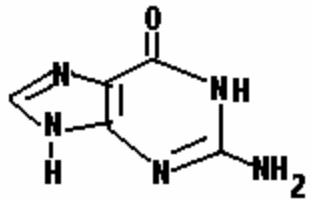
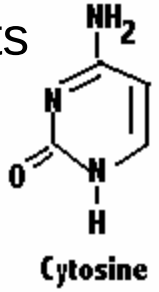
DNA

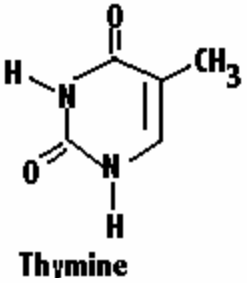
Branden & Tooze, Ch. 7

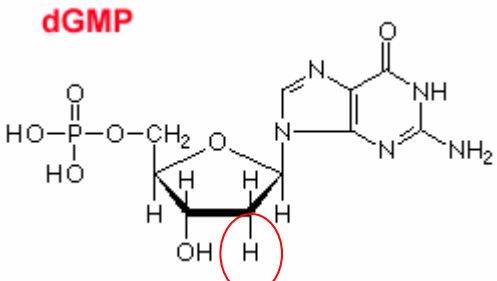
Deoxyribose nucleic acids are made of three parts

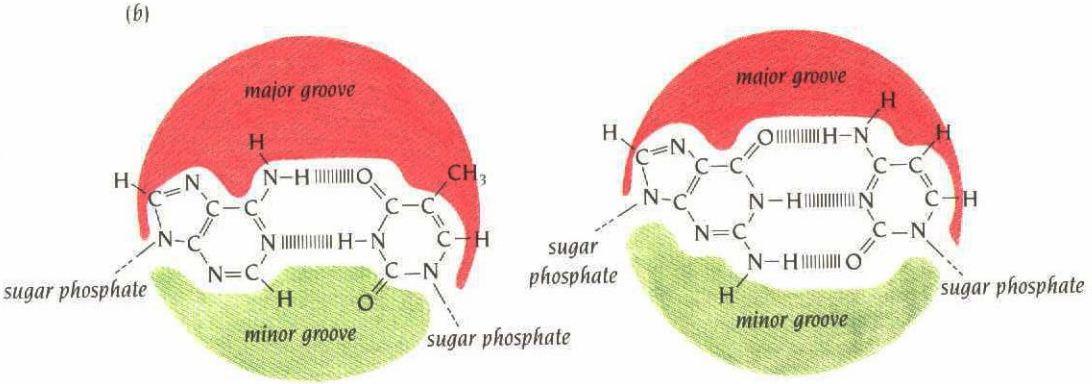
- base: adenine, cytosine, guanine, thymine
- sugar: deoxyribose
- phosphate: will form the phosphate backbone



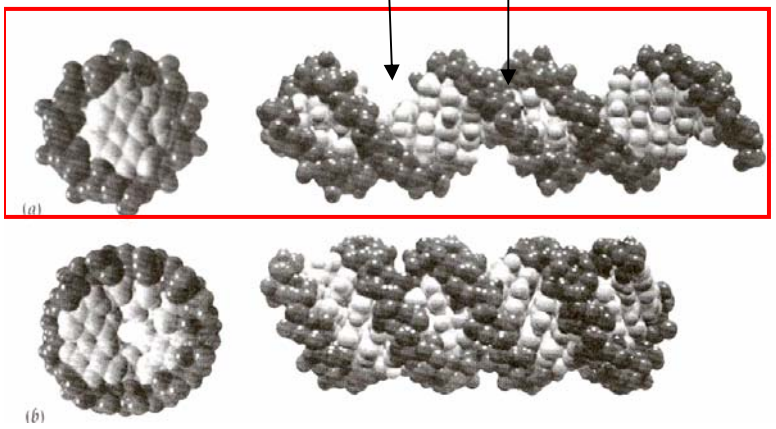


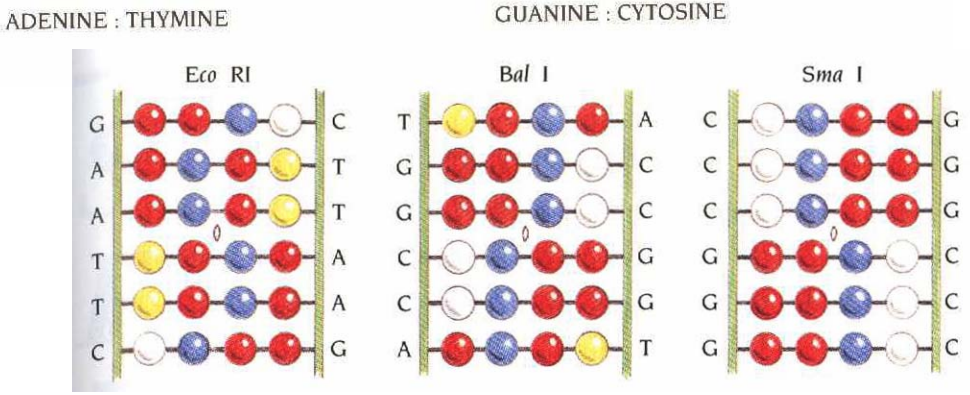






wide narrow

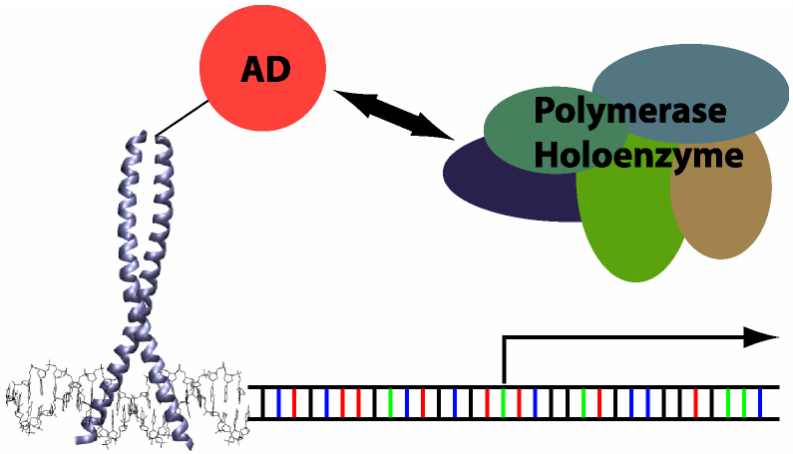


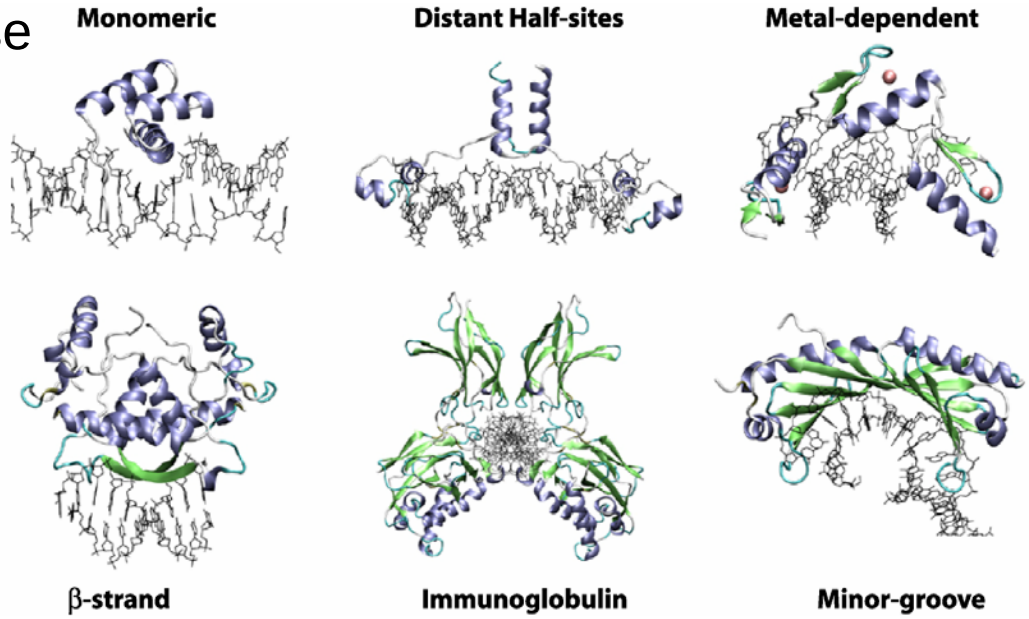


DNA binding protein

- Sequence-specific recognition of DNA epitomizes macromolecular interaction
  - transcription factors are proteins that bind to the promoter region of a gene and regulate gene expression
  - restriction endonuclease are enzymes that cleave specific DNA sequences

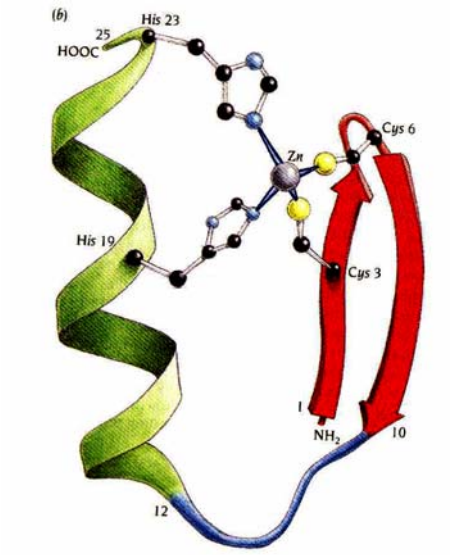
- DNA binding activity resides within the DNA binding domain of a transcription factor while the activation domain mediates protein-protein interaction
- DBD's are structurally diverse

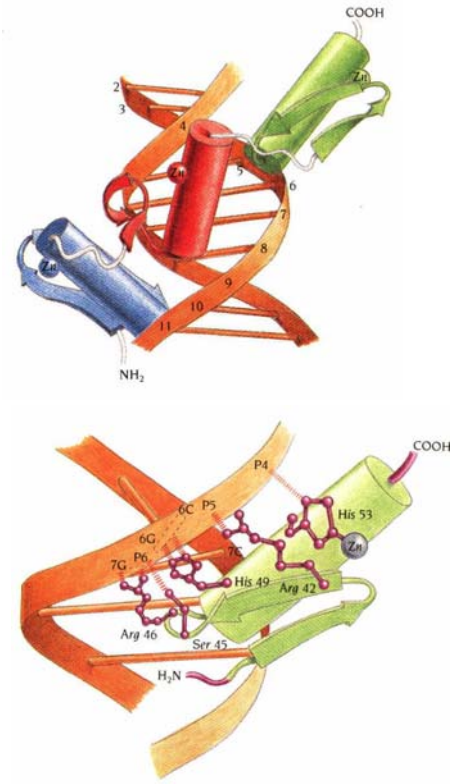


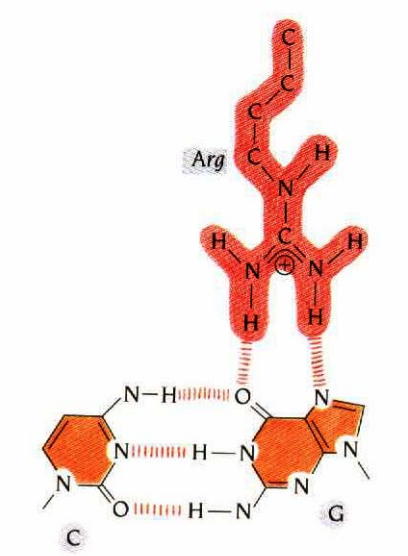


Zinc finger proteins

- Zinc finger proteins comprise multiple copies of a small beta-beta-alpha domain stabilized by a bound zinc atom
- Each module in the protein functions independently and recognizes ~ 3 – 4 DNA base pairs
- Select residues in each domain are responsible for interacting with DNA (the rest provide structural scaffold)



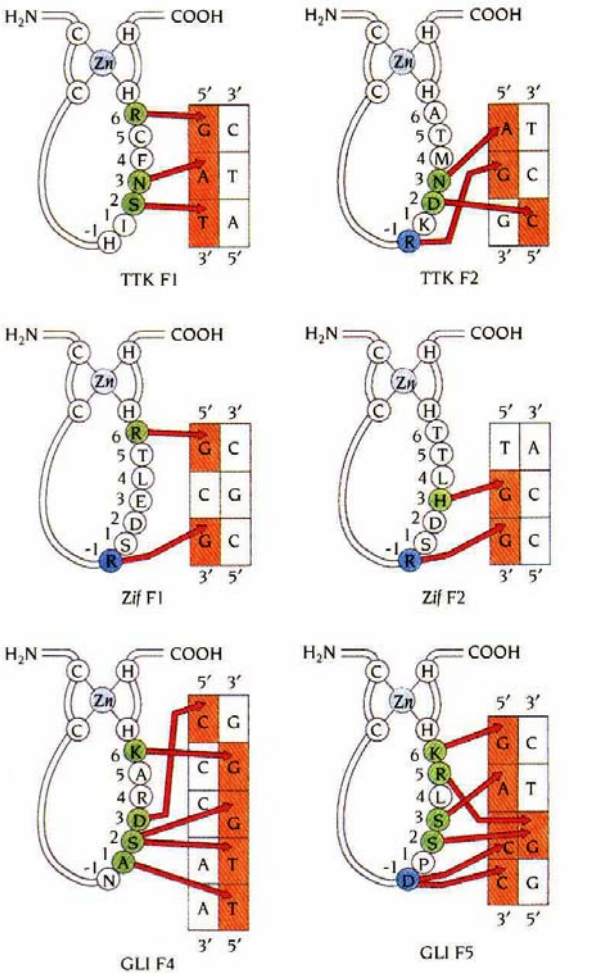


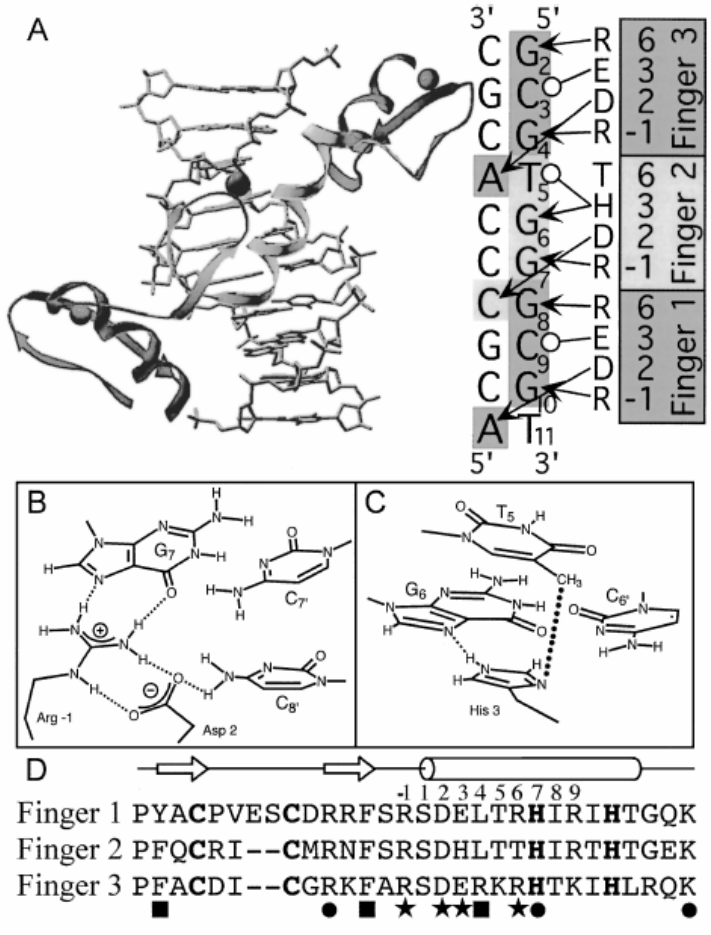


B&T, Ch 10

- Polydactyl ZFP with 6 domains can recognize 18 bp, which are statistically unique in the human genome

$$4^{18} > 3 \times 10^{9}$$





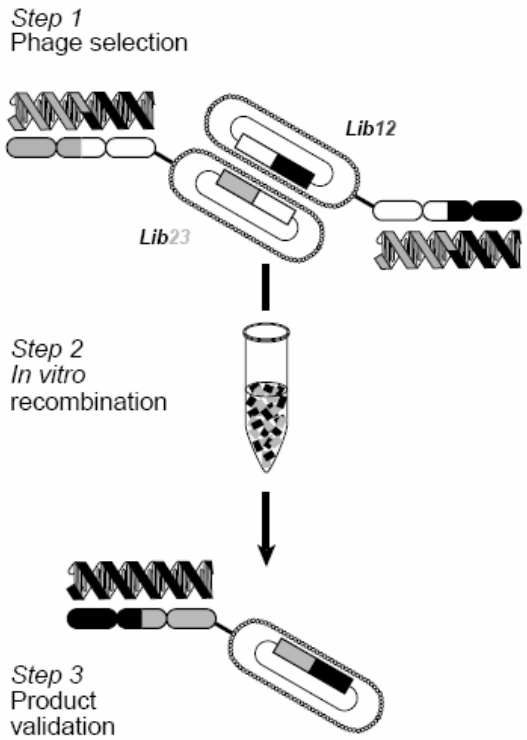
B&T, Ch 10

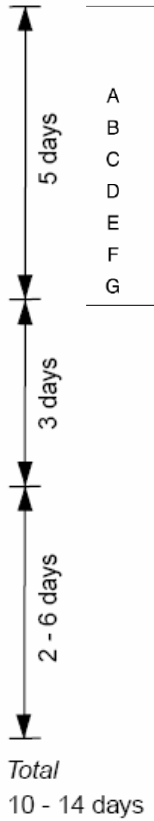
Wolfe et al, Ann Rev Biophy Biomol Struct 3, 183 (2000)

Engineering ZFP Specificity

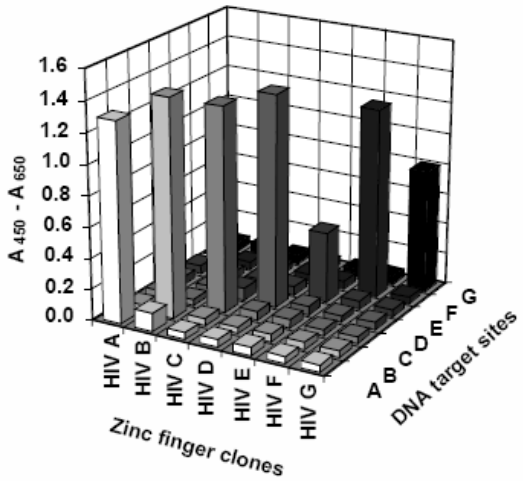
Phage display of randomized ZFP (one and a half finger at a time)

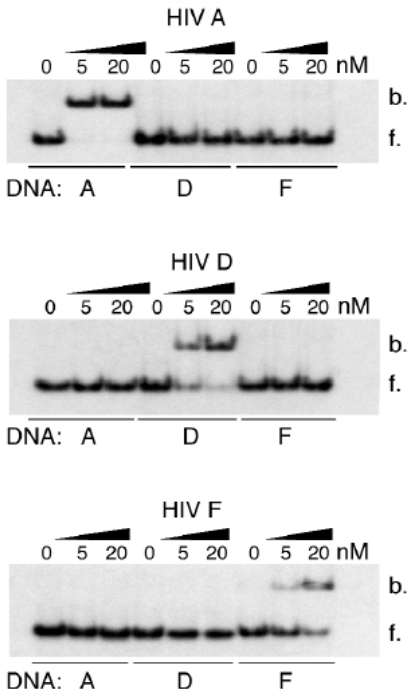
Simultaneous randomization of residues in more than one domain is important to optimize binding to long DNA sequences



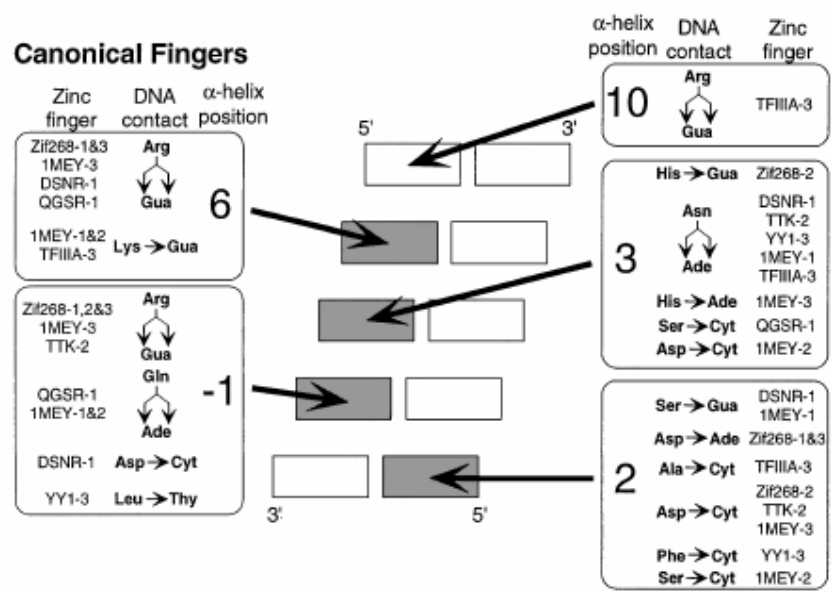


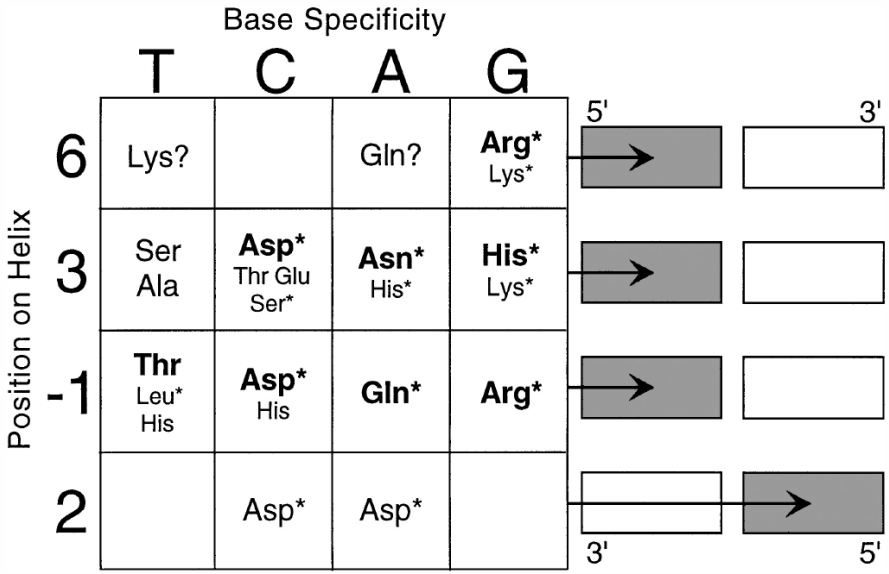
Clone	DNA target sequence[a]	Position of base Q in LTR	Zinc finger sequence[b]		
	F1 F2 F3		F1	F2	F3
	3'-H IJK LMN OPQ -5'		-1123456	-1123456	-1123456
A	T GCG GAG GGA	-79	RSDELTR	RSDNLST	RRDHRTT
B	G AGG GGT CAG	-58	DSAHLTR	RSDHLST	DSANRTK
C	T ACG TCG TAG	-36	ASADLTR	NRSDLSR	TSSNRKK
D	T TCG TCG ACG	-22	HSSDLTR	QSSDLSK	QNATRKR
E	T CCG AGT CTA	+22	DSSSLTK	QSAHLST	DSSSRTK
F	T CTC TCG AGG	+33	ASDDLTQ	RSSDLSR	QSAHRTK
G	G GAT CAA TCG	+44	RSDALIQ	DRANLST	ASSTRTK



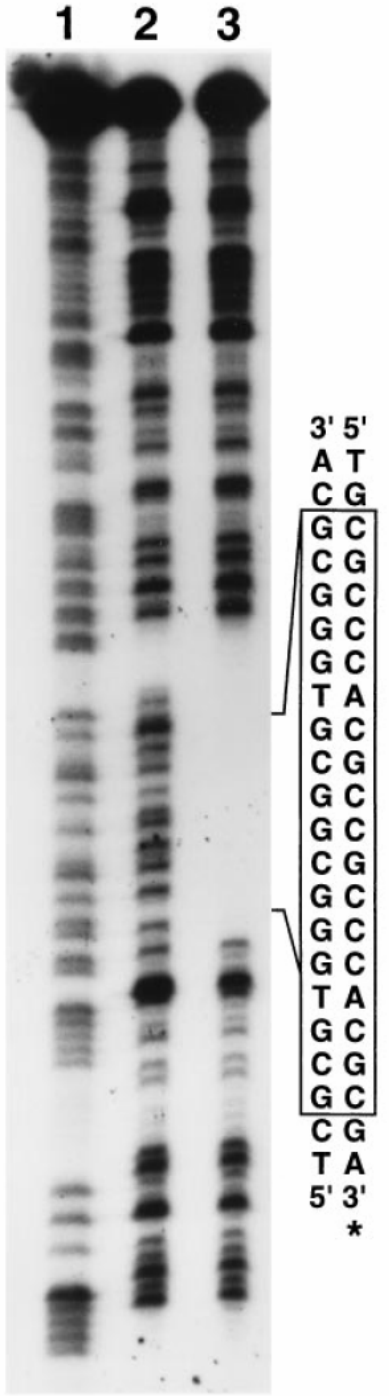


Isalan et al, Nat Biotech 19, 656 (2001)





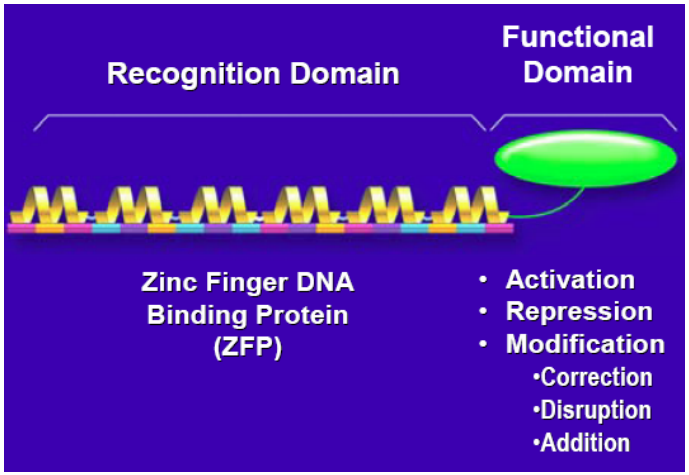
Wolfe et al, Ann Rev Biophy Biomol Struct 3, 183 (2000)



Liu et al, PNAS 94, 5525 (1997)

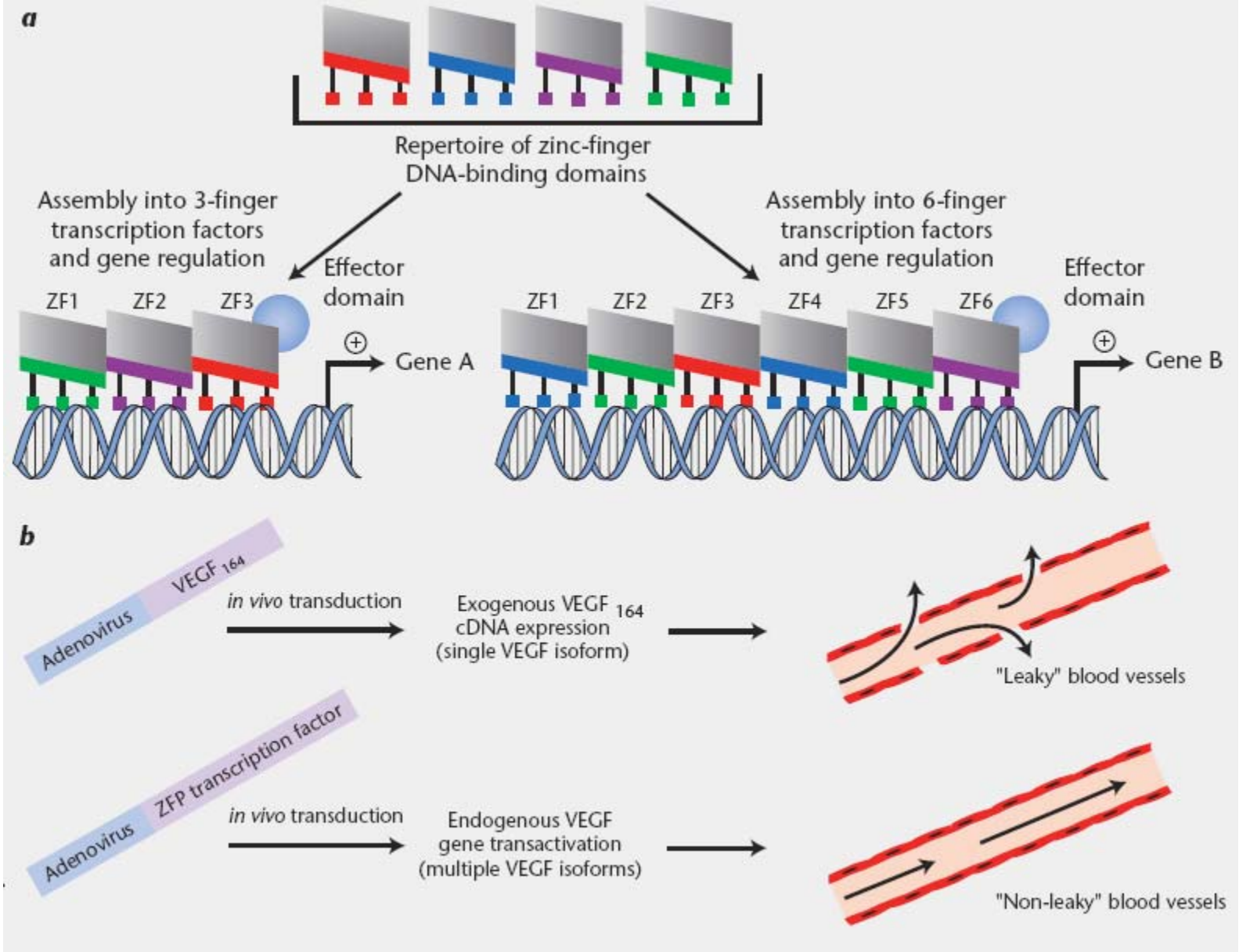
What to do with engineered ZFP

- Regulate gene expression by fusing DBD with a functional domain
- The functional domain can be an activator or a repressor
- Can target the localization through addition of nuclear localization signal
- Combine with gene therapy to effect changes



Sangamo Biosciences pipeline

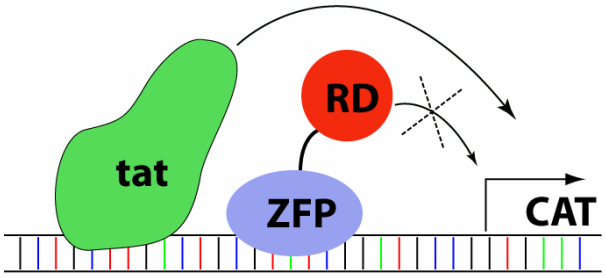
Growing blood vessels

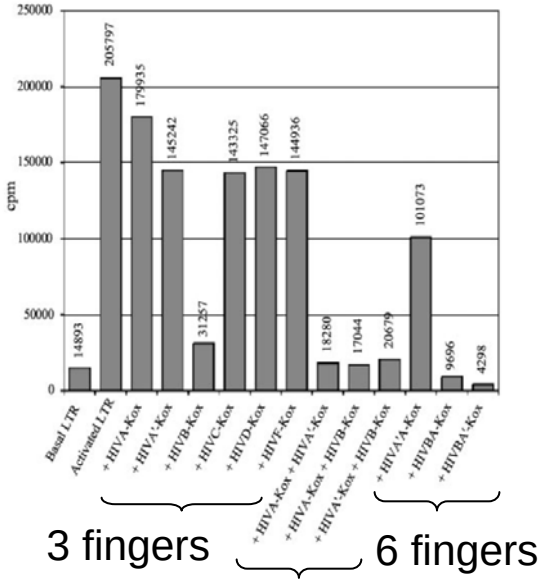


Pasqualini et al, Nat Med 8, 1353 (2002)

Inhibiting HIV-1 replication

- Gene transcription can be repressed by fusing a ZFP with a repressor domain, e.g. Kruppel-associated box (KRAB) repressor (KOX1)
- HIV-1 encodes two regulatory proteins, Tat and Rev
- Engineered ZFPs can bind the Rev response element, raising the question whether viral replication may be controlled using these proteins





3 fingers

6 fingers

multiple
3 fingers

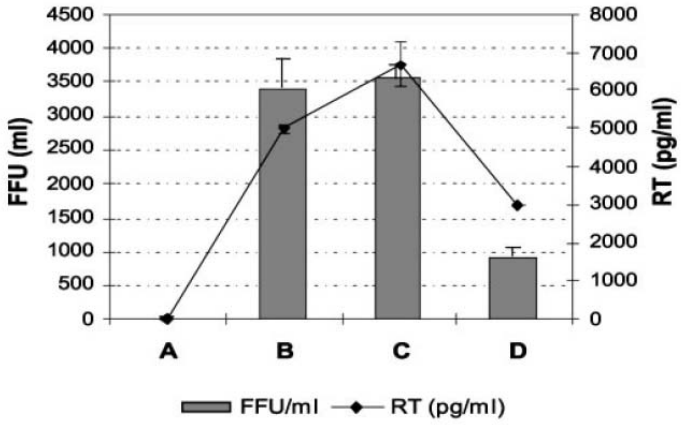
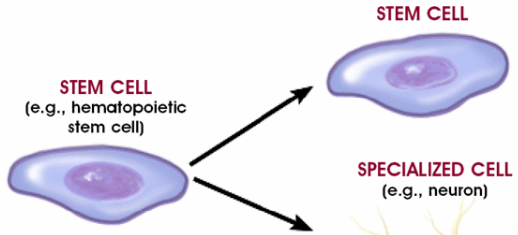


Fig. 5. Assays to demonstrate inhibition of HIV-1 replication. The bars show HIV-1 focus-forming units (FFU)/ml, and the line shows the level of HIV-1 reverse transcriptase (RT) in the culture supernatant. The negative control contains pcDNA3.1 alone (A), whereas positive controls contain pcDNA3.1 + CXCR4 (B) or TFZ-KOX + CXCR4 (C). HIVBA′-KOX was tested and showed a reduction in the number of foci and the levels of viral RT (D).

Reynolds et al, PNAS 100, 1615 (2003)

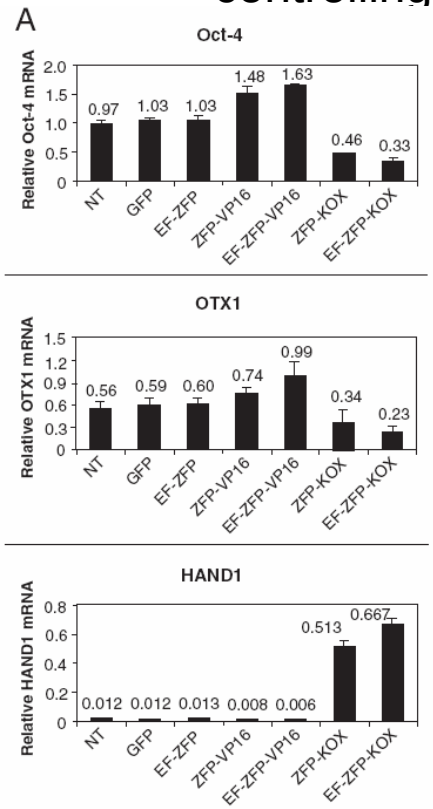
Controlling stem cell fate



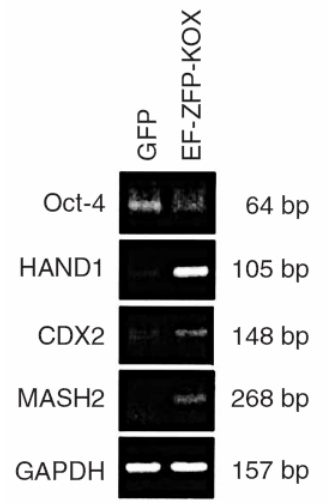
Stem cells are progenitor cells that can differentiate into specialized cells

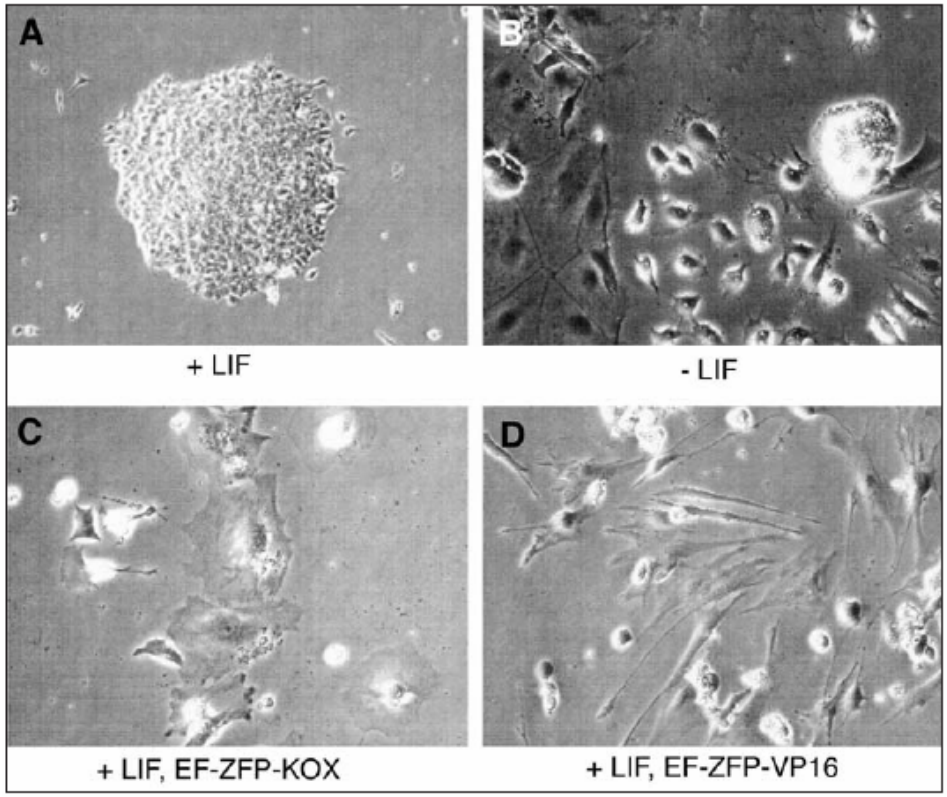
Differentiation and cell longevity are controlled via signaling pathways and transcriptional regulation

- Oct-4 gene is important for self-renewal and pluripotency
- controlling Oct-4 has an effect on other downstream gene expression



VP16=activator
KOX=repressor





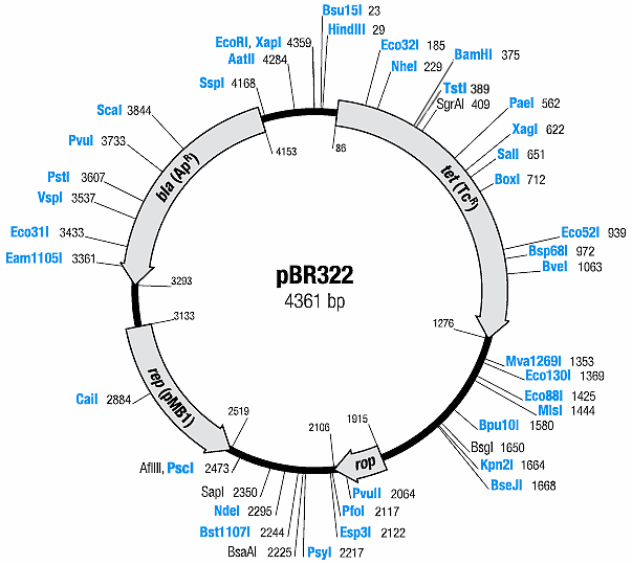
Bartsevich et al, Stem Cells 21, 632 (2003)

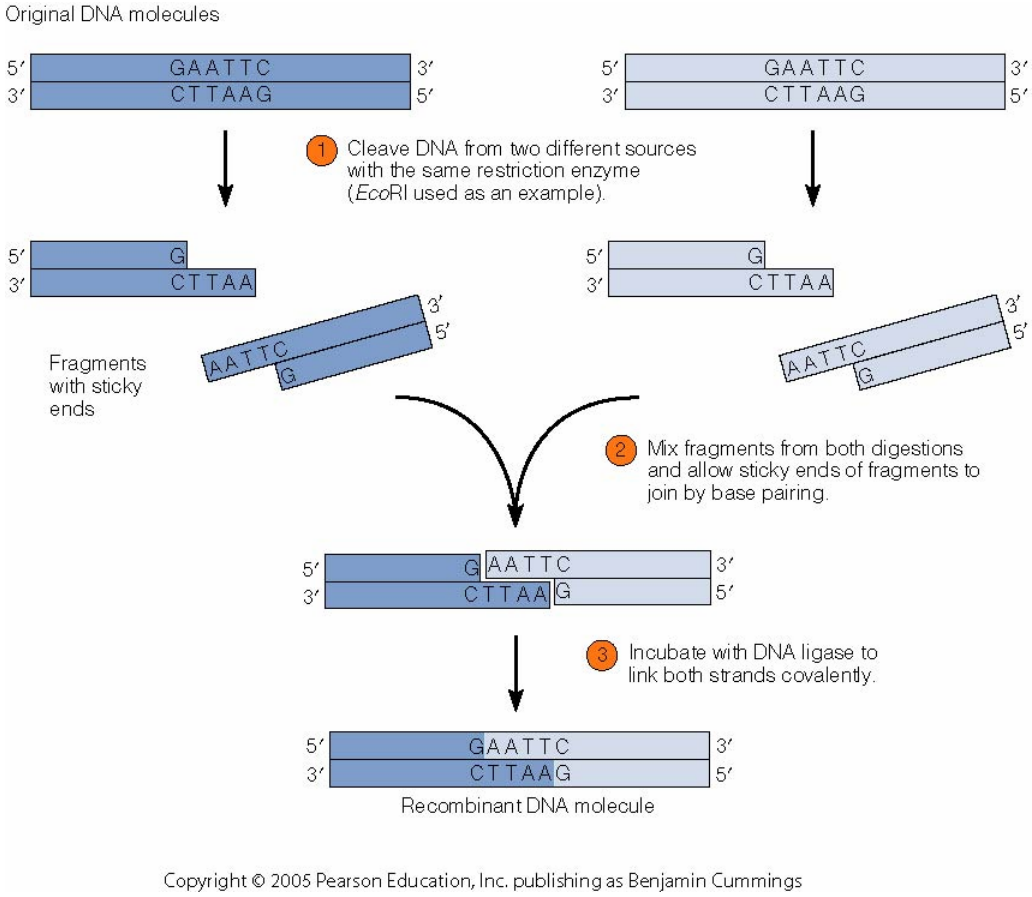
Restriction enzymes

- Restriction enzymes recognize specific DNA sequences and hydrolyze the phosphate backbone
- Used in molecular biology to “sub-clone” DNA

EcoR I

Sma I

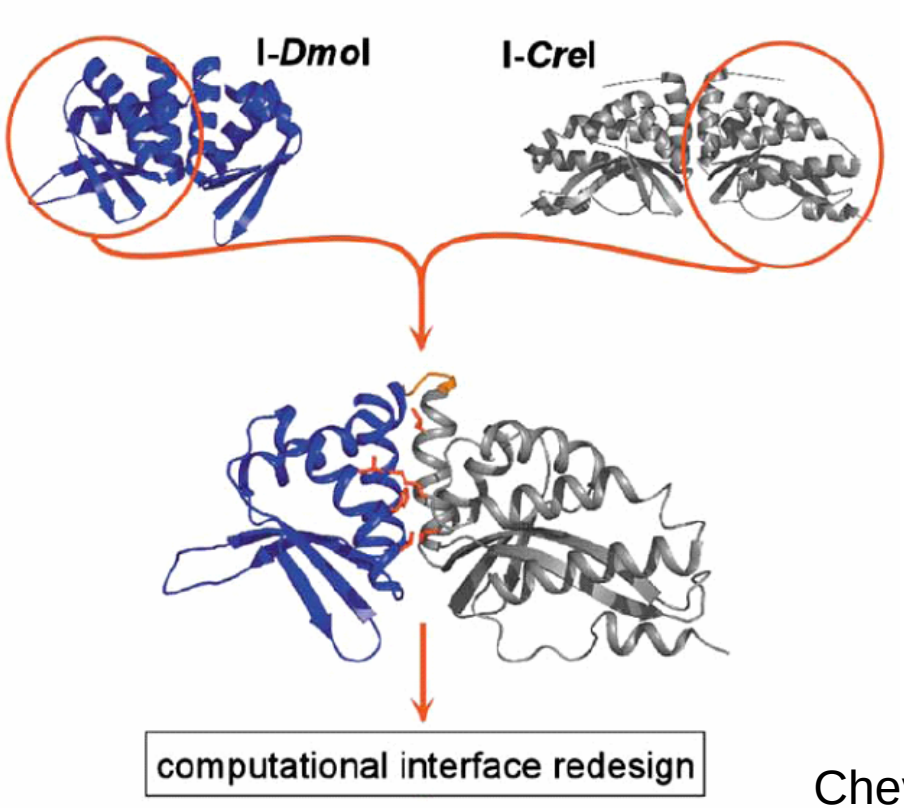




restriction digest followed by ligation

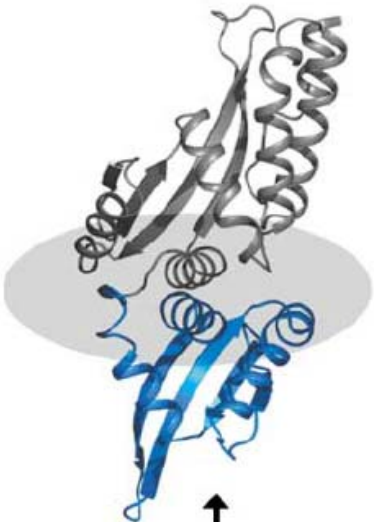
Computation design of a homing endonuclease

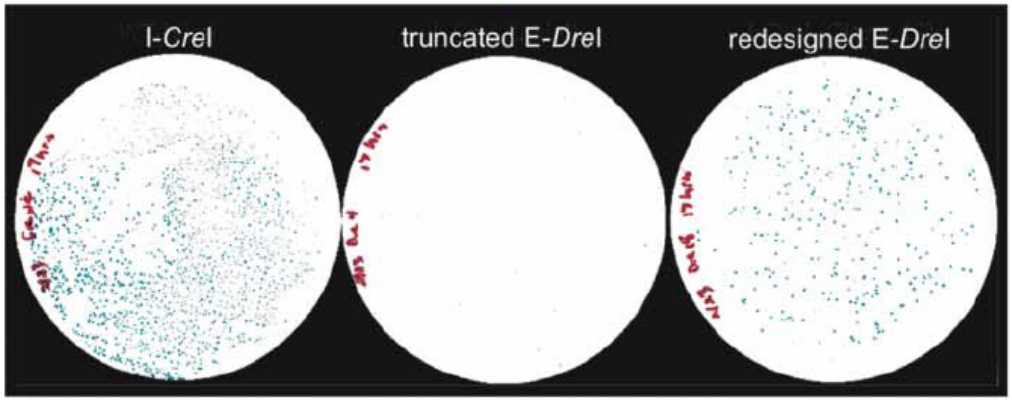
- Domain that binds DNA sequence-specifically (e.g. ZFP) can be fused to a catalytic domain that modifies DNA non-specifically (e.g. nuclease) to target covalent modification
- Interdependence of structure, substrate recognition and catalysis makes designing new restriction enzyme a challenge

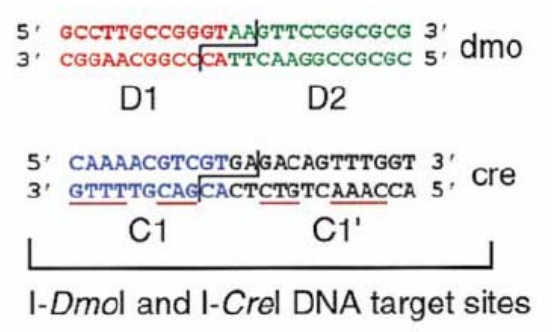


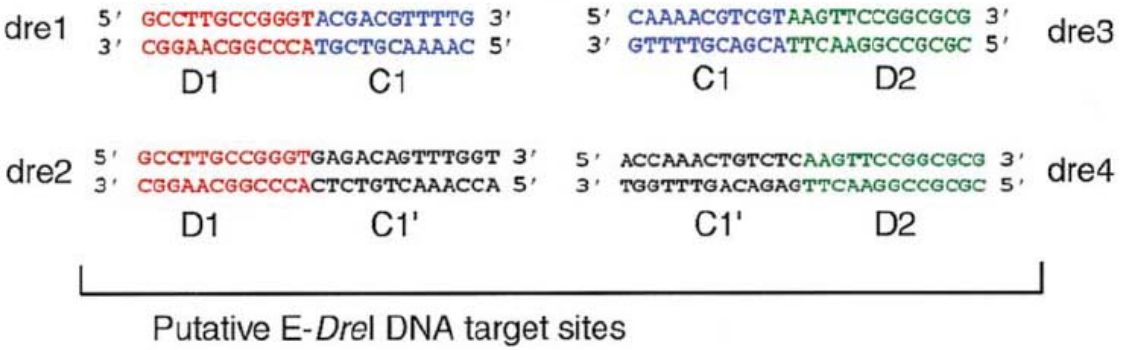
- use conserved “LAGLIDADG” helix to orient the domains
- first model based on ala scanning
- optimize the interface by including more residues in the calculation (total of 14 residues, of which 8 were ultimately changed)
- introduce a short peptide linker between the two domains to generate a monomeric protein

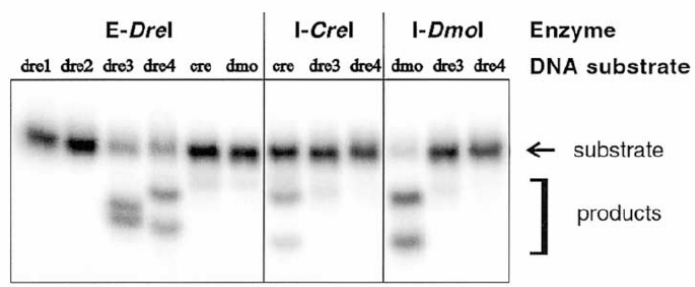
Chevalier et al, Mol Cell 10, 895 (2002)

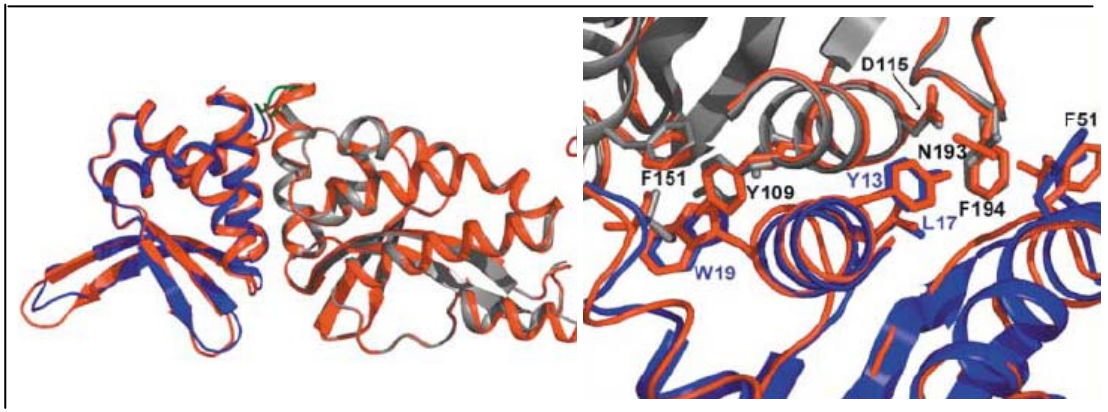



I-*Cre*I
truncated E-*Dre*I
redesigned E-*Dre*I


5′ GCCTTGCCGGGTAAGTTCCGGCGCG 3′
3′ CGGAACGGCCCATTCAAGGCCGCGC 5′ dmo
D1 D2
5′ CAAAACGTCGTGAGACAGTTTGGT 3′
3′ GTTTTGCAGCACTCTGTCAAACCA 5′ cre
C1 C1′
I-*Dmo*I and I-*Cre*I DNA target sites


dre1
5′ GCCTTGCCGGGTACGACGTTTTG 3′
3′ CGGAACGGCCCATGCTGCAAAAC 5′
D1 C1
dre3
5′ CAAAACGTCGTAAGTTCCGGCGCG 3′
3′ GTTTTGCAGCATTCAAGGCCGCGC 5′
C1 D2
dre2
5′ GCCTTGCCGGGTGAGACAGTTTGGT 3′
3′ CGGAACGGCCCACTCTGTCAAACCA 5′
D1 C1′
dre4
5′ ACCAAACTGTCTCAAGTTCCGGCGCG 3′
3′ TGGTTTGACAGAGTTCAAGGCCGCGC 5′
C1′ D2
Putative E-*Dre*I DNA target sites


E-*Dre*I
I-*Cre*I
I-*Dmo*I
Enzyme
dre1 dre2 dre3 dre4 cre dmo
cre dre3 dre4
dmo dre3 dre4
DNA substrate
substrate
products


D115
F51
N193
F151
Y109
Y13
F194
L17
W19