# Gene Editing for neXt-CP50 and neXt-MP50 challenges

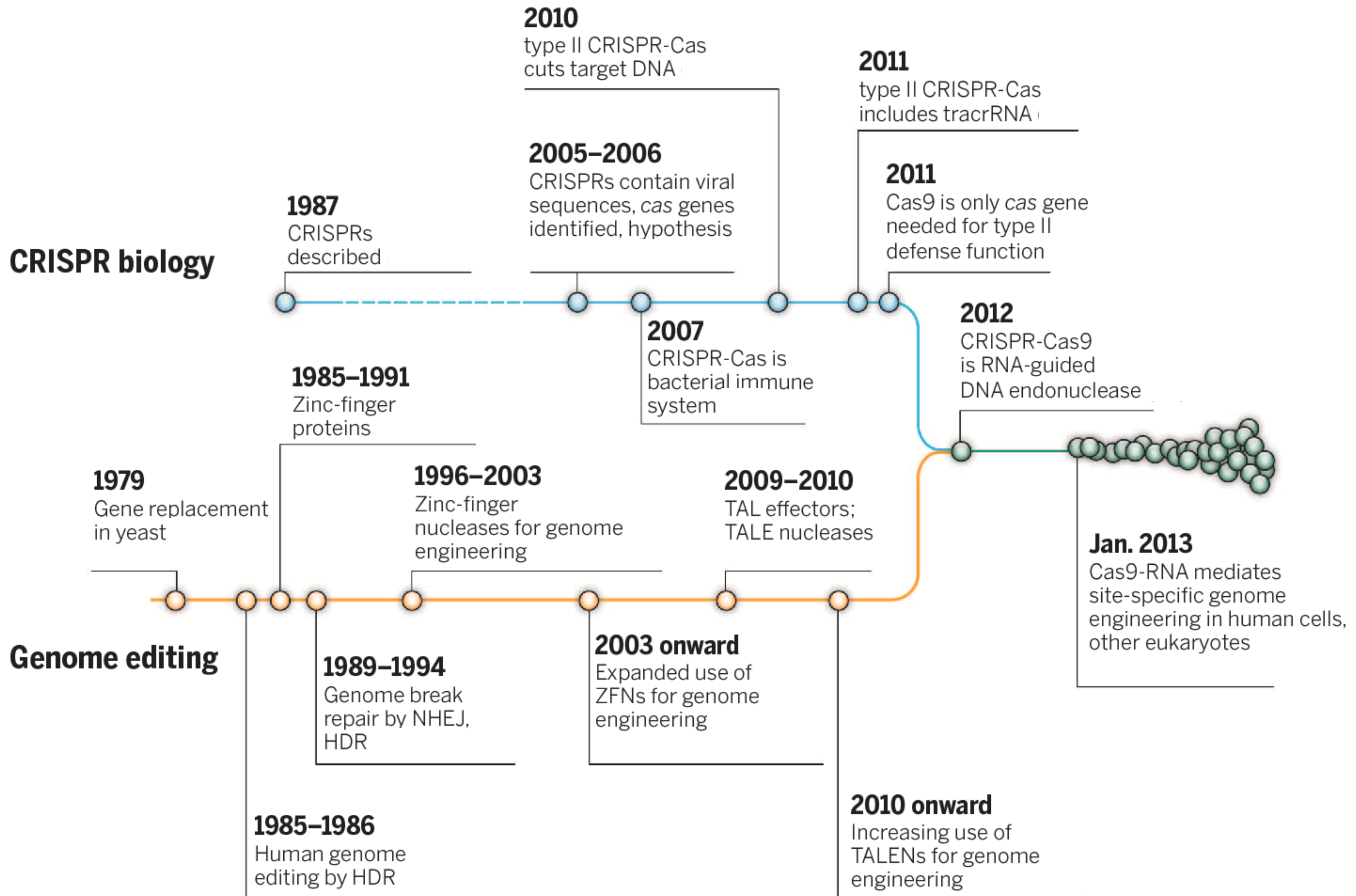
**<u>Katerina Poverennaya</u>, Leonid Kurbatov, Andrey Lisitsa**

INSTITUTE OF BIOMEDICAL CHEMISTRY

MOSCOW, RUSSIA

www.ibmc.msk.ru

# HISTORY OF GENE EDITING

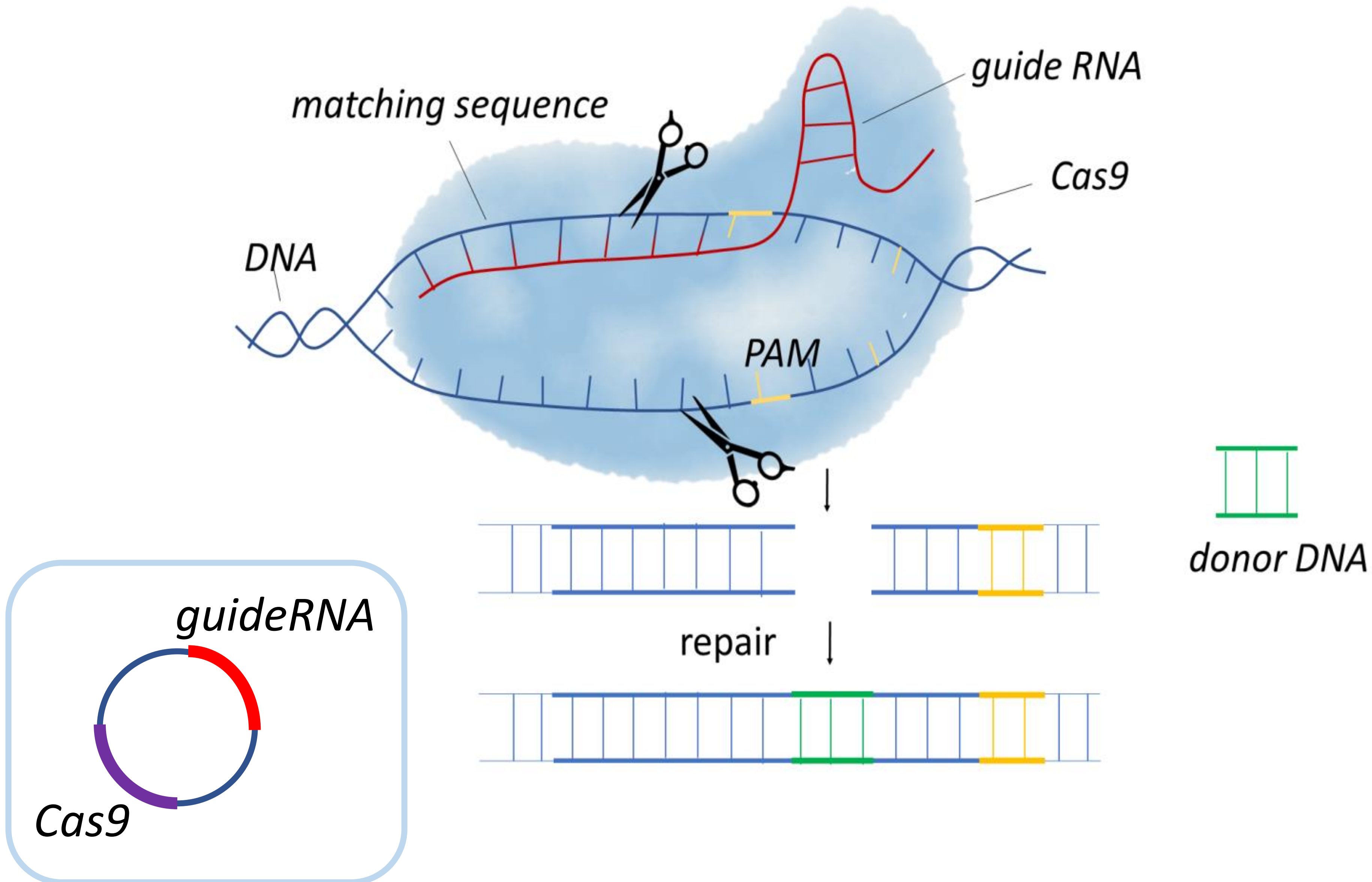



*Doudna & Charpentier, 2014*

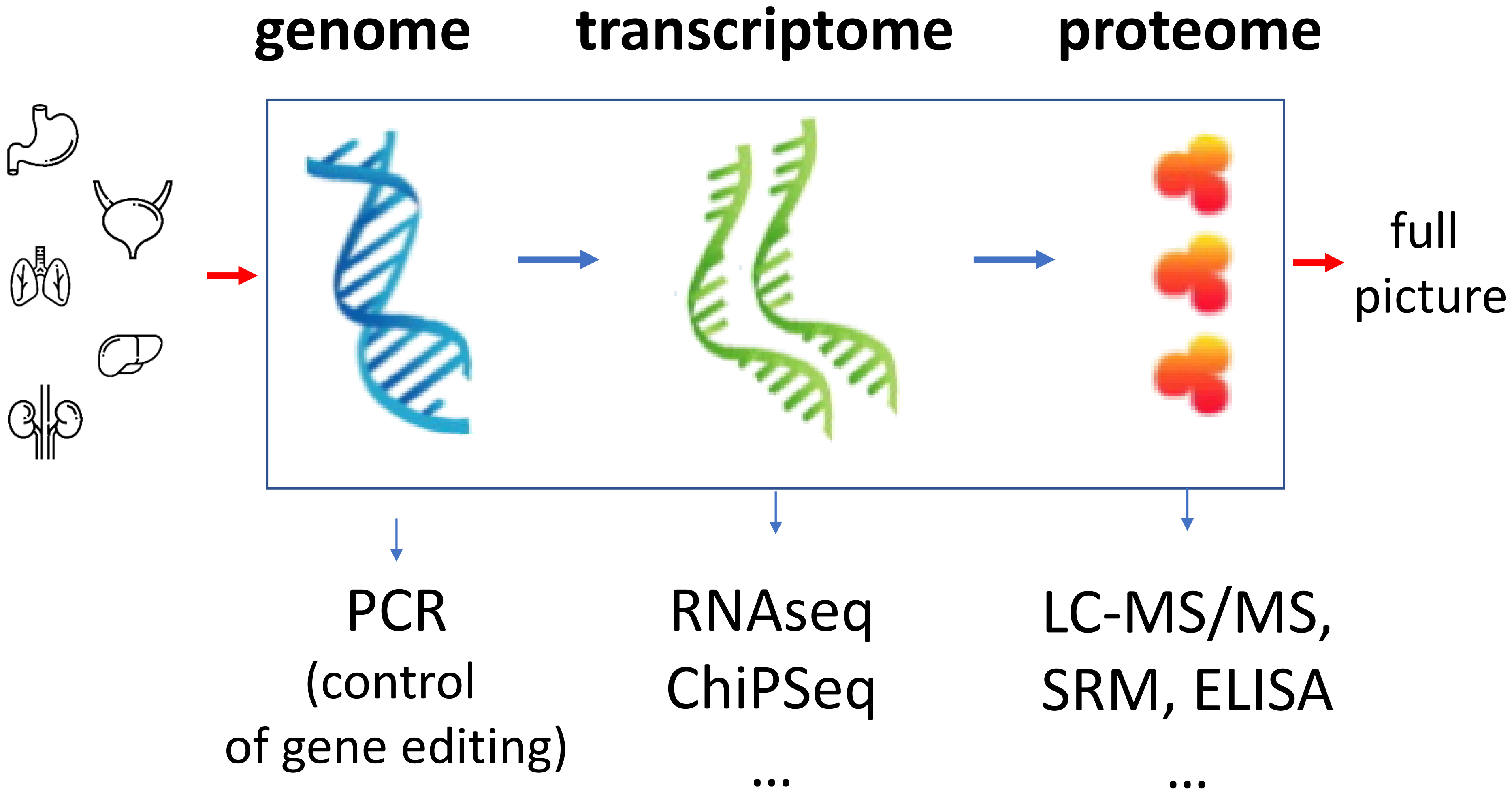
# COMPARISON OF METHODS

| | ZNFs | TALENs | CRISPR-Cas9 |
|---|---|---|---|
| **Target site length** | 18-36 bp per ZNF pair | 28-40 bp per TALEN pair | 19-22 bp |
| **Off-target effects** | Some mismatches are tolerated | Some mismatches are tolerated | Tolerant even of several mismatches |
| **Targeting constraints** | G-rich regions are challenging | 5’T for each TALEN in required | PAM is required |
| **Ease of design** | Difficult | Moderate | Easy |
| **Multiplexing** | Challenging | Challenging | Easy |
| **Ease of *in vivo* delivery** | Small size allows use of many viral vectors | Large size of each TALEN limits viral vectors | *S. Pyogenes* Cas9 is too large for smaller capacity viral vector |
| **Ease of *ex vivo* delivery** | Relatively easy | Relatively easy | Respectively easy |

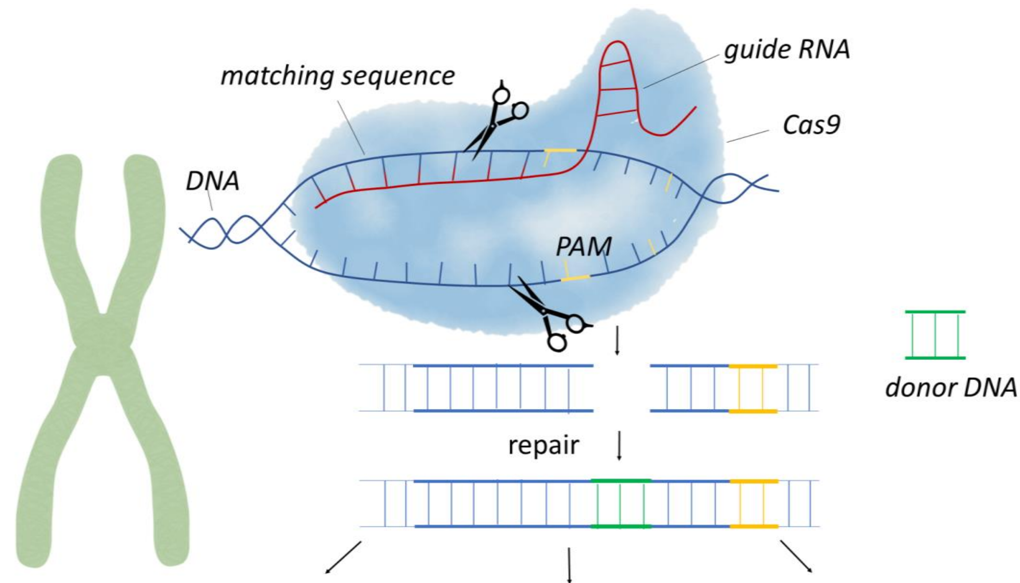
# SCHEME of CRISPR-Cas9 TECHNOLOGY



# EXPLORATION OF BIOLOGICAL INFORMATION



# APPLICATION FOR C-HPP

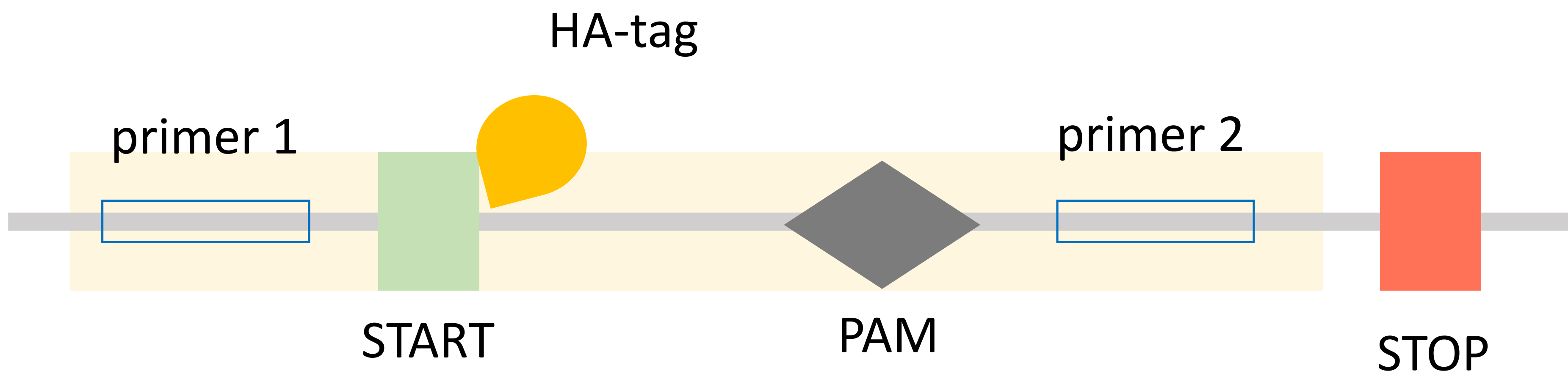



| challenge | knock-out | knock-down | knock-in | tagging |
|---|---|---|---|---|
| neXt-MP50 | - | - | + | + |
| neXt-CP50 | + | + | + | + |

# FUNCTIONAL ANNOTATION (PPI): INSERT TAG

TRANSCRIPTOME → prevailing transcript of target gene

GENOME → searching primers

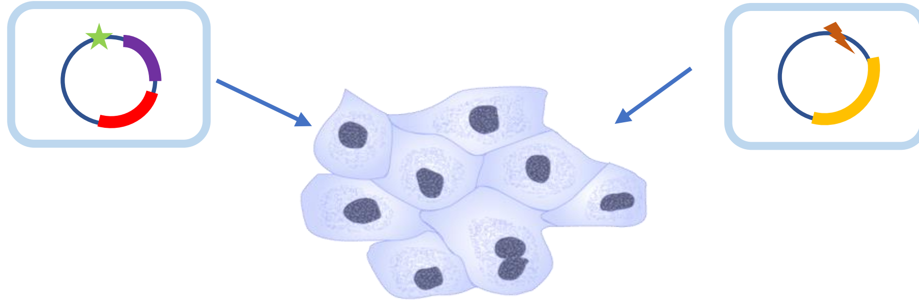


# GENE EDITING + AP-MS

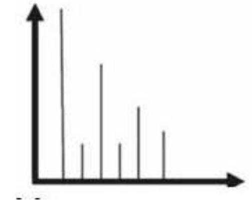
*transfection*

*Plasmid 1*
*(with guideRNA-Cas9 complex)*

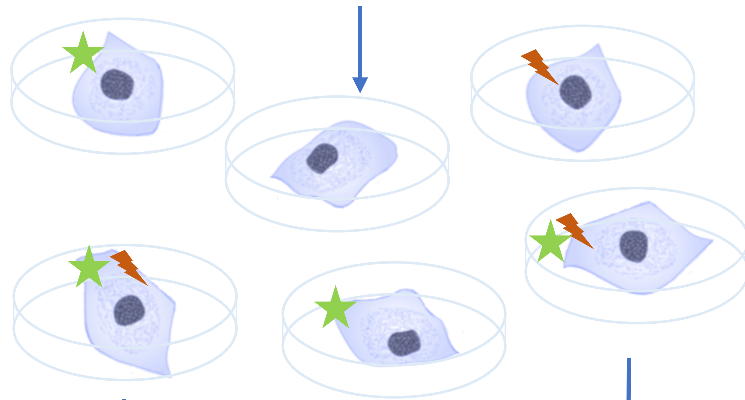
*Plasmid 2*
*(with changed sequence)*



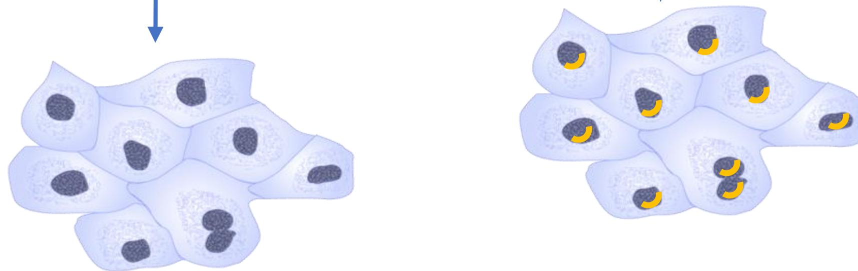
*MS-analysis*



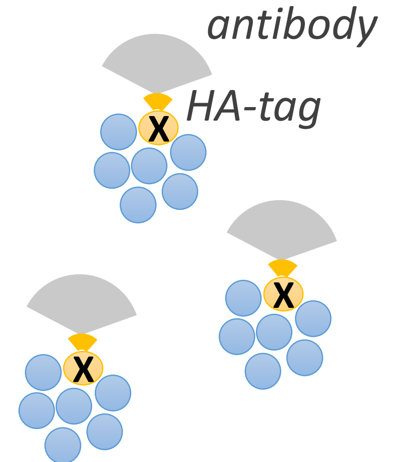
*selection*



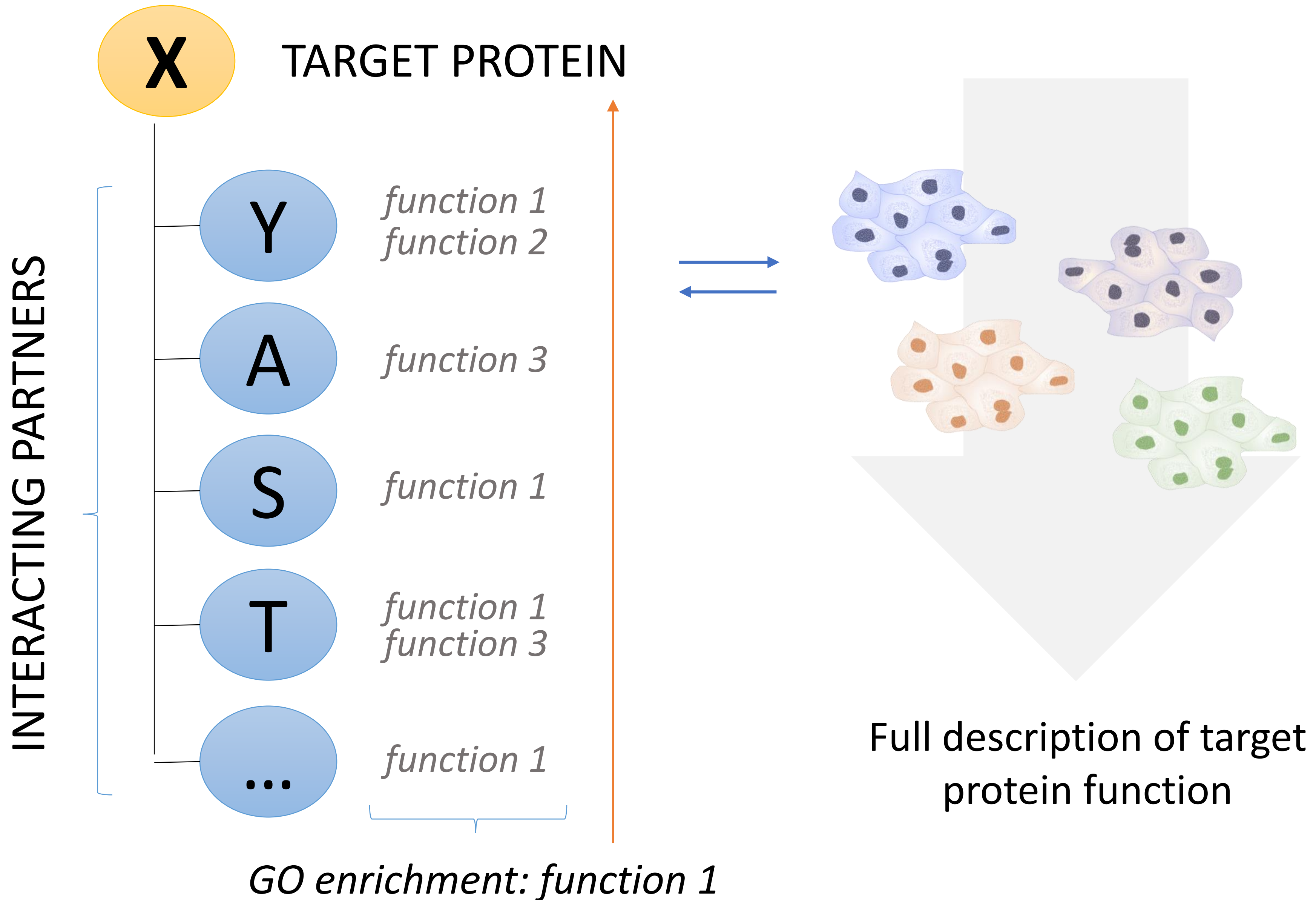
*growing*



*fishing*



# FUNCTION: GUILT-BY-ASSOCIATION



# THANK YOU FOR ATTENTION!

**Monument to the laboratory mouse** editing DNA itself.
Akademgorodok, Novosibirsk, Russia