

Novel Ligands and Modalities for Purifying Gene-editing Products: CRISPR and Viral Vectors

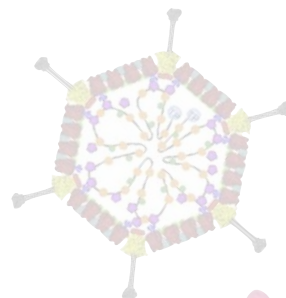
Stefano Menegatti & BioPep Team

Chemical and Biomolecular Engineering

NC Initiative in Viral Vector Research and Learning

NC State University

NCVVIRAL



Recovery of Biological Products XIX
Roma, July 10-15, 2022

COI Disclosure

Stefano Menegatti



Associate Professor, University Faculty Scholar
Chemical and Biomolecular Engineering
Biomanufacturing Training and Education Center (BTEC)
NC State University



Chief Technical Officer
LigaTrap Technologies

To the best of my knowledge and belief any actual, perceived or potential conflicts between my duties as an employee and my private and/or business interests are fully disclosed in accordance with the requirements of NC State University's Conflict of Interest Policy.



GENE-EDITING
TOOLBOX

NEW PURIFICATION
PARADIGMS

CRISPR
CAS

VIRAL
VECTORS

NC-VVIRAL

CRISPR CAS

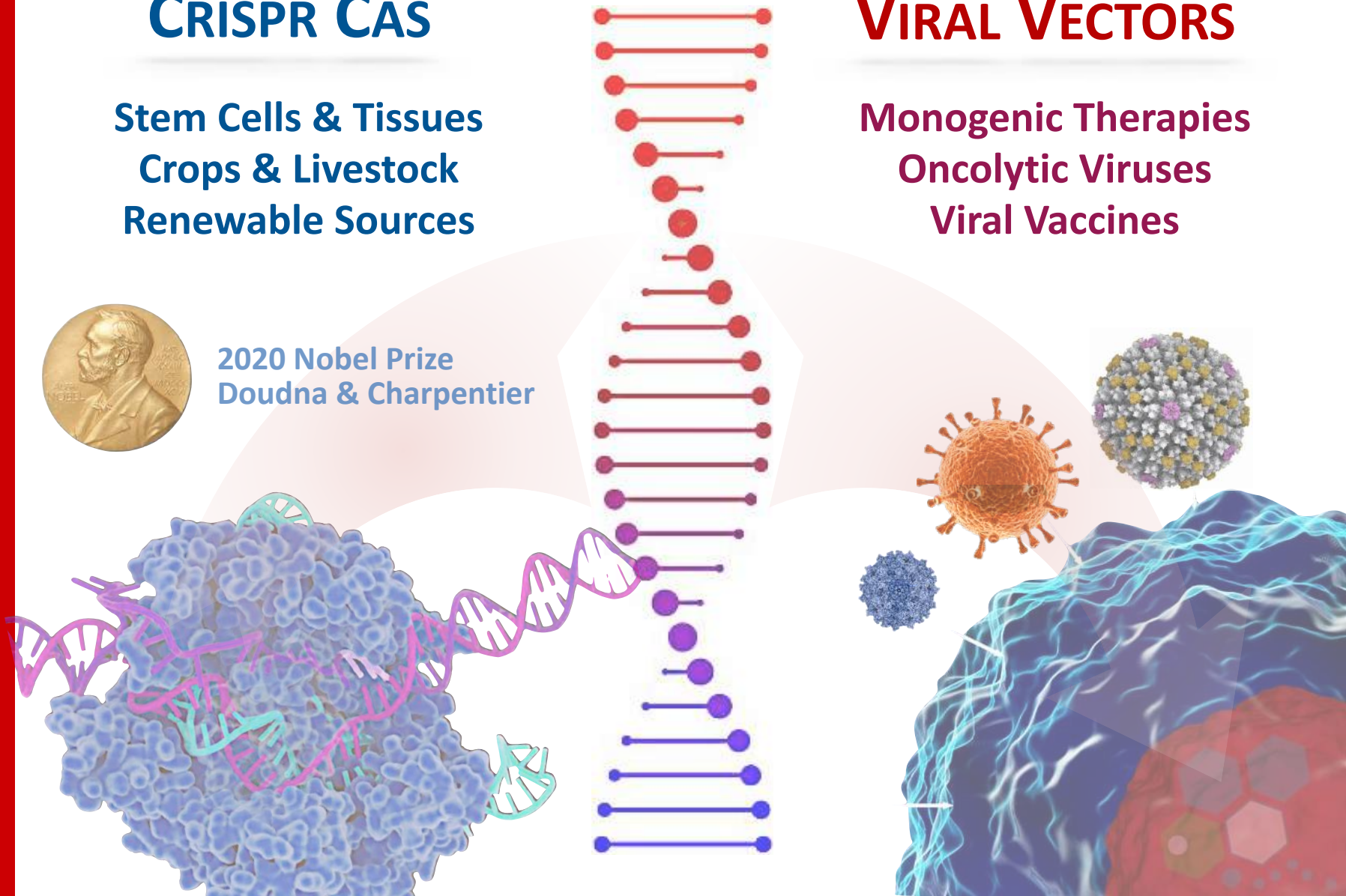
Stem Cells & Tissues
Crops & Livestock
Renewable Sources



2020 Nobel Prize
Doudna & Charpentier

VIRAL VECTORS

Monogenic Therapies
Oncolytic Viruses
Viral Vaccines



GENE-EDITING
TOOLBOX

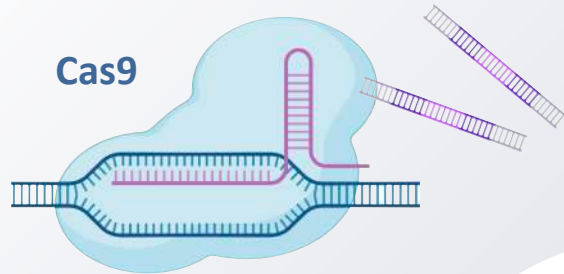
NEW PURIFICATION
PARADIGMS

CRISPR
CAS

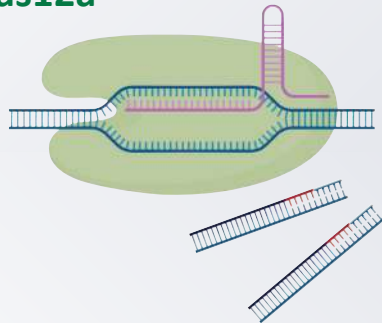
VIRAL
VECTORS

NC-VVIRAL

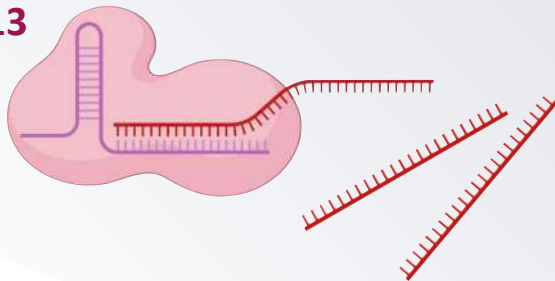
CRISPR CAS



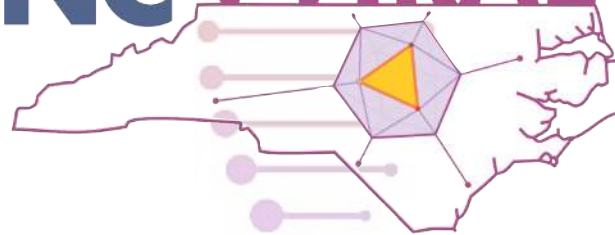
Cas12a



Cas13



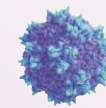
NC VVIRAL



VIRAL VECTORS



Lentivirus (LV)



AdenoAssociated
Virus (AAV)

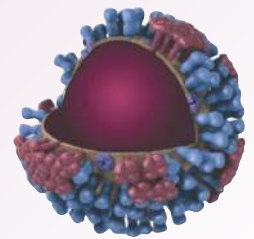


Adenovirus
(Ad)

OTHER VECTORS



Exosomes



VLPs

GENE-EDITING
TOOLBOX

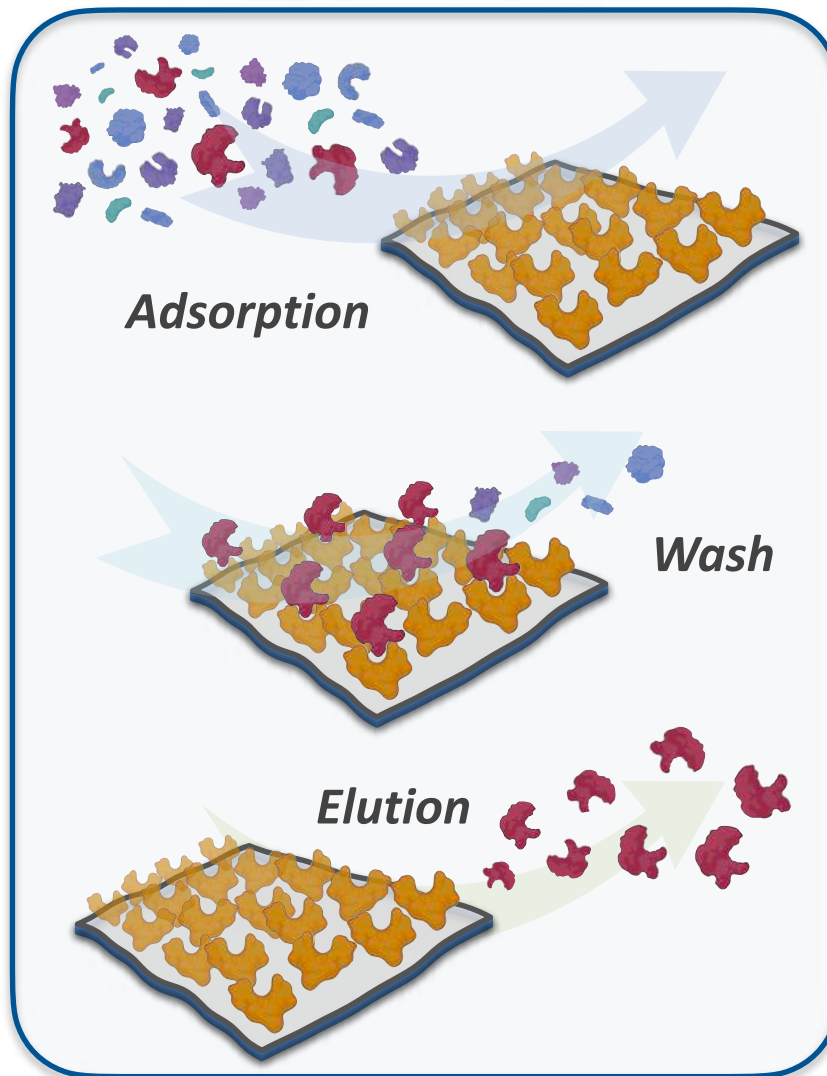
NEW PURIFICATION
PARADIGMS

CRISPR
CAS

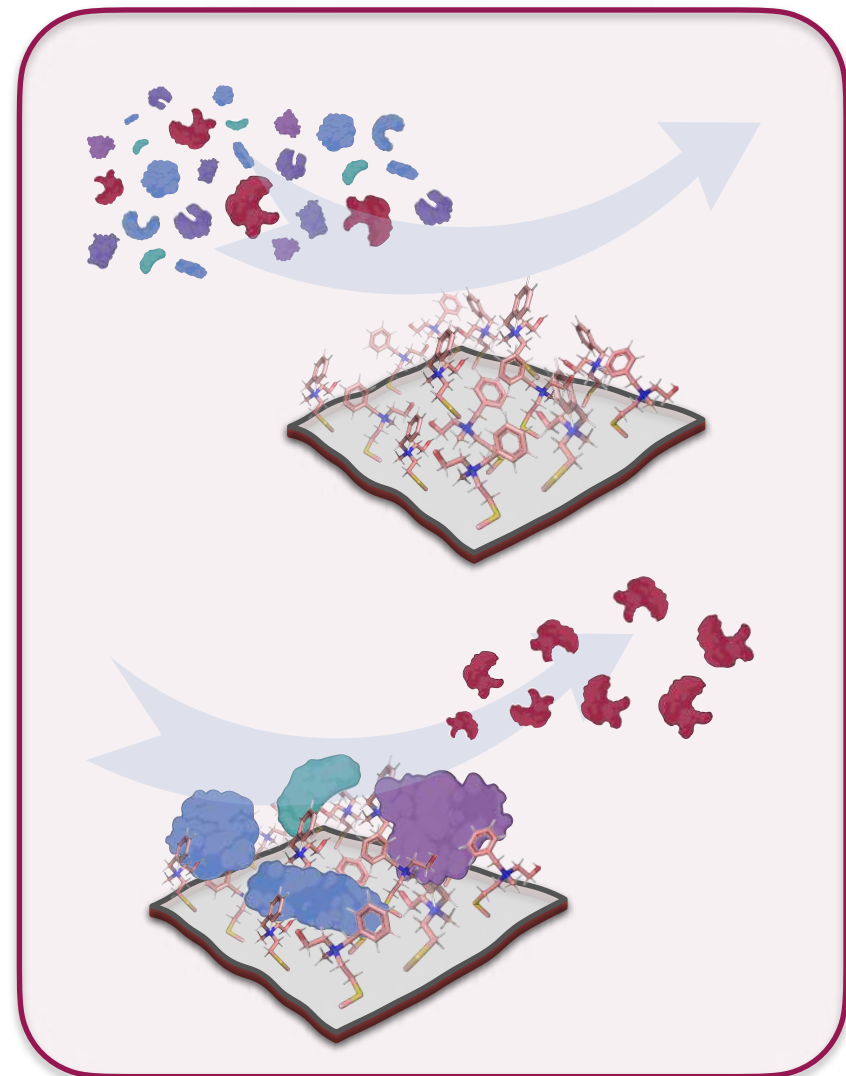
VIRAL
VECTORS

NC-VVIRAL

BIND-&-ELUTE



FLOW-THROUGH



GENE-EDITING
TOOLBOX

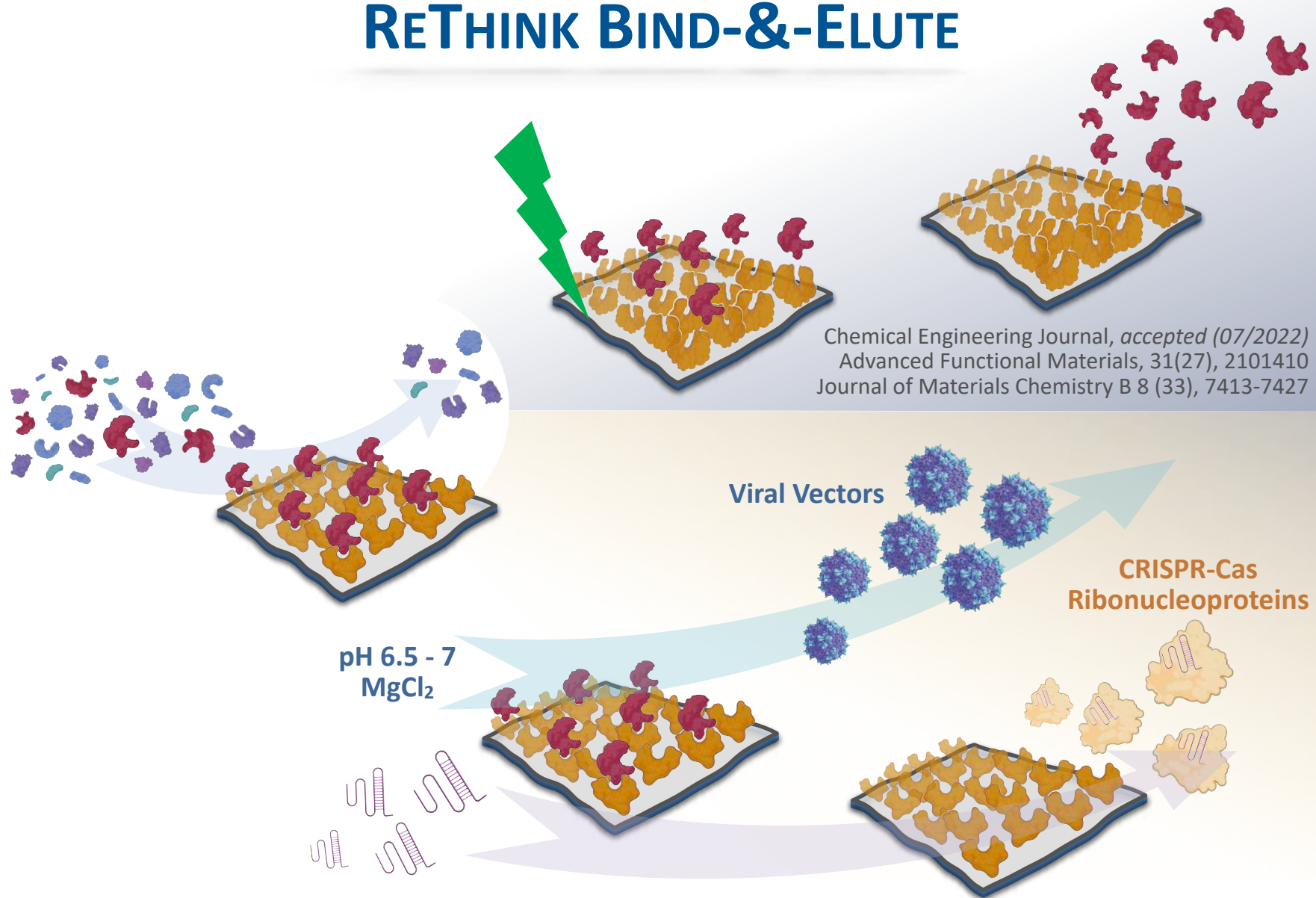
NEW PURIFICATION
PARADIGMS

CRISPR
CAS

VIRAL
VECTORS

NC-VVIRAL

RETHINK BIND-&-ELUTE



GENE-EDITING
TOOLBOX

NEW PURIFICATION
PARADIGMS

CRISPR
CAS

VIRAL
VECTORS

NC-VVIRAL

RETHINK FLOW-THROUGH: LIGAGUARD™

BACTERIAL



E. coli cells

Cas9
Cas12a
Cas13

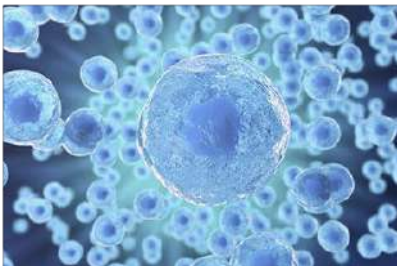
P. pastoris cells

scFv
Cas9
Cas12a

FUNGAL

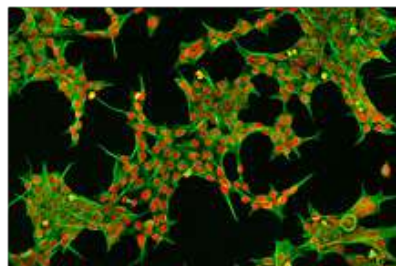


MAMMALIAN



CHO cells

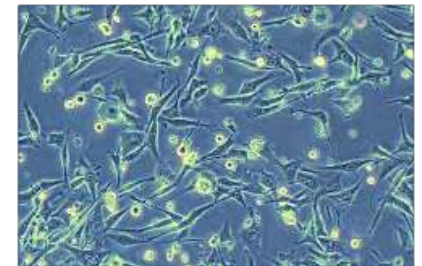
mAbs
Blood Factors



HEK cells

Vero cells

Ad, AAV, LV, ...



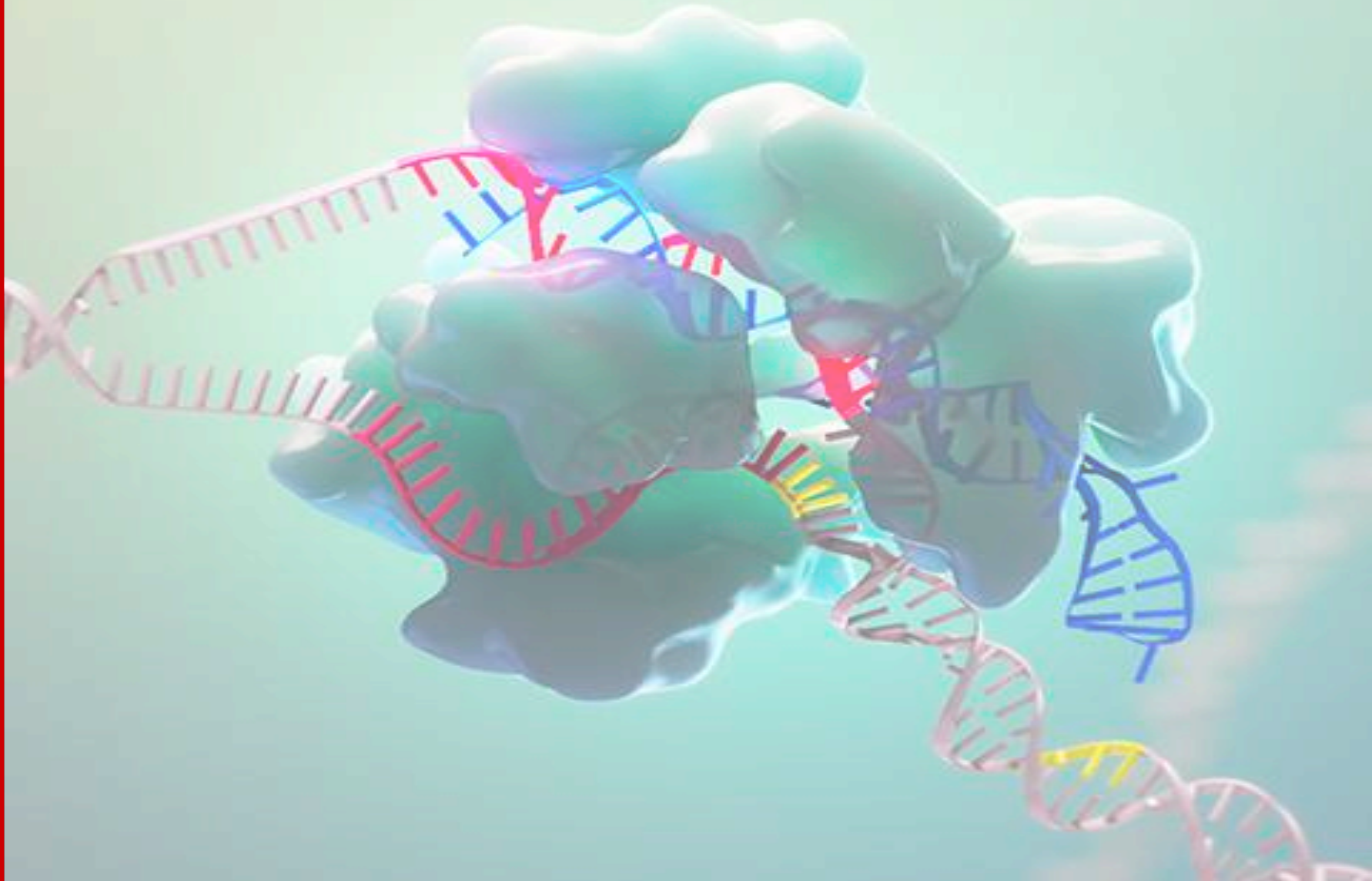
GENE-EDITING
TOOLBOX

NEW PURIFICATION
PARADIGMS

CRISPR
CAS

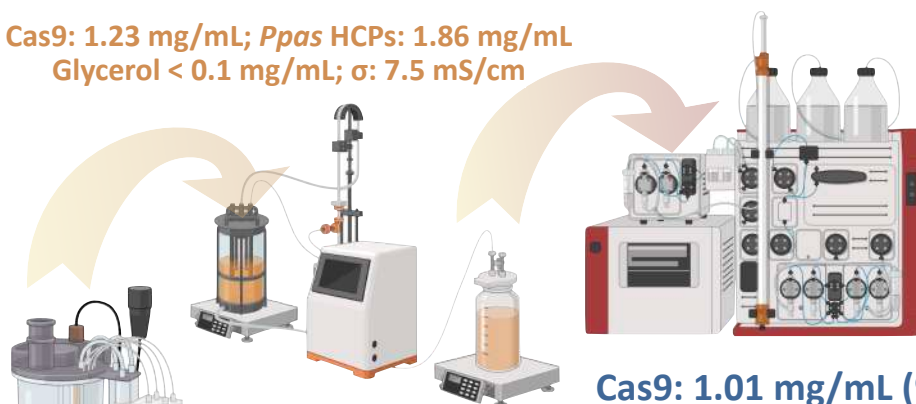
VIRAL
VECTORS

NC-VVIRAL



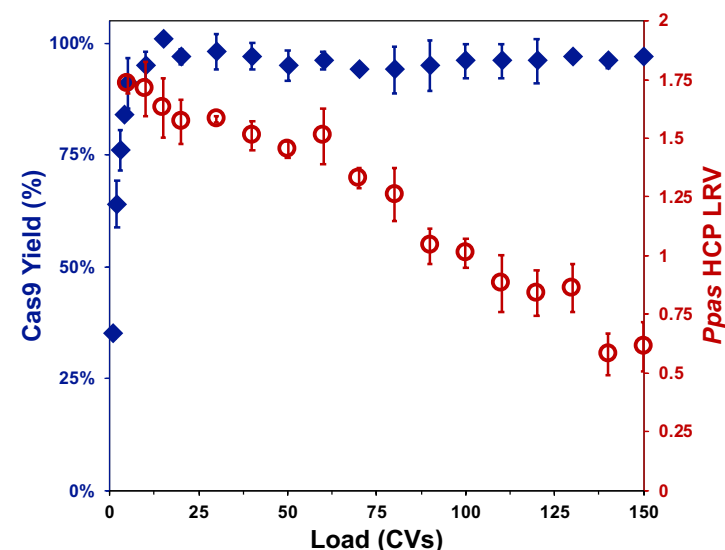
PPas LIGAGUARD™ FOR CRISPR-Cas9 PURIFICATION

Cas9: 1.23 mg/mL; Ppas HCPs: 1.86 mg/mL
Glycerol < 0.1 mg/mL; σ : 7.5 mS/cm



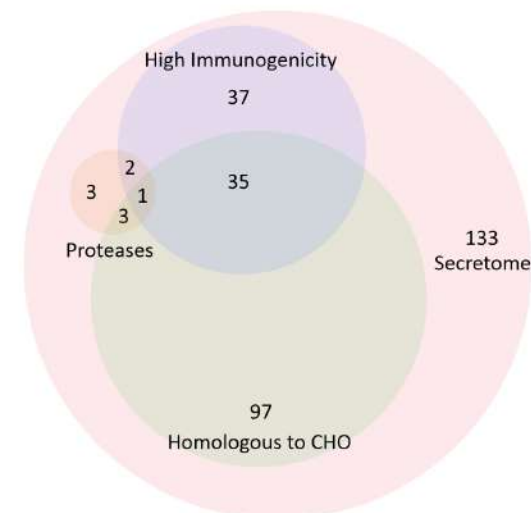
Cas9: 0.75 mg/mL
Ppas HCPs: 1.1 mg/mL
Glycerol: 5% v/v
 σ : 81.6 mS/cm

Cas9: 1.01 mg/mL (94.5%)
Ppas HCPs < 0.06 mg/mL
(75 CVs)



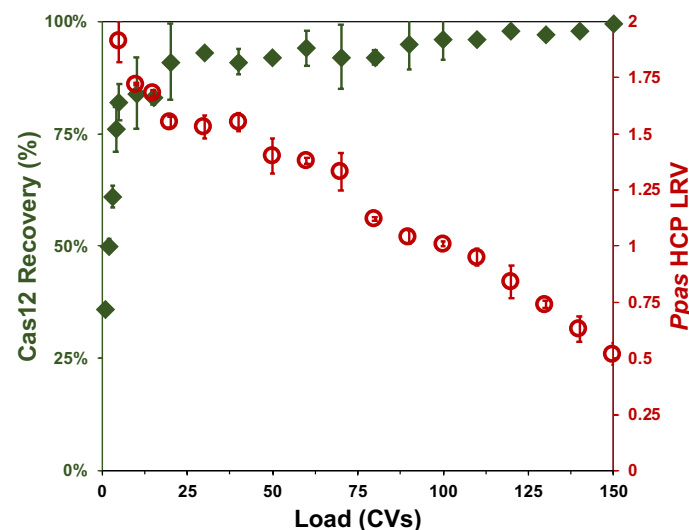
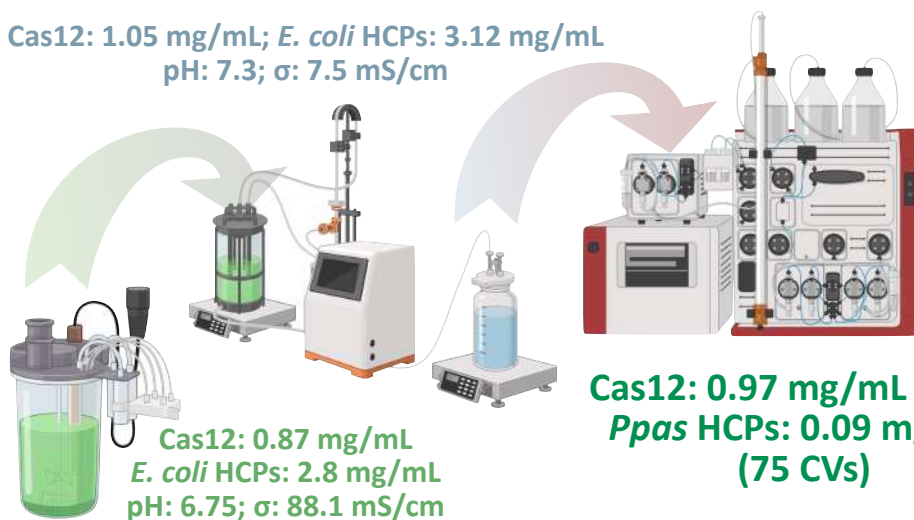
Cleared Ppas HCP classes

- A. **Proteases:** Proteinase A, Peptidase A1, ...
- B. **Immunogenic:** Plasma Membrane ATPase, 60s Ribosomal protein, ...
- C. **CHO Homologs:** Histone H3, Thioredoxin, ...
- D. **Immunogenic Proteases:** Lysophospholipase, Aspartic protease, ...
- E. **CHO-Homolog Proteases:** Aspartyl protease, Ubiquitin-specific protease
- F. **CHO Homologous Immunogenic Proteases:** Lon protease homolog, ...



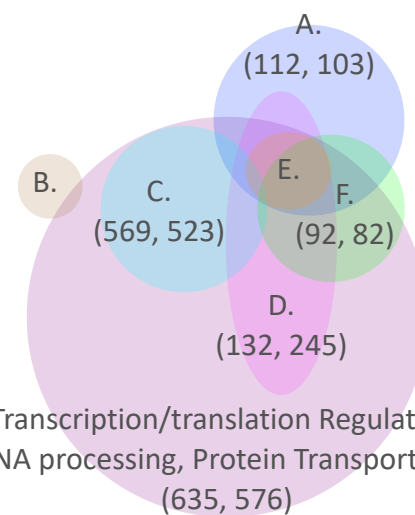
E. COLI LIGAGUARD™ FOR CRISPR-Cas12 PURIFICATION

Cas12: 1.05 mg/mL; *E. coli* HCPs: 3.12 mg/mL
pH: 7.3; σ : 7.5 mS/cm



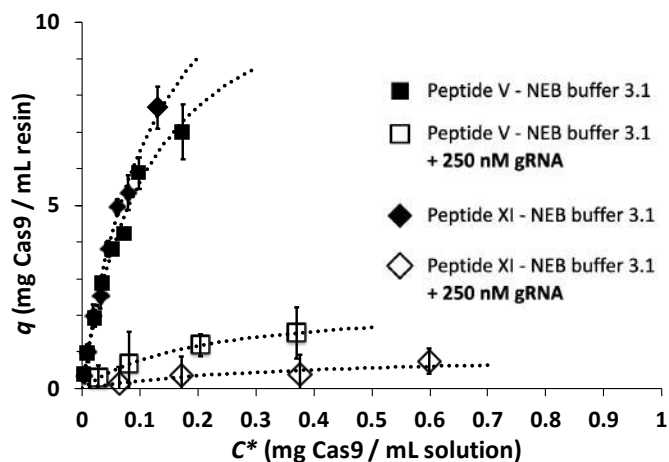
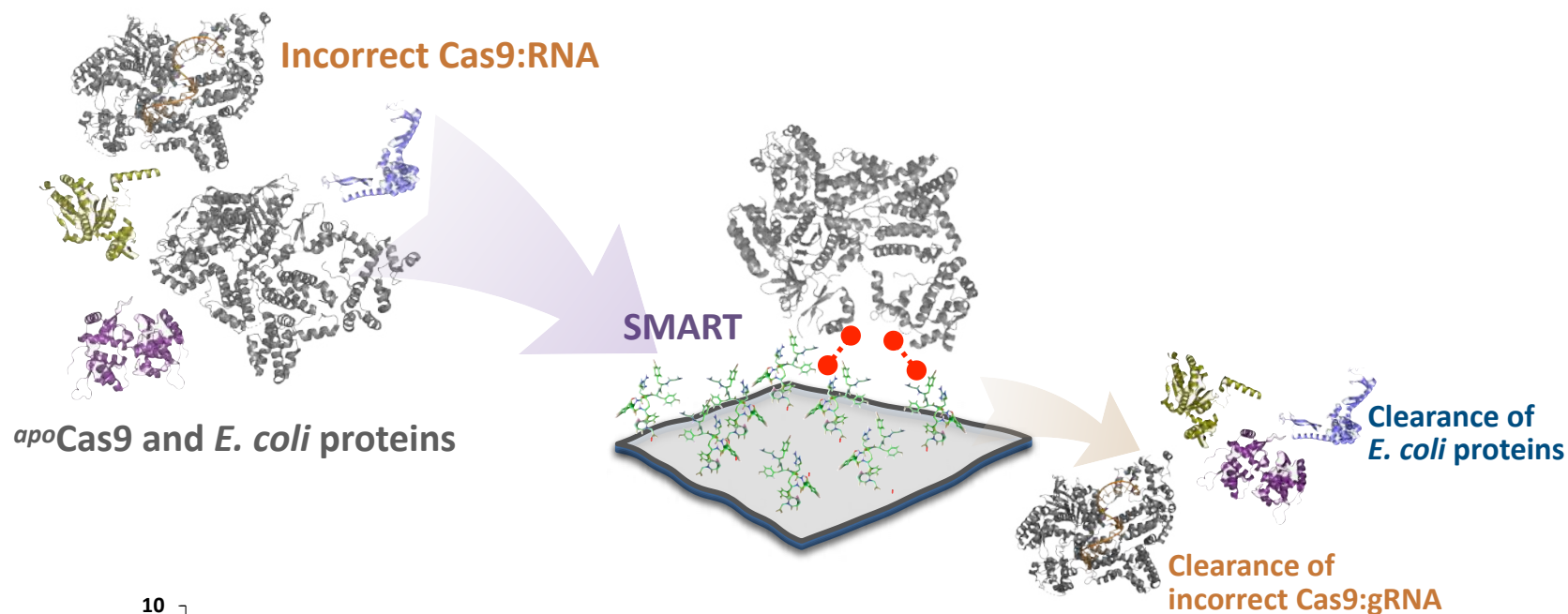
Cleared *E. coli* process-related impurities

- A. Proteases:** Aminopeptidase N, Lysine decarboxylase, ...
- B. Toxins:** Lipopolysaccharide (LPS), ...
- C. Metabolism/biosynthesis:** Pyruvate Dehydrogenase, Pyruvate kinase I, ...
- D. Immunogenic:** Enolase, Flagellin, ...
- E. Immunogenic Proteases:** Pyruvate Dehydrogenase and Pyruvate kinase I
- F. CHO-Homolog Proteases:** Xylanase, Aspartic protease, ...
- G. CHO Homologous Immunogenic Proteases:** Glyc-3-P dehydrogenase A, Heat shock protein 60, ...

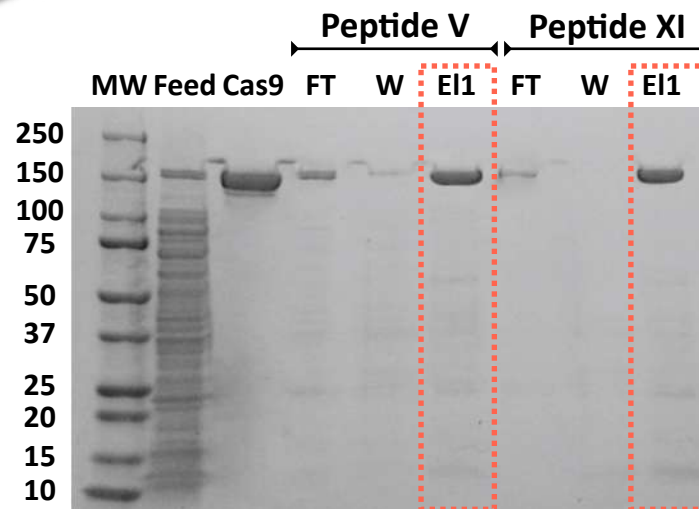
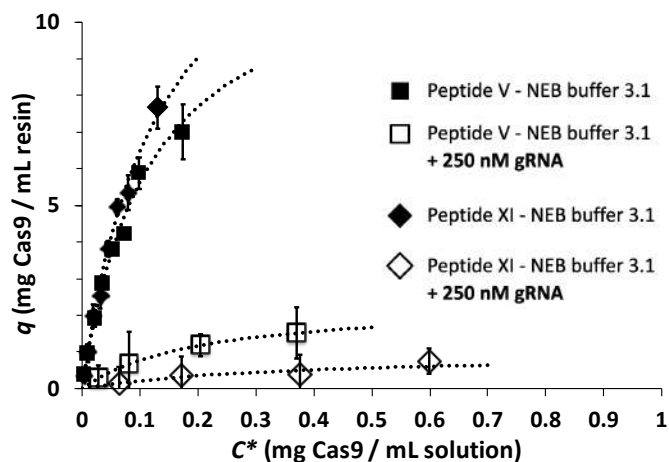
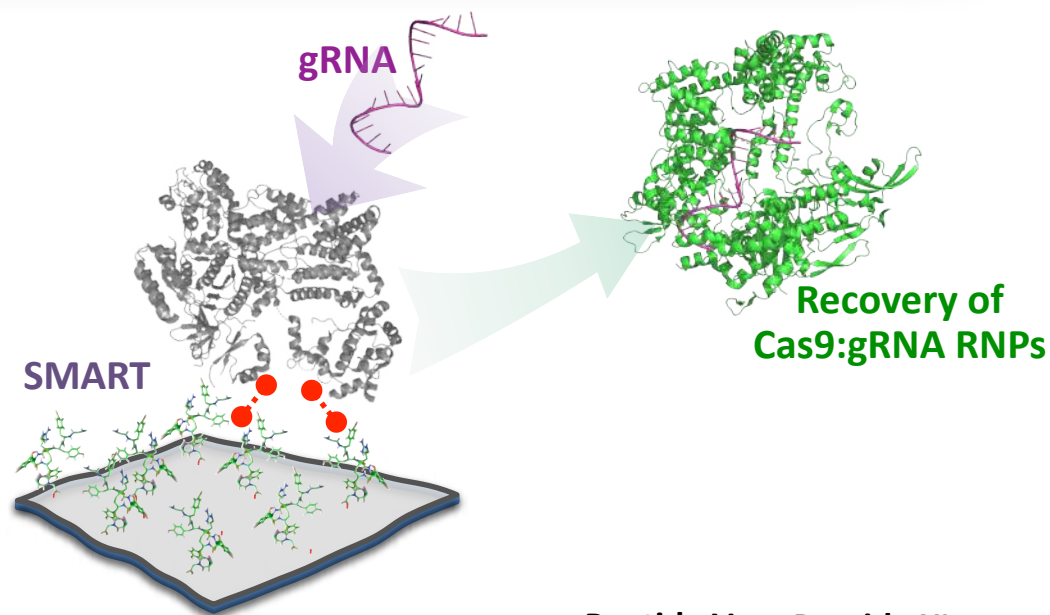


Transcription/translation Regulators,
DNA processing, Protein Transport, etc.
(635, 576)

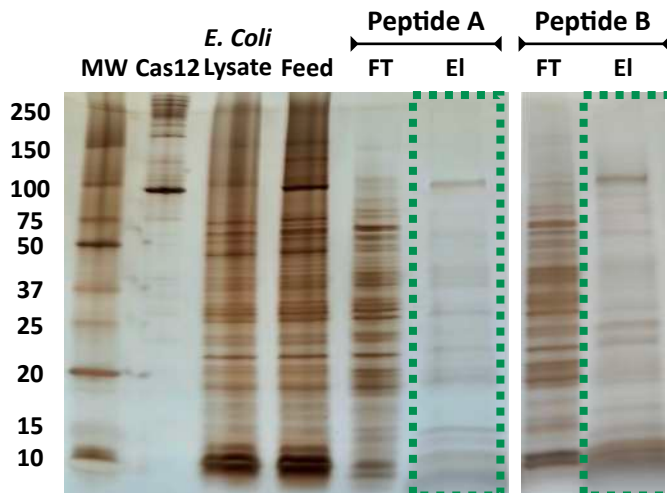
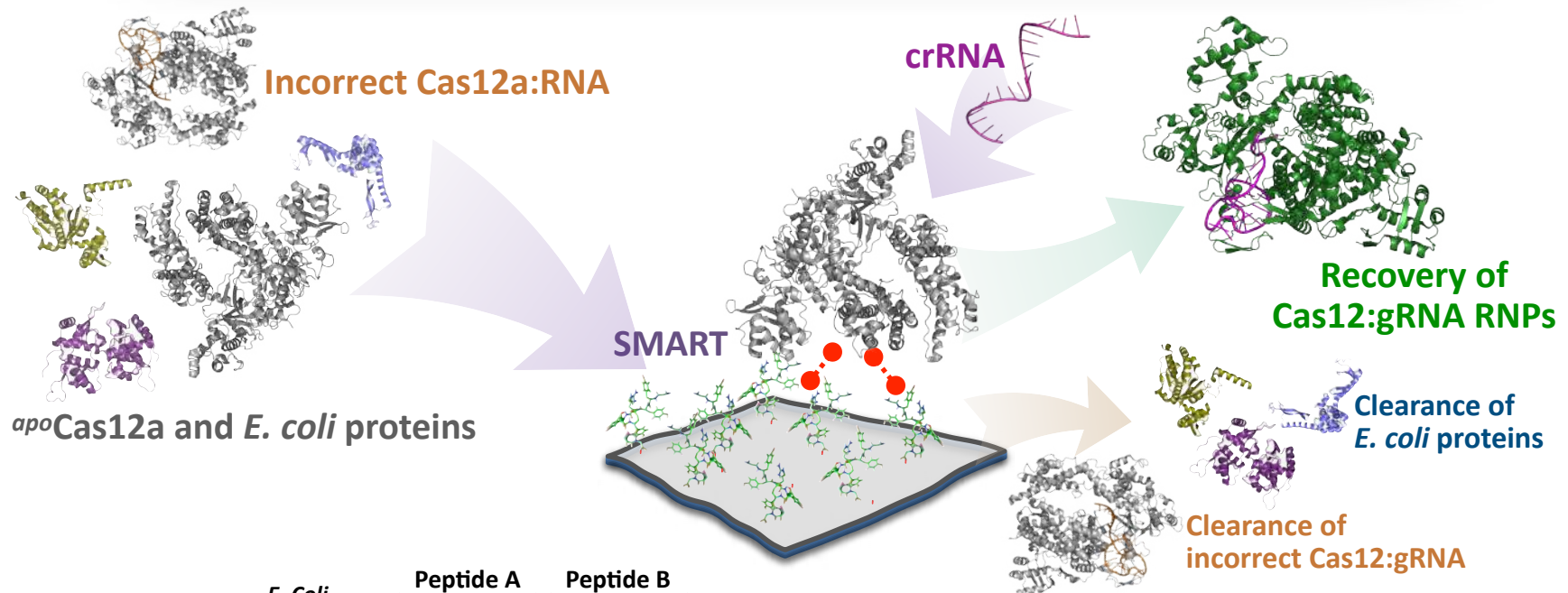
SMART - STERICALLY-MODULATED AFFINITY BY RNA TRIGGER



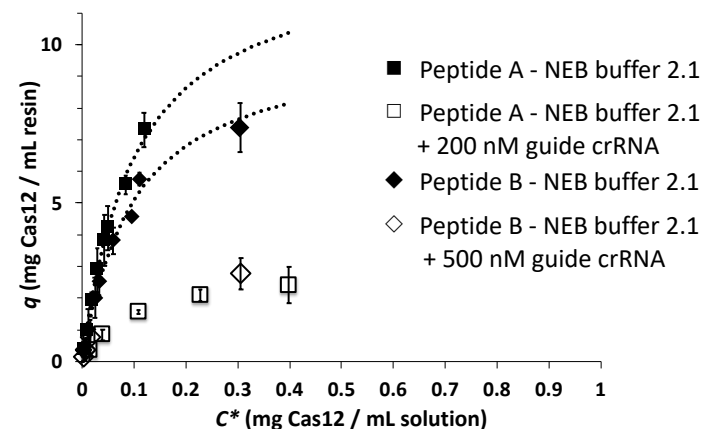
SMART - STERICALLY-MODULATED AFFINITY BY RNA TRIGGER



SMART - STERICALLY-MODULATED AFFINITY BY RNA TRIGGER



Cas12 SMART Binding Isotherms



GENE-EDITING
TOOLBOX

NEW PURIFICATION
PARADIGMS

CRISPR
CAS

VIRAL
VECTORS

NC-VVIRAL



GENE-EDITING
ToolBox

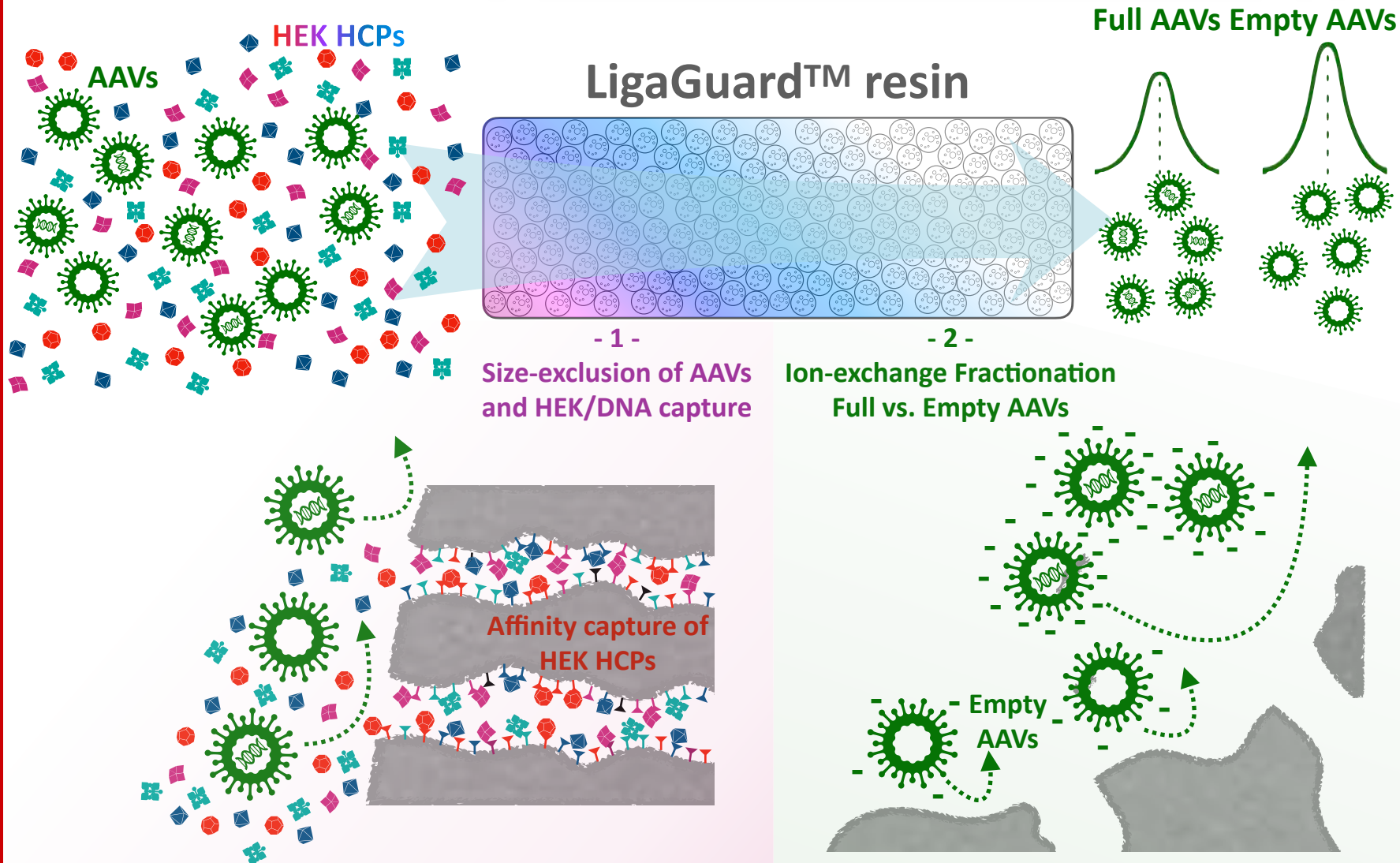
NEW PURIFICATION
PARADIGMS

CRISPR
CAS

VIRAL
VECTORS

NC-VVIRAL

LigaTrap[®] LIGA GUARD[™] FOR VIRAL VECTOR PURIFICATION



GENE-EDITING
TOOLBOX

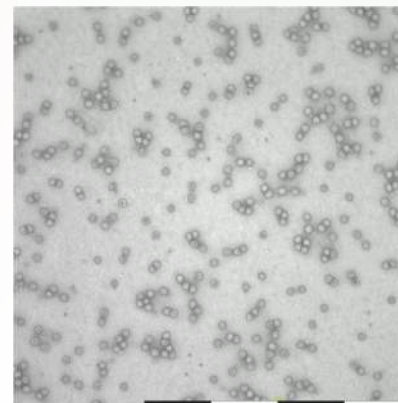
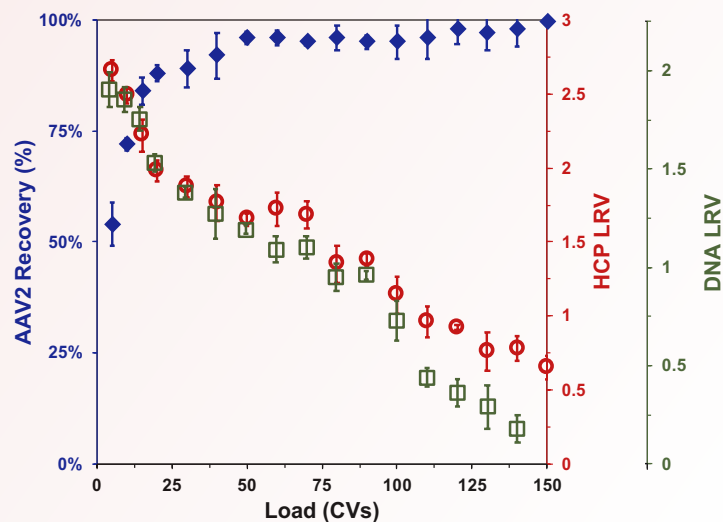
NEW PURIFICATION
PARADIGMS

CRISPR
CAS

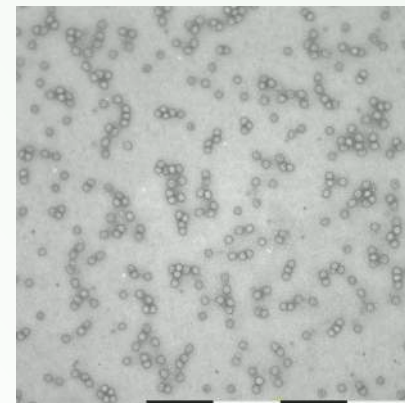
VIRAL
VECTORS

NC-VVIRAL

LigaTrap[®] LIGA GUARD[™] FOR VIRAL VECTOR PURIFICATION



Empty-rich peak



Full-rich peak

Cleared HEK293 HCP classes

A. Apoptosis regulators: Caspases, Kinases, ...

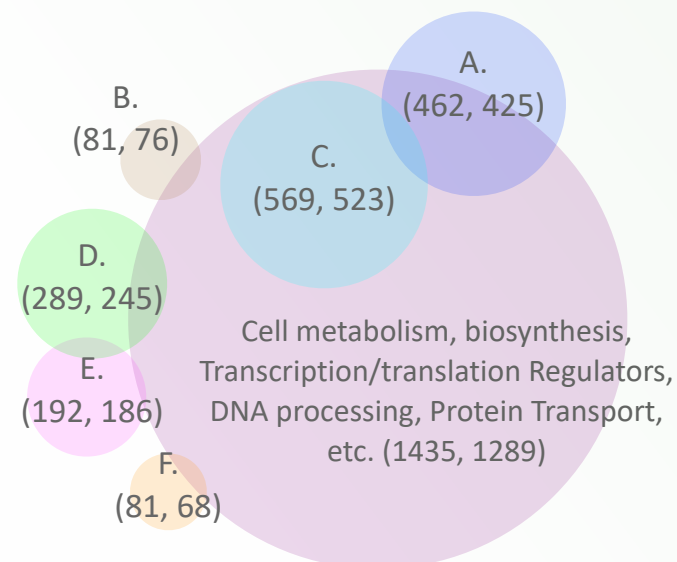
B. Stress response: Heat shock proteins, mitogen-activated kinases, ...

C. Proteases: Cathepsins, Carboxypeptidases, Matrix metalloproteases, Serine proteases, Serpins, Cystatins, ...

D. Chromatin regulators: Histone cluster members, histone deacetylase, Lysine demethylase / methyltransferase, ...

E. Chaperones: Clusterin, Peptidylprolyl isomerases, Proteasome assem.,

F. Lipid degradation: Lipases, Lipoprotein lipase, Phospholipases



GENE-EDITING
TOOLBOX

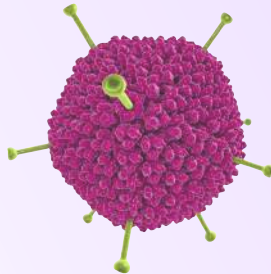
NEW PURIFICATION
PARADIGMS

CRISPR
CAS

VIRAL
VECTORS

NC-VVIRAL

NCVVIRAL



- Lentivirus -
LV
(VSV-G or full envelope)

DBC_{10%}: $5.8 \cdot 10^9$ - $1.2 \cdot 10^{11}$ TU/mL

El: $4.7 \cdot 10^7$ - $1.1 \cdot 10^9$ TU/mL

0.2 NaCitr + 0.5 M MgCl₂, pH 6.5

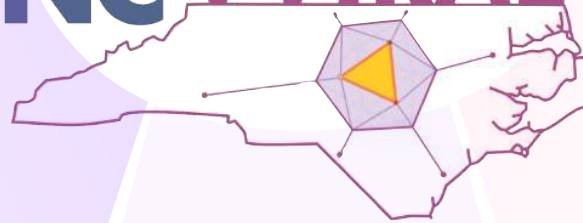
0.15 M NaCl in PBS, pH 6.0

Yield: 57 - 68% (qPCR)

Yield: 46 - 58% (TA)

HCP LRV: 1.75 - 2.01

pDNA LRV: 0.92 - 1.62



- Adenoassociated Virus -
AAV1 - AAV12 (VP2-VP3)
rAAV2, 6, 8 and retroAAV2

DBC_{10%}: $5 \cdot 10^{13}$ - $3 \cdot 10^{14}$ vp/mL

El: $2.1 \cdot 10^{13}$ - $7.5 \cdot 10^{13}$ vp/mL

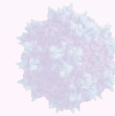
0.5 M MgCl₂, pH 6.5

0.1 M Arg in PBS, pH 6.8

Yield: 65 - 81% (ELISA)

HCP LRV: 2.14 - 2.85 (ELISA)

pDNA LRV: 1.51 - 1.92



- Adenovirus -
Ad5

Ad17, Ad11, Ad35

DBC_{10%}: TBD

El: $3.1 \cdot 10^8$ - $7.1 \cdot 10^8$ TU/mL

0.35 M NaCl in PBS, pH 7.4

Yield: 22 - 43% (ELISA)

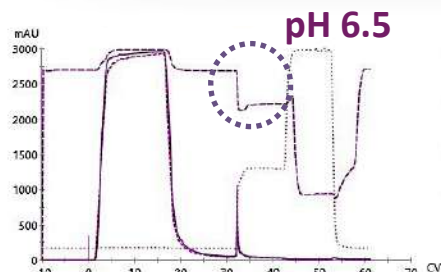
Yield: 13 - 22% (qPCR)

HCP LRV: 1.55 - 2.05 (ELISA)

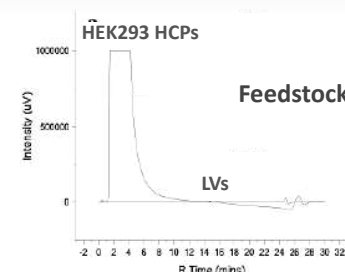
pDNA LRV: TBD

LENTIVIRUS PURIFICATION IN BIND-AND-ELUTE MODE

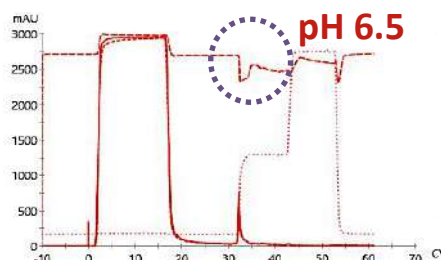
PLenty
#1



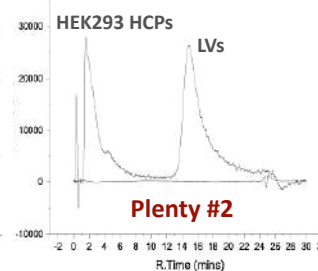
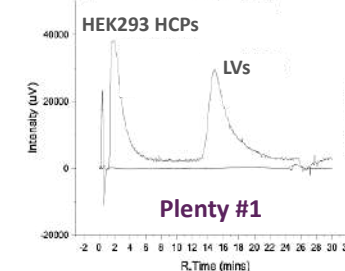
DBC_{10%}: $4.5 \pm 0.3 \cdot 10^{10}$ TU/mL
 El: $1.1 \pm 0.8 \cdot 10^9$ TU/mL
0.2 NaCitrate + 0.5 M MgCl₂, pH 6.5
 Yield: 62.0 ± 3.5 % (qPCR)
 Yield: 57.1 ± 2.8 % (Transduction)
 HCP LRV: 1.81 ± 0.23
 pDNA LRV: 1.62 ± 0.05



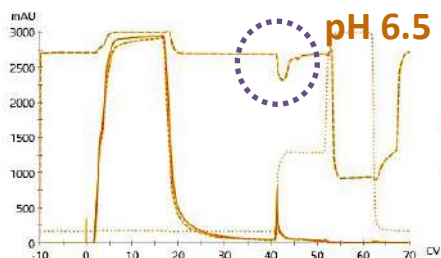
PLenty
#2



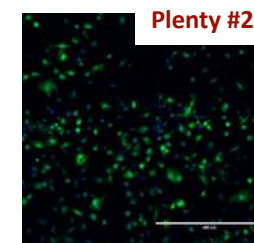
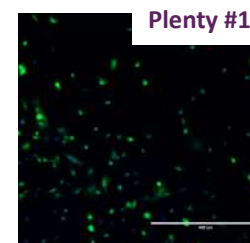
DBC_{10%}: $1.8 \pm 0.2 \cdot 10^9$ TU/mL
 El: $6.7 \pm 0.9 \cdot 10^8$ TU/mL
0.2 NaCitrate + 0.5 M MgCl₂, pH 6.5
 Yield: 59.1 ± 0.6 % (qPCR)
 Yield: 50.6 ± 1.0 % (Transduction)
 HCP LRV: 1.73 ± 0.3
 pDNA LRV: 1.32 ± 0.20



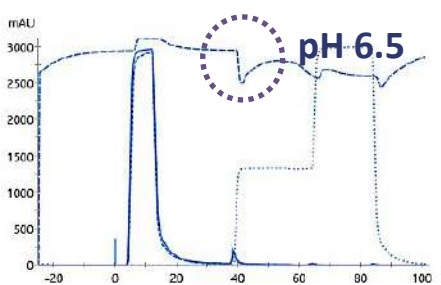
PLenty
#3



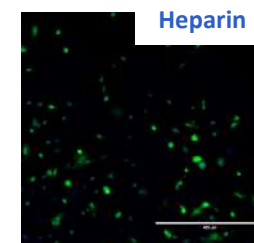
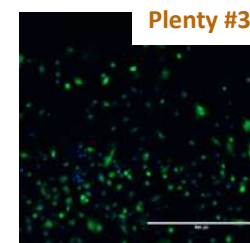
DBC_{10%}: $6.2 \pm 0.4 \cdot 10^9$ TU/mL
 El: $5.8 \pm 0.2 \cdot 10^8$ TU/mL
0.15 M NaCl in PBS, pH 6.0
 Yield: 57.8 ± 4.5 % (qPCR)
 Yield: 51.2 ± 3.8 % (Transduction)
 HCP LRV: 1.79 ± 0.1
 pDNA LRV: 1.48 ± 0.07



Heparin
Poros



DBC_{10%}: $1.8 \pm 0.6 \cdot 10^9$ TU/mL
 El: $6.7 \pm 0.6 \cdot 10^7$ TU/mL
0.35 M NaCl in PBS, pH 6.0
 Yield: 57 ± 0.6 % (qPCR)
 Yield: 56 ± 0.5 % (Transduction)
 HCP LRV: 1.38 ± 0.05
 pDNA LRV: 0.92 ± 0.07



GENE-EDITING
TOOLBOX

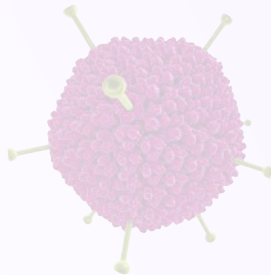
NEW PURIFICATION
PARADIGMS

CRISPR
CAS

VIRAL
VECTORS

NC-VVIRAL

NCVVIRAL



- Lentivirus -
LV
(VSV-G or full envelope)

DBC_{10%}: $5.8 \cdot 10^9$ - $1.2 \cdot 10^{11}$ TU/mL

El: $4.7 \cdot 10^7$ - $1.1 \cdot 10^9$ TU/mL

0.2 NaCitr + 0.5 M MgCl₂, pH 6.5

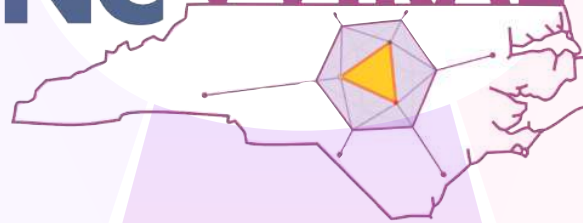
0.15 M NaCl in PBS, pH 6.0

Yield: 57 - 68% (qPCR)

Yield: 46 - 58% (TA)

HCP LRV: 1.75 - 2.01

pDNA LRV: 0.92 - 1.62



- Adenoassociated Virus -
AAV1 - AAV12 (VP2-VP3)
rAAV2, 6, 8 and retroAAV2

DBC_{10%}: $5 \cdot 10^{13}$ - $3 \cdot 10^{14}$ vp/mL

El: $2.1 \cdot 10^{13}$ - $7.5 \cdot 10^{13}$ vp/mL

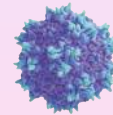
0.5 M MgCl₂, pH 6.5

0.1 M Arg in PBS, pH 6.8

Yield: 65 - 81% (ELISA)

HCP LRV: 2.14 - 2.85 (ELISA)

pDNA LRV: 1.51 - 1.92



- Adenovirus -
Ad5

Ad17, Ad11, Ad35

DBC_{10%}: TBD

El: $3.1 \cdot 10^8$ - $7.1 \cdot 10^8$ TU/mL

0.35 M NaCl in PBS, pH 7.4

Yield: 22 - 43% (ELISA)

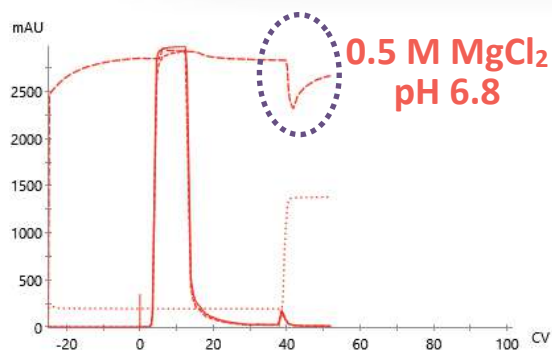
Yield: 13 - 22% (qPCR)

HCP LRV: 1.55 - 2.05 (ELISA)

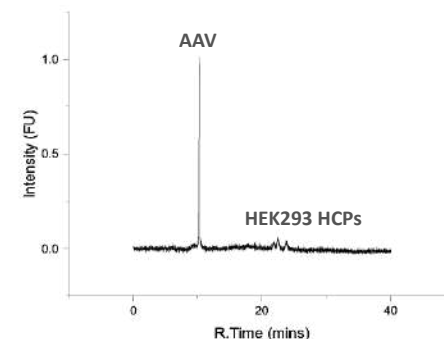
pDNA LRV: TBD

AAV PURIFICATION IN BIND-AND-ELUTE MODE

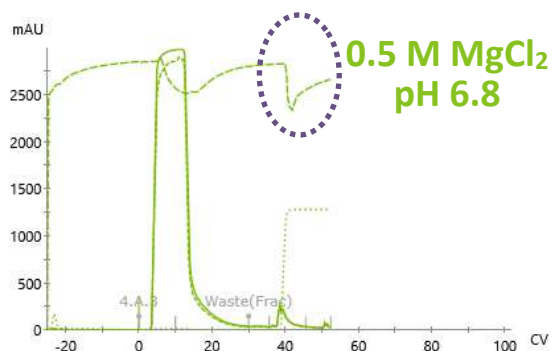
AAV5



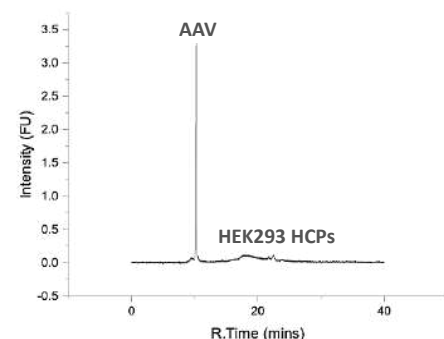
El: $3.82 \pm 0.31 \cdot 10^{13}$ VGs/mL
Yield: 52.4 ± 1.4 % (qPCR)
Yield: 48.5 ± 1.1 % (Transduction)
HCP LRV: 2.81 ± 0.08
pDNA LRV: 1.51 ± 0.15



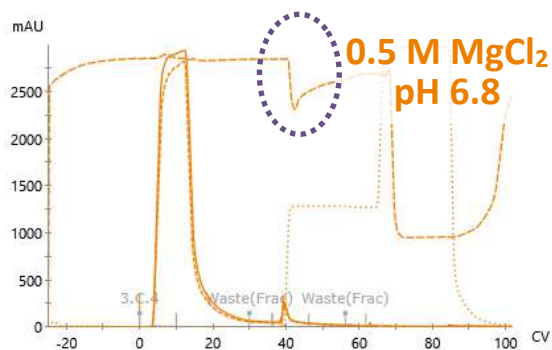
AAV8



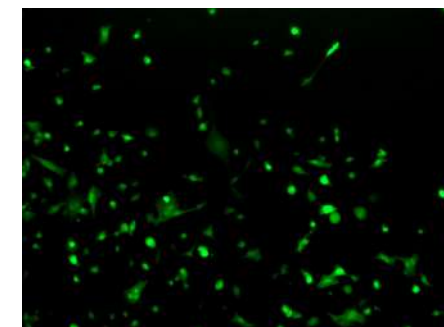
El: $6.34 \pm 0.66 \cdot 10^{13}$ VGs/mL
Yield: 71.0 ± 0.7 % (qPCR)
Yield: 70.3 ± 0.8 % (Transduction)
HCP LRV: 2.67 ± 0.22
pDNA LRV: 1.62 ± 0.12



AAV9



El: $3.82 \pm 0.13 \cdot 10^{13}$ VGs/mL
Yield: 68.2 ± 1.4 % (qPCR)
Yield: 48.3 ± 0.6 % (Transduction)
HCP LRV: 2.88 ± 0.12
pDNA LRV: TBD



GENE-EDITING
TOOLBOX

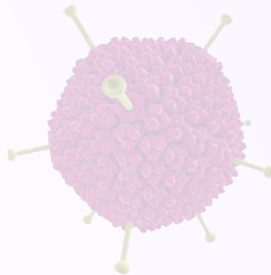
NEW PURIFICATION
PARADIGMS

CRISPR
CAS

VIRAL
VECTORS

NC-VVIRAL

NCVVIRAL



- Lentivirus -
LV

(VSV-G or full envelope)

DBC_{10%}: $5.8 \cdot 10^9$ - $1.2 \cdot 10^{11}$ TU/mL

El: $4.7 \cdot 10^7$ - $1.1 \cdot 10^9$ TU/mL

0.2 NaCitr + 0.5 M MgCl₂, pH 6.5

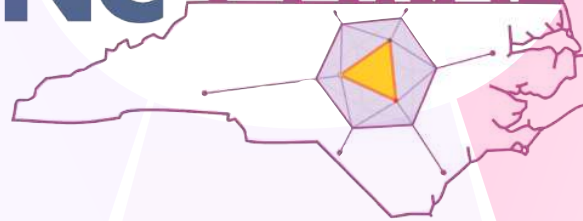
0.15 M NaCl in PBS, pH 6.0

Yield: 57 - 68% (qPCR)

Yield: 46 - 58% (TA)

HCP LRV: 1.75 - 2.01

pDNA LRV: 0.92 - 1.62



- Adenoassociated Virus -
AAV1 - AAV12 (VP2-VP3)
rAAV2, 6, 8 and retroAAV2

DBC_{10%}: $5 \cdot 10^{13}$ - $3 \cdot 10^{14}$ vp/mL

El: $2.1 \cdot 10^{13}$ - $7.5 \cdot 10^{13}$ vp/mL

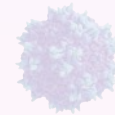
0.5 M MgCl₂, pH 6.5

0.1 M Arg in PBS, pH 6.8

Yield: 65 - 81% (ELISA)

HCP LRV: 2.14 - 2.85 (ELISA)

pDNA LRV: 1.51 - 1.92



- Adenovirus -
Ad5

Ad17, Ad11, Ad35

DBC_{10%}: TBD

El: $3.1 \cdot 10^8$ - $7.1 \cdot 10^8$ TU/mL

0.35 M NaCl in PBS, pH 7.4

Yield: 22 - 43% (ELISA)

Yield: 13 - 22% (qPCR)

HCP LRV: 1.55 - 2.05 (ELISA)

pDNA LRV: TBD

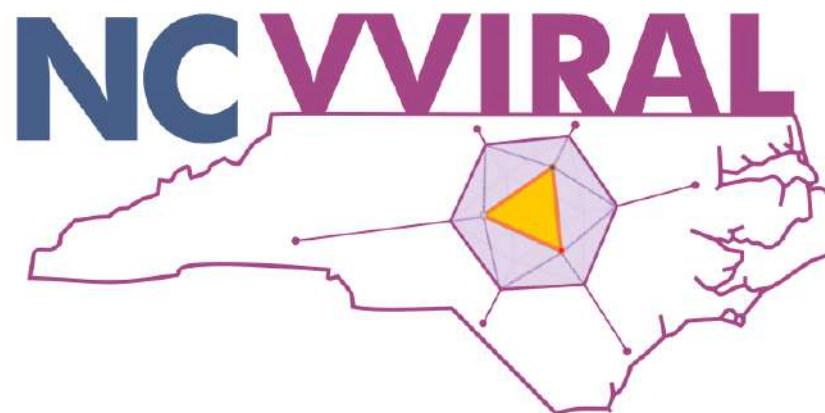
GENE-EDITING
TOOLBOX

NEW PURIFICATION
PARADIGMS

CRISPR
CAS

VIRAL
VECTORS

NC-VVIRAL



NC-VVIRAL: An Academic-Industrial Partnership in the Research Triangle to Forge the Next-Generation Biomanufacturing of Gene Therapy Products



THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL



Duke
UNIVERSITY



NC Central
UNIVERSITY



JSNN

Joint School of
Nanoscience and Nanoengineering



NORTH CAROLINA
DEPARTMENT
COMMERCE



North Carolina
Biotechnology Center



KENAN INSTITUTE
ENGINEERING, TECHNOLOGY & SCIENCE
NC STATE UNIVERSITY

GENE-EDITING
TOOLBOX

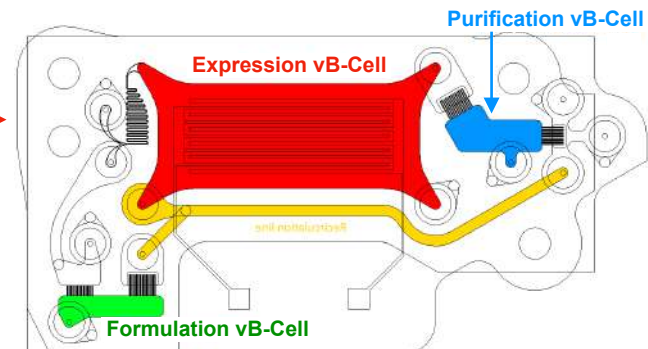
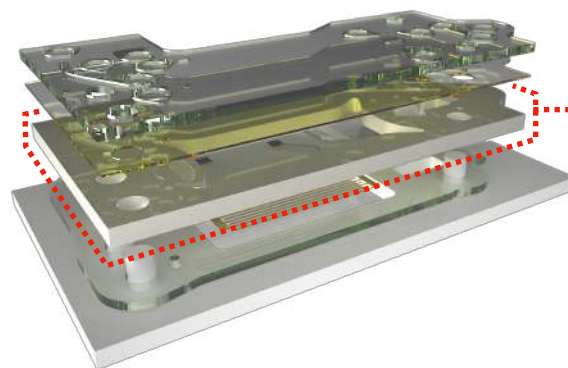
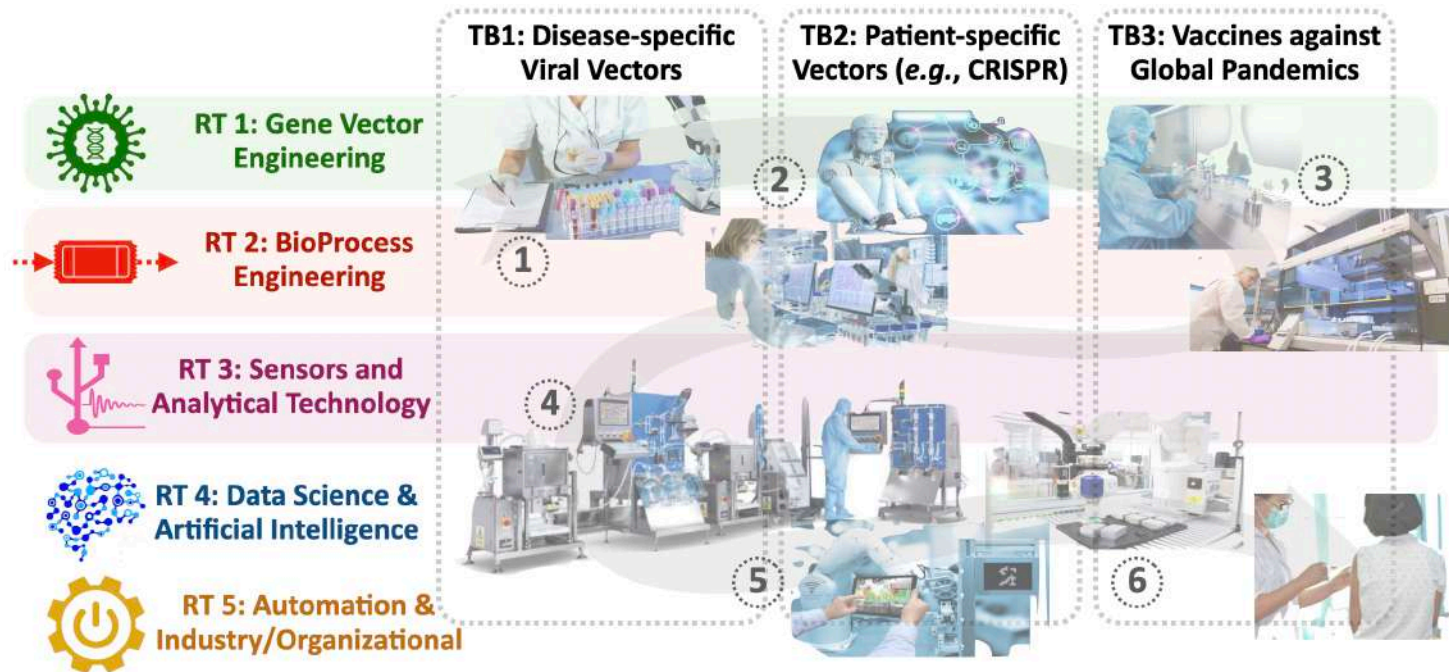
NEW PURIFICATION
PARADIGMS

CRISPR
CAS

VIRAL
VECTORS

NC-VVIRAL

NC-VVIRAL: A Technology Foundry for VV Manufacturing



GENE-EDITING
TOOLBOX

NEW PURIFICATION
PARADIGMS

CRISPR
CAS

VIRAL
VECTORS

NC-VVIRAL

Co-founders:



Stef



Mike



Y'all come visit, ya hear?!?

Inauguration: July 22nd, NCSU Centennial Campus, EB3 - 4025

Contacts: smenega@ncsu.edu & mdaniel6@ncsu.edu

GENE-EDITING
TOOLBOX

NEW PURIFICATION
PARADIGMS

CRISPR
CAS

VIRAL
VECTORS

NC-VVIRAL

Acknowledgements



KENAN INSTITUTE
ENGINEERING, TECHNOLOGY & SCIENCE

NC STATE UNIVERSITY



**THE NONWOVENS
INSTITUTE**



MERCK



KBI
BIOPHARMA

novo
nordisk
fonden

Genentech

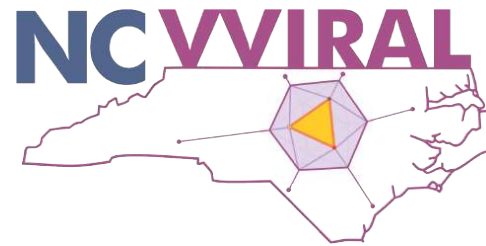


Ligand Trap
TECHNOLOGIES

CSL Behring

Biotherapies for Life™

EASTMAN



Thank you!



GENE-EDITING
TOOLBOX

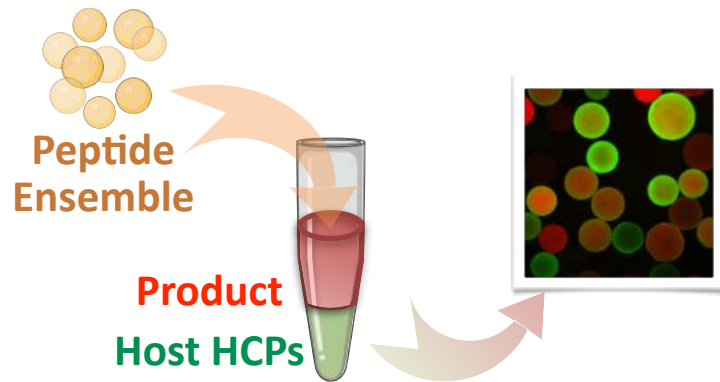
NEW PURIFICATION
PARADIGMS

CRISPR
CAS

VIRAL
VECTORS

NC-VVIRAL

LIGAND DEVELOPMENT



GENE-EDITING
TOOLBOX

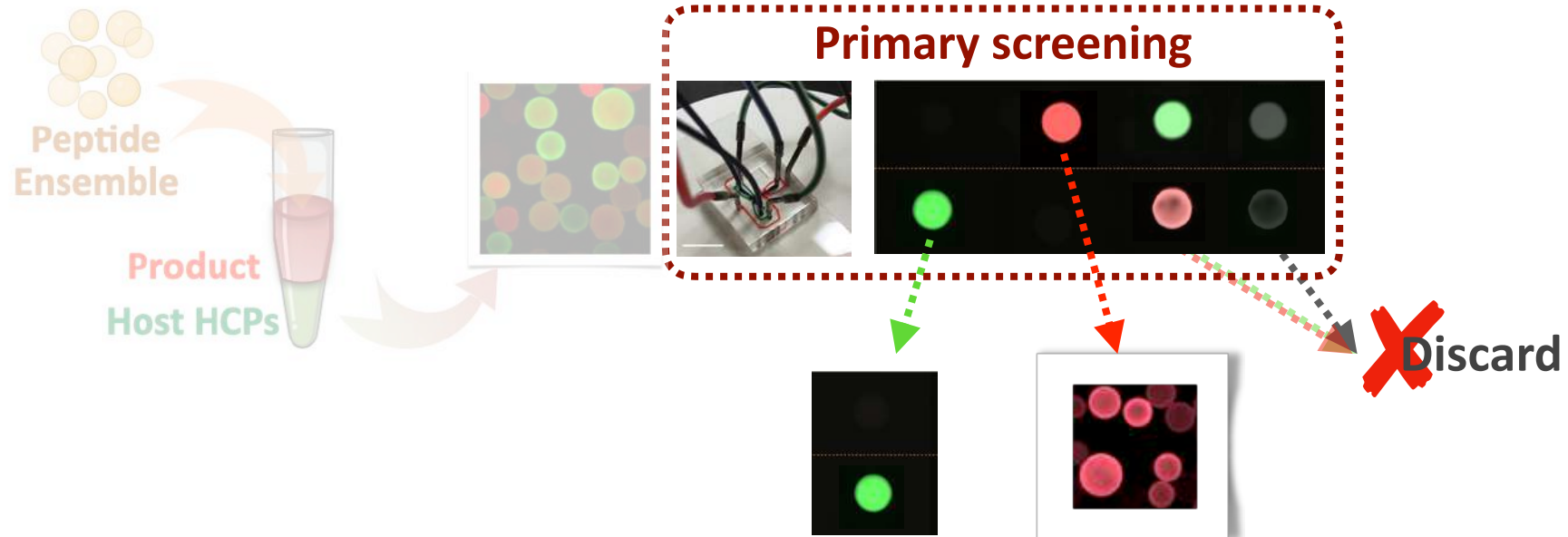
NEW PURIFICATION
PARADIGMS

CRISPR
CAS

VIRAL
VECTORS

NC-VVIRAL

LIGAND DEVELOPMENT



GENE-EDITING
TOOLBOX

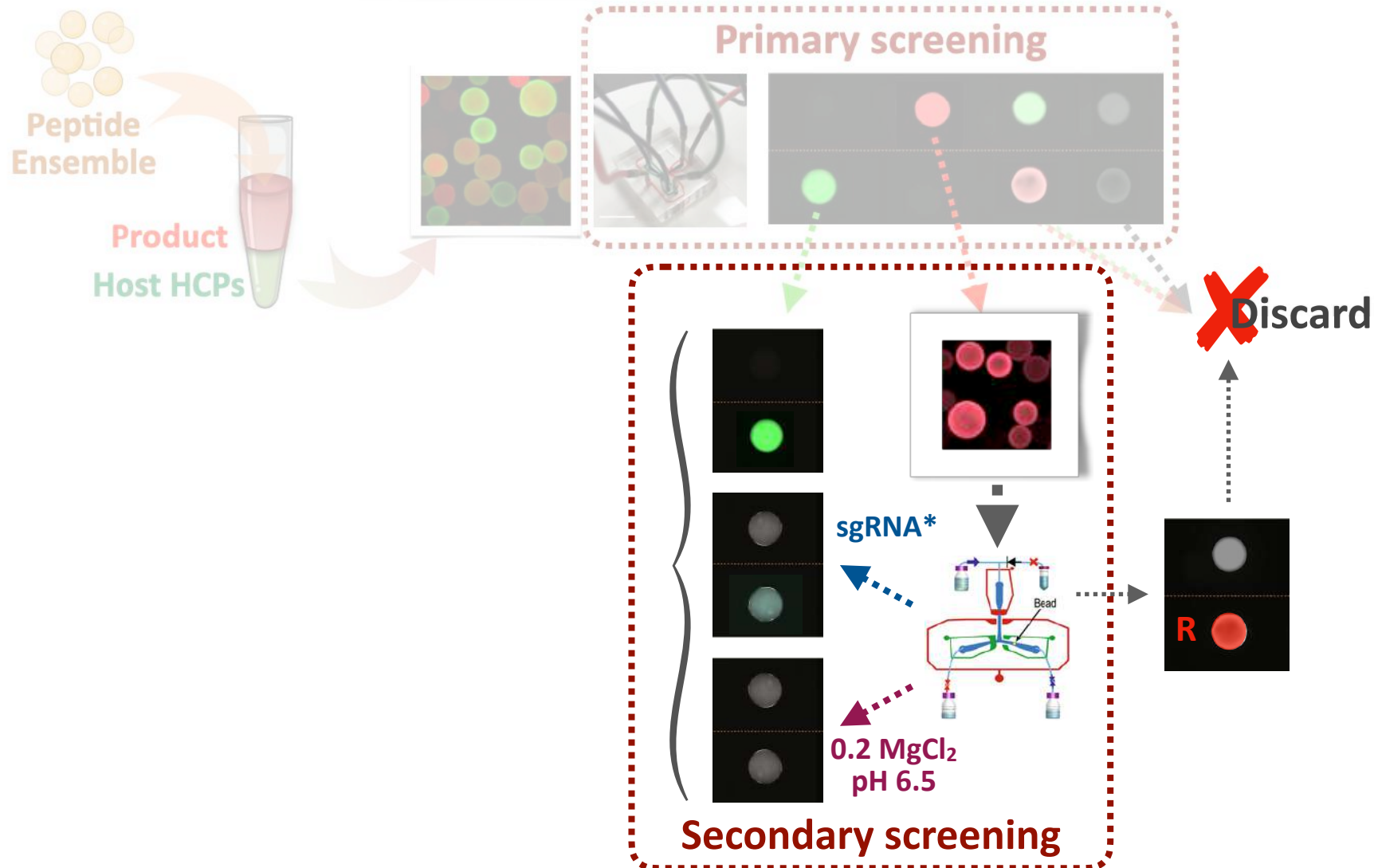
NEW PURIFICATION
PARADIGMS

CRISPR
CAS

VIRAL
VECTORS

NC-VVIRAL

LIGAND DEVELOPMENT



GENE-EDITING
TOOLBOX

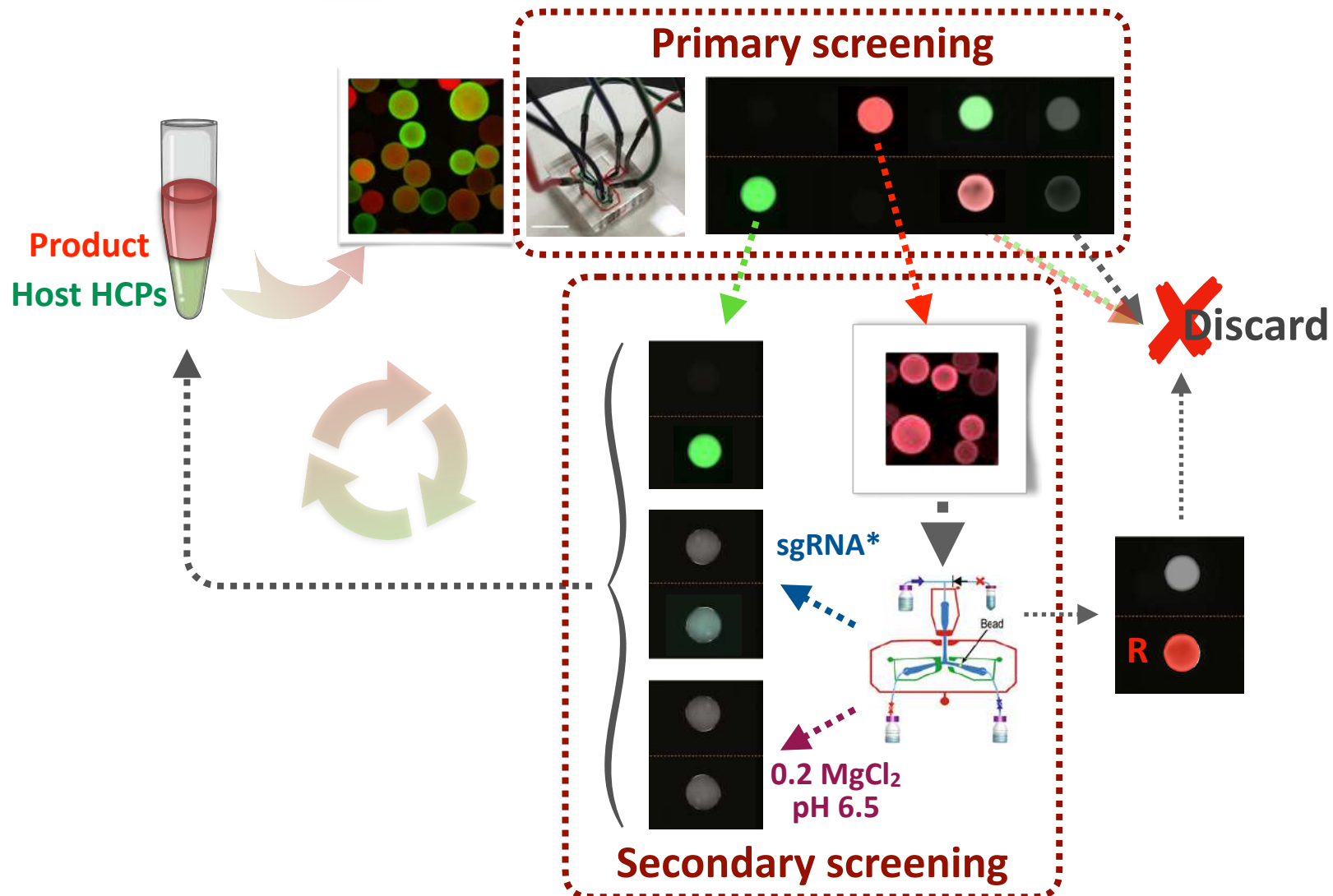
NEW PURIFICATION
PARADIGMS

CRISPR
CAS

VIRAL
VECTORS

NC-VVIRAL

LIGAND DEVELOPMENT



GENE-EDITING
TOOLBOX

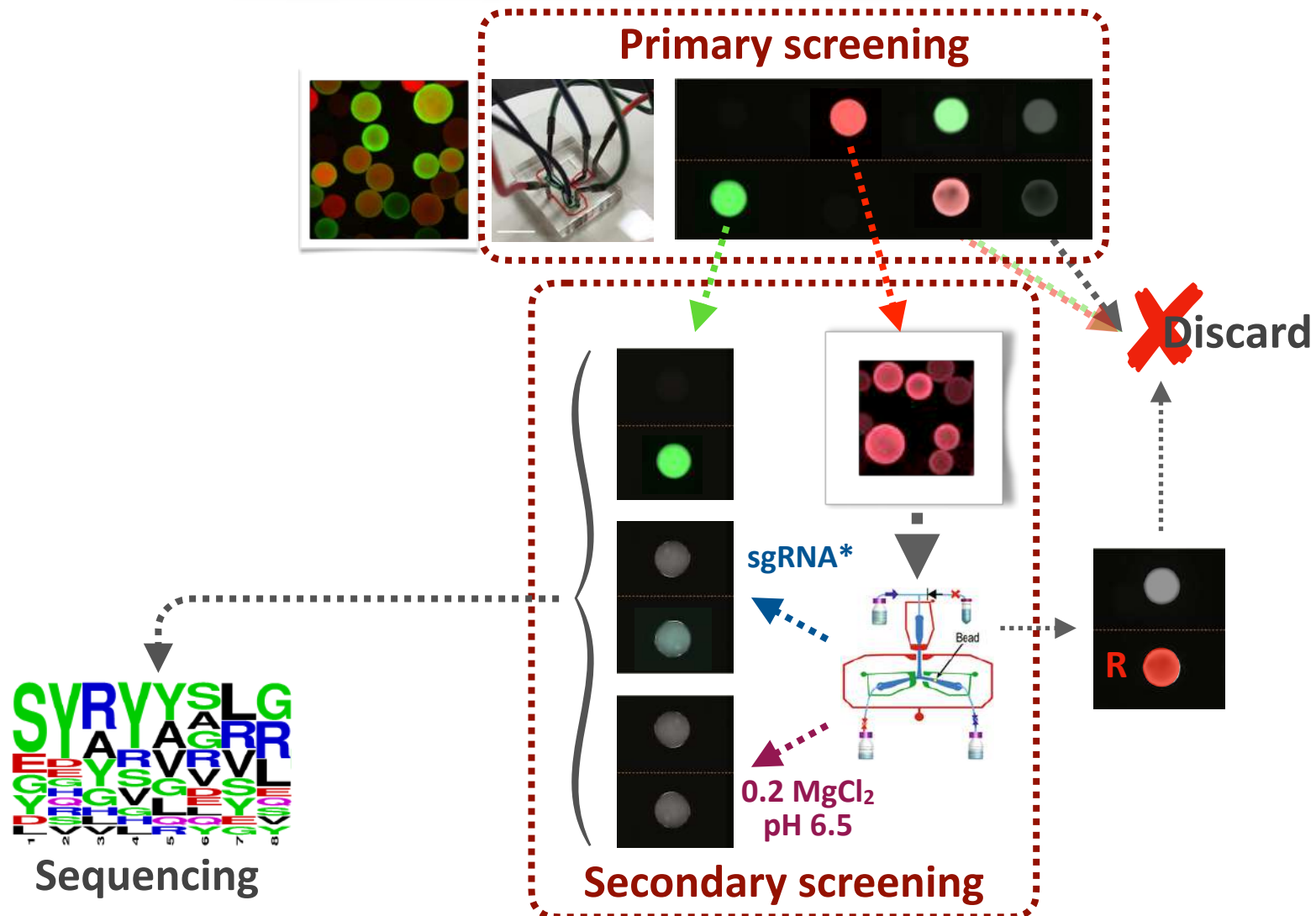
NEW PURIFICATION
PARADIGMS

CRISPR
CAS

VIRAL
VECTORS

NC-VVIRAL

LIGAND DEVELOPMENT



GENE-EDITING
ToolBox

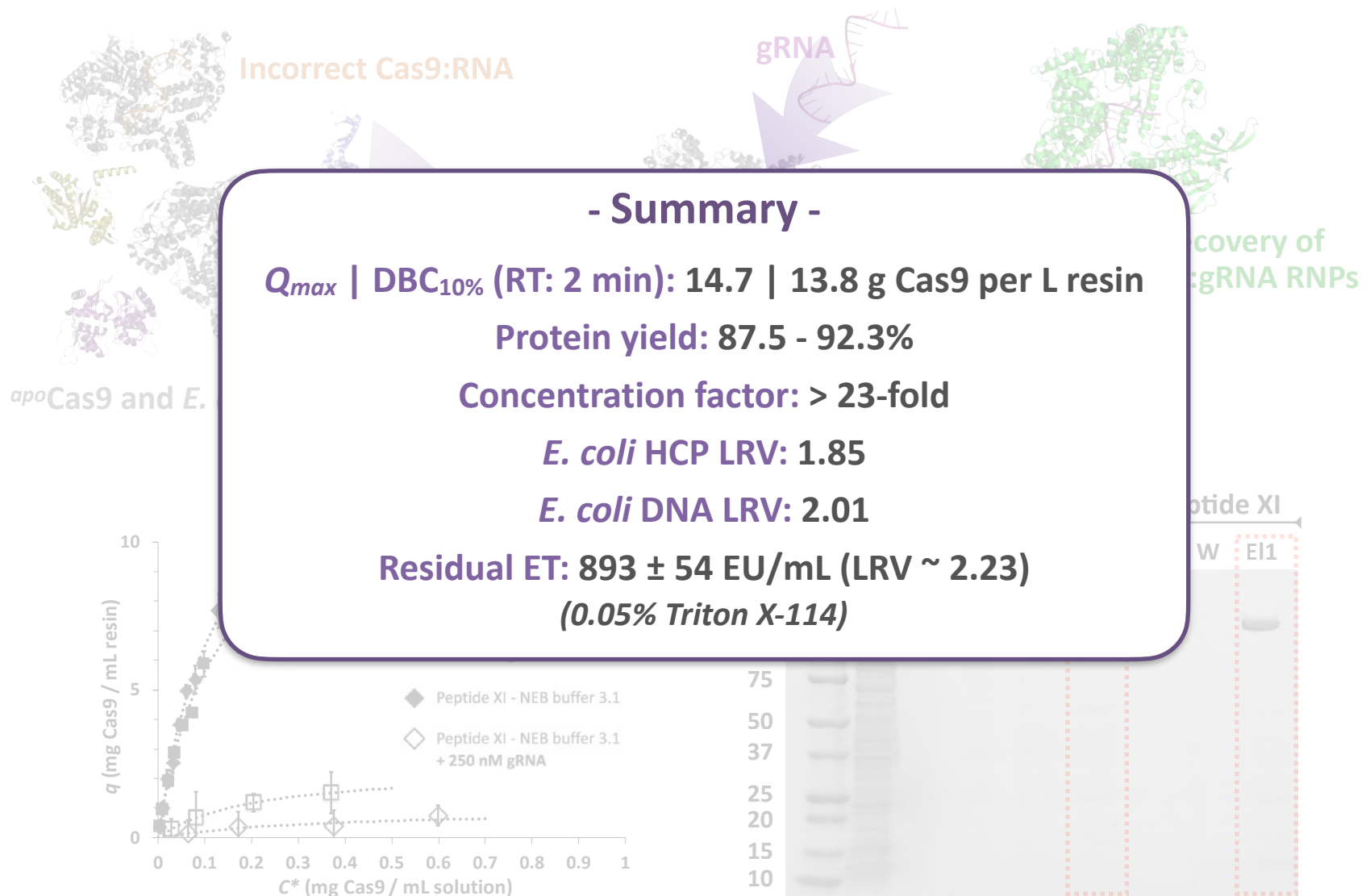
NEW PURIFICATION
PARADIGMS

CRISPR
Cas

VIRAL
VECTORS

NC-VVIRAL

SMART - STERICALLY-MODULATED AFFINITY BY RNA TRIGGER



GENE-EDITING
TOOLBOX

NEW PURIFICATION
PARADIGMS

CRISPR
CAS

VIRAL
VECTORS

NC-VVIRAL

SMART - STERICALLY-MODULATED AFFINITY BY RNA TRIGGER

Incorrect Cas12a:RNA

crRNA

- Summary -

Q_{max} | DBC_{10%} (RT: 2 min): 11.2 | 10.4 g Cas12 per L resin

Protein yield: 62.4 - 66.0%

Concentration factor: > 25-fold

E. coli HCP LRV: 0.91

E. coli DNA LRV: 1.87

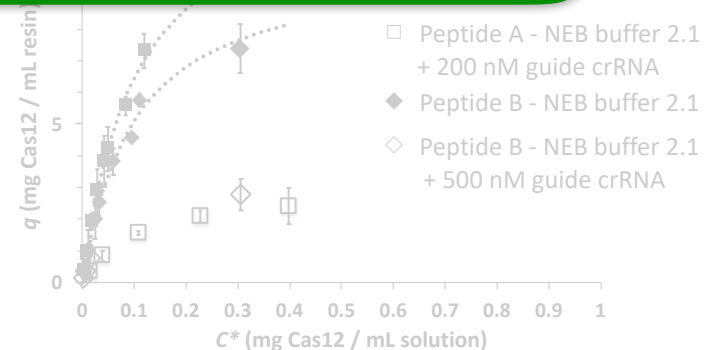
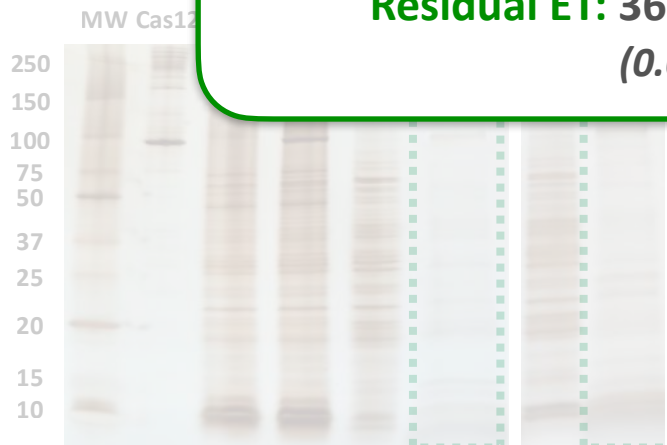
Residual ET: 3664 ± 138 EU/mL (LRV ~ 1.72)
(0.05% Triton X-114)

recovery of
2:gRNA RNP

Clearance of
E. coli proteins

12:gRNA

apoCas12a and E



GENE-EDITING
TOOLBOX

NEW PURIFICATION
PARADIGMS

CRISPR
CAS

VIRAL
VECTORS

NC-VVIRAL

LIGA GUARD™ FOR VIRAL VECTOR PURIFICATION

- Summary (AAV2) -

DBC_{10%}: 23 - 25 g HEK293 HCPs per L of resin (RT: 1 min)
25 - 28 g/L (RT: 2 min)

HEK293 HCP LRV: > 2 (25 CVs)
> 1.5 (75 CVs); > 1 (100 CVs)

HEK293 DNA LRV: > 1.55 (25 CVs);
> 1.25 (75 CVs); > 0.55 (100 CVs)

pDNA removal > 75% at 50 CVs (no benzonase)

Product Yield: > 96% VGs; > 67% VPs

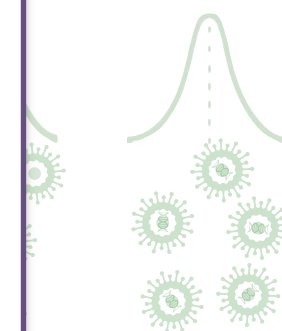
Full/Empty (10 - 13 mS/cm):

11.3/88.7 - 24.5/75.5 (feed) → 33.5/66.5 - 40.1/59.9 (feed)

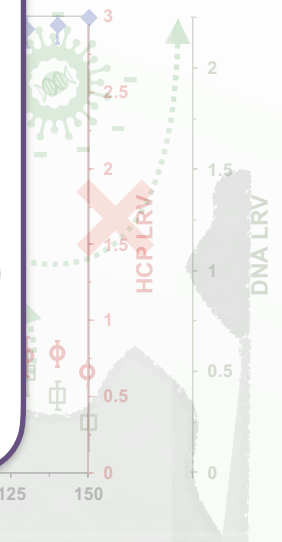
Transduction activity: 45.7 - 61.0% → 42.1 - 59.9%
(%GFP⁺ HuH7 cells at 10⁵ vg/cell)



AAVs Full AAVs



Purity



GENE-EDITING
TOOLBOX

NEW PURIFICATION
PARADIGMS

CRISPR
CAS

VIRAL
VECTORS

NC-VVIRAL

ADENOVIRUS PURIFICATION IN FLOW-THROUGH MODE

ViroGuard™ resin

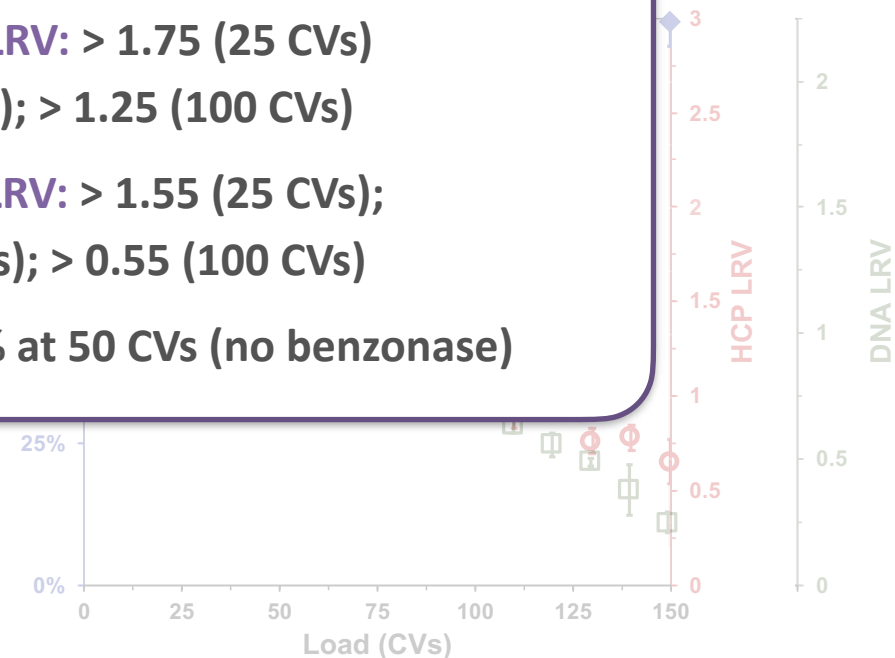
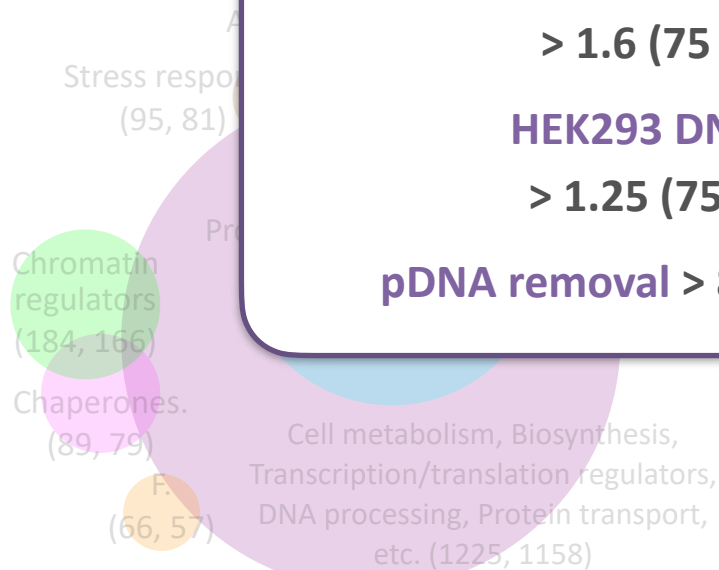
- Summary (Ad5) -

DBC_{10%}: 24 - 27 g HEK293 HCPs per L of resin (RT: 1 min)
24 - 28 g/L (RT: 2 min)

HEK293 HCP LRV: > 1.75 (25 CVs)
> 1.6 (75 CVs); > 1.25 (100 CVs)

HEK293 DNA LRV: > 1.55 (25 CVs);
> 1.25 (75 CVs); > 0.55 (100 CVs)

pDNA removal > 83% at 50 CVs (no benzonase)



GENE-EDITING
TOOLBOX

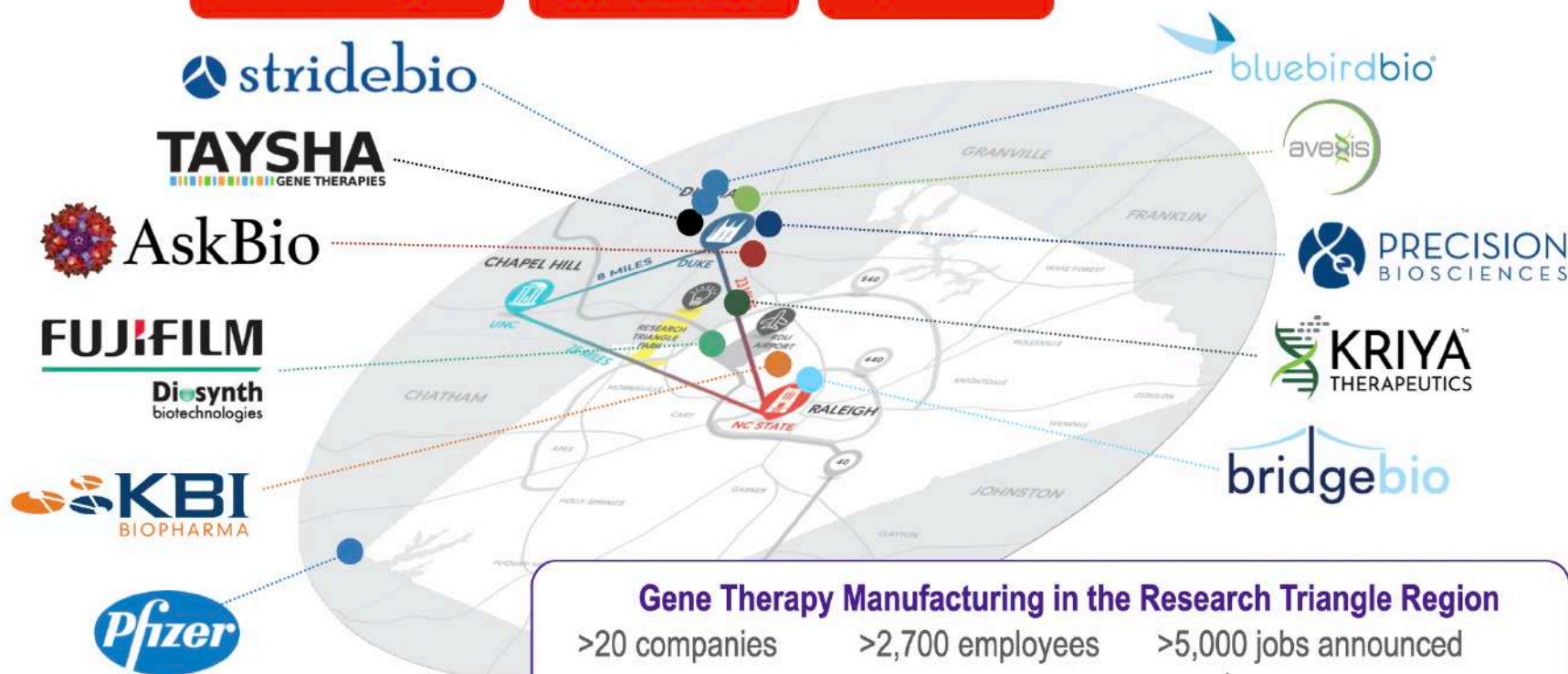
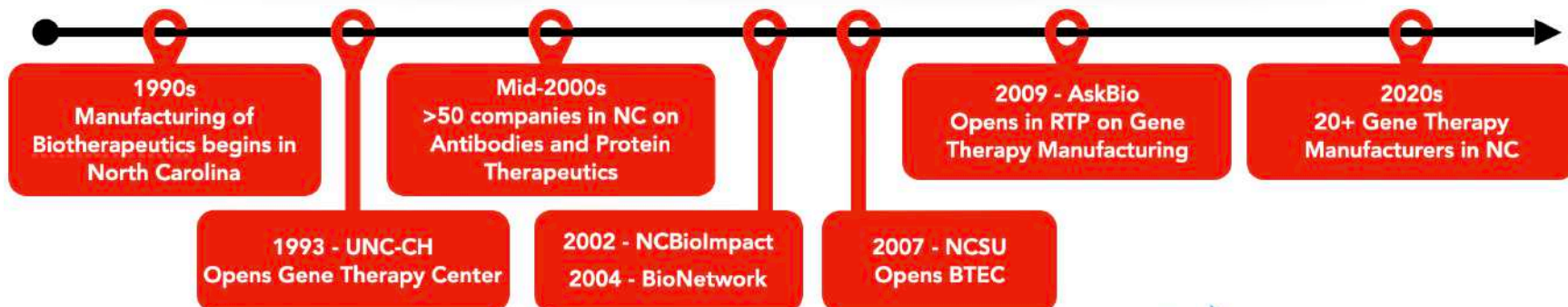
NEW PURIFICATION
PARADIGMS

CRISPR
CAS

VIRAL
VECTORS

NC-VVIRAL

NC-VVIRAL: A North Carolina Network



Gene Therapy Manufacturing in the Research Triangle Region

>20 companies
>8M Ft.² Facilities

>2,700 employees
>500 patents

>5,000 jobs announced
>\$4B investment

GENE-EDITING
TOOLBOX

NEW PURIFICATION
PARADIGMS

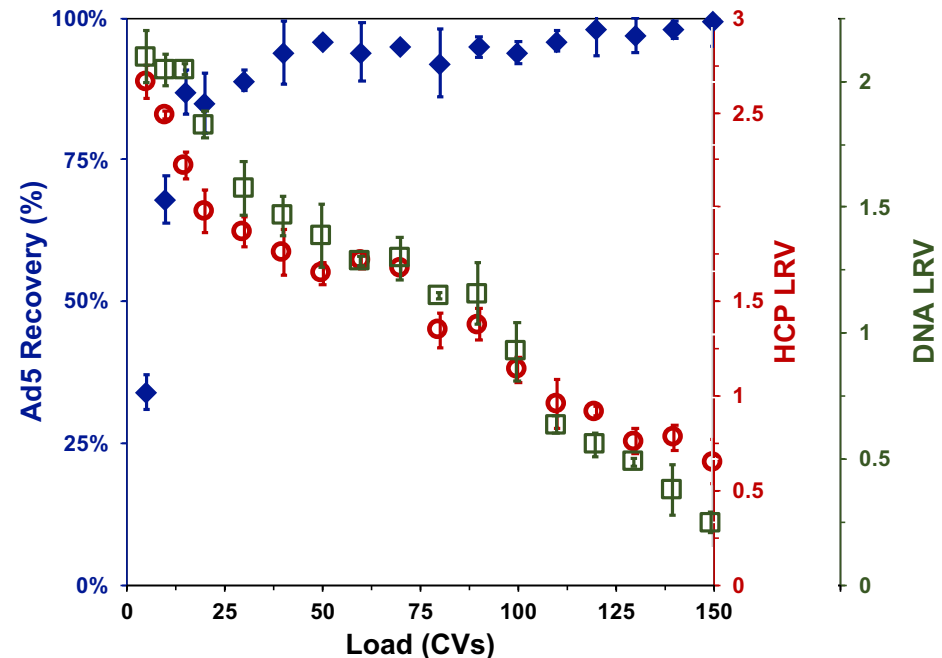
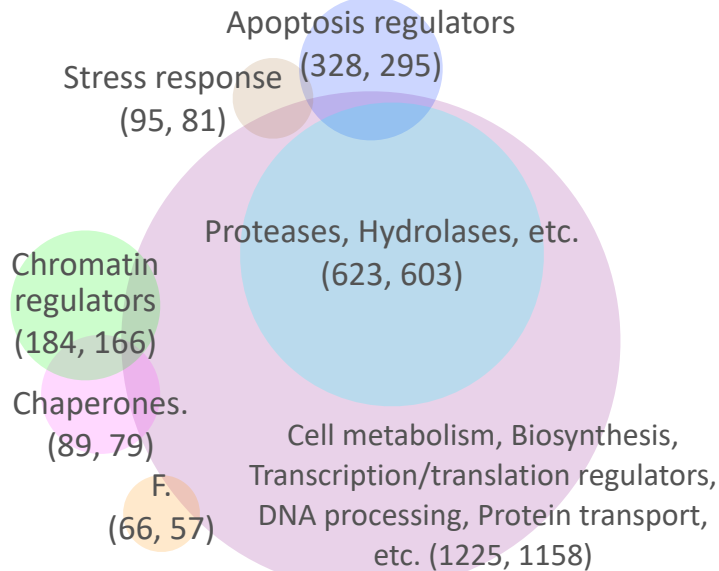
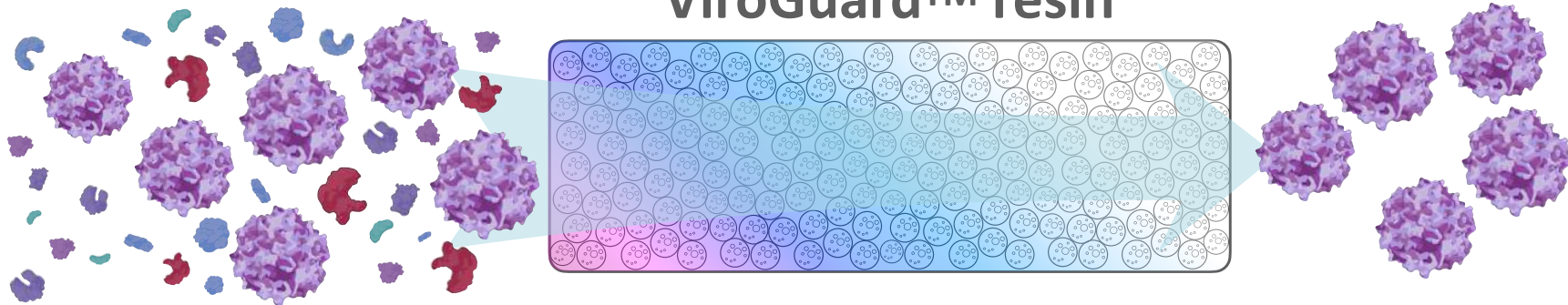
CRISPR
CAS

VIRAL
VECTORS

NC-VVIRAL

ADENOVIRUS PURIFICATION IN FLOW-THROUGH MODE

ViroGuard™ resin



GENE-EDITING
ToolBox

NEW PURIFICATION
PARADIGMS

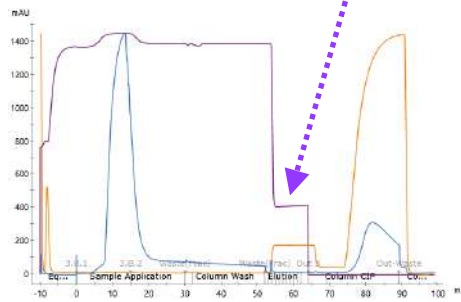
CRISPR
CAS

VIRAL
VECTORS

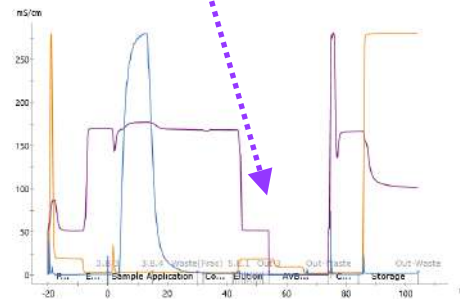
NC-VVIRAL

AAV PURIFICATION IN BIND-AND-ELUTE MODE

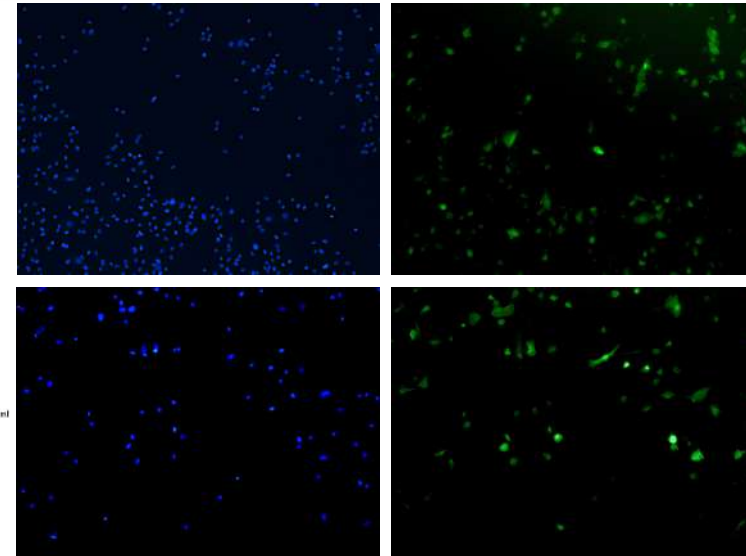
Elution: 0.5 M MgCl₂, pH ~ 3.0



AAVx-Poros



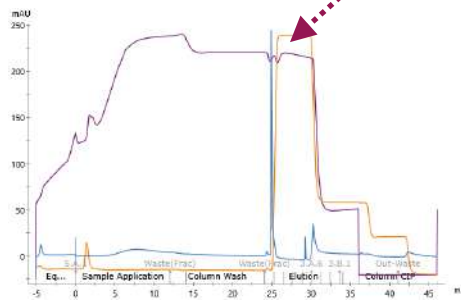
ABV-Sepharose



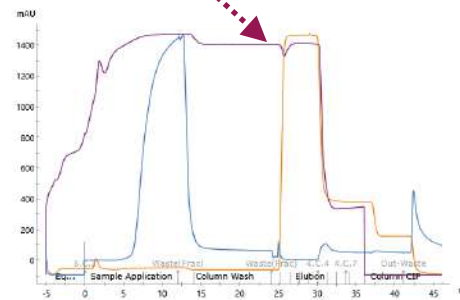
DAPI

GFP

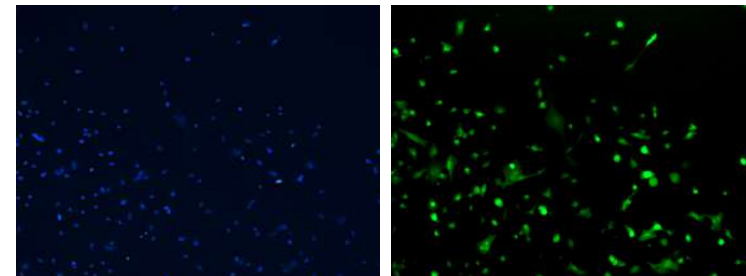
Elution: 0.5 M MgCl₂, pH 6.5



LigaGuard → AAVidity

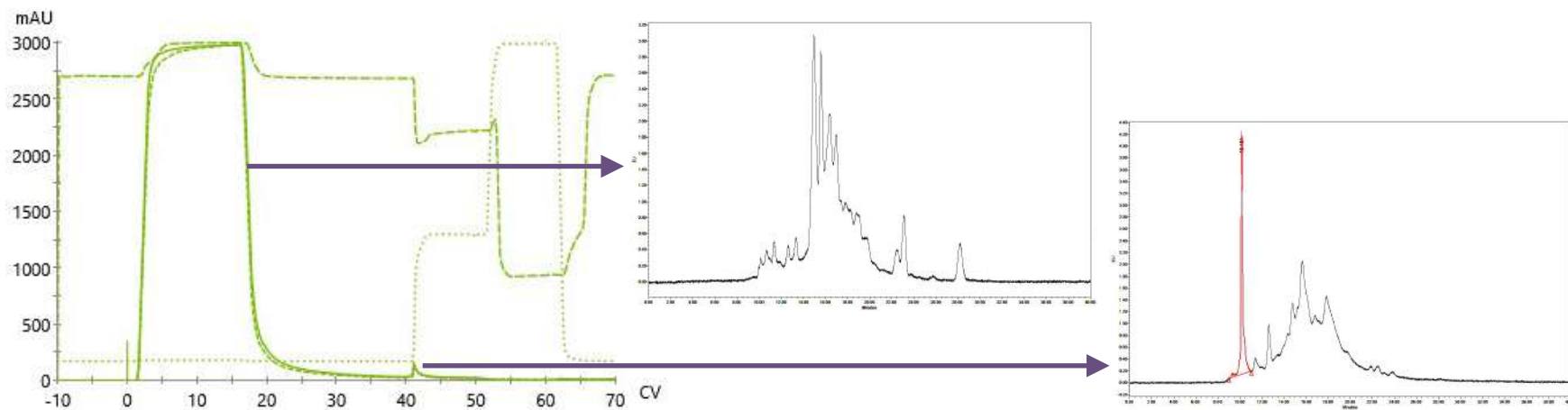


AAVidity



HuH7 cells at 10⁵ vg/cell

ADENOVIRUS PURIFICATION IN BIND-AND-ELUTE MODE



El: $3.7 \pm 0.53 \cdot 10^8$ TU/mL

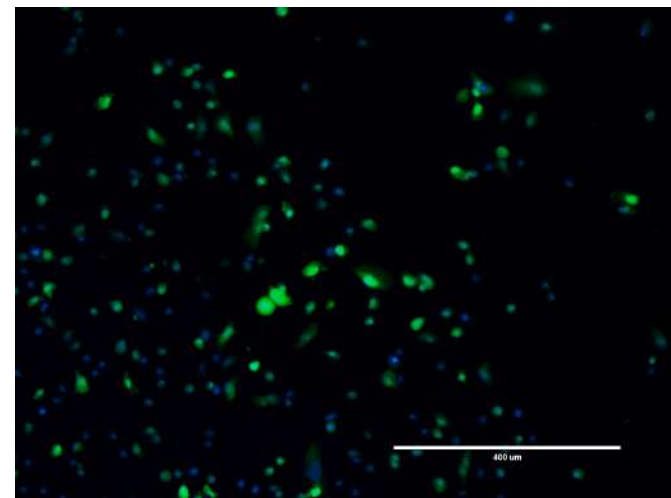
0.35 M NaCl in PBS, pH 7.4

Yield: $26.1 \pm 4.43\%$ (ELISA)

Yield: $19.8 \pm 2.22\%$ (qPCR)

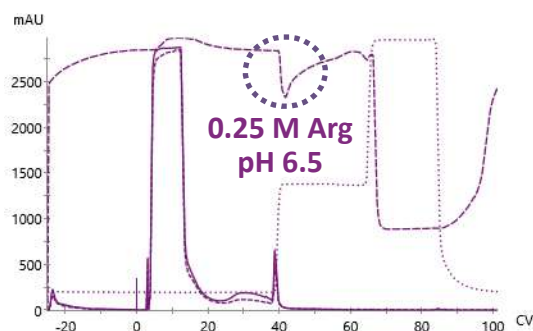
HCP LRV: 1.55 ± 0.07 (ELISA)

pDNA LRV: TBD

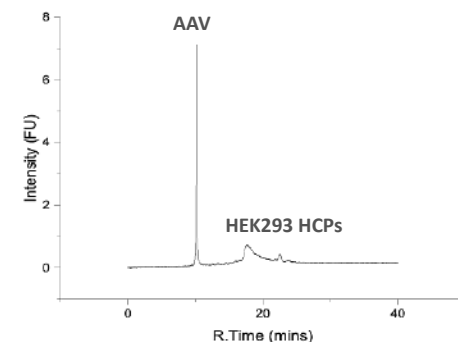


AAV PURIFICATION IN BIND-AND-ELUTE MODE

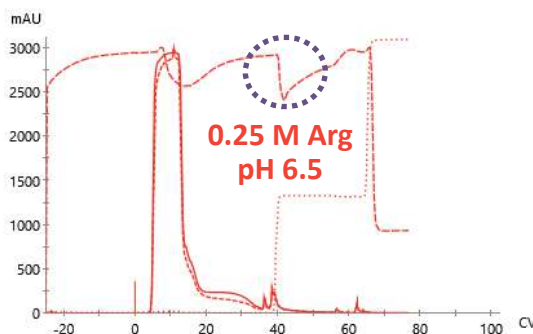
rAAV2



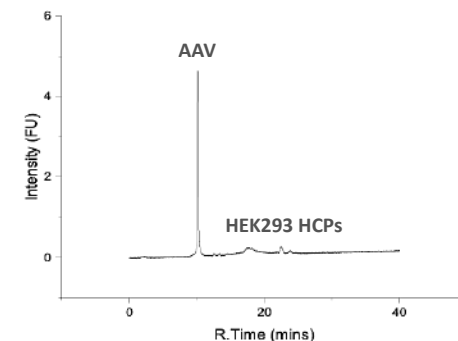
El: $7.48 \pm 0.53 \cdot 10^{13}$ VGs/mL
Yield (qPCR): 93.3 ± 3.5 %
Yield (Transduction): 73.3 ± 4.2 %
HCP LRV: 2.14 ± 0.26
pDNA LRV: 1.81 ± 0.22



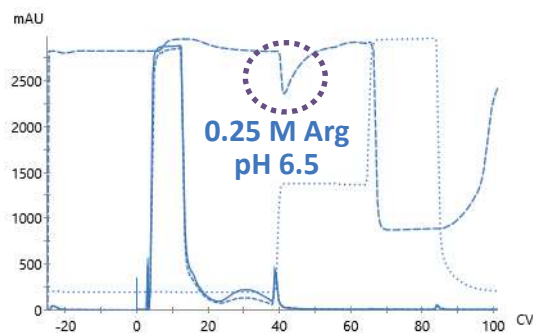
retro
AAV2



El: $4.16 \pm 0.55 \cdot 10^{13}$ VGs/mL
Yield (qPCR): 52.2 ± 5.2 %
Yield (Transduction): 26.6 ± 2.6 %
HCP LRV: 2.44 ± 0.35
pDNA LRV: 1.89 ± 0.21



AAV-DJ



El: $5.77 \pm 0.72 \cdot 10^{13}$ VGs/mL
Yield (qPCR): 72.1 ± 5.6 %
Yield (Transduction): TBD %
HCP LRV: 2.27 ± 0.27
pDNA LRV: TBD

