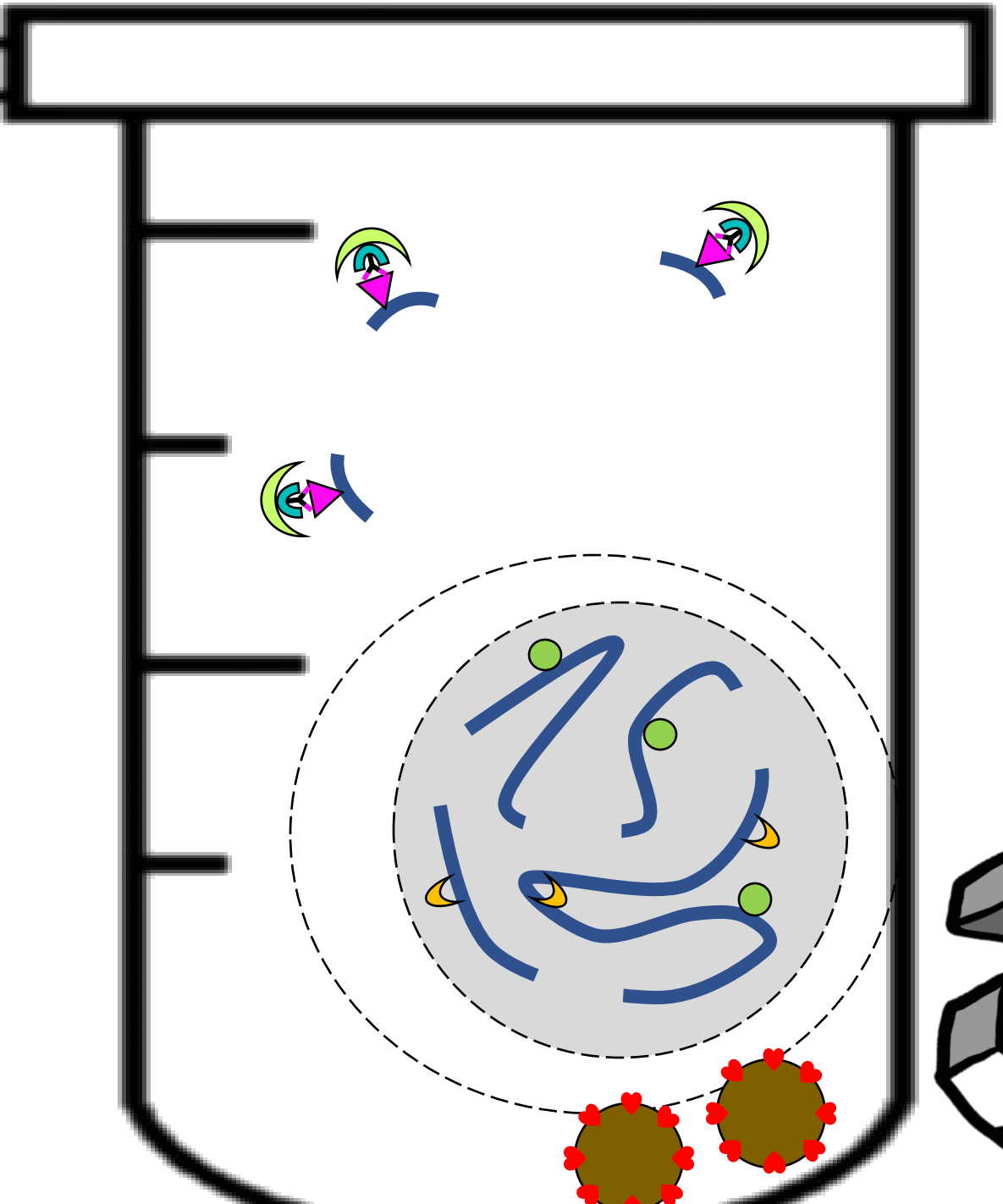

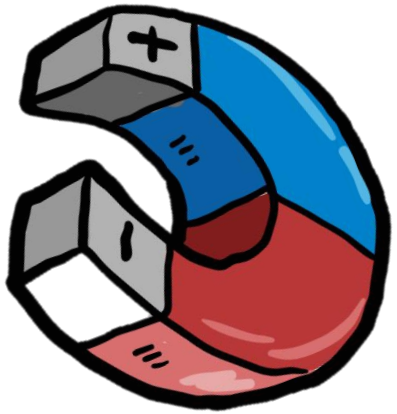

- Recover supernatant with our DNA fragments of interest, leaving behind the cells and the rest of the genomic DNA

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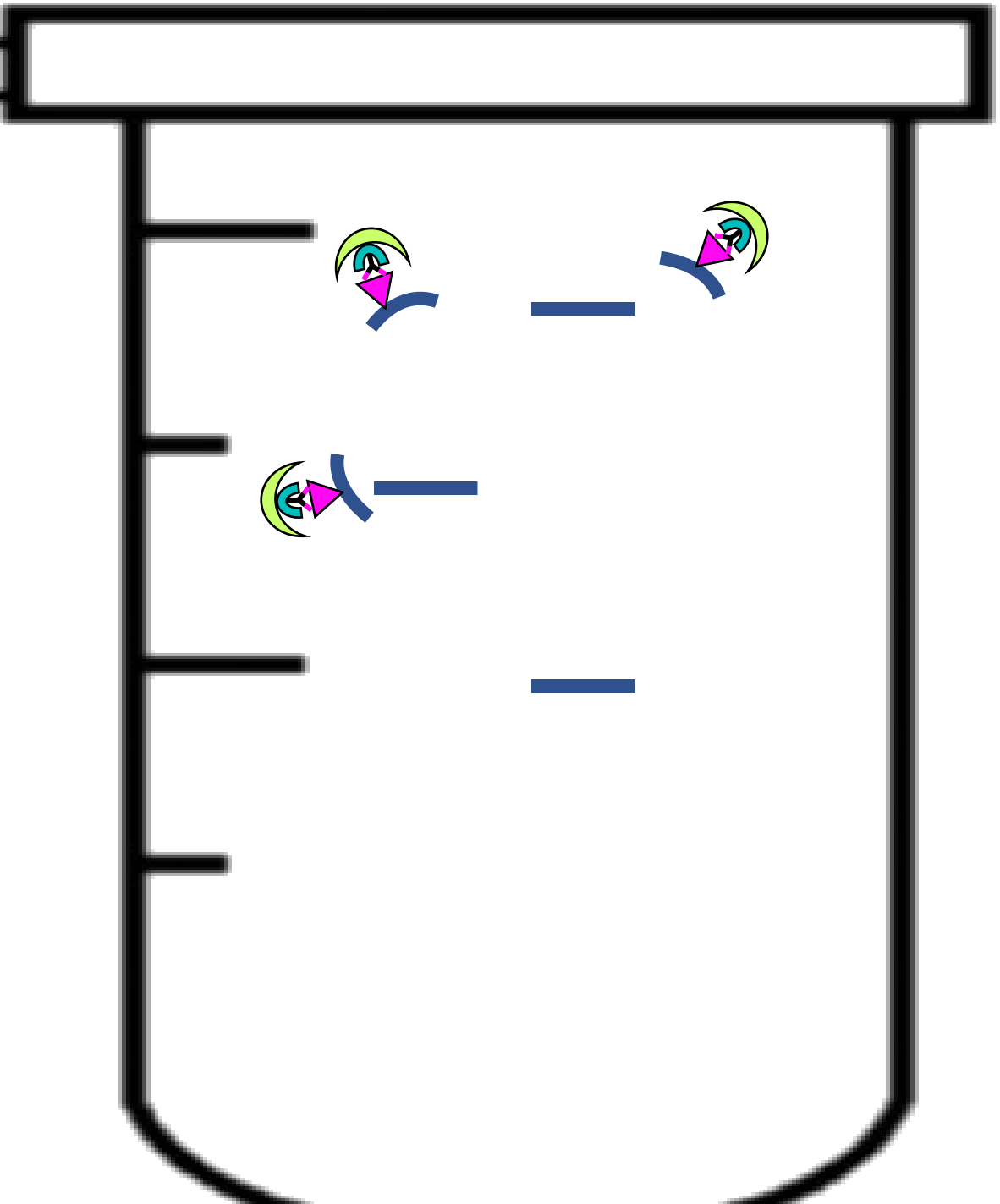

- Purify DNA

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CUT&RUN is ChIPseq on STEROIDS

The technique is performed on native chromatin, which ensures **preservation of the protein epitope**

CUT&RUN

No need for crosslinking or sonication

WAAAY less cells needed

Improved signal and less background

ChIPseq

Basically the same lame technique since 1985 (Solomon and Varshavsky)

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CUT&RUN is ChIPseq on STEROIDS

Standard ChIPseq protocols require millions of cells; CUT&RUN developers claim it can work with works with **as little as 100 cells for abundant histone marks or 1000 cells for TFs**

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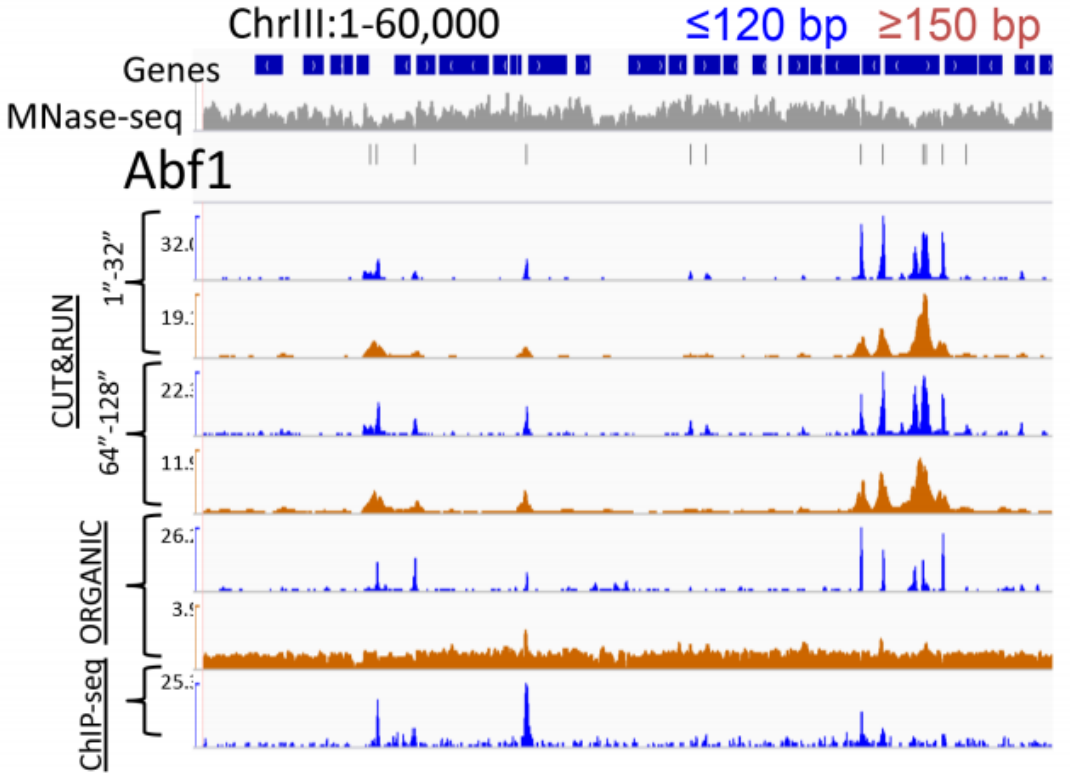
CUT&RUN is ChIPseq on STEROIDS



Adapted from Skene and Henikoff (2017)

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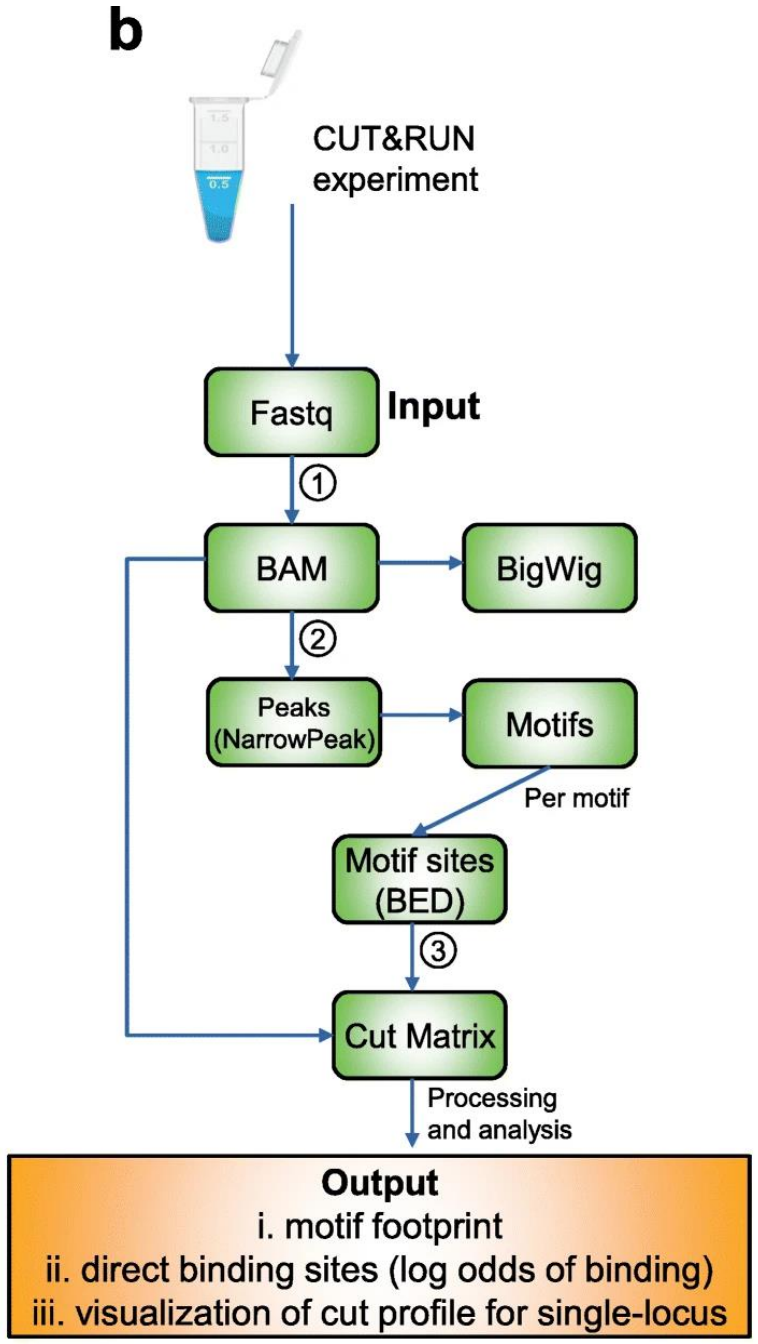
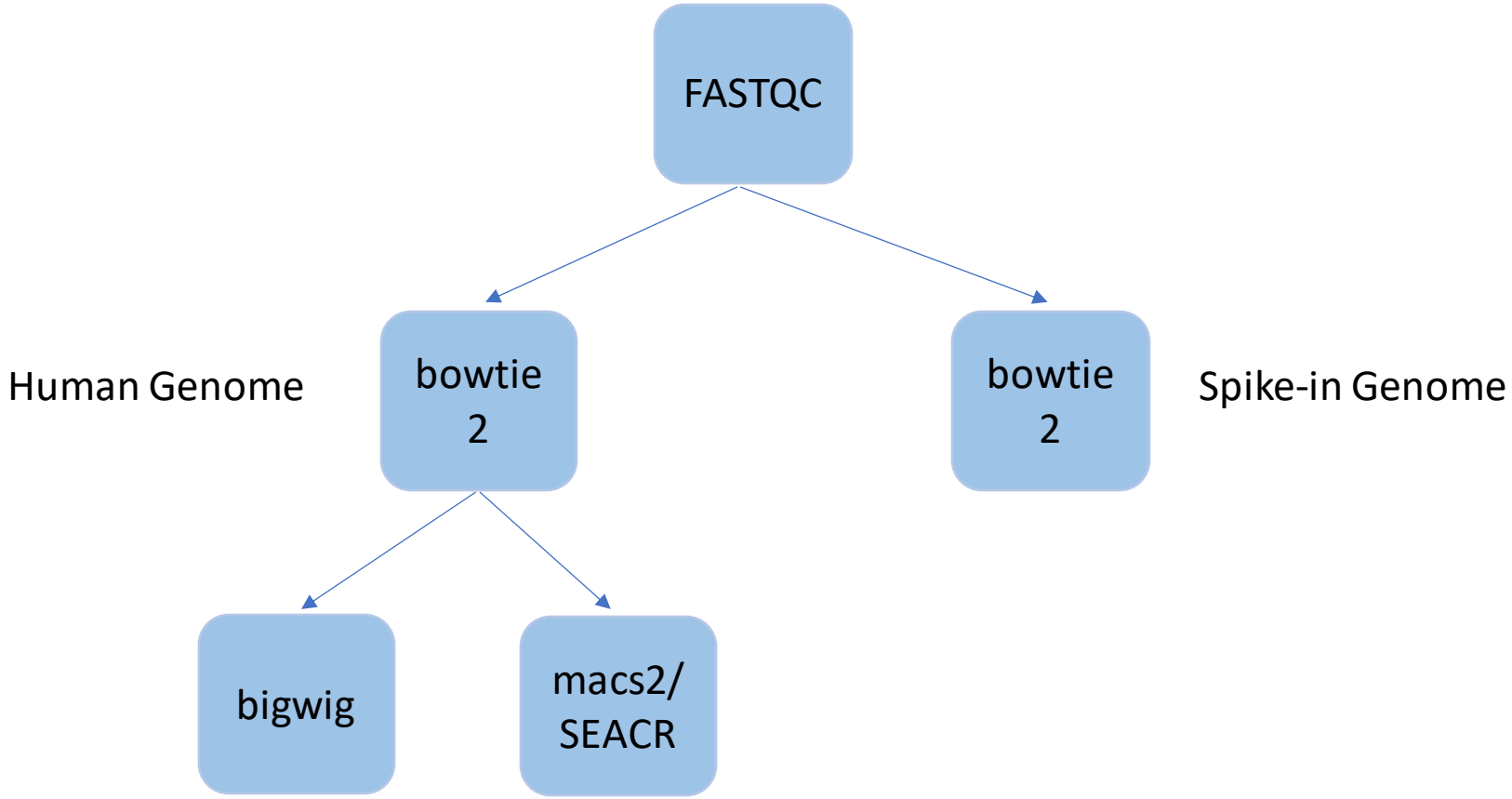
CUT&RUN Tools

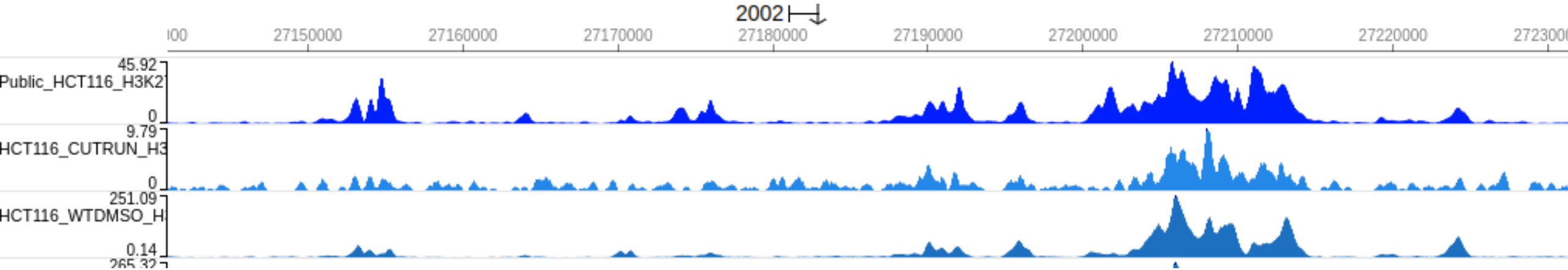


Zhu, Q., Liu, N., Orkin, S.H. *et al.* (2019). CUT&RUNTools: a flexible pipeline for CUT&RUN processing and footprint analysis. *Genome Biol*

Our (preliminary) CUT&RUN pipeline



Our (preliminary) result



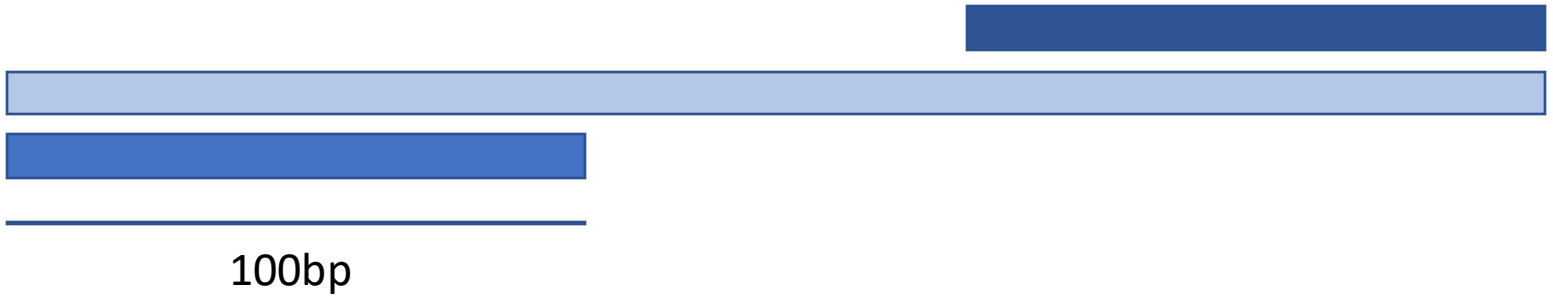
Have you thought "oh it seems really easy"?

SPOILER:

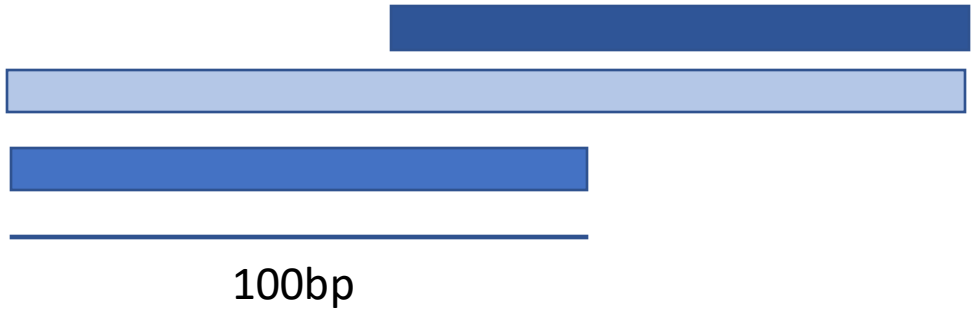
It's not that easy

(yet)

Critical Step 1: Alignment



ChIP-seq
Fragments



CUT&RUN
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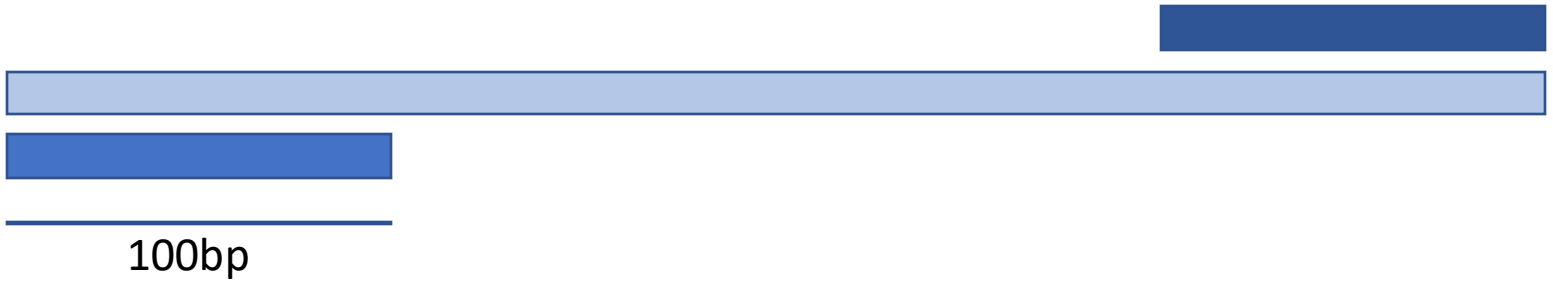

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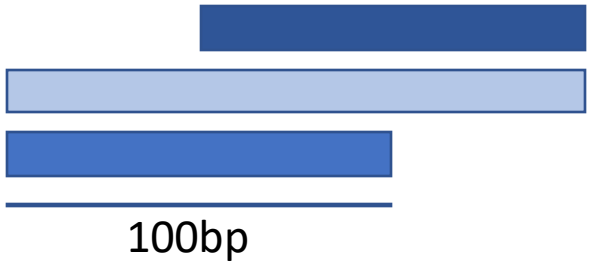
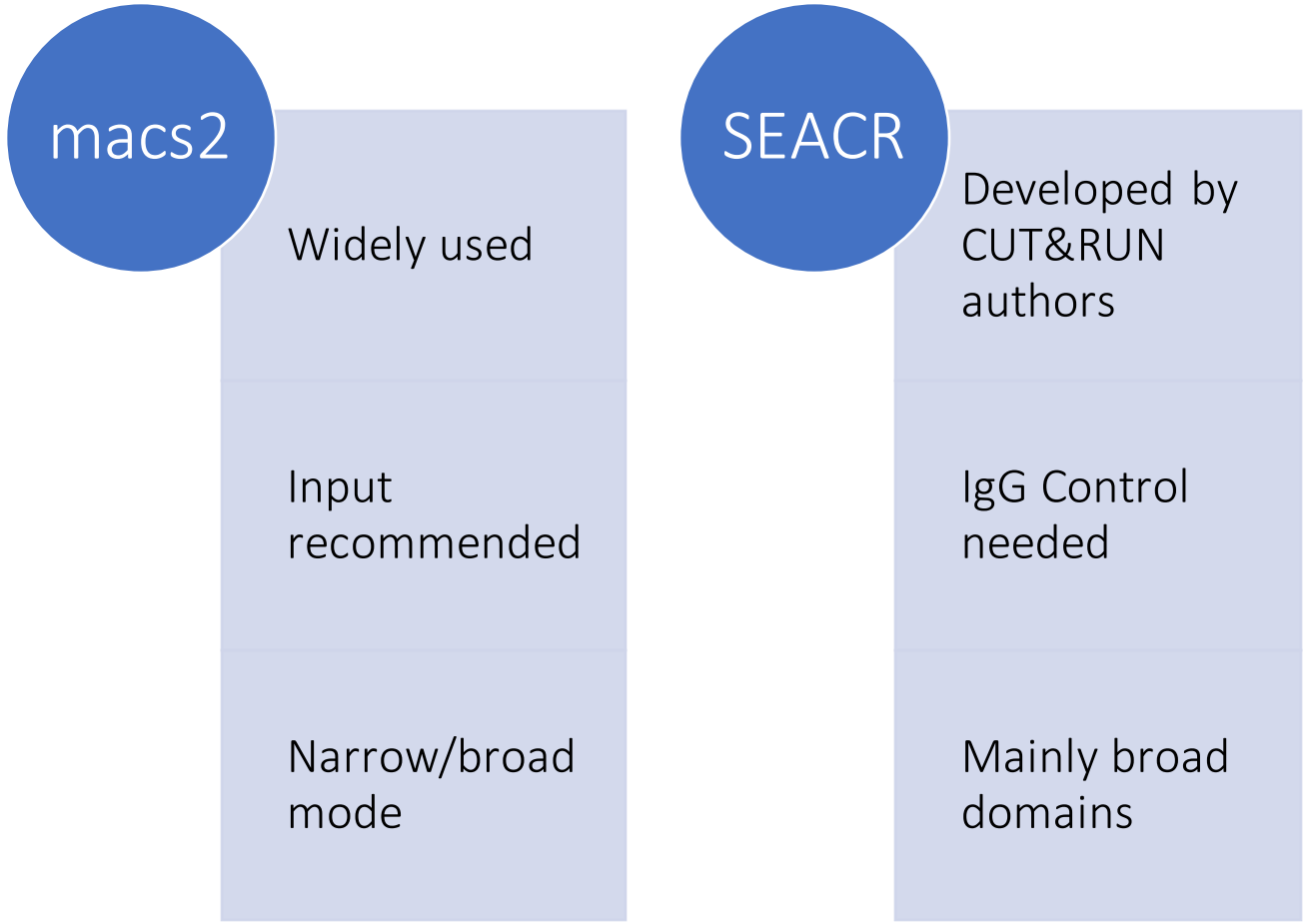


`--dovetail`

CUT&RUN
Fragments

Critical Step 2: Peak Calling



Critical Step 3: Normalization – Spike-in

Normalization 1:

Treatment spike-in = 100,000 spike-in reads in 5,000,000 uniquely aligned reads = 2%
Untreated spike-in = 30,000 spike-in reads in 3,000,000 uniquely aligned reads = 1%

Ratio total/spike-in

Treatment normalization factor = 1 / 2% spike-in = **0.5**
Untreated normalization factor = 1/ 1% spike-in = **1.0**

Normalization 2:

Treatment spike-in = 100,000 spike-in reads
Untreated spike-in = 30,000 spike-in reads

Absolute spike-in reads

Treatment normalization factor = 30,000 / 100,000 = **0.3**
Untreated normalization factor = 30,000/ 30,000 = **1.0**

Critical Step 3: Normalization – Background

Suggested by: Samuel Collombet

1. Merge peak-calling from conditions
2. Mask genome with 1.

} Define background

3. Coverage in 10Kb windows
4. Compute scaling factor with DESeq2

Take home message

- Both ChIPseq and CUT&RUN aim to answer the same biological question: **where does a certain protein bind in the genome**??

HOWEVER

- CUT&RUN has **lower cell input and sequencing requirements**
- CUT&RUN gives **lower background**
- CUT&RUN **maintains epitope integrity** due to lack of crosslink and sonication

Slide from: Llorenç Rovirosa

Thanks for your attention

Any question?

Laureano Tomás-Daza
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