Genome Editing and Engineering

Course No: BT-637

LECTURE-12

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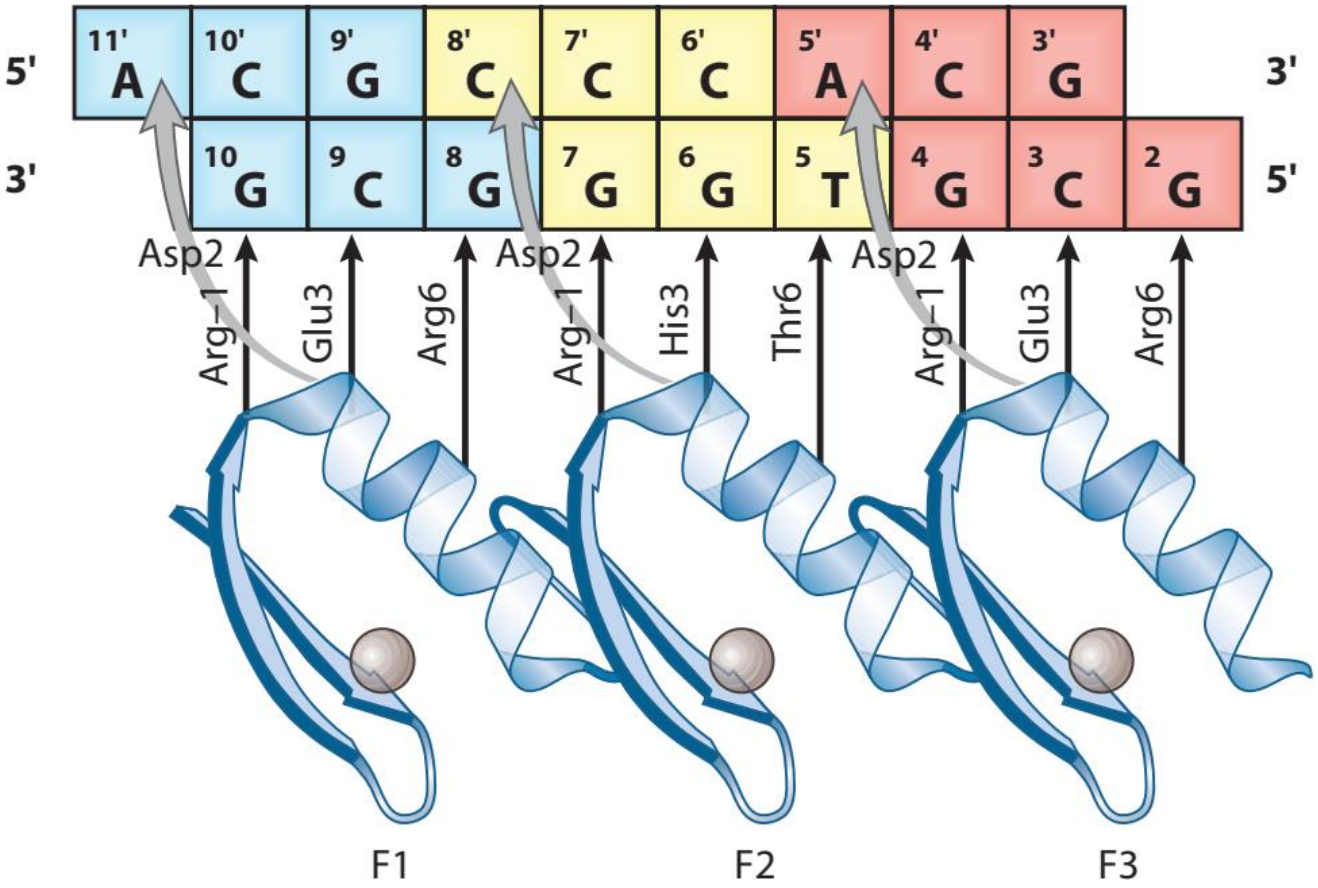
Indian Institute of Technology Guwahati

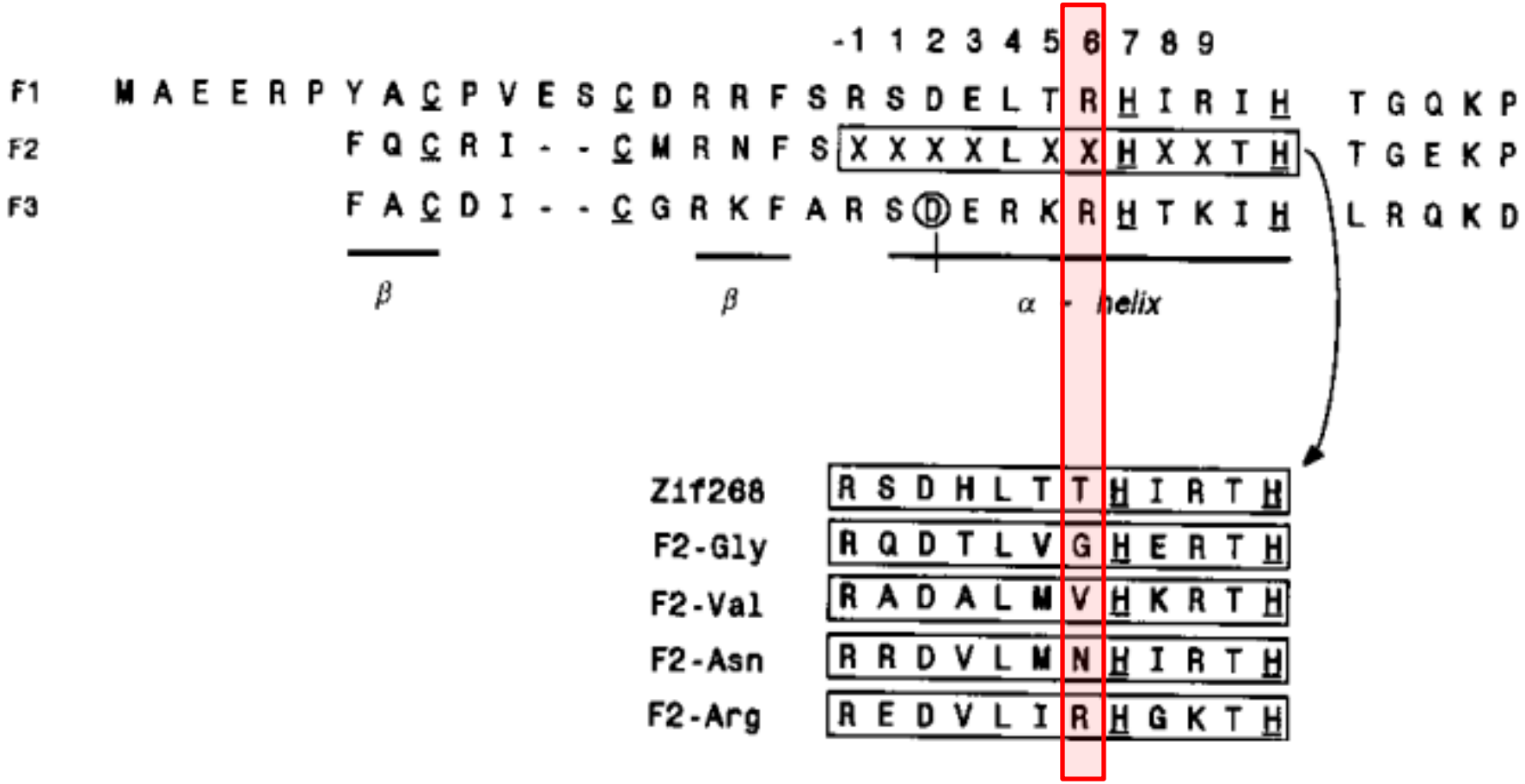
Synergy between adjacent zinc fingers in sequence-specific DNA recognition

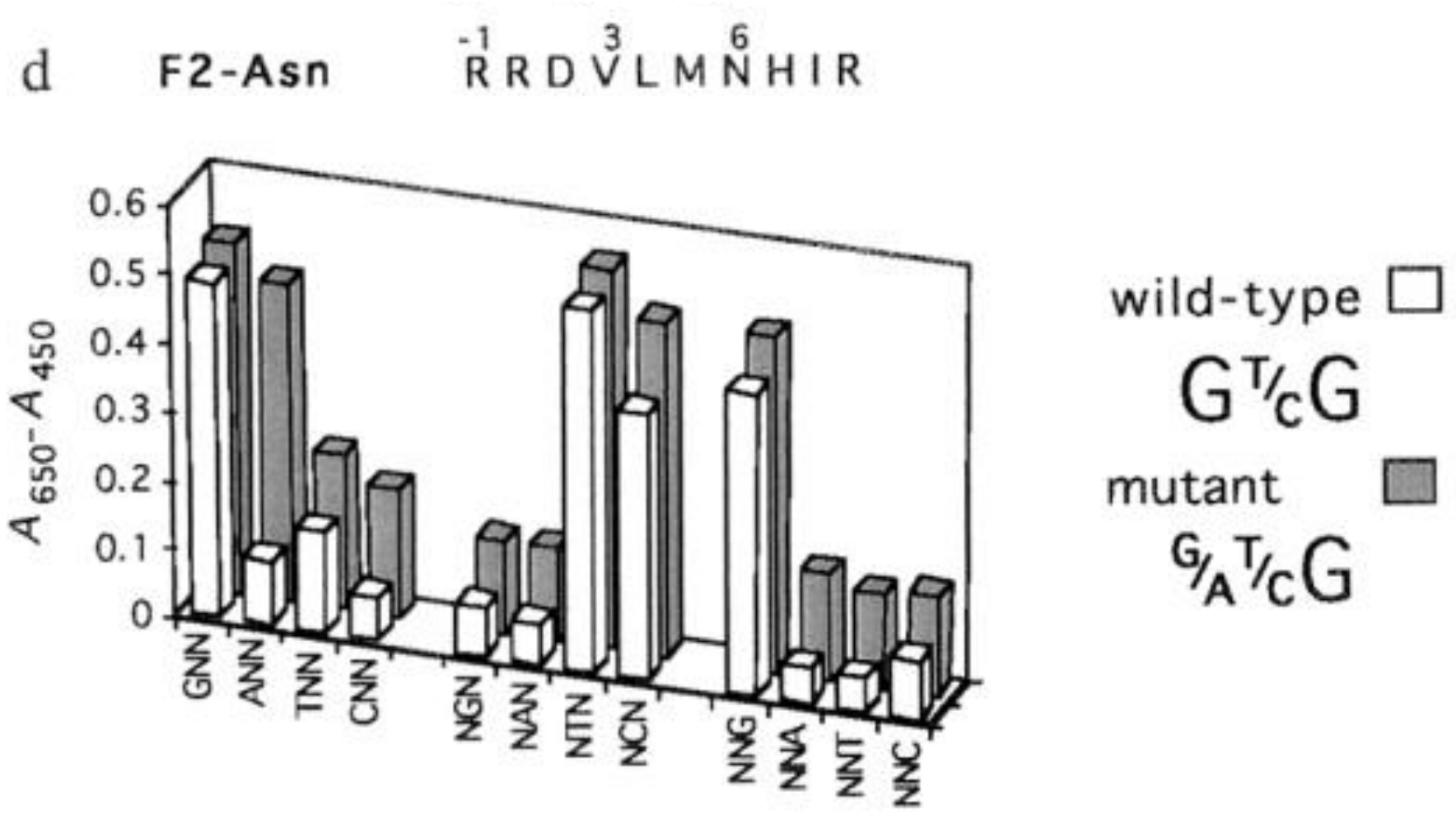
MARK ISALAN, YEN CHOO*, AND AARON KLUG



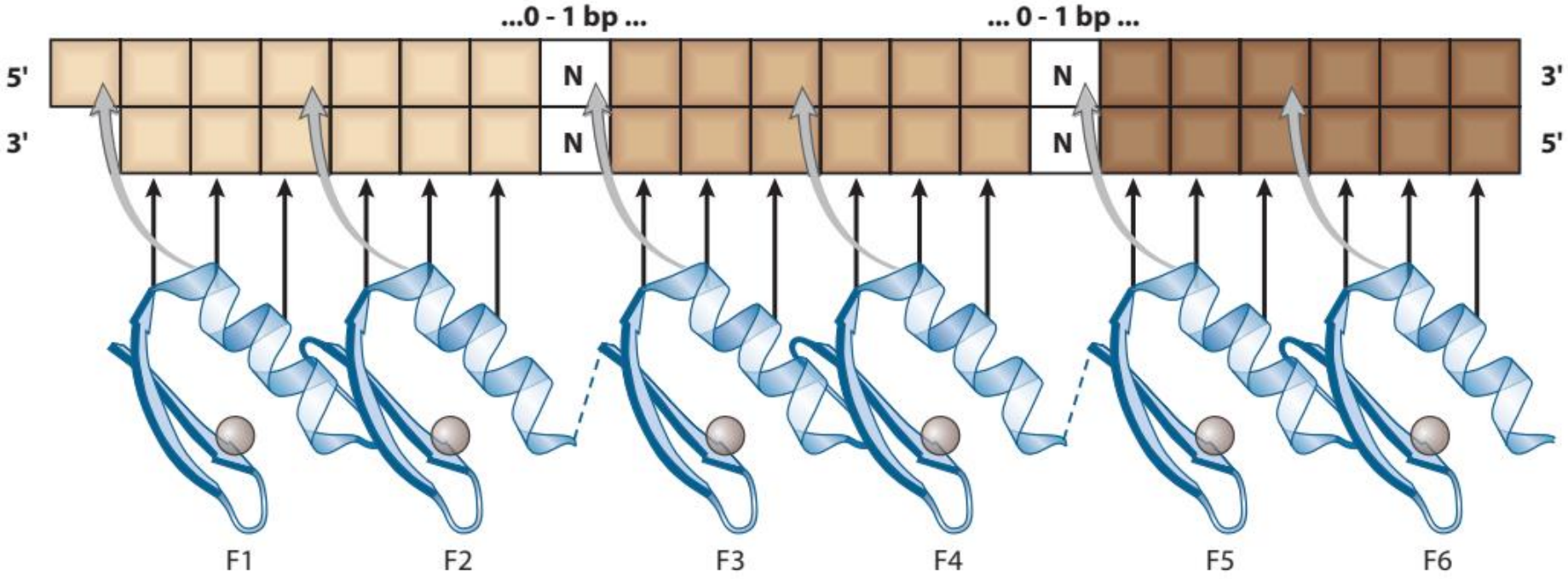
ZF for each codon

1st letter	Second Letter: U	Second Letter: C	Second Letter: A	Second Letter: G	3rd letter
U	UUU Phe UUC UUA Leu UUG	UCU UCC Ser UCA UCG	UAU Tyr UAC UAA Stop UAG Stop	UGU Cys UGC UGA Stop UGG Trp	U C A G
C	CUU CUC Leu CUA CUG	CCU CCC Pro CCA CCG	CAU His CAC CAA Gln CAG	CGU CGC Arg CGA CGG	U C A G
A	AUU AUC Ile AUA AUG Met	ACU ACC Thr ACA ACG	AAU Asn AAC AAA Lys AAG	AGU Ser AGC AGA Arg AGG	U C A G
G	GUU GUC Val GUA GUG	GCU GCC Ala GCA GCG	GAU Asp GAC GAA Glu GAG	GGU GGC Gly GGA GGG	U C A G


-1 1 2 3 4 5 6 7 8 9
F1 M A E E R P Y A C P V E S C D R R F S R S D E L T R H I R I H T G Q K P
F2 F Q C R I - - C M R N F S X X X X L X X H X X T H T G E K P
F3 F A C D I - - C G R K F A R S D E R K R H T K I H L R Q K D
β
β
α - helix
Zif268 R S D H L T T H I R T H
F2-Gly R Q D T L V G H E R T H
F2-Val R A D A L M V H K R T H
F2-Asn R R D V L M N H I R T H
F2-Arg R E D V L I R H G K T H

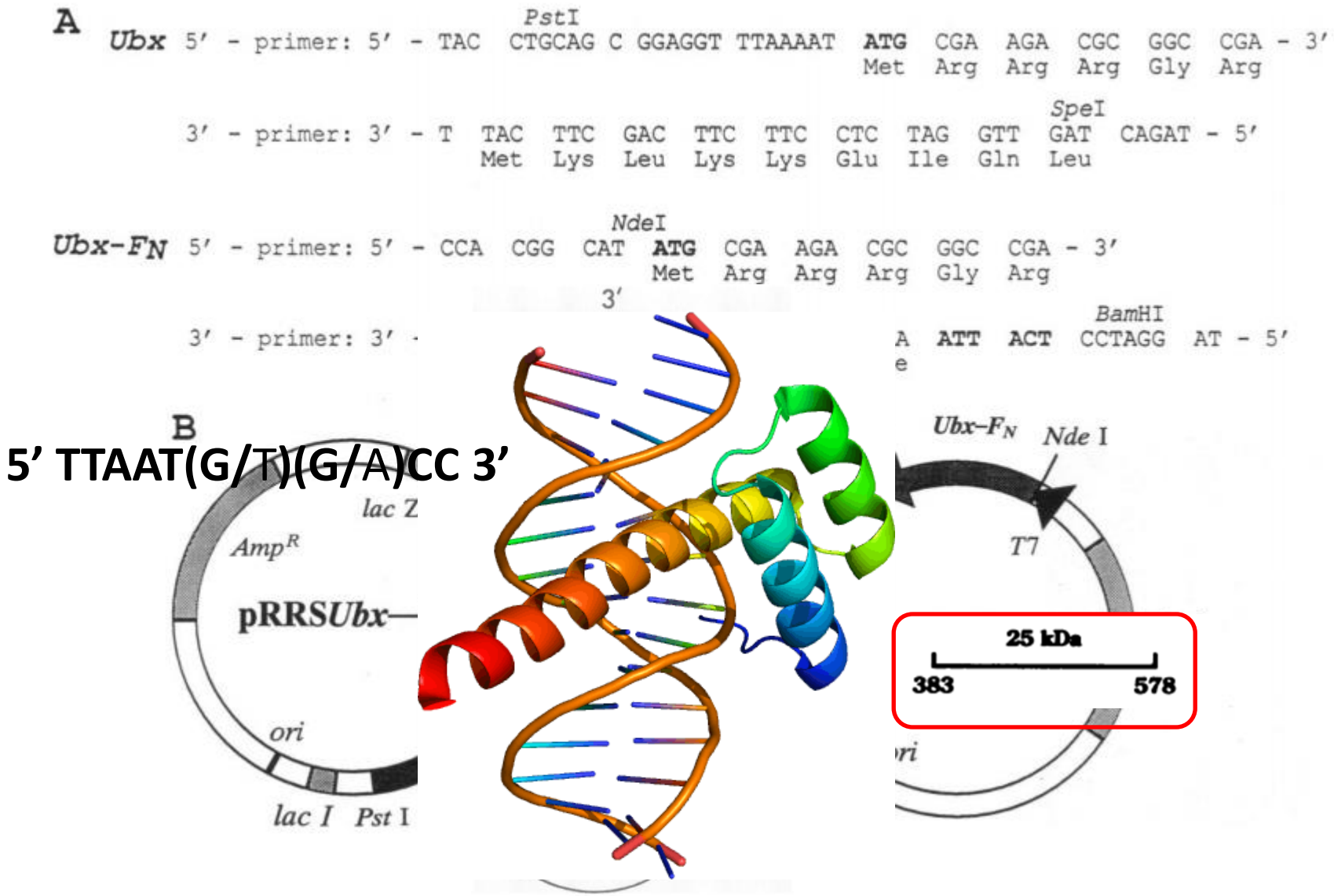

d
F2-Asn
-1 3 6
R R D V L M N H I R
0.6
0.5
0.4
0.3
0.2
0.1
0
A_{650}-A_{450}
GNN
ANN
TNN
CNN
NGN
NAN
NTN
NCN
NNG
NNA
NNT
NNC
wild-type
G T/C G
mutant
G/A T/C G

Designing of Seq. Spec. Recog. of DNA

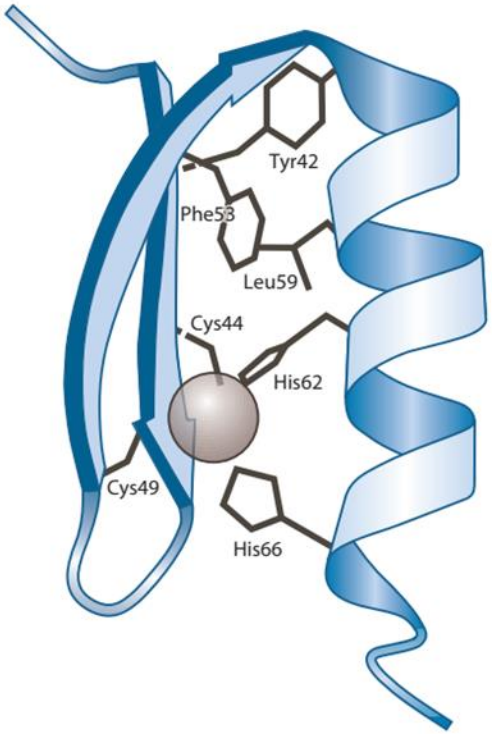


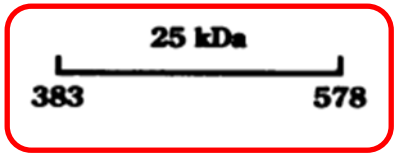
fused together using an extended canonical linker (2 × 3F scheme). (*b*) Three two-finger peptides linked using canonical linkers extended by an insertion of either a glycine residue or a glycine-serine-glycine sequence in the canonical linkers between fingers 2 and 3 and fingers 4 and 5, respectively.

Chimeric restriction endonuclease



Artificial Nucleases

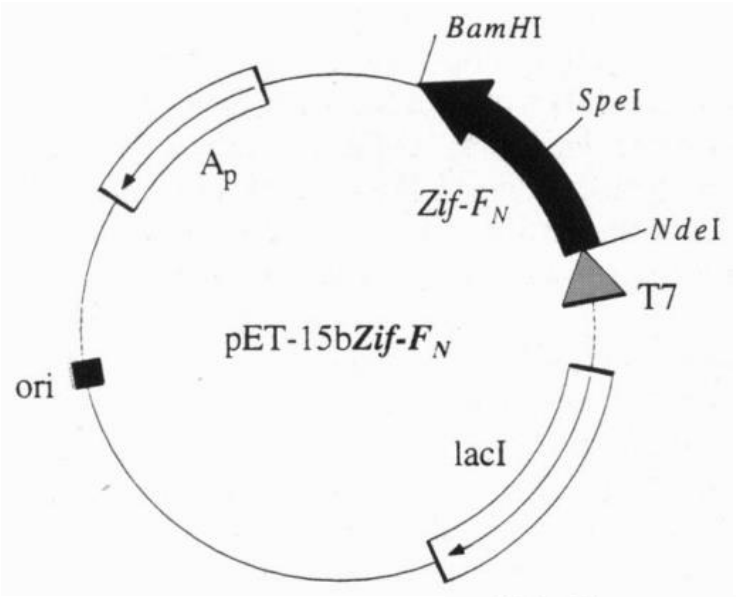


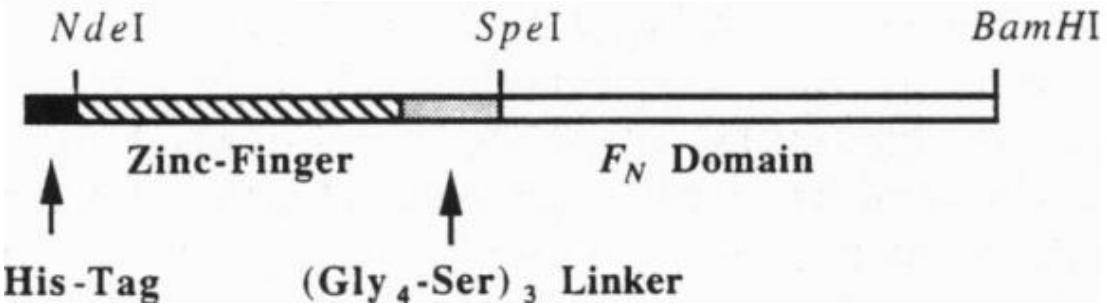


Hybrid restriction enzymes: Zinc finger fusions to *Fok* I cleavage domain

(*Flavobacterium okeanokoites*/chimeric restriction endonuclease/protein engineering/recognition and cleavage domains)

YANG-GYUN KIM, JOOYEUN CHA, AND SRINIVASAN CHANDRASEGARAN*





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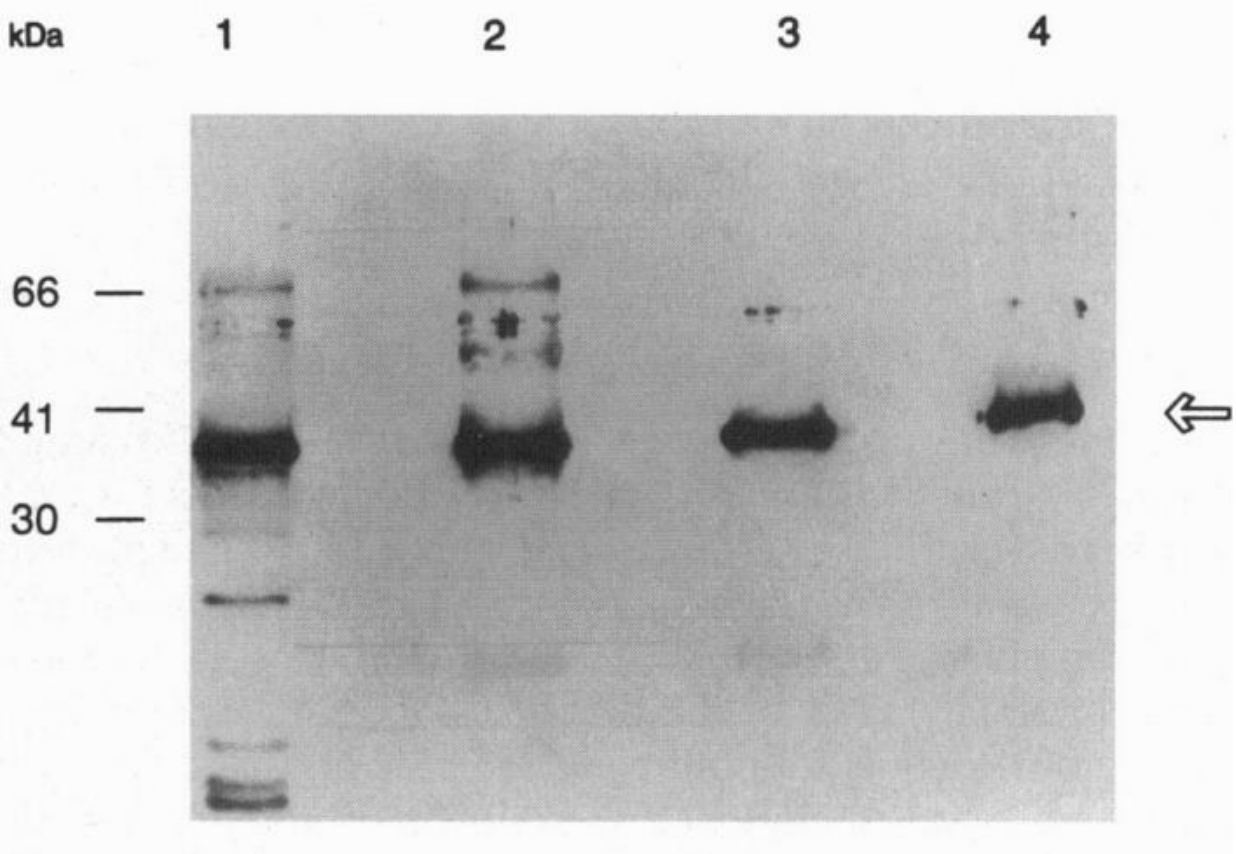

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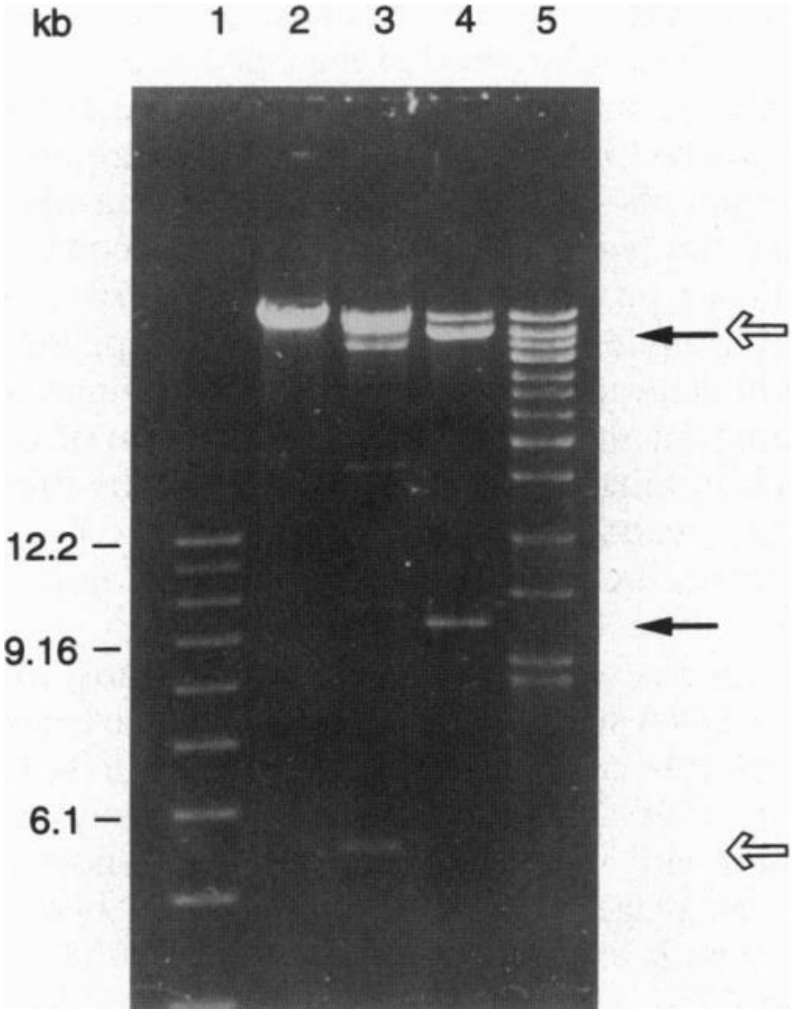

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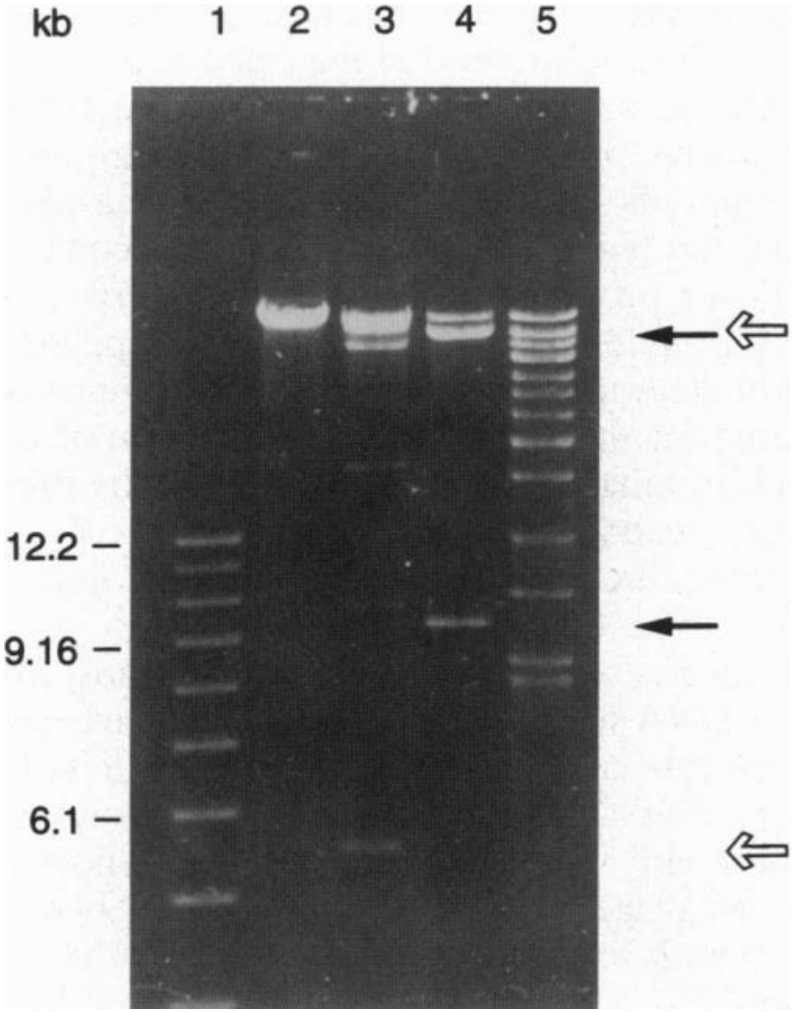
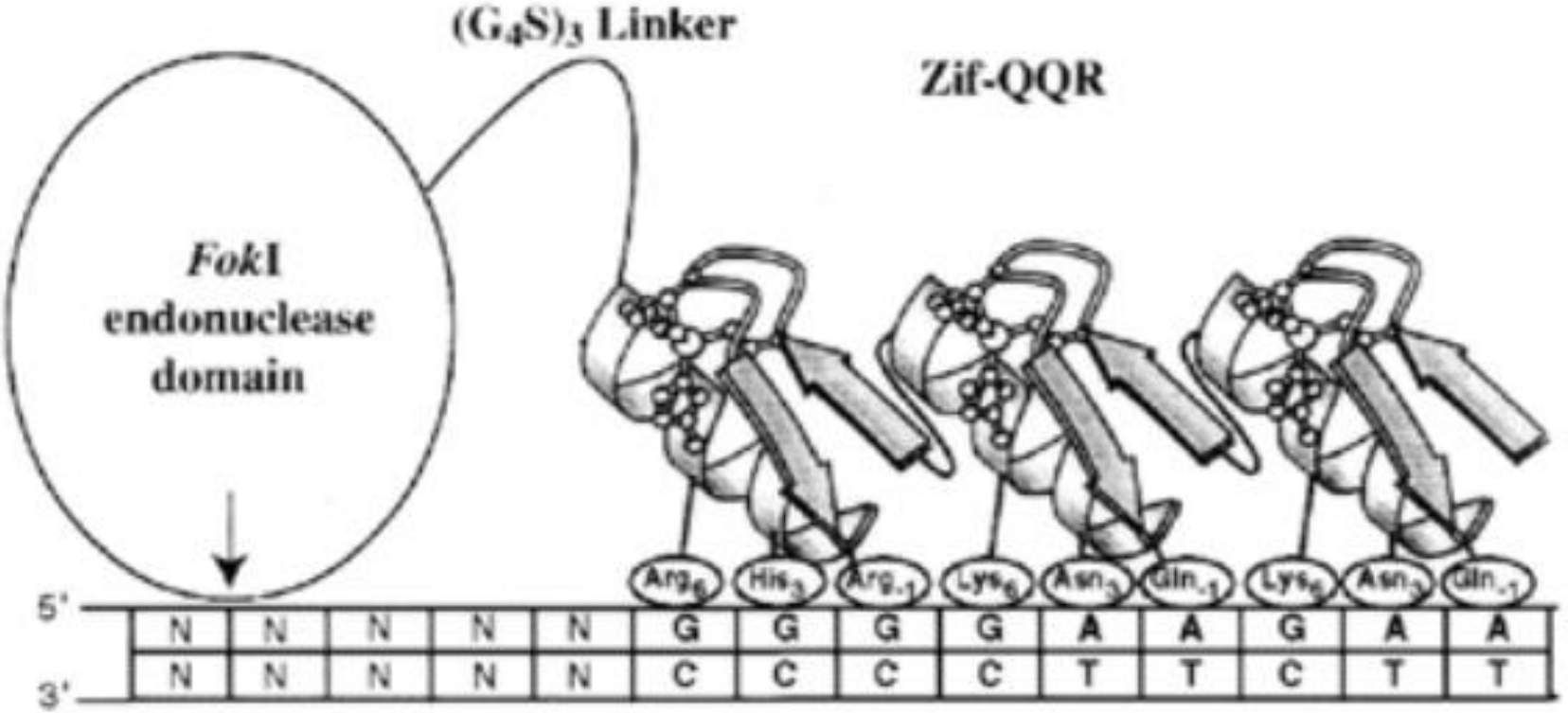
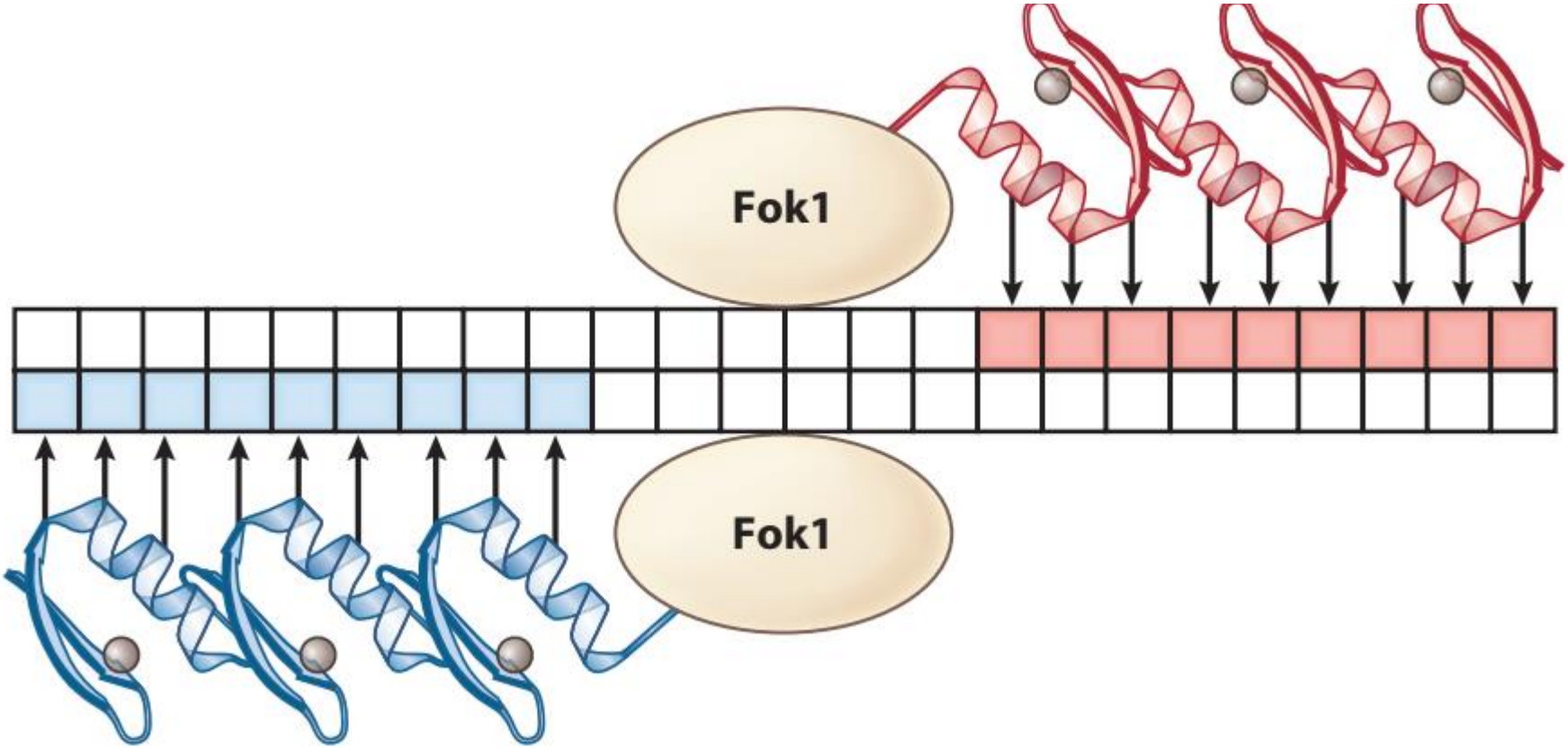
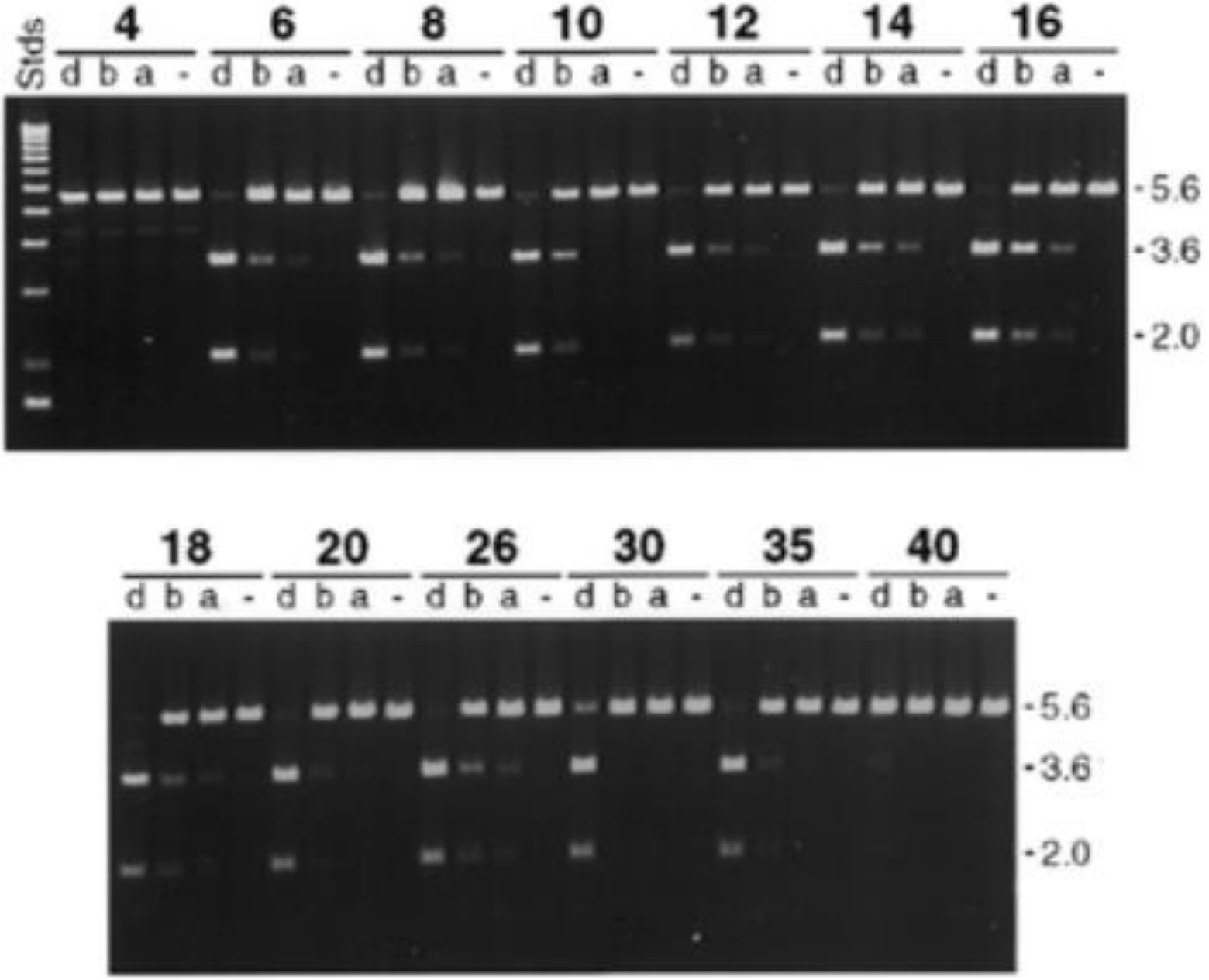



(G4S)3 Linker
Zif-QQR
FokI endonuclease domain
Arg6 His3 Arg-1 Lys6 Asn3 Gln-1 Lys6 Asn3 Gln-1
5'
3'
N N N N N G G G G A A G A A
N N N N N C C C C T T C T T

Requirements for double-strand cleavage by chimeric restriction enzymes with zinc finger DNA-recognition domains

Jeff Smith[1,2], Marina Bibikova[3], Frank G. Whitby[3], A. R. Reddy[1,4], Srinivasan Chandrasegaran[1] and Dana Carroll[3,*]




Stds
4
6
8
10
12
14
16
d b a -
5.6
3.6
2.0
18
20
26
30
35
40

Limitations

- **Targeted Genome editing in various organisms**
- **Two major limitations:**

1. **Zinc finger domains have limited modularity.**
2. **Lack of specificity of some ZF domains = off target cleavage.**
3. **Tedious, elaborate and time consuming process.**

Conclusions of Lecture-12

- Today we saw the First Zinc finger Nuclease (ZNFs).
- Use of hybrid nuclease by various lab. groups.
- The foundation of Sangamo Therapeutic company.
- Limitations: a) Limited modularity

  b) off-targets

  c) Lengthy process

Questions?