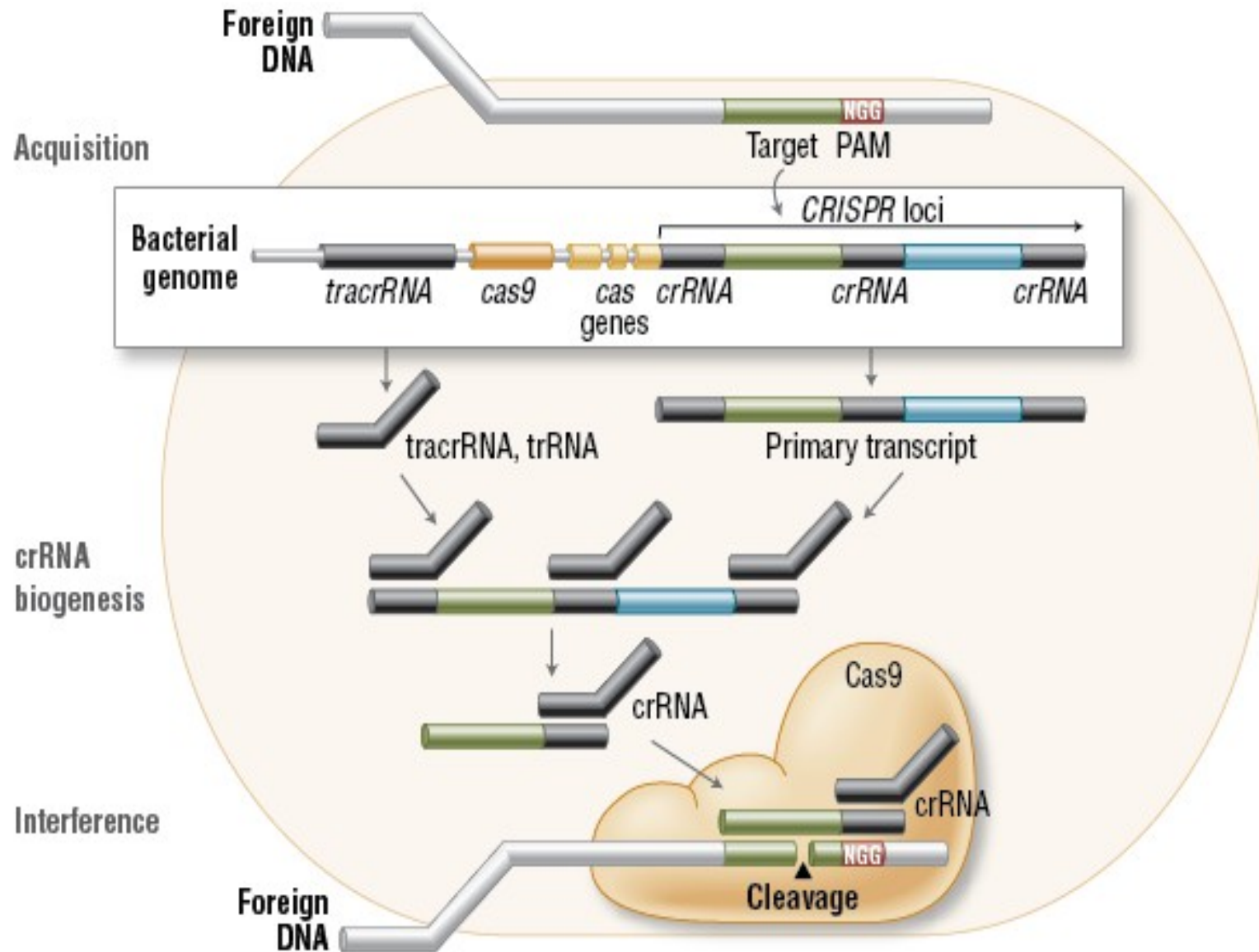
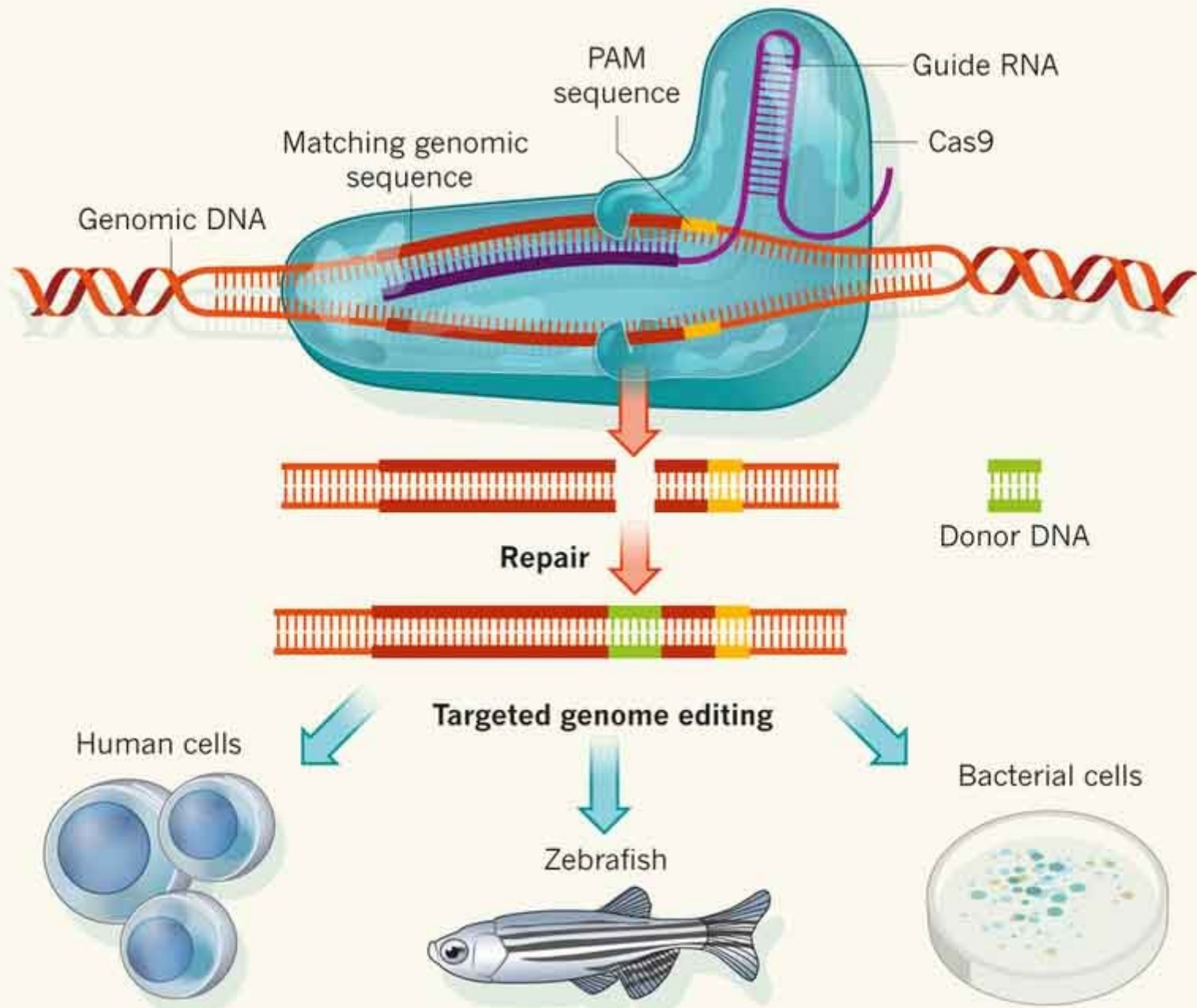


CRISPR-Cas9

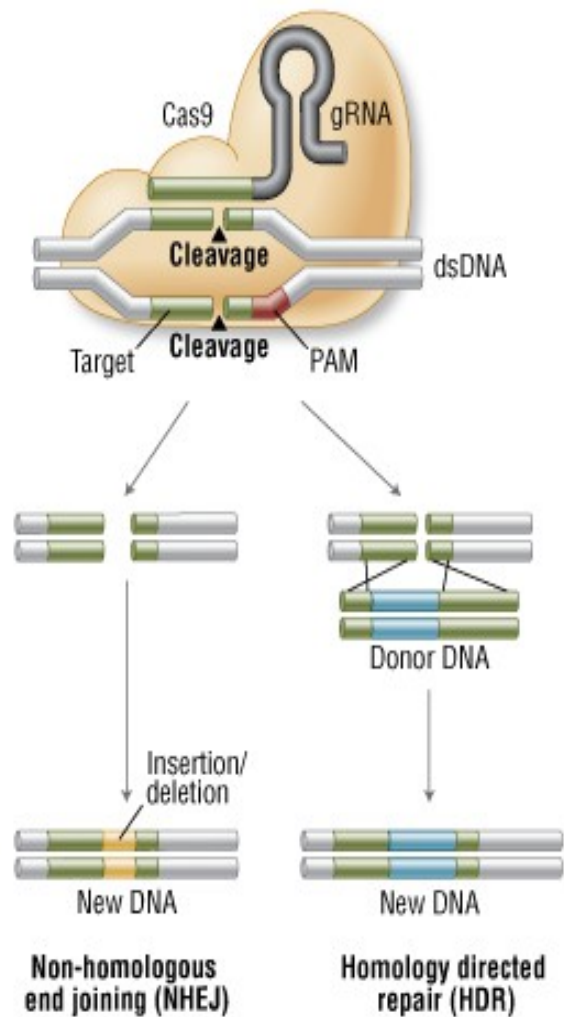
CRISPR (Clustered Regularly Interspaced Short
Palindromic Repeats)

CRISPR-associated (Cas) enzyme

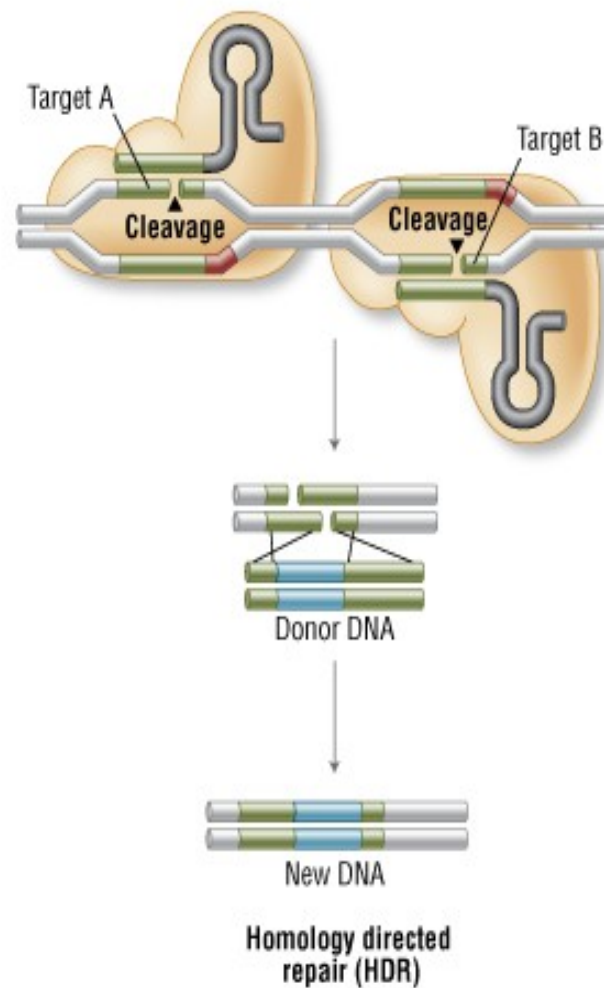




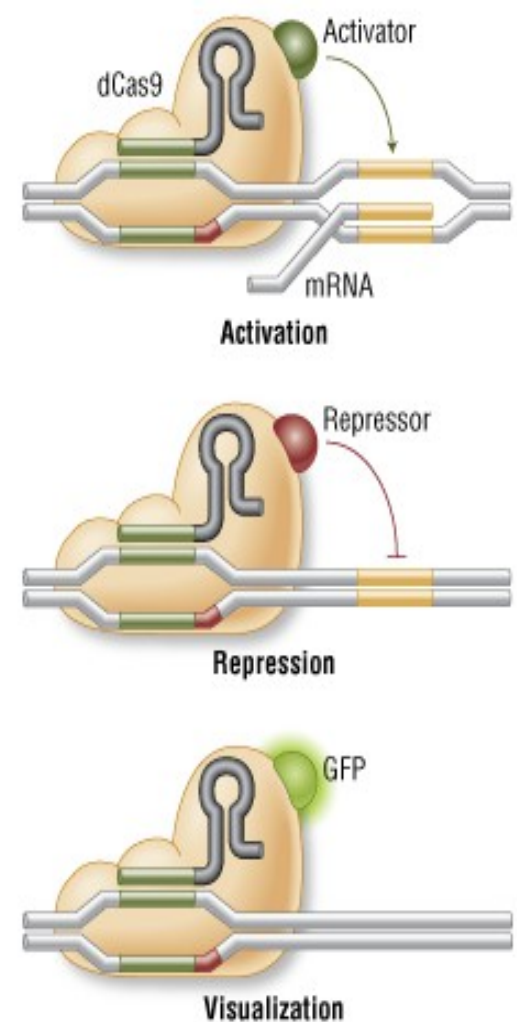
A. Genome Engineering With Cas9 Nuclease



B. Genome Engineering By Double Nicking With Paired Cas9 Nickases



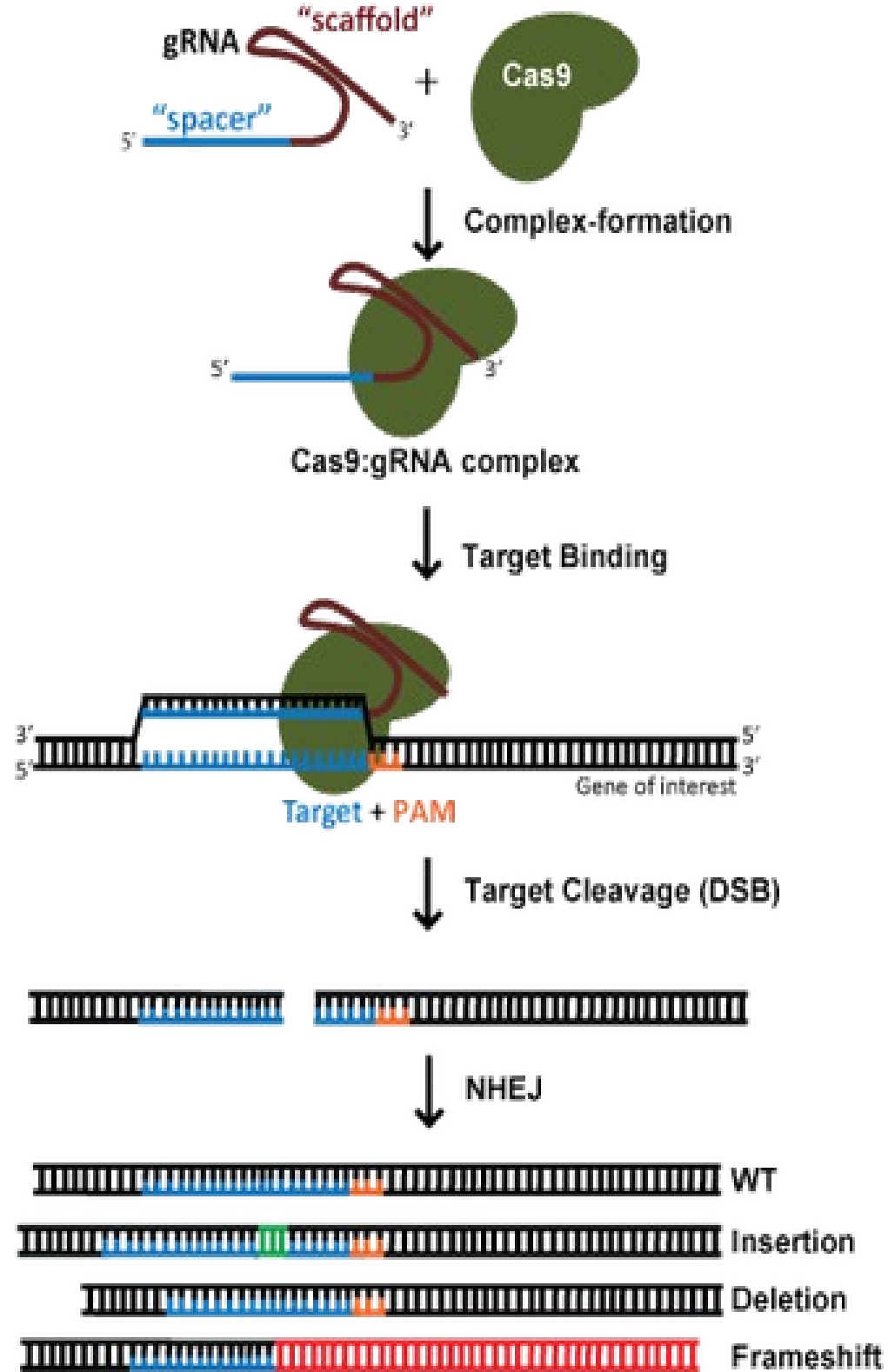
C. Localization With Defective Cas9 Nuclease



CRISPR

Why is this technology so exciting and with such great potential?

What are the main concerns with the technology and its application to human biology?



Does your target sequence

have GGN19GG or CCN19CC sequence?

target within an exon?

target both a and b (long and short genes)?

NOT have 100% identity to any human
sequence?

NOT have Dral site?

T7 RNA pol promoter site

→ txn initiation site

5' - **TAATACGACTCACTATA**GGAGAGACCGAGAGAGGGTCTCAGT
coding strand

3' - **ATTATGCTGAGTGATAT**CCTCTCTGGCTCTCTCCCAGAGTCA
template strand

5' - GGAGAGACCGAGAGAGGGUCUCAGU RNA

Oligos will always look like this

F1 5' TAGGN18

R1 5' AAACN18

N18 is gene specific sequence

Lab Safety

Good Lab Practices for uniformity, consistency, reliability, reproducibility, quality, and integrity of your experiments and safety of you, your friends and your things.

Keep yourself safe.

Keep your friends safe.

Keep your things safe.

Dress Code

What type of shoes are frog favorites?

Closed-toed shoes

Must wear

Closed-toed shoes

Lab coats

Goggles

Gloves

Abbreviated Rules

- A) **STOP** and **ASK** if you are uncertain of ANYTHING.
- B) **NO FOOD** or **DRINKS** in the lab.
- C) Follow the **dress code** and all **safety** rules and guidelines.
- D) Keep your **bench clean and tidy**.
- E) **Wash hands** frequently.
- F) **STOP** and **ASK** if you are uncertain of ANYTHING.

Safe from hazards

Physical:

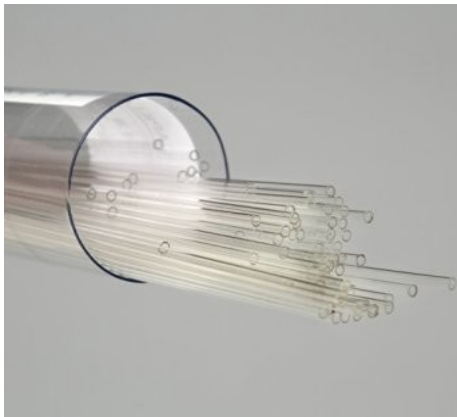
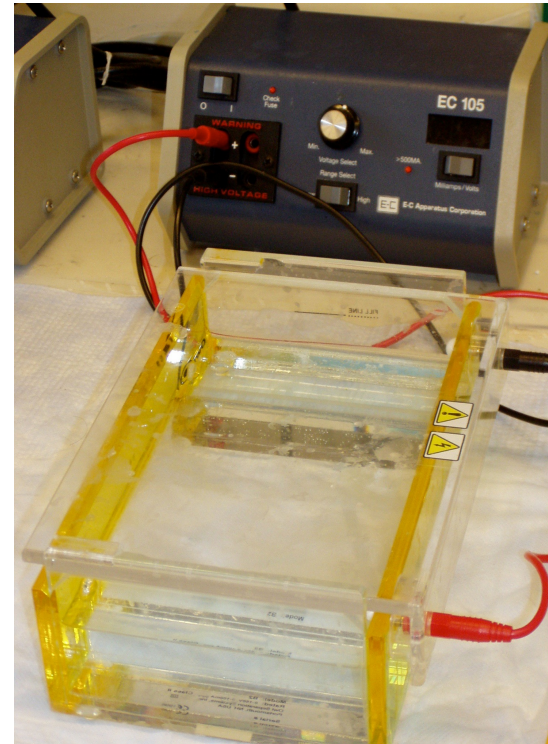
Fire

Electrical

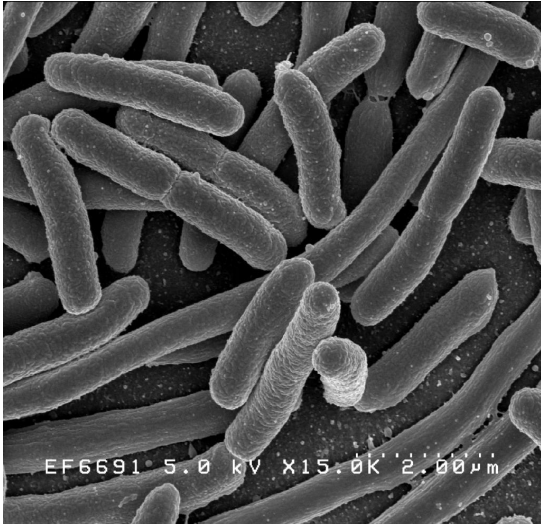
Sharps

High pressure cylinder

Aerosol and splash risk w
centrifuges and pipetman



Safe from hazards



Biological:

non-pathogenic *E. coli*
(BSL-1: no known health risk)



Safe from hazards

Chemical:

Recombinant or synthetic Nucleic Acids
(rsNA)

Ethidium Bromide (EtBr)

Formaldehyde (FA)

Methanol (MeOH)

Ethanol (EtOH)

Phenol

Chloroform

Hydrogen peroxide (H₂O₂)

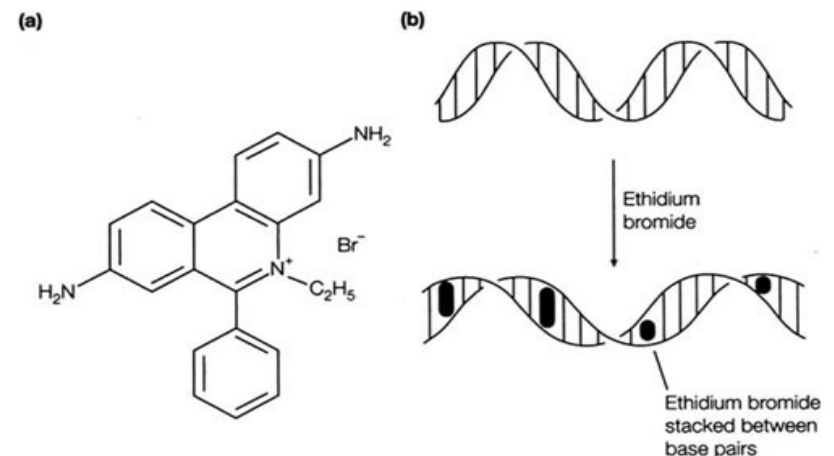
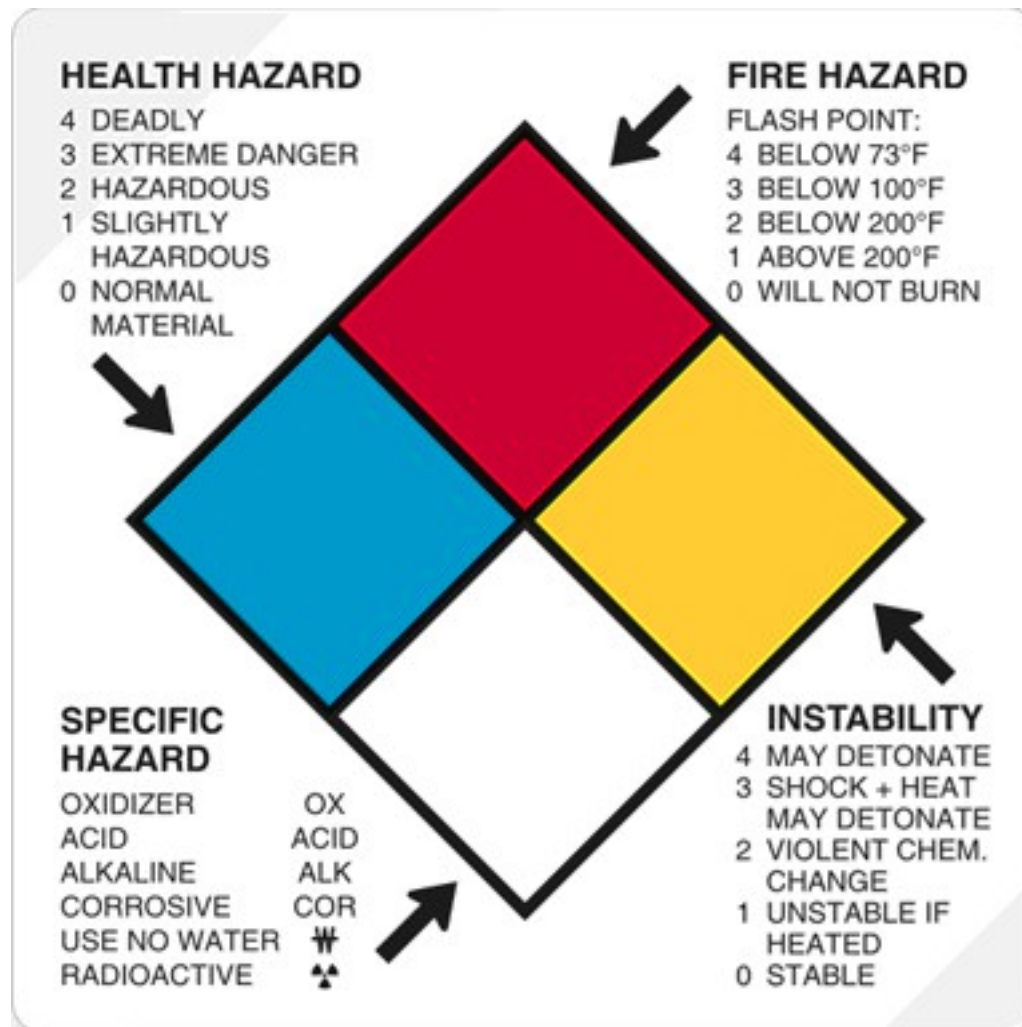


Fig. 3. (a) Ethidium bromide; (b) the process of intercalation, illustrating the lengthening and untwisting of the DNA helix.



For all the hazardous chemicals, we will go over their Material Safety DataSheets (MSDS)

<https://www.osha.gov/chemicaldata/>

Waste Disposal

Always read the label on the waste container and dispose of waste accordingly.

Gloves contaminated with bacteria go into biohazardous solid waste container.

Gloves contaminated with hazardous chemicals are discarded depending on the type of hazardous material. Follow labels.

Glass uncontaminated with any hazardous materials: Uncontaminated Glass trash

Glass contaminated with hazardous materials: Contaminated glass containers

Serological pipettes and micropipette tips are considered sharps. They will be disposed of into puncture proof cardboard boxes depending on the type of hazardous material. Follow labels.

Waste Disposal continued...

The following hazardous chemicals will be collected according to physical state of the contaminated item (e.g. solid, liquid, gel). Follow labels and ask your instructors when in doubt.

Ethidium bromide

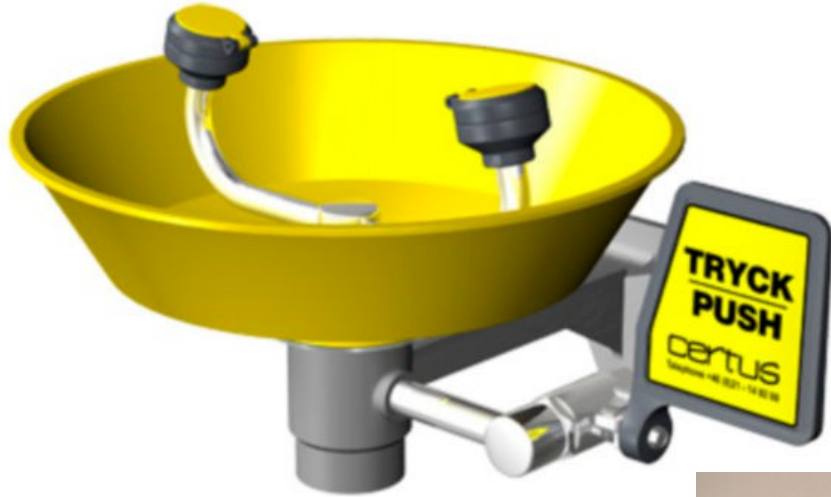
Ethanol

Methanol

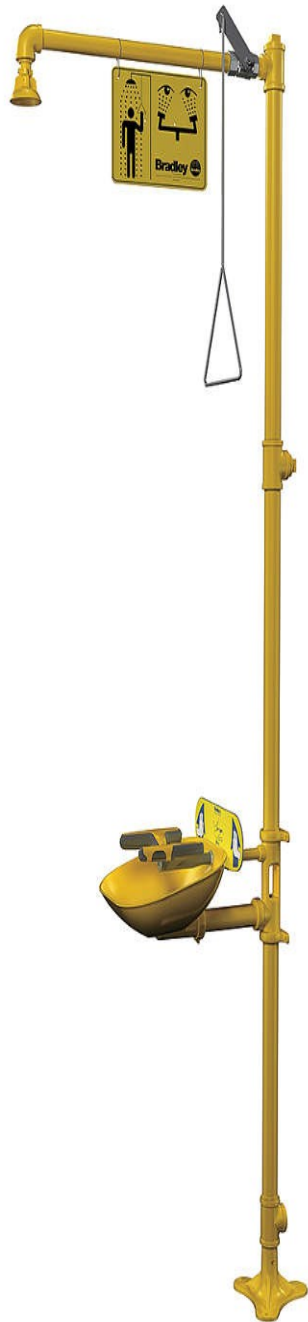
Phenol

Chloroform

Eye Wash Station



Shower



Check out the locations

Eye wash station in the lab

Shower in the hallway

Review the Lab Rules Sheet

Safety Quiz