Breakout Session

To develop base editing platforms

Xingxu Huang
ShanghaiTech University
Huangxx@shanghaitech.edu.cn

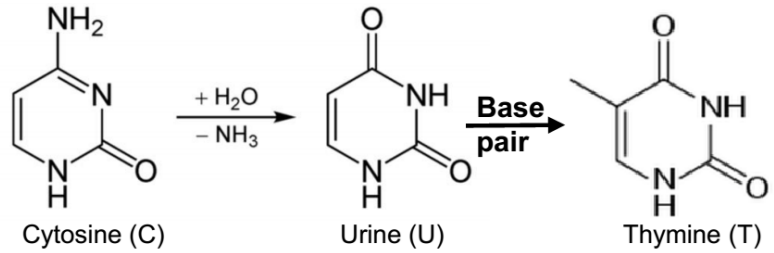
立志成才 报国裕民

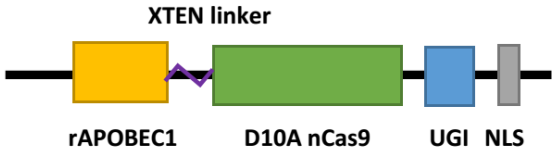
Base editing provides a more precise strategy

CBE: cytosine base editor

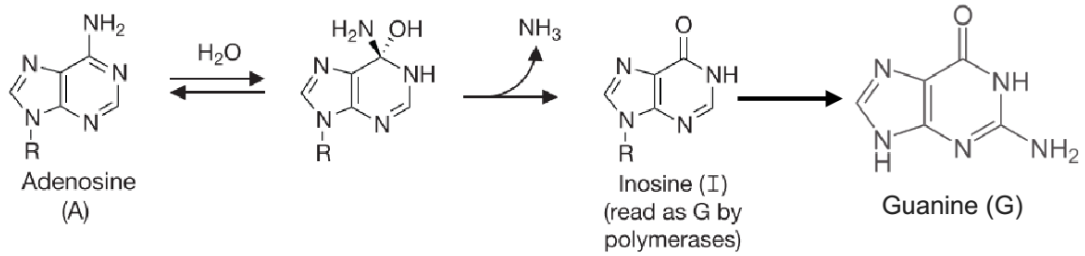
ABE: adenine base editor

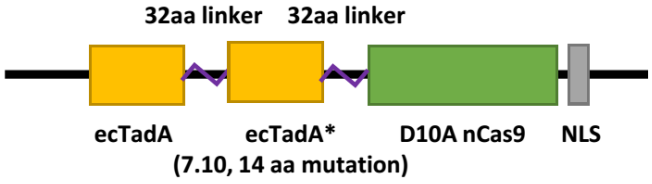
BE3





ABE

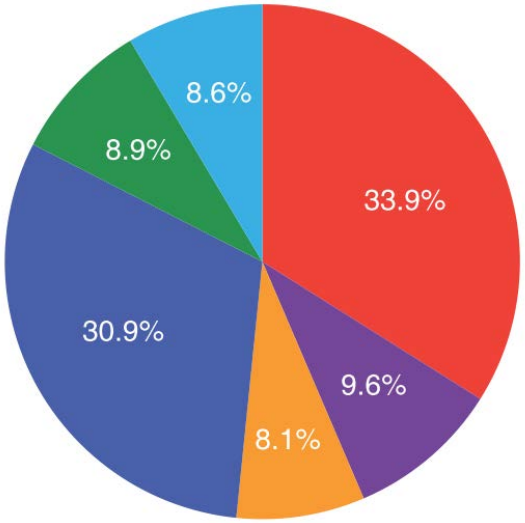




Perform programmable editing of a target base in genome by deamination without double-stranded DNA cleavage.

Komor et al. *Nature* 2016; Gaudelli et al. *Nature* 2017

Base pair changes required to model naturally-occurring human SNPs



Modeled by A·T →G·C (67.0% by ABE)
Modeled by A·T →C·G
Modeled by A·T →T·A
Modeled by C·G→T·A (71.2% by CBE)
Modeled by C·G→G·C
Modeled by C·G→A·T

(Total 555,109,482)

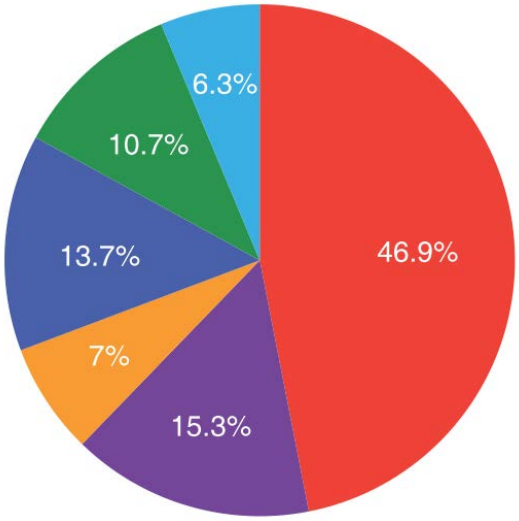
dbSNP: https://www.ncbi.nlm.nih.gov/SNP/

Current editable naturally-occurring human SNPs

PAM	Corrected by ABE	Precise correction by ABE (10.5%)	Corrected by CBE	Precise correction by CBE (11.9%)
Total	126,225,392 (100%)	58,434,362 (100%)	122,110,677 (100%)	66,062,154 (100%)
NG (xCas9/SpCas9-NG)	111,793,856 (88.6%)	50,642,619 (86.7%)	106,222,425 (87.0%)	55,003,882 (83.3%)
NGG (SpCas9)	36,118,948 (28.6%)	15,657,121 (26.8%)	31,682,039 (25.9%)	15,035,152 (22.8%)
NGA (VQR SpCas9)	44,155,390 (35.0%)	16,213,326 (27.7%)	45,180,539 (37.0%)	21,815,188 (33.0%)
NGAG (EQR SpCas9)	14,192,236 (11.2%)	5,208,662 (8,9%)	13,033,871 (10.7%)	6,175,091 (9.3%)
NGCG (VRER SpCas9)	2,240,558 (1.8%)	977,152 (1.7%)	1,667,471 (1.4%)	673,810 (1.0%)
NNNRRT (KKH SaCas9)	46,024,868 (36.5%)	17,655,489 (30.2%)	54,115,184 (44.3%)	26,168,072 (39.6%)
NNGRRT (SaCas9)	11,402,588 (9.0%)	4,744,162 (8.1%)	12,468,625 (10.2%)	5,973,411 (9.0%)

Precise correction: only 1 editable base in edit window (from positions 4 to 7 for ABE, or 4 to 8 for CBE) **(22.4%)**

Base pair changes required to correct pathogenic human SNPs



- Corrected by A·T →G·C (71.4% by ABE)
- Corrected by A·T →C·G
- Corrected by A·T →T·A
- Corrected by C·G→T·A (79.1% by CBE)
- Corrected by C·G→G·C
- Corrected by C·G→A·T

(Total 31,743)

ClinVar: https://www.ncbi.nlm.nih.gov/clinvar/

Current editable pathogenic human SNPs

PAM	Corrected by ABE	Precise correction by ABE (21.5%)	Corrected by CBE	Precise correction by CBE (4.1%)
Total	10,636 (100%)	6,810 (100%)	3,437 (100%)	1,312 (100%)
NG (xCas9/SpCas9-NG)	9,806 (92.2%)	6,148 (90.3%)	3,183 (92.6%)	1,140 (86.9%)
NGG (SpCas9)	3,853 (36.2%)	2,357 (34.6%)	1,236 (36.0%)	383 (29.2%)
NGA (VQR SpCas9)	3,524 (33.1%)	1,916 (28.1%)	1,395 (40.6%)	416 (31.7%)
NGAG (EQR SpCas9)	1,139 (10.7%)	611 (9.0%)	446 (13.0%)	116 (8.8%)
NGCG (VRER SpCas9)	394 (3.7%)	235 (3.5%)	178 (5.2%)	51 (3.9%)
NNNRRT (KKH SaCas9)	3,311 (31.1%)	1,786 (26.2%)	1,287 (37.4%)	425 (32.4%)
NNGRRT (SaCas9)	846 (8.0%)	439 (6.4%)	319 (9.3%)	102 (7.8%)

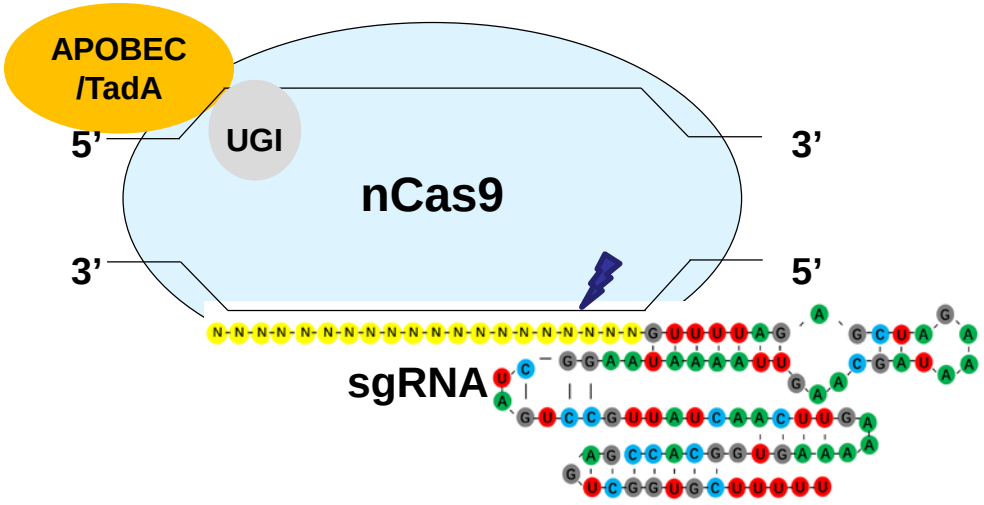
Precise correction: only 1 editable base in edit window (from positions 4 to 7 for ABE, or 4 to 8 for CBE) **(25.6%)**

Challenges for BE

- BE limitations
  - Base-editing product purity
  - Generation of indels
  - Off-target editing
  - Editing window and bystander edits
- Delivery
- Detection
- Preparation
- … …

Yin et al. *Nat Rev Drug Dis.* 2017, 16: 387-399.
Rees & Liu. *Nat Rev Genet.* 2018, 19(12): 770-788.

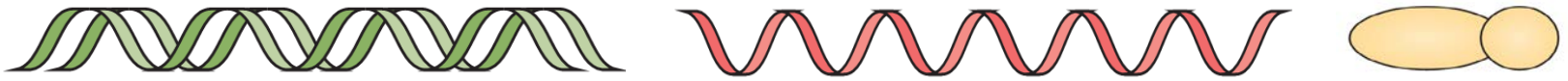
To improve BE



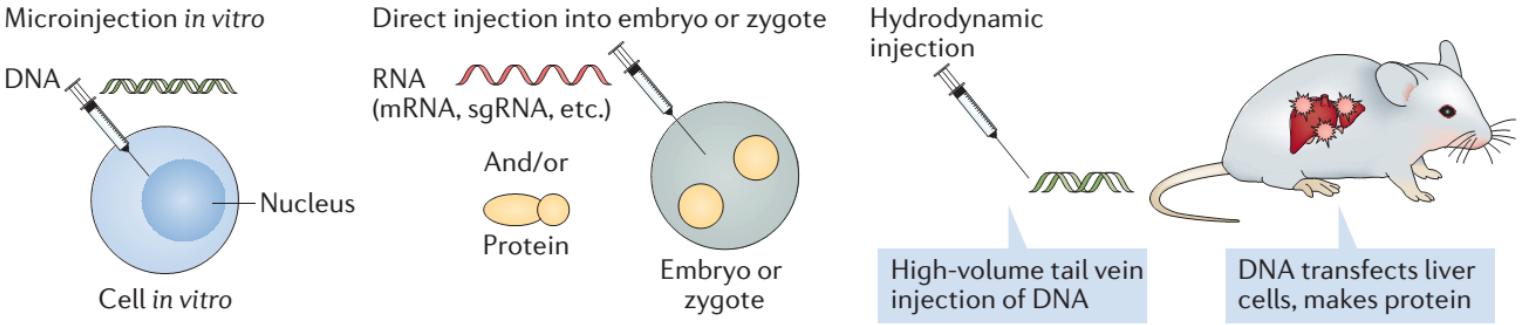
Rees & Liu. *Nat Rev Genet.* 2018, 19(12): 770-788.

To test different delivery strategies

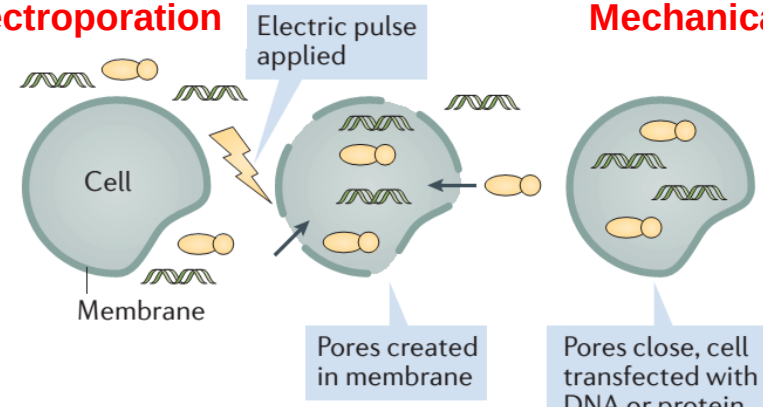
1. DNA **2. mRNA** **3. RNP**



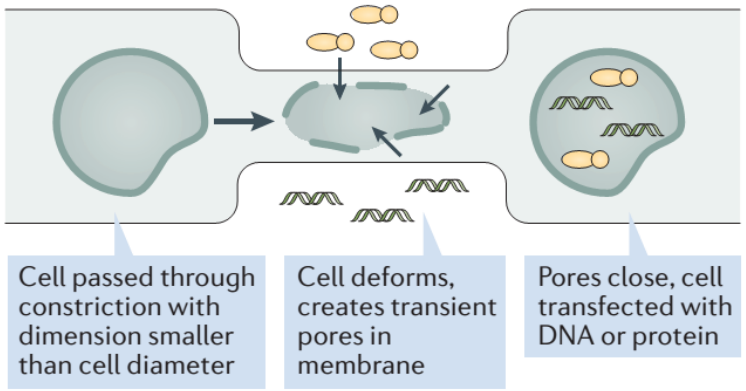
Injection



Electroporation

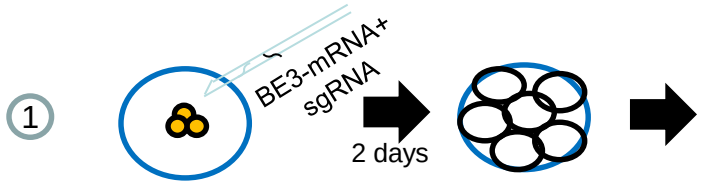


Mechanical deformation



Yin et al. *Nat Rev Drug Dis.* 2017, 16: 387-399.

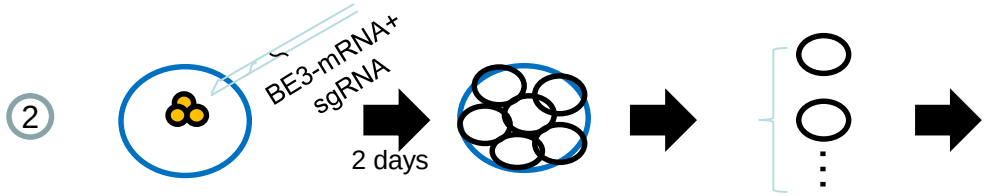
To develop different detections & analysis



Tripronuclear embryos

Collection & Detection

- ◆Genotyping
- ◆Phenotyping

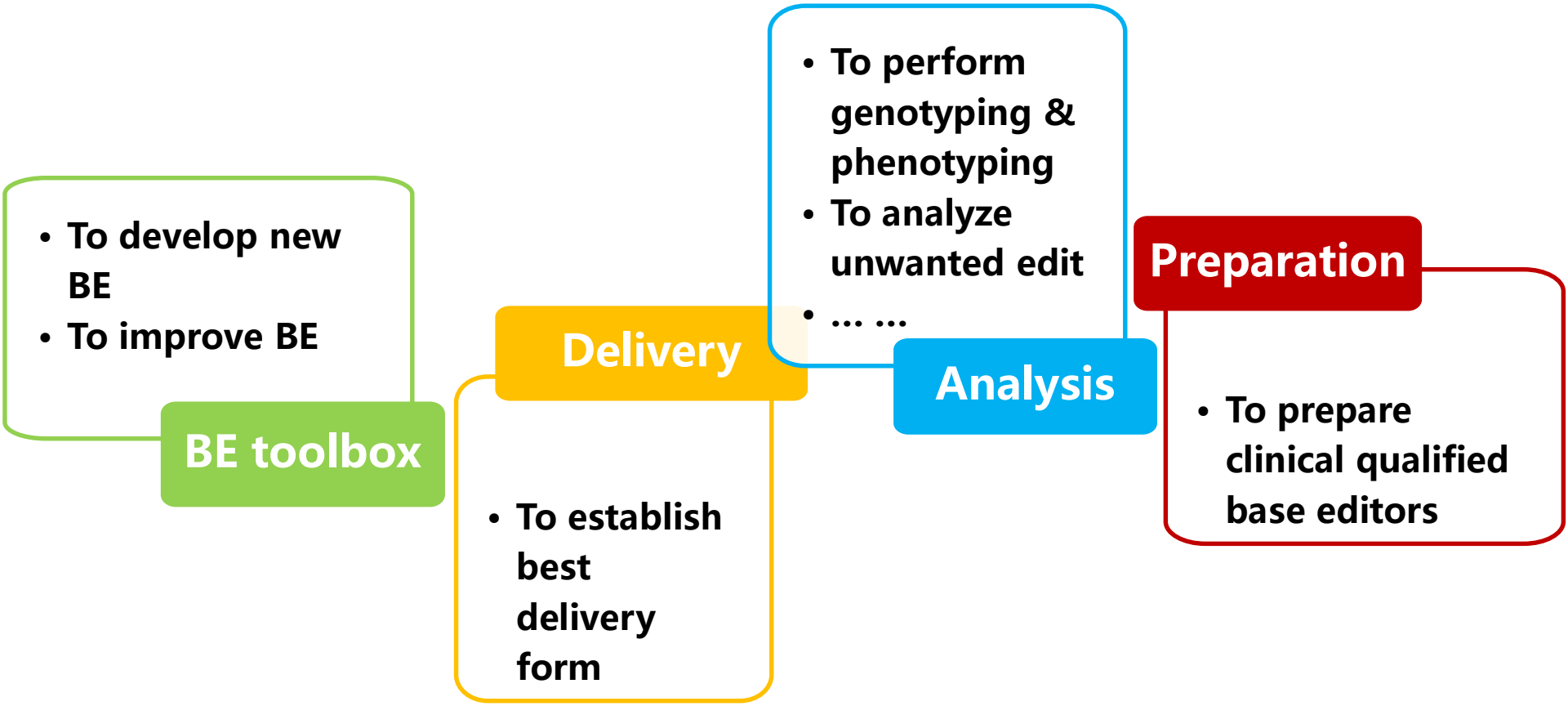


Tripronuclear embryos

Individual blastomere

Collection & Detection

To develop base editing platforms



上海科技大学
ShanghaiTech University

THANK YOU

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