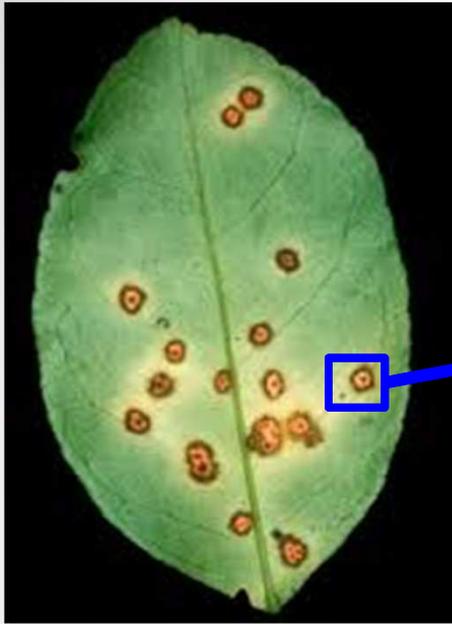
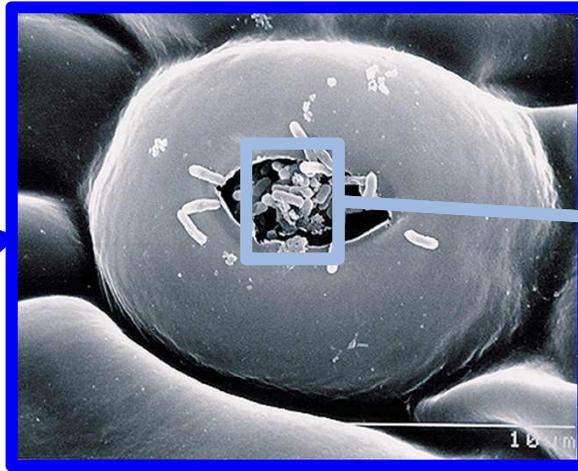


TRANSCRIPTIONAL
ACTIVATOR-LIKE
EFFECTORS
(TALEs)

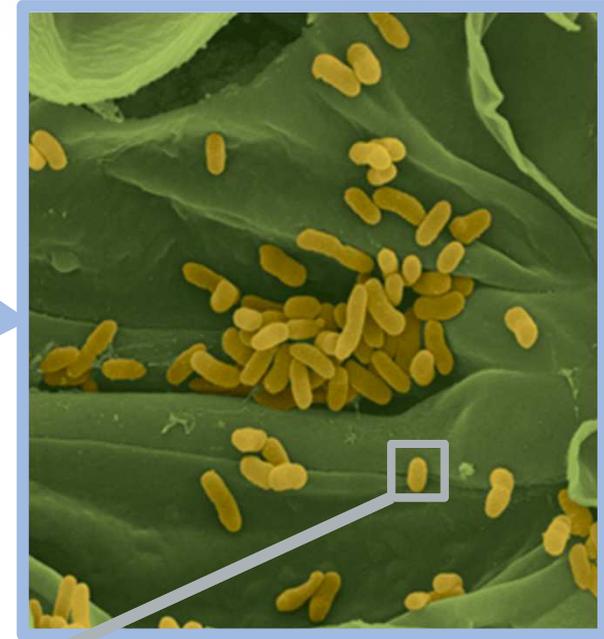
Origine



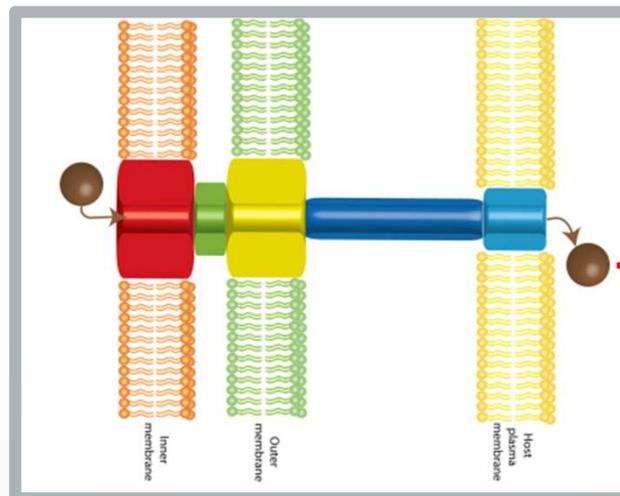
Infezione



Stoma



Xanthomonas
(Gram -)

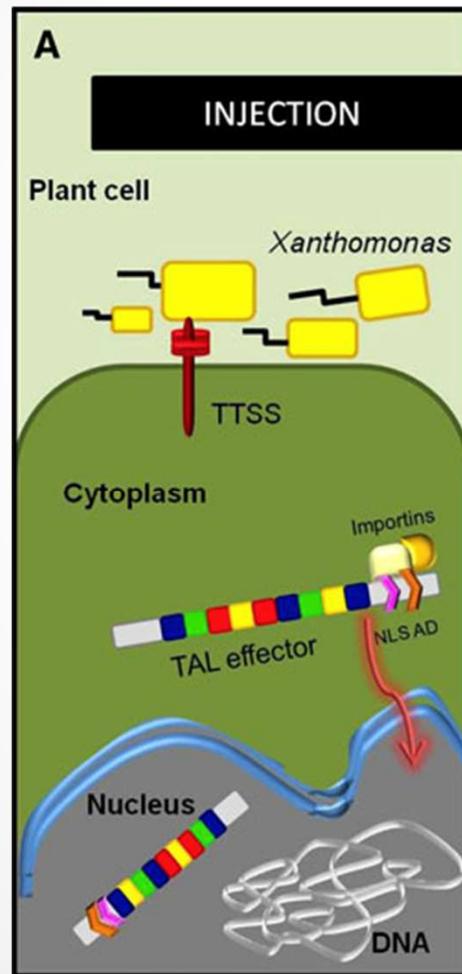


TALE

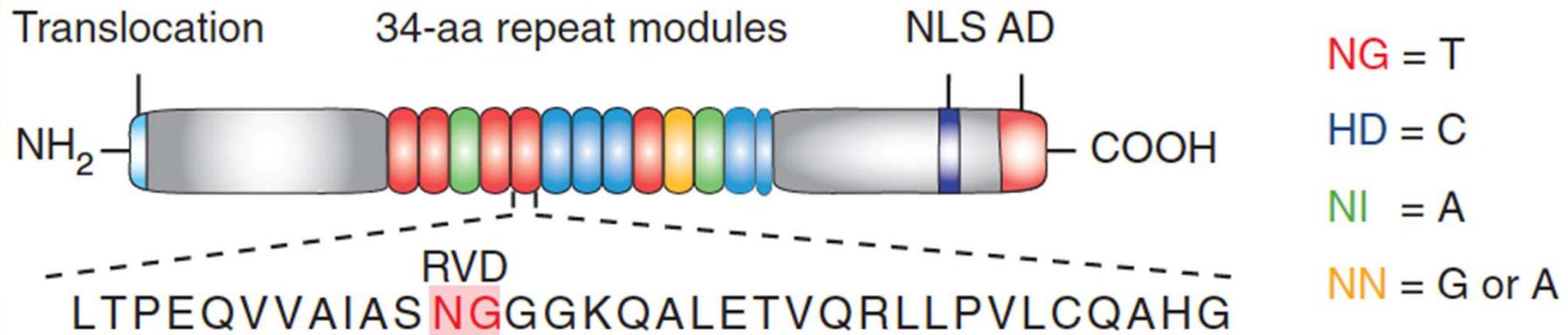
Sistema di secrezione di tipo III

Funzione

TALE: Transcription **A**ctivator-**L**ike **E**ffector, TALE possono legare e regolare in modo specifico alcuni geni nelle piante durante la patogenesi.



TALE: Struttura Proteica

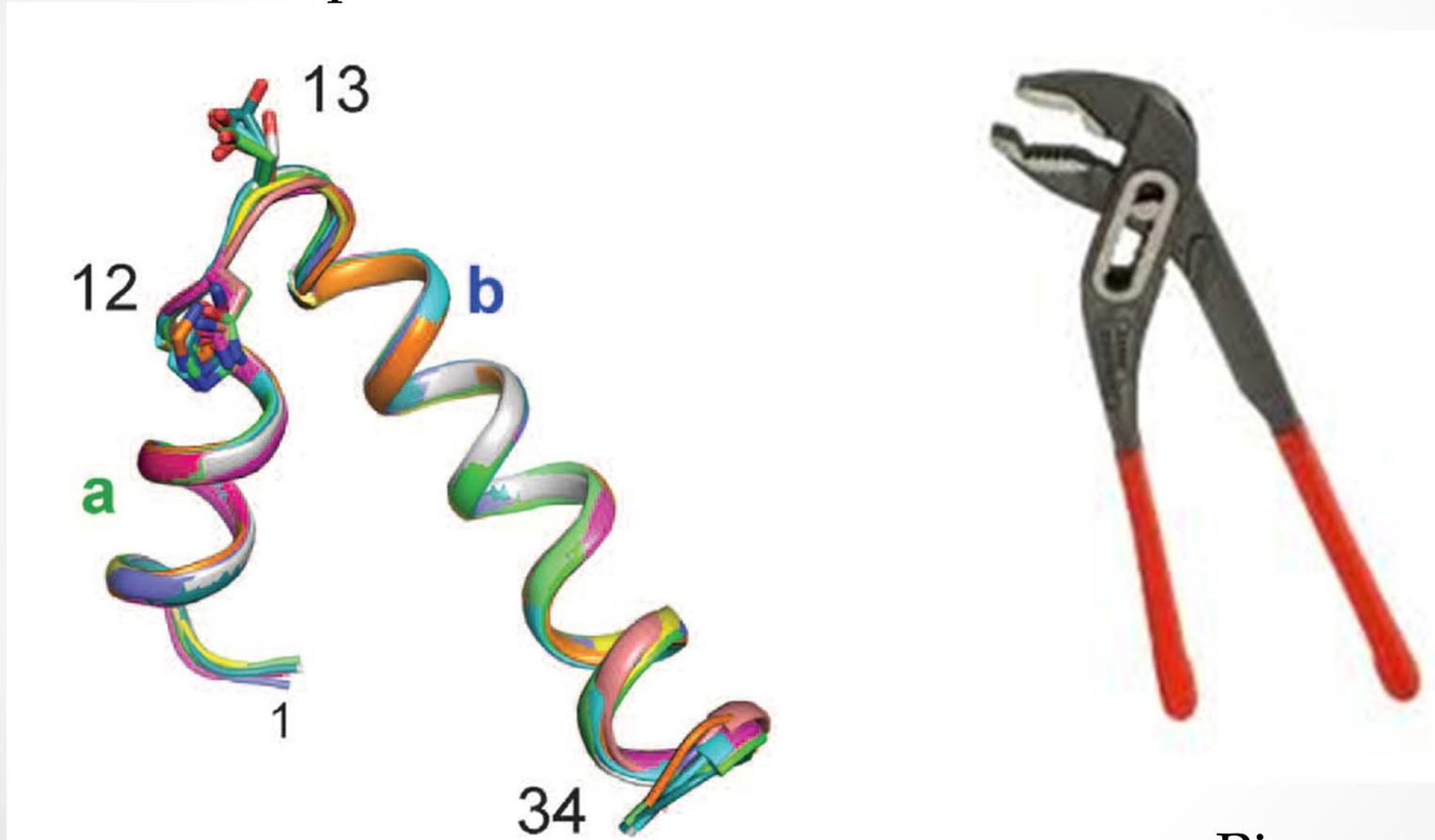


Caratteristiche Principali:

- Regione Amminoternale: dominio di traslocazione
- Regione centrale composta da tandem repeat di 34 aa (monomeri)
- RVD (repeat variable diresidues): in ogni monomero gli aa nelle posizioni 12 e 13 mediano il riconoscimento di uno specifico nucleotide
- Regione carbossiterminale: NLS (nuclear localization signal) + dominio effettore

Struttura Proteica

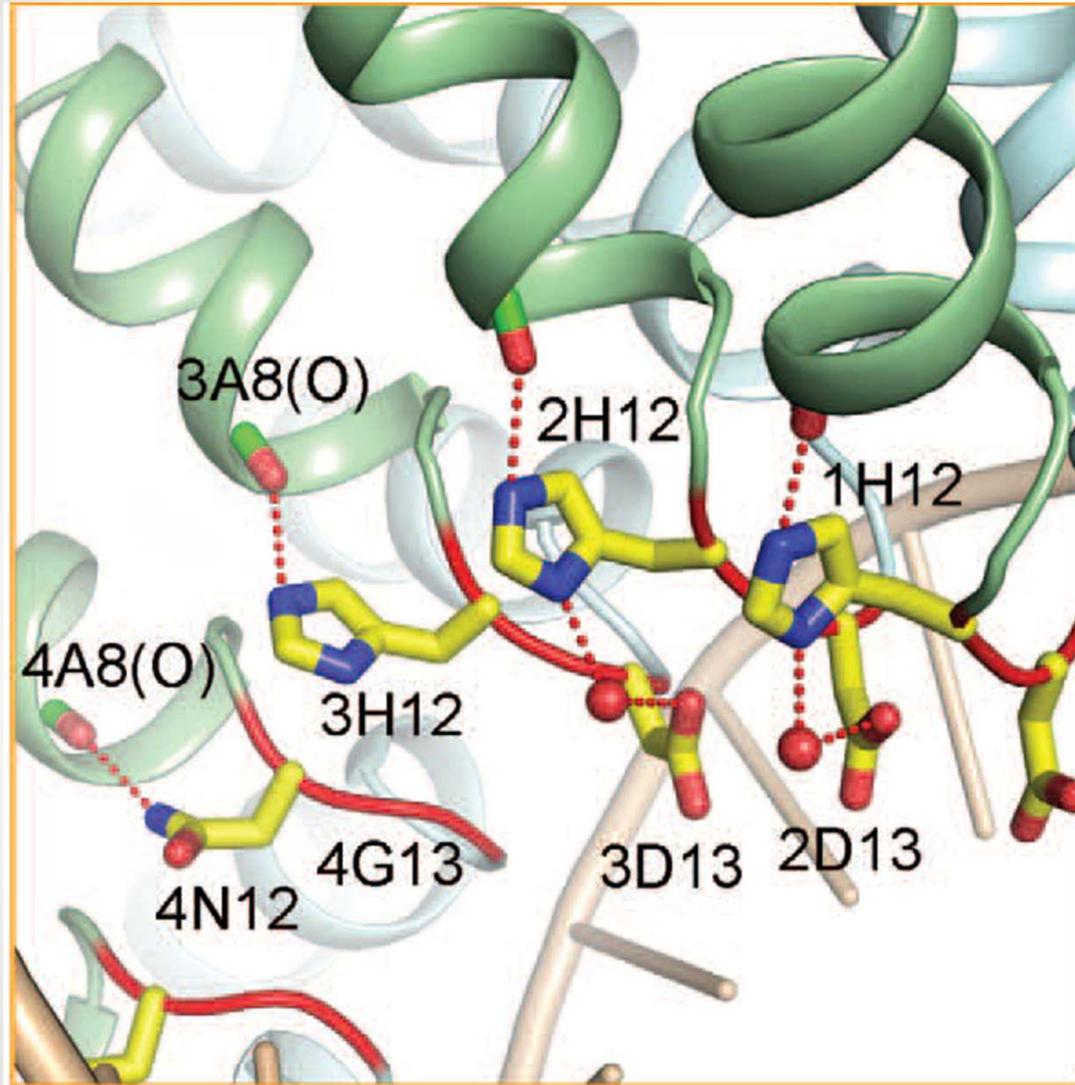
Helix-loop-helix



Struttura terziaria di un monomero

Pinza

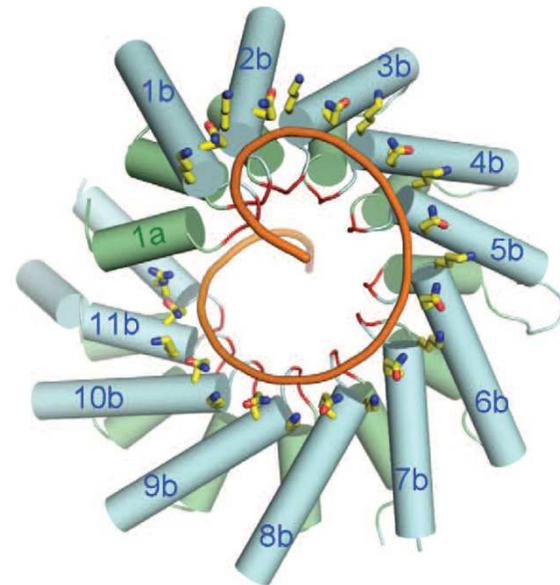
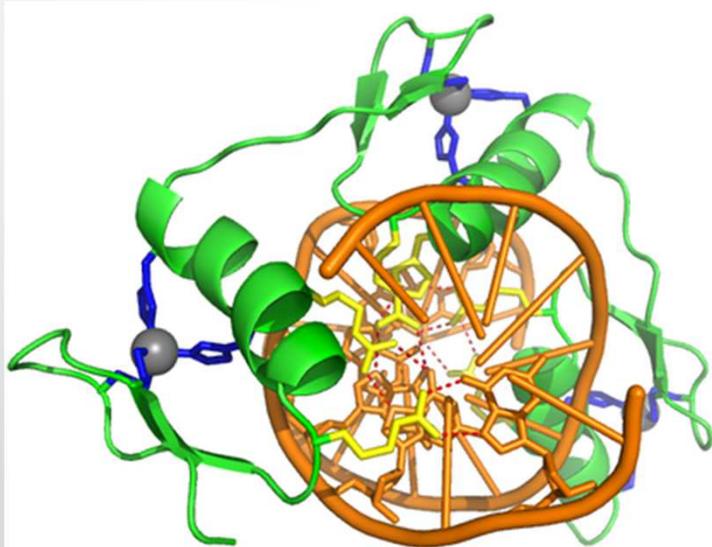
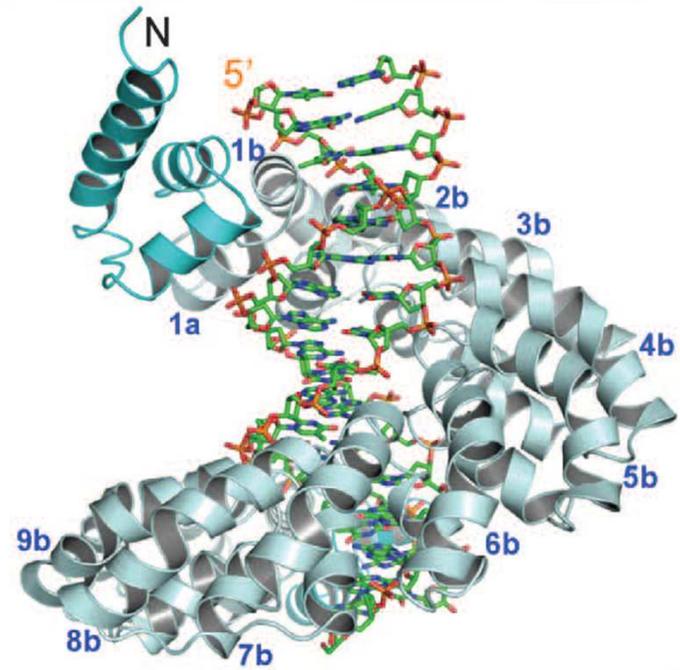
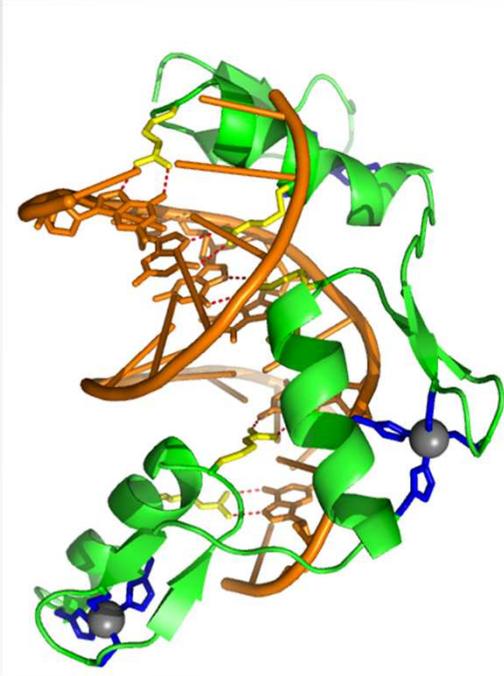
INTERAZIONE TALE-DNA



L'aminoacido in posizione 12 stabilizza il loop interagendo con l'alfa-elica minore (alanina in posizione 8).

L'interazione TALE/DNA avviene ad esclusivo carico dell'aminoacido in posizione 13 che interagisce direttamente con il nucleotide formando **legami idrogeno**. -----

ZFPs Versus TALEs



ZFPs Versus TALEs

ZFPs

ATGGGCTATCTCAAGTA
TACCCGATAGAGTTCAT



Monomero: 28-30 aa

Target: NNN

Totale per il
riconoscimento di 15
nucleotidi: 150 aa

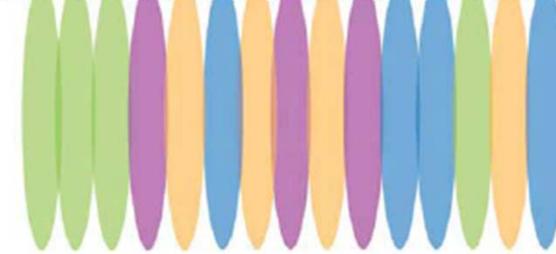
Design complicato: non esistono
ZFP specifiche per tutte le triplette

Tecnologia brevettata:



TALEs

TACCCGATAGAGTTCAT
ATGGGCTATCTCAAGTA



Monomero: 34 aa

Target: N

Totale per il riconoscimento
di 15 nucleotidi: 510 aa

Design semplice: il nucleotide
prima della sequenza target
riconosciuta deve essere una
Timina



Metodo di assemblaggio

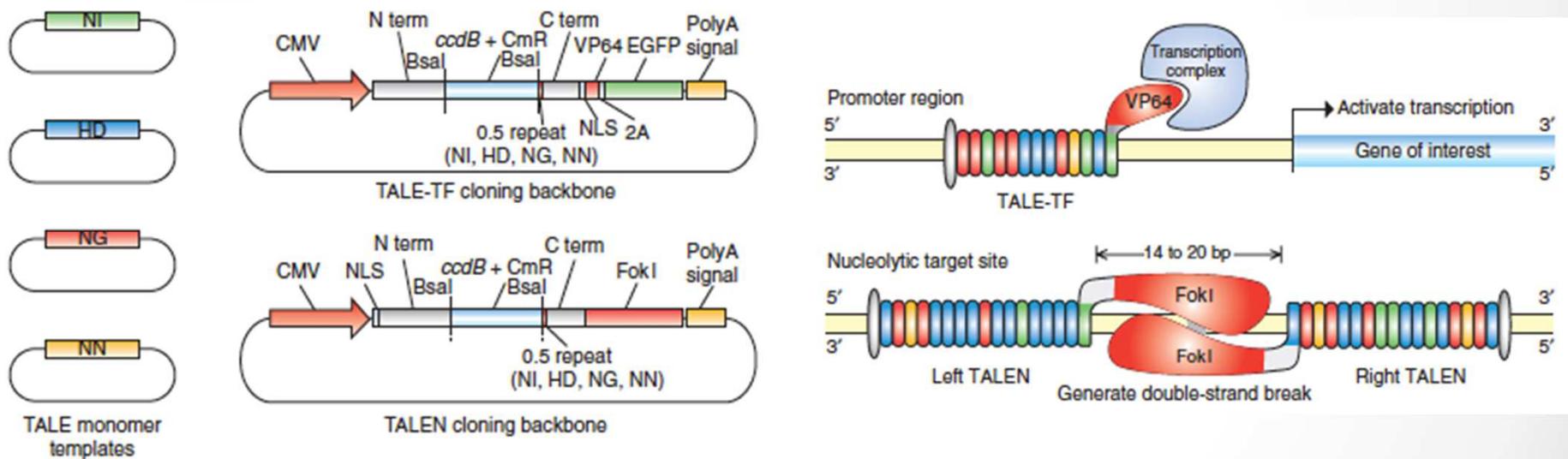
NATURE PROTOCOLS

VOL.7 NO.1 | 2012 | 171

A transcription activator-like effector toolbox for genome engineering

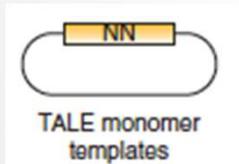
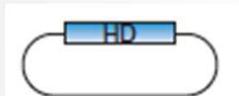
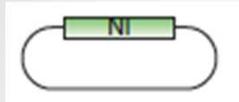
Neville E Sanjana^{1,2,4}, Le Cong¹⁻⁴, Yang Zhou^{1,2,4}, Margaret M Cunniff^{1,2}, Guoping Feng^{1,2} & Feng Zhang^{1,2}

¹Broad Institute of Massachusetts Institute of Technology and Harvard, Cambridge, Massachusetts, USA. ²Department of Brain and Cognitive Sciences, McGovern Institute for Brain Research, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA. ³Program in Biological and Biomedical Sciences, Harvard Medical School, Boston, Massachusetts, USA. ⁴These authors contributed equally to this work. Correspondence should be addressed to F.Z. (zhang_f@mit.edu).

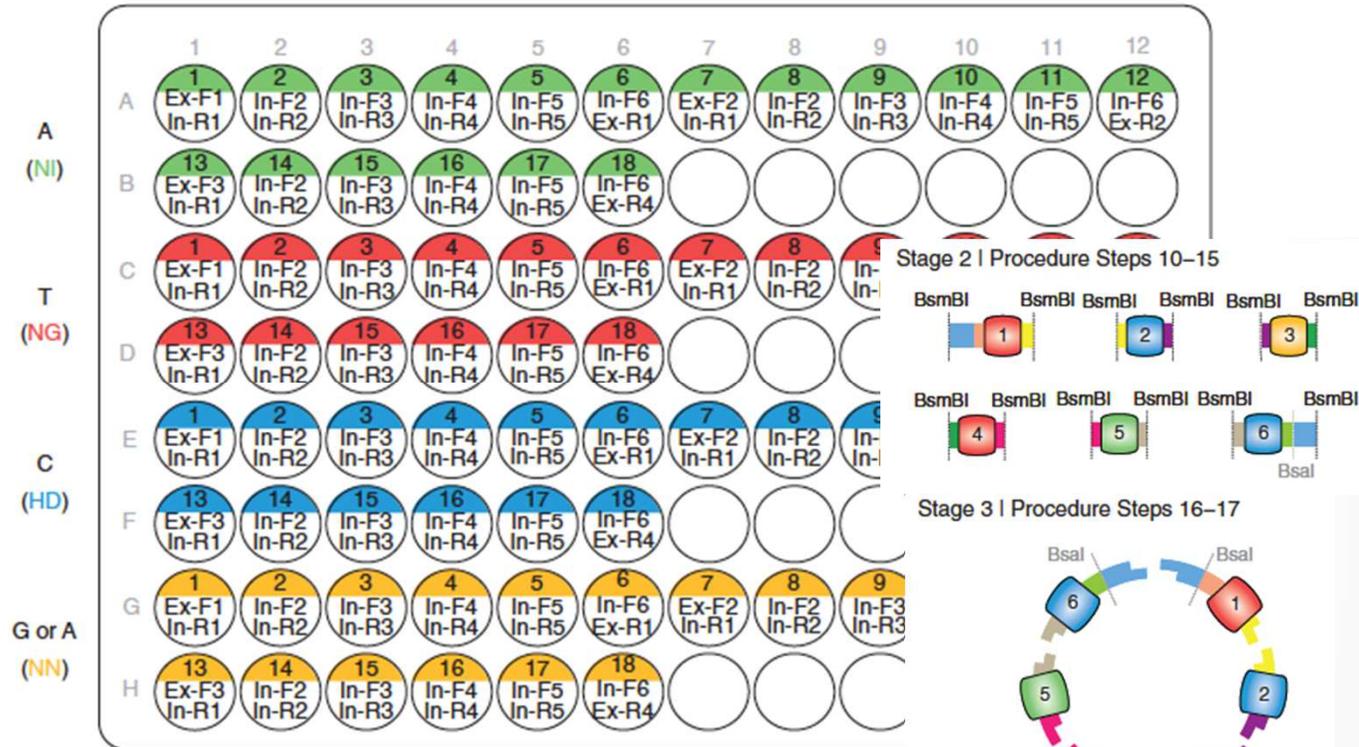


Target Sequence

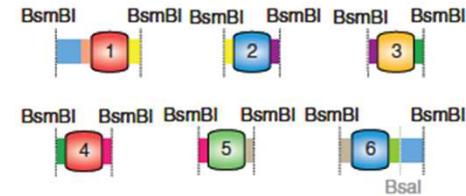
T C G T A C



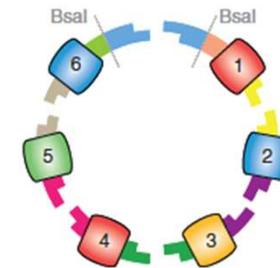
TALE monomer templates



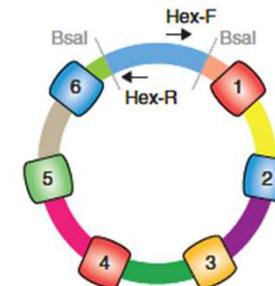
Stage 2 | Procedure Steps 10-15



Stage 3 | Procedure Steps 16-17



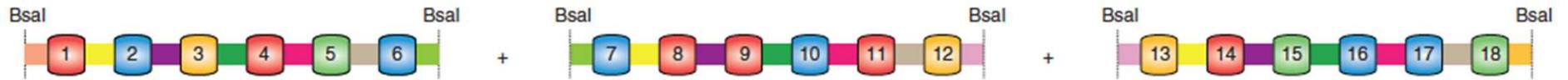
Stage 4 | Procedure Steps 18-25



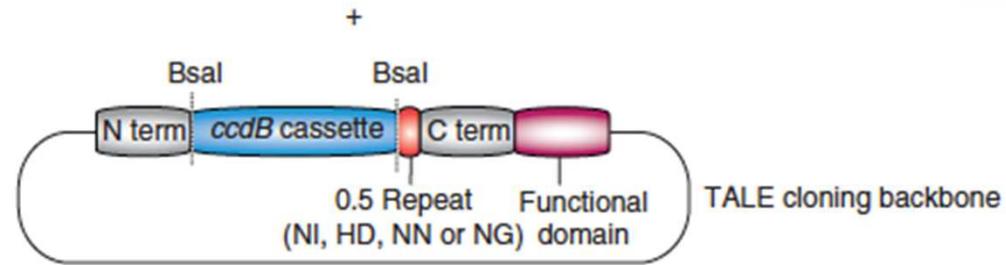
1. Amplificazione monomeri con primer modificati
2. Selezione prodotti PCR in base alla sequenza target
3. Golden Gate: restrizione e ligazione simultanea
4. Amplificazione esameri con primers Hex-F e Hex-R

5. Golden Gate: Restrizione e ligazione con esameri successivi

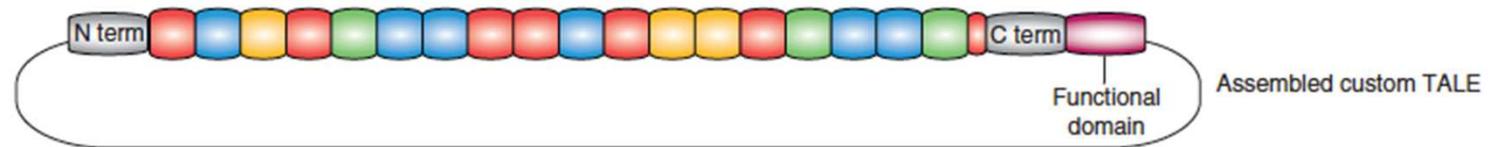
Stage 5 | Procedure Steps 26–28



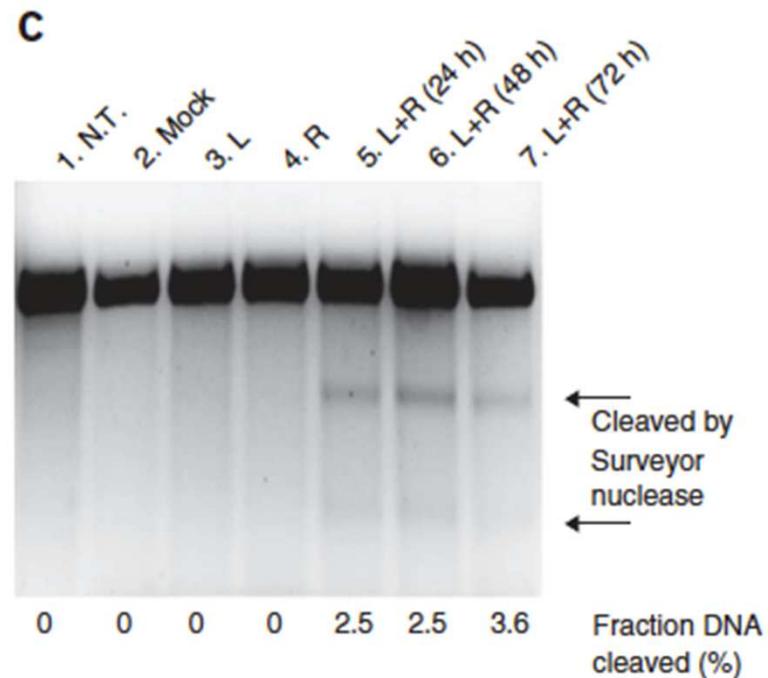
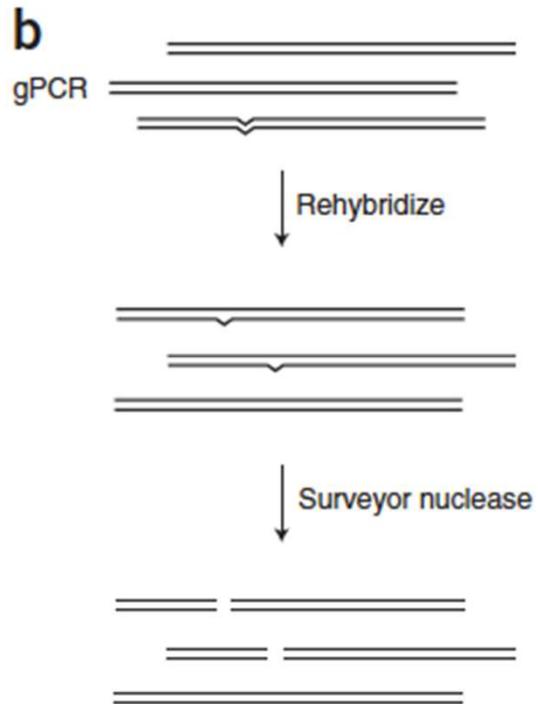
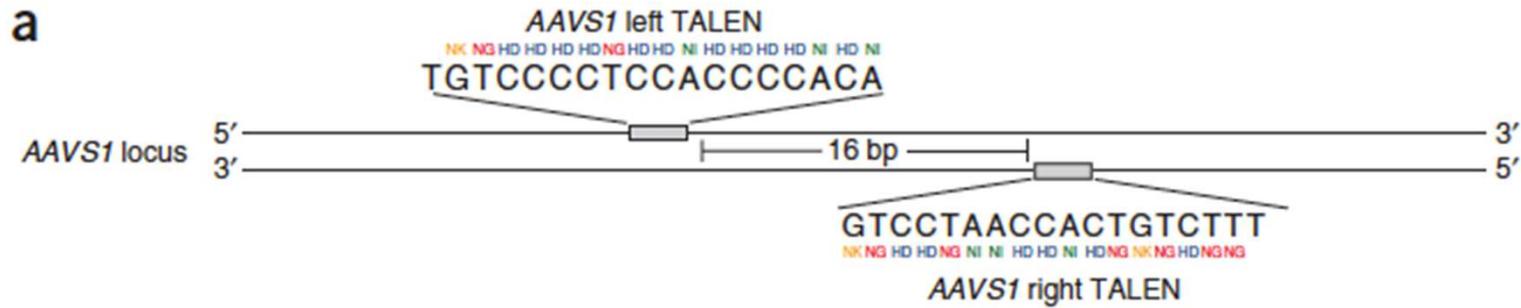
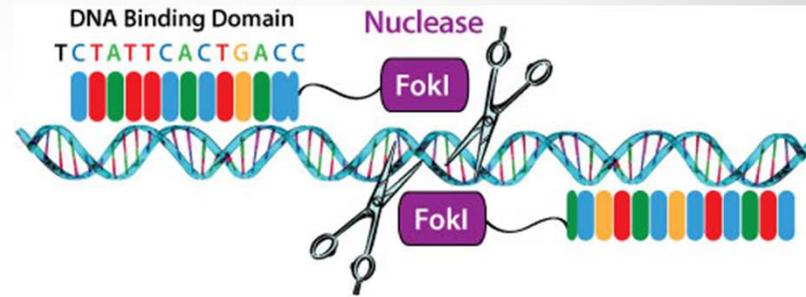
6. Ligazione nel vettore finale



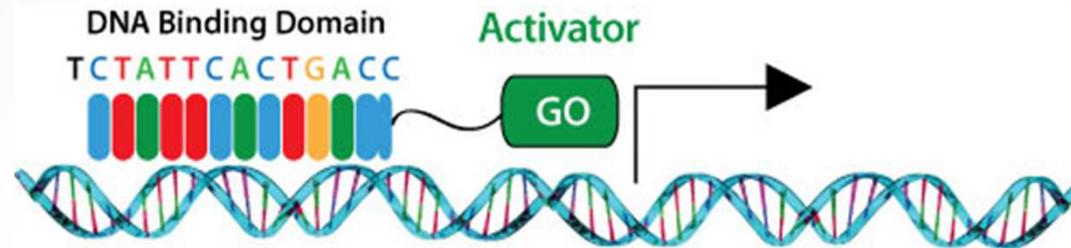
Stage 6 | Procedure Steps 29–38



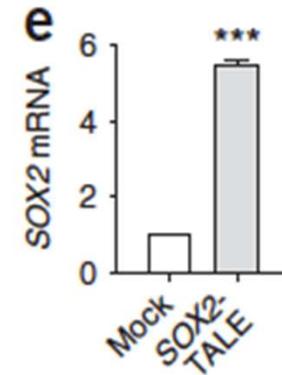
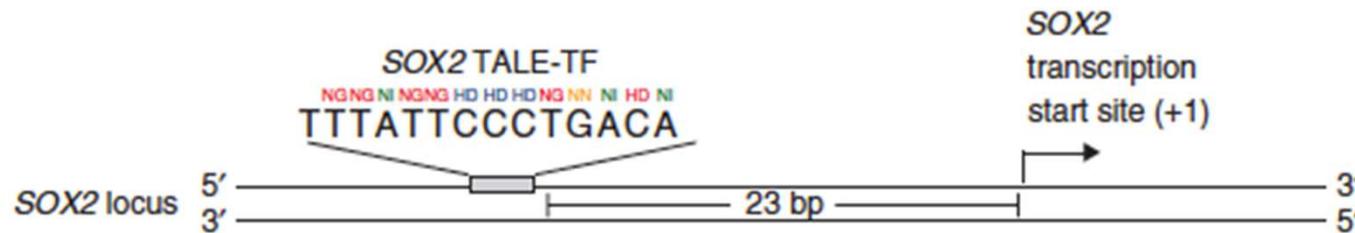
TALEN: TALE-Nuclease



TALE-TF: TALE-Transcription Factor



d



Referenze

Tell Me a Tale of TALEs.

Muñoz Bodnar A, Bernal A, Szurek B, López CE.
Mol Biotechnol. 2012 Nov 1. 10.1007/s12033-012-9619-3

Structural basis for sequence-specific recognition of DNA by TAL effectors.

Deng D, Yan C, Pan X, Mahfouz M, Wang J, Zhu JK, Shi Y, Yan N.
Science. 2012 Feb 10;335(6069):720-3. doi: 10.1126/science.1215670. Epub 2012 Jan 5.

A transcription activator-like effector toolbox for genome engineering.

Sanjana NE, Cong L, Zhou Y, Cunniff MM, Feng G, Zhang F.
Nat Protoc. 2012 Jan 5;7(1):171-92. doi: 10.1038/nprot.2011.431.

Domande

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bvlmtt@unife.it