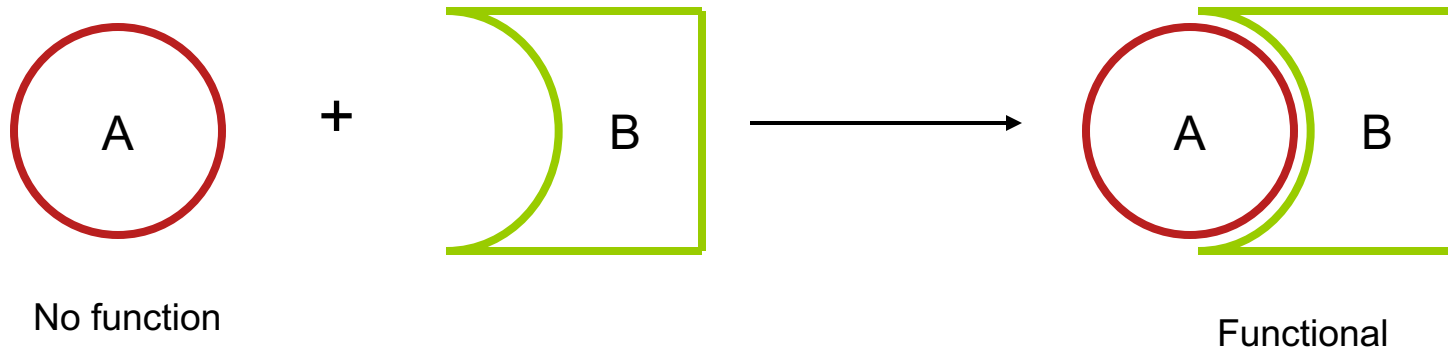


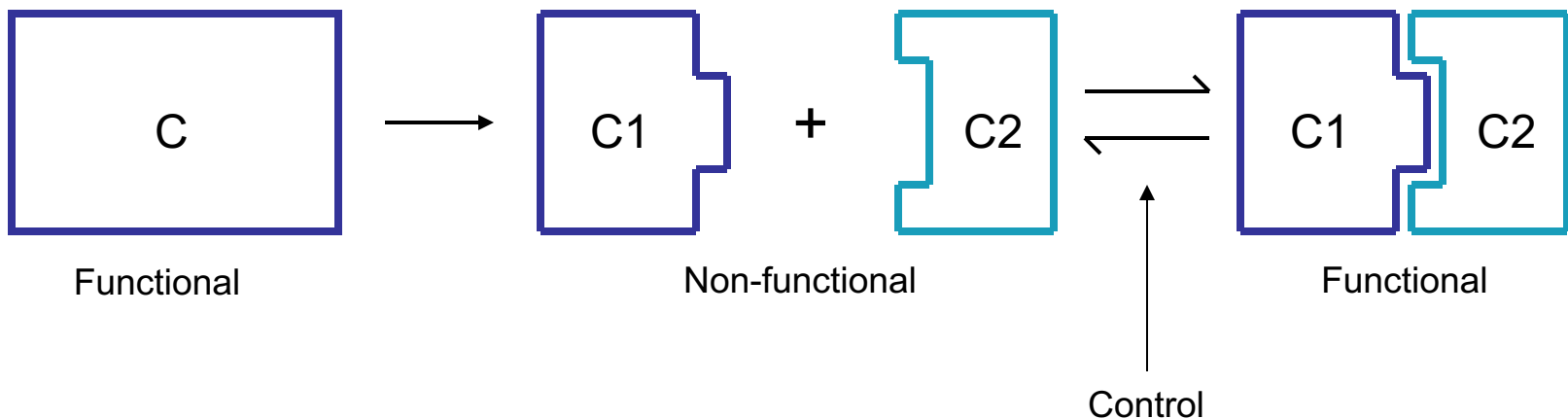
Directed evolution in protein engineering

Control of Protein Function

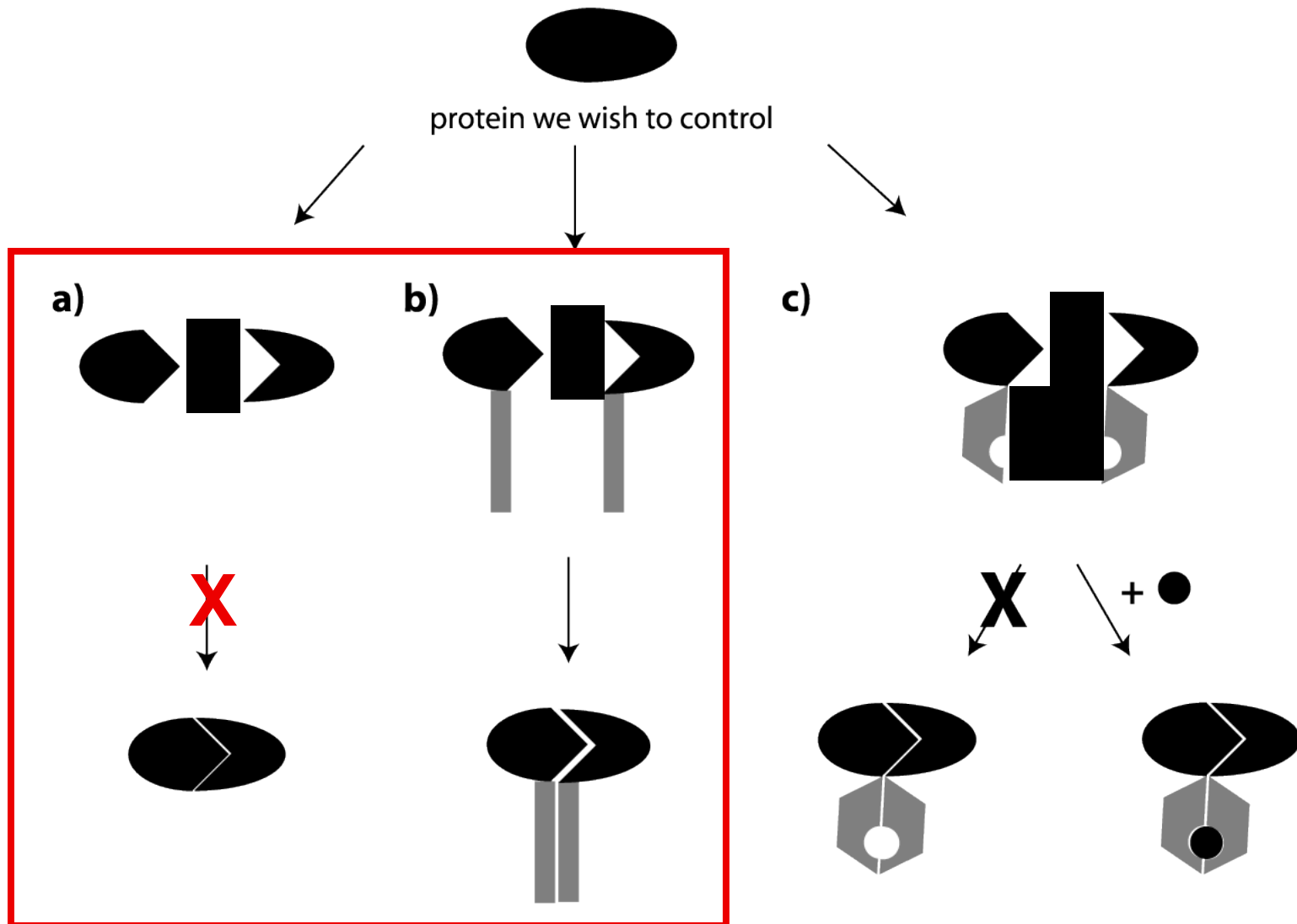
- Nature



- Engineered Control



Protein Fragment Complementation and Assisted Protein Reassembly



Engineering proteins with improved properties

Molecular evolution works through cycles of

- (a) creating a library of gene variations and
- (b) identifying by selection or screening those rare members of the library which code for proteins that have an improvement in function.

Depending on the method of library construction and the methods available for selection and screening, libraries of variants can be constructed and evaluated.

Engineering proteins with improved properties

Common methods of library construction, including error-prone PCR and DNA shuffling, create diversity by changing the amino acid sequences.

In contrast, **incremental truncation**, a method for creating a library of every one base truncation of dsDNA, creates diversity by changing the length of a gene.

Incremental truncation libraries can be created by time dependent *Exo III* digestions

Error Prone PCR

PCR



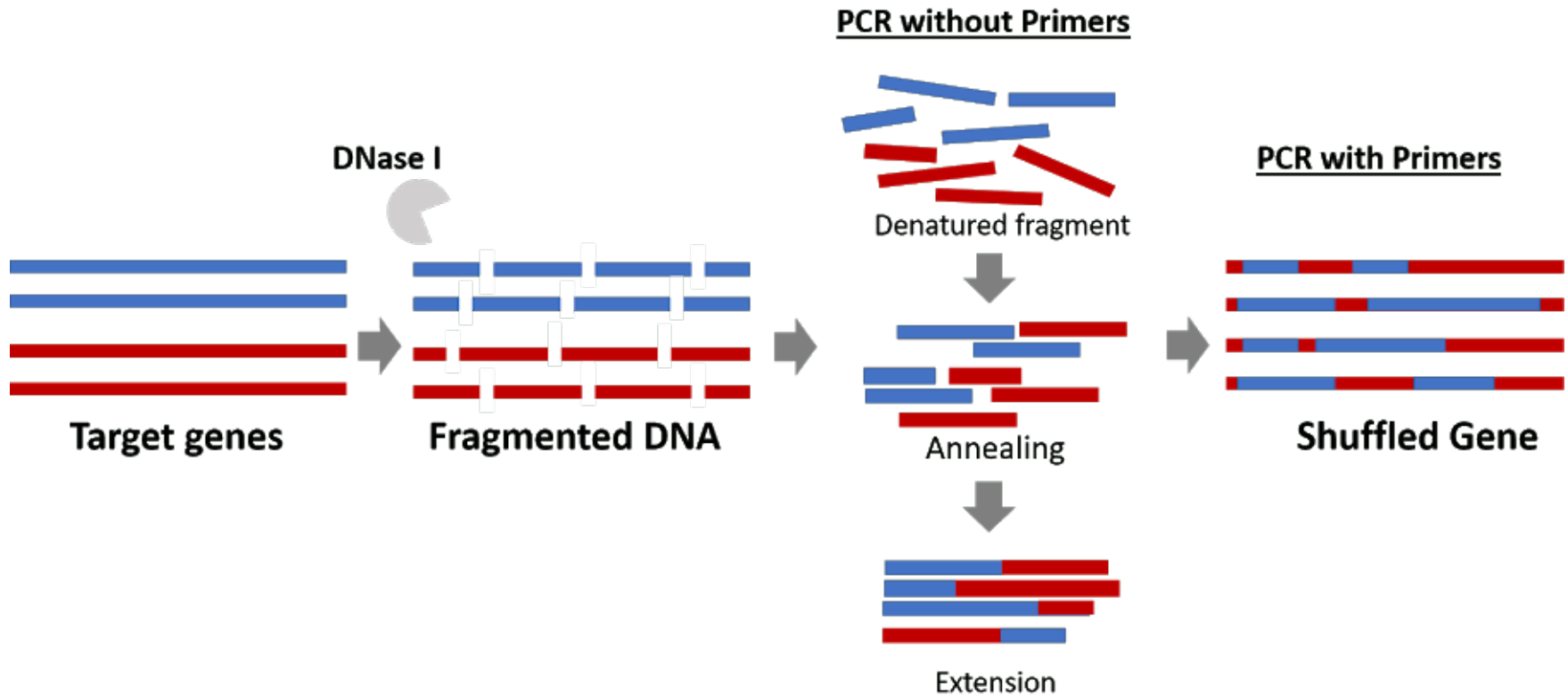
dCTP, dTTP
dGTP, dATP
 Mg^{2+}

Error-Prone PCR



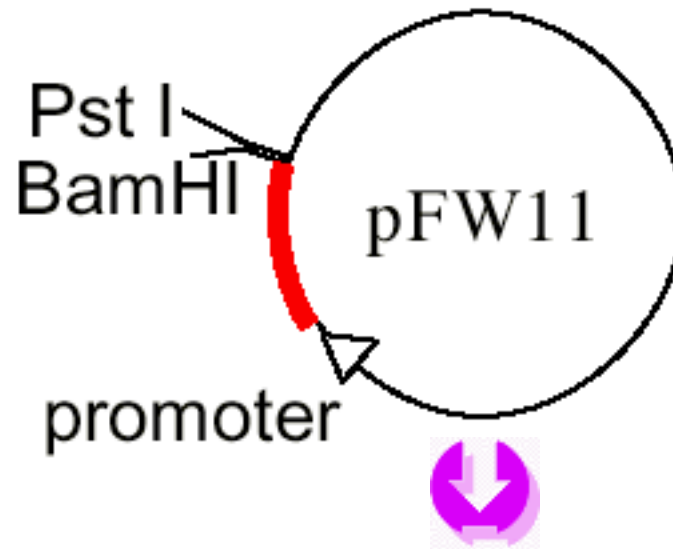
dCTP, dTTP ↑
dGTP, dATP ↓
 Mg^{2+} ↑
 Mn^{2+}

DNA Shuffling

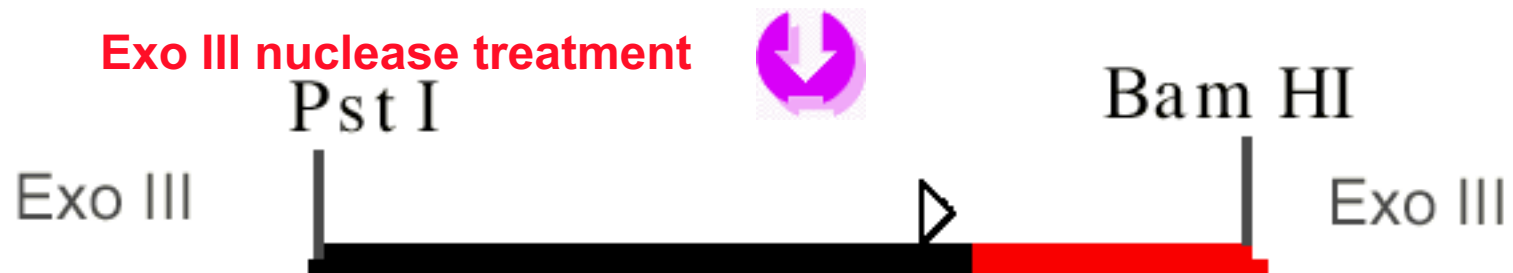


Method for in vitro recombination of homologous genes

Incremental Truncation



Linearize vector by double digestion



Exo III nuclease treatment

Salt concentration (NaCl)

Exo III – helps stepwise removal of mononucleotides from 3'-hydroxyl termini of duplex DNA

Time-dependent sampling !!

PstI

Recognition Sequence

5'CTGCAG 3'
3'GACGTC 5'

Cut Site

5'--CTGCA G--3'
3'--G ACGTC--5'

BamHI

Recognition

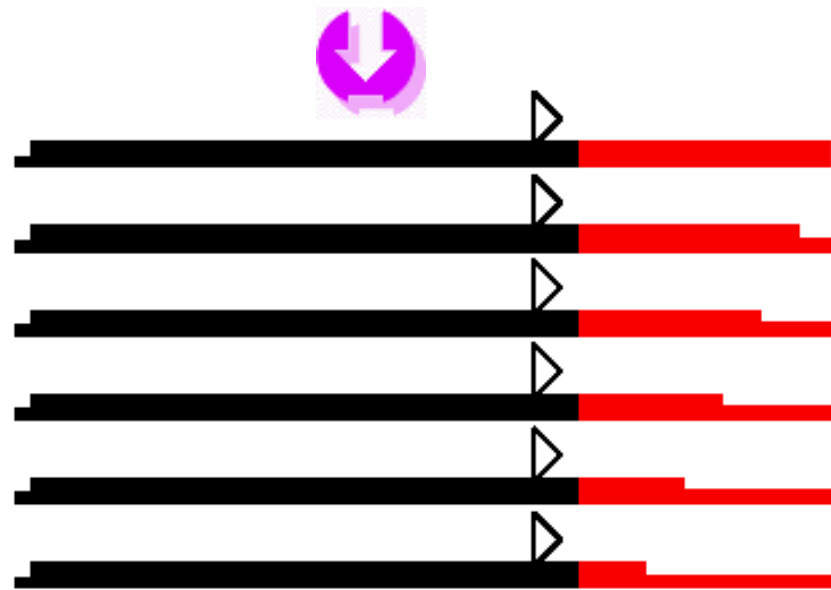
5' -GGATTC-3'
3' -CCTAAG-5'

Cut site

5' -G **GATTC**-3'
3' -CCTAA **G**-5'

The 3' overhangs are resistant to Exo III digestion
whereas the 5' overhangs are SUSCEPTIBLE.

resistant

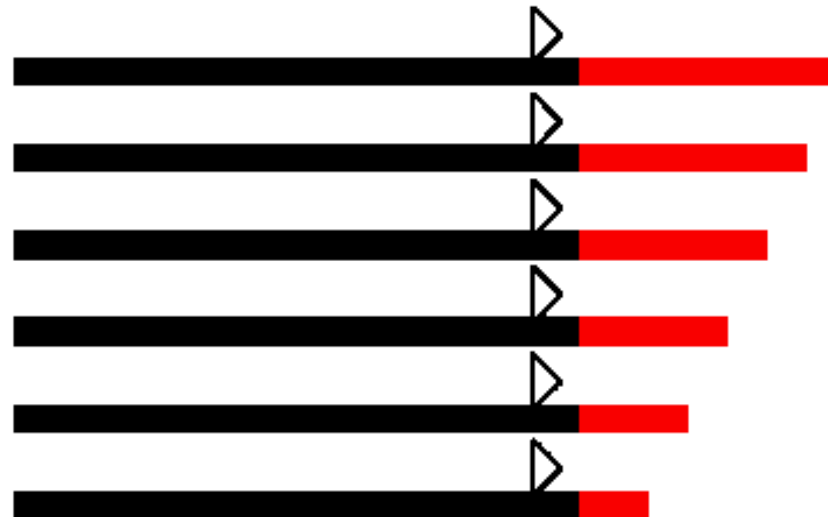


susceptible

Removal of single-stranded
extensions (3' and 5') to leave
ligatable blunt ends



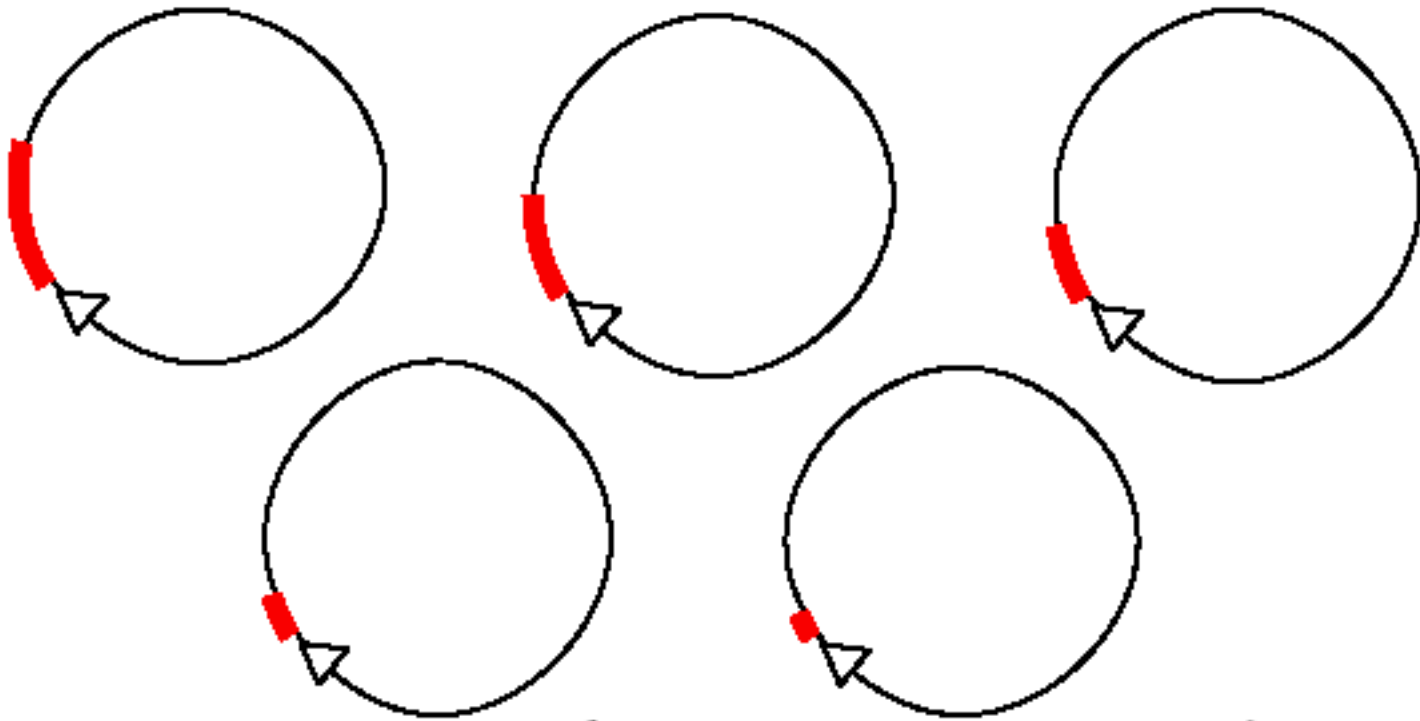
Mung bean nuclease treatment



Klenow treatment

5'-3' polymerase
3'-5'-exonuclease

NO 5'-3' exonuclease



Diverse Library with every one base deletion

Combining Incremental Truncation Libraries

