Efficient construction of sequence-specific TAL effectors for modulating mammalian transcription

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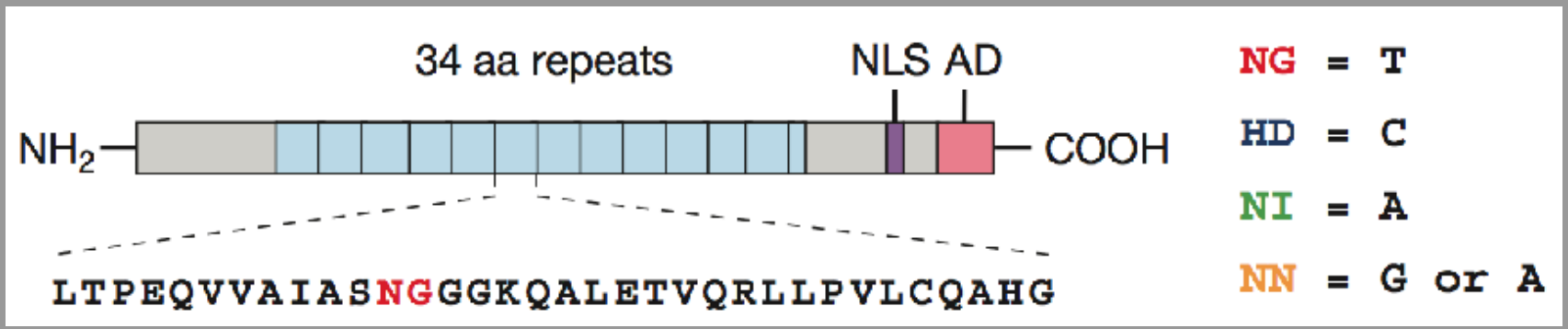
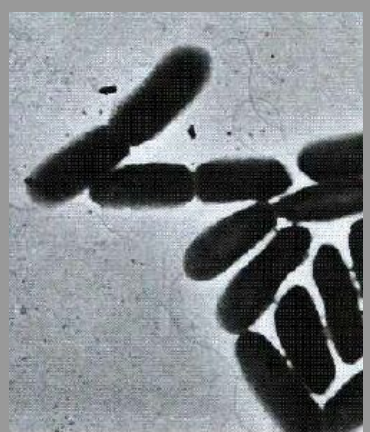
Introduction

- Directing proteins to DNA efficiently and precisely for various biological manipulations is one of the goals of biological engineering
- Polydactyl zinc fingers and meganucleases have been engineered to enable sequence-specific DNA perturbation. However, they suffer from two drawbacks:
  1. Lack of a simple correspondence between amino acid sequence and DNA recognition
  2. Difficult and expensive of its design and development
- TALEs are hence introduced as a direct and simpler alternative for DNA-targeting protein domains

What are TALEs?

Transcription Activator–Like Effectors (TALEs)





Naturally produces

Modulates gene expression

Xanthomonas

Host plant

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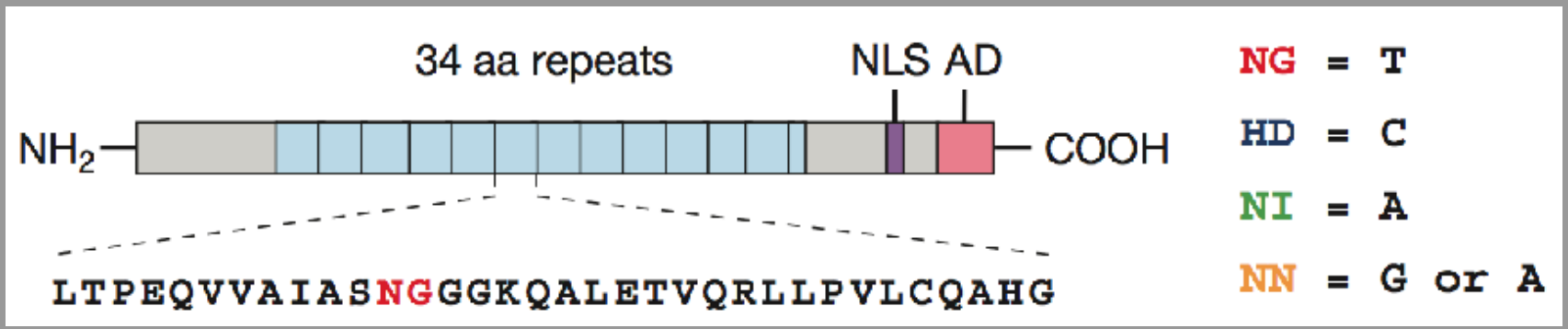


0	1	2	3	4	5	6	7	8	9	10	11	12	12.5
	HD	HD	NN	HD	HD	NG	HD	HD	HD	NG	HD	NG	HD
T	C	C	G	C	C	T	C	C	C	T	C	T	C
	NN	HD	NN	NN	HD	NG	HD	NN	HD	NG	NN	NG	NN
T	G	C	G	G	C	T	C	G	C	T	G	T	G
	NI	NI	NN	NI	NI	NG	NI	NI	NI	NG	NI	NG	NI
T	A	A	G	A	A	T	A	A	A	T	A	T	A
	NI	NG	NN	NI	NG	NG	NG	NI	NG	NG	NI	NG	NI
T	A	T	G	A	T	T	T	A	T	T	A	T	A
	NI	NN	NN	NI	NN	NG	NN	NI	NN	NG	NI	NG	NI
T	A	G	G	A	G	T	G	A	G	T	A	T	A

- Strong correlation between amino acids at positions 12 and 13 and the corresponding bases in the TALE-binding site
- Potentially designable protein with sequence-specific DNA-binding capabilities

How can we construct designer TALEs effectively?

Possible TALEs constructions

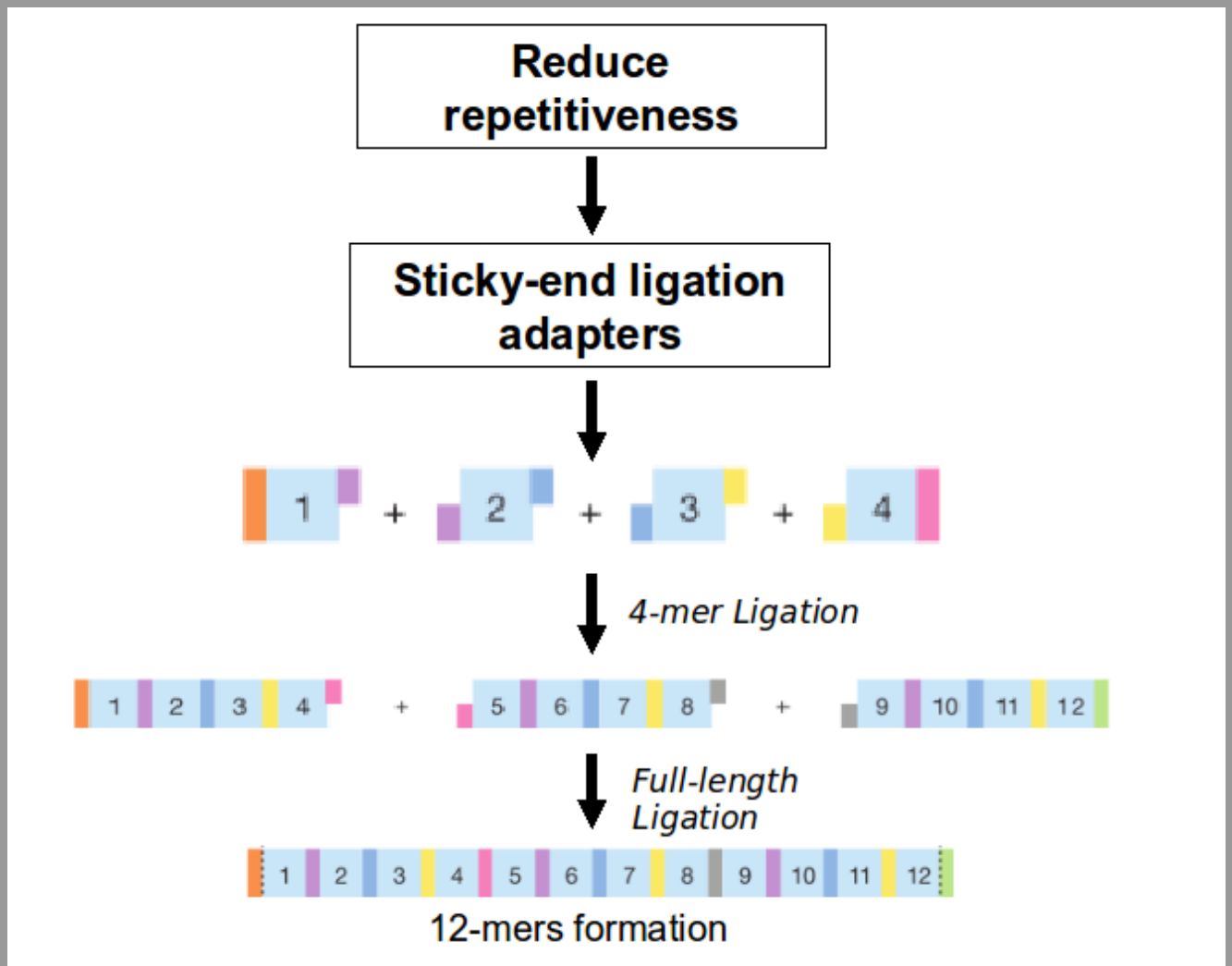
Old methods:

- PCR gene assembly & series ligations
- commercial services

Not high-throughput & cost-prohibitive

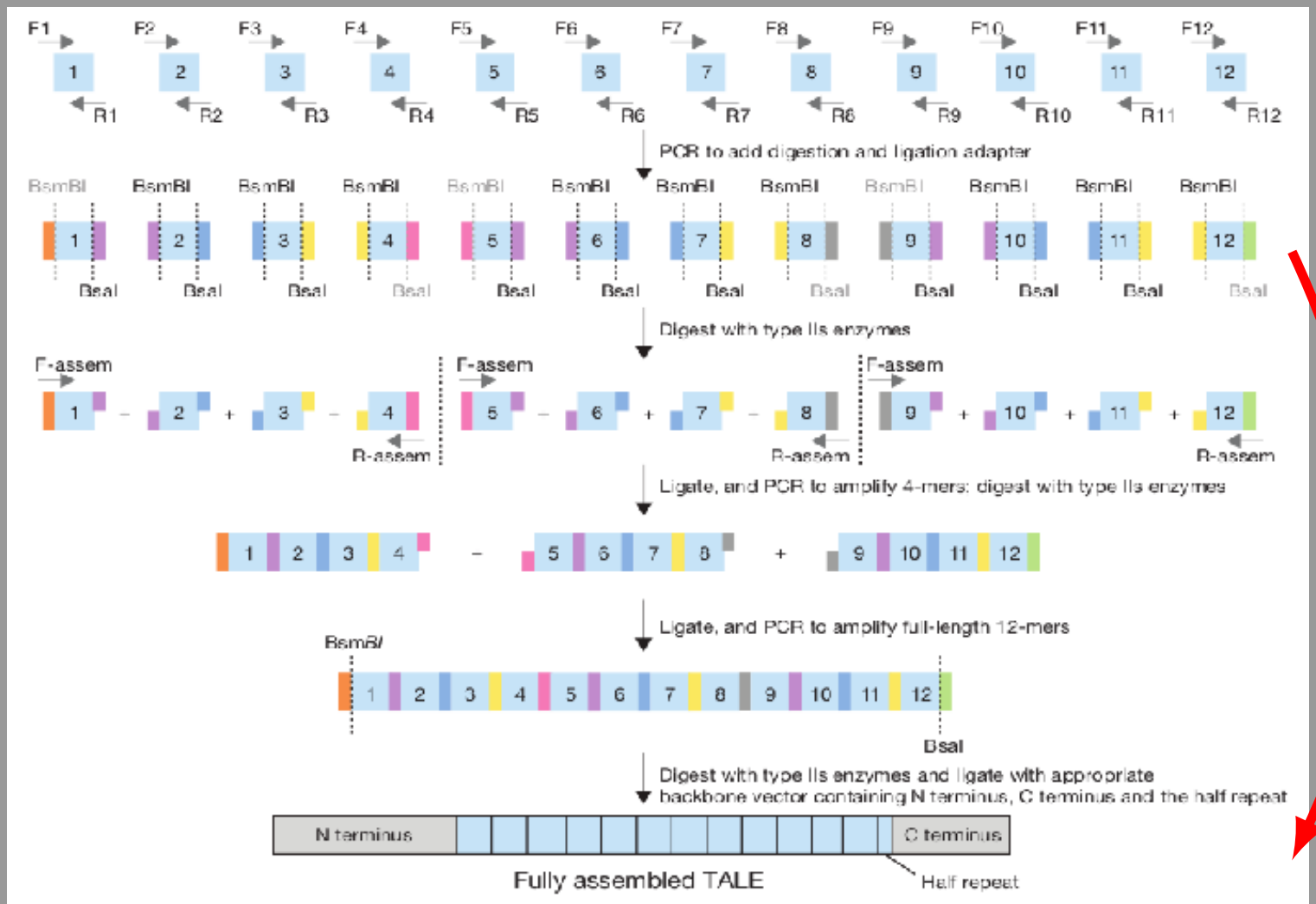
Construction overview

New method:

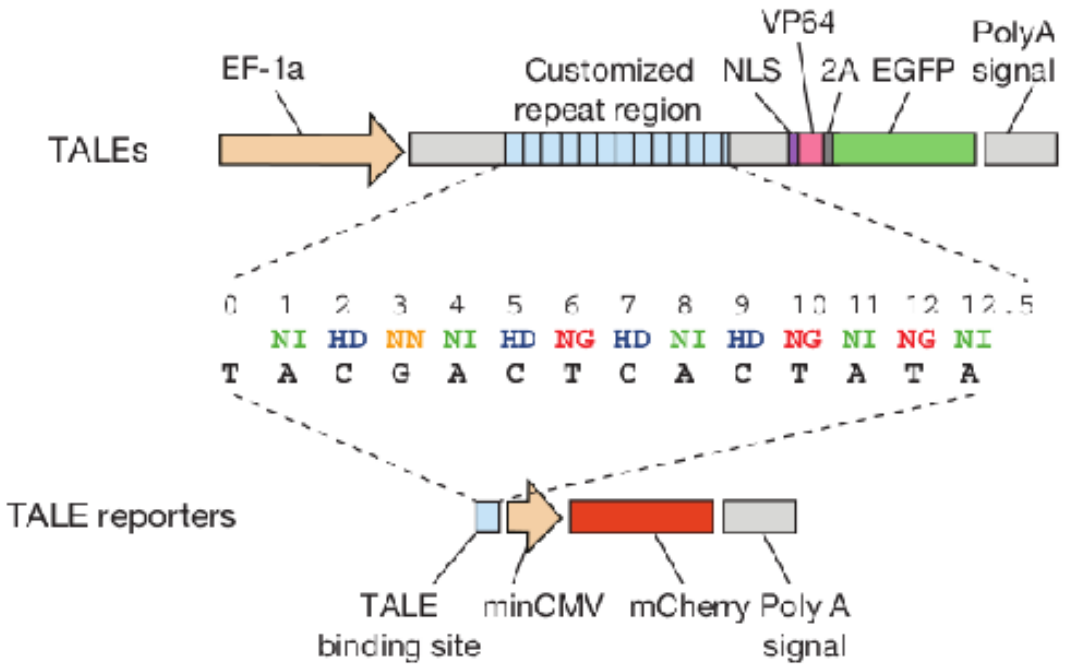


Hierarchical Ligation Assembly

Construction details



Fluorescence-based reporter strategy



GFP (*expression*)

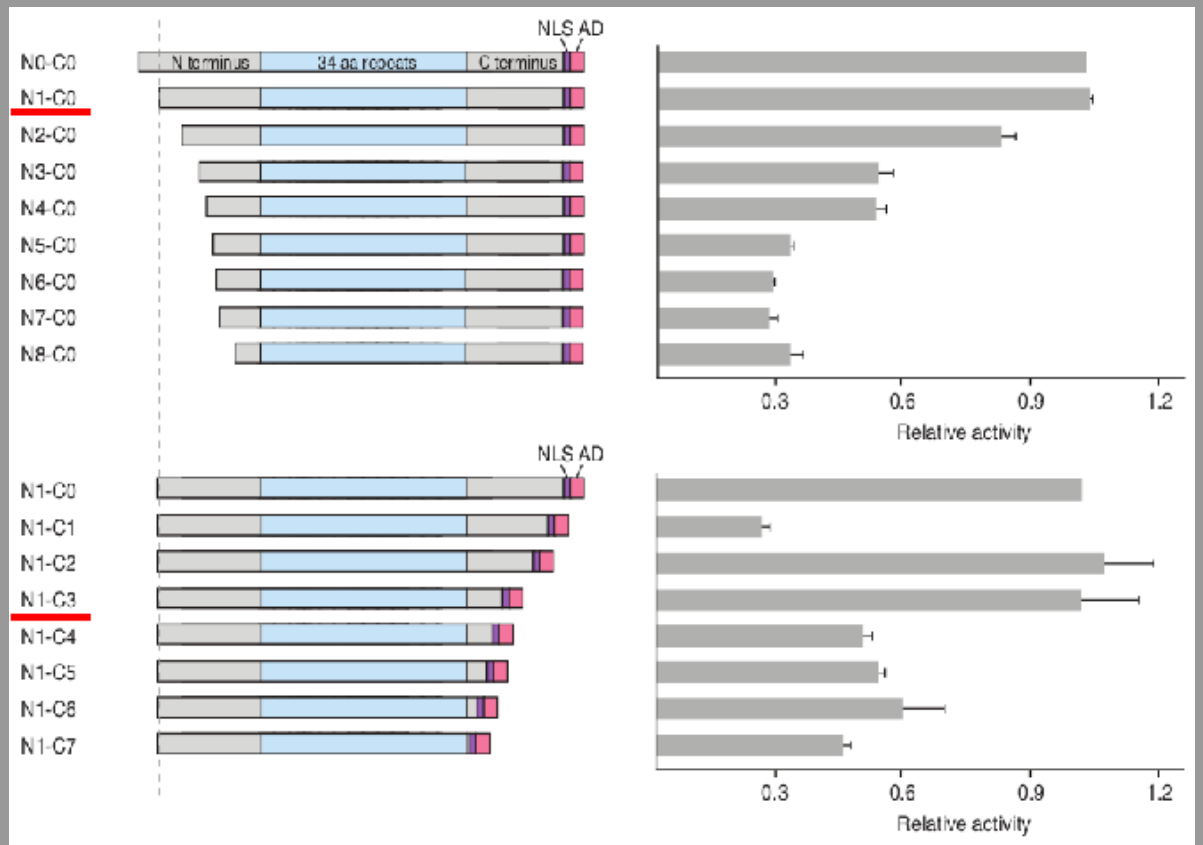
mCherry (*hybridization*)

GFP + mCherry → **Quantitative approach**

Sequence adjustments

Protein structure

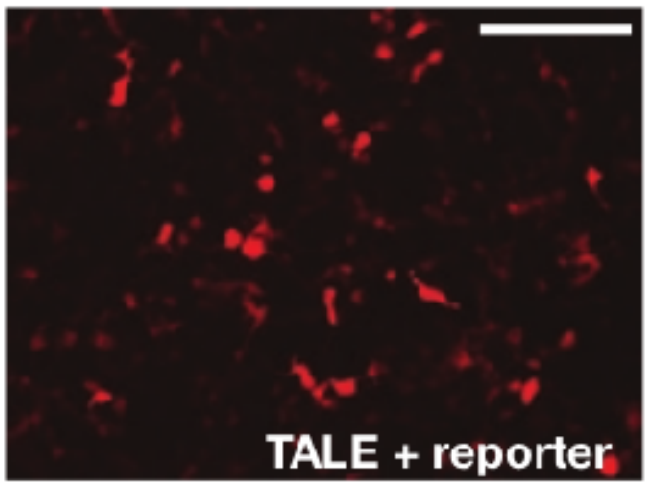
Serial truncations



In vitro & *in vivo* validations

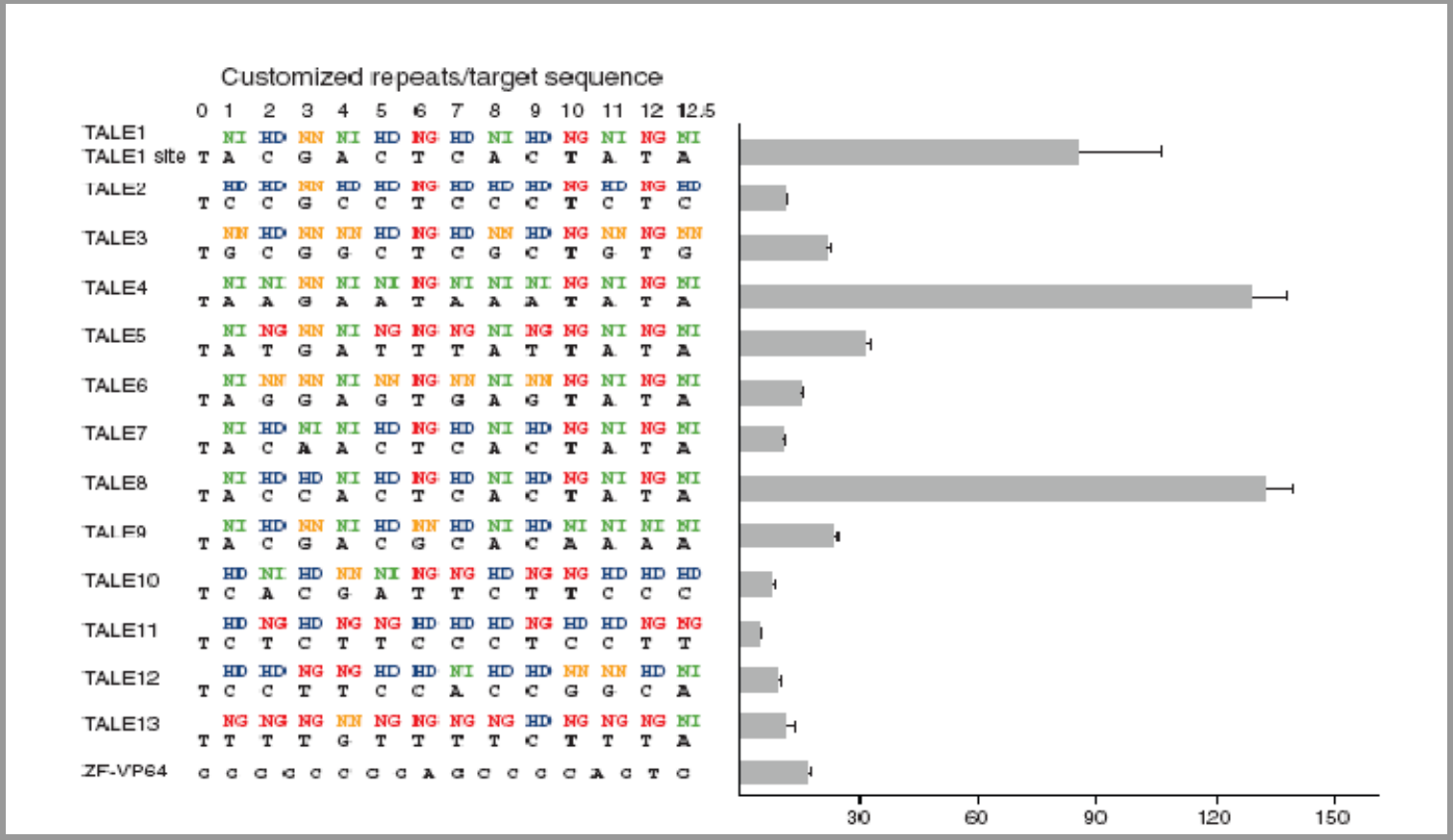
TALEs in mammalian cells

Expression only successful on cotransfection with both TALE and Reporter Plasmid



- TALEs facilitated the binding of the transcription factors
- Thus initiate the expression of the genes

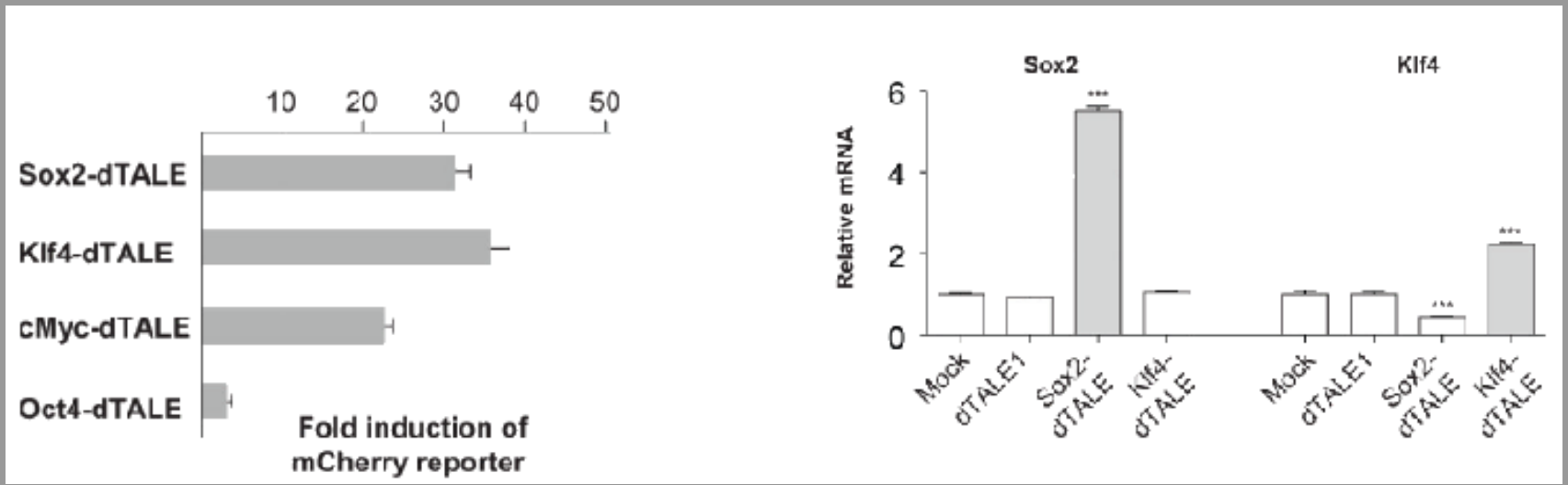
TALEs binding affinity



- Binding affinity not affected by GC content
- What affects the binding affinity?

TALEs applied to iPSCs

Reporter mCherry overexpression



- Failure for Oct4?

Endogeneous genes overexpression

- Side effects
- Failures for Oct4 and cMyc

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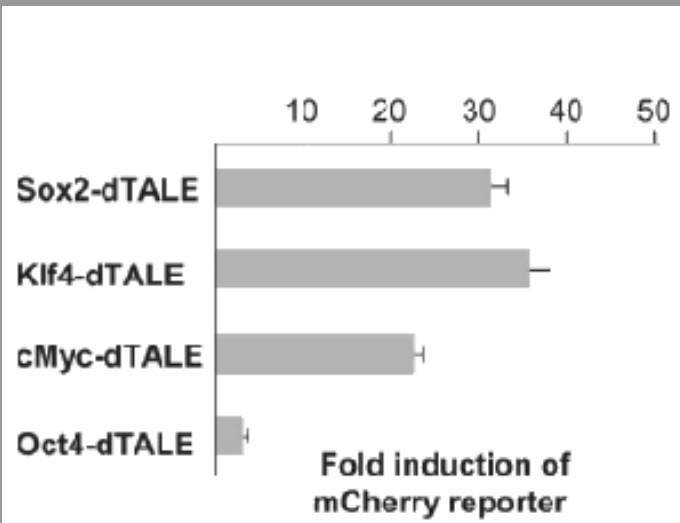

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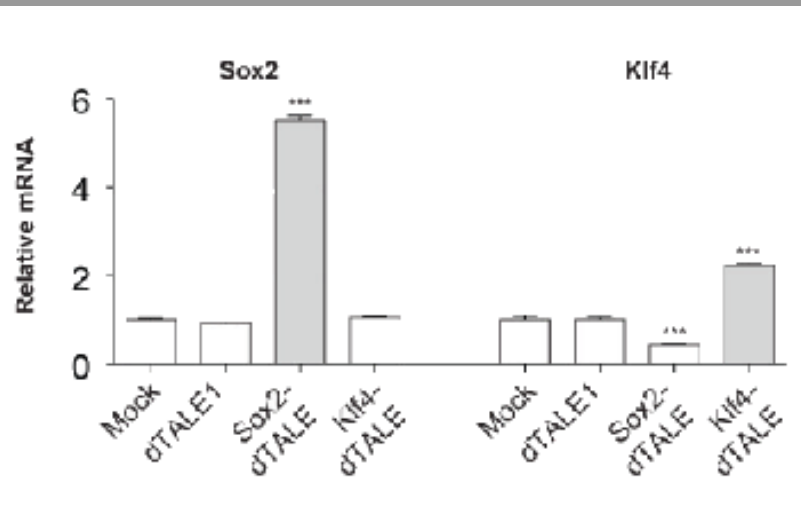

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Epigenetic states

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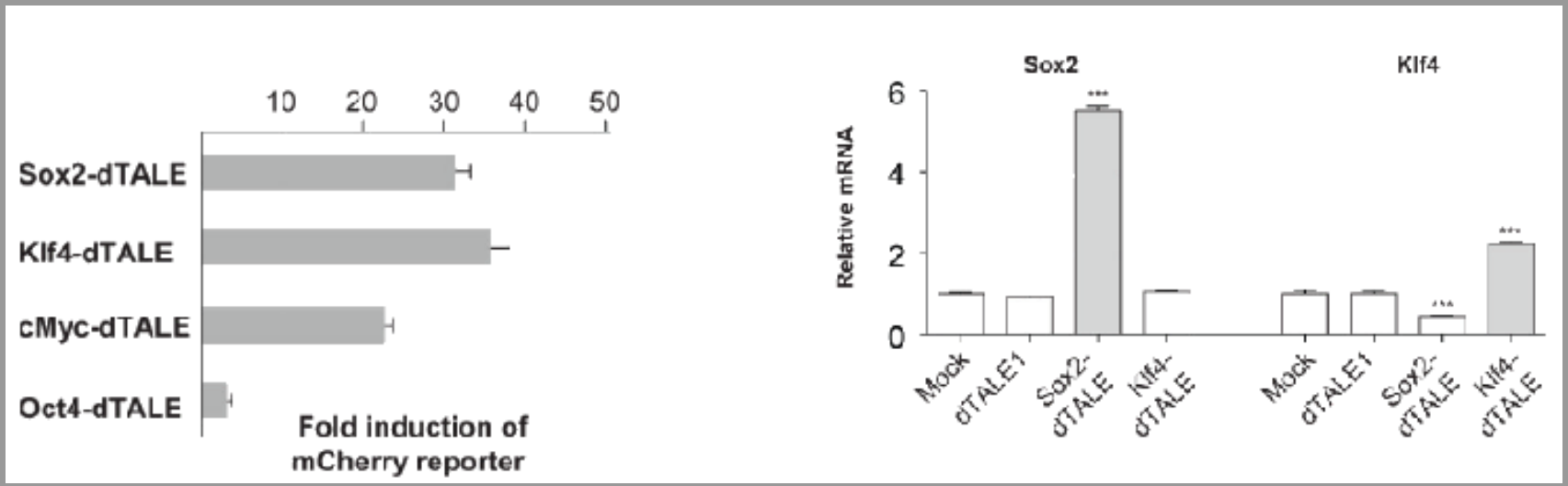

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Epigenetic states

$\Rightarrow$ *Combination with Chromatin-remodeling agents?*

Conclusions & Expectations

Key points of the article:

- Economical and more efficient way to construct customized TALEs
- Successful TALEs usage for overexpressing genes

Challenges and future works:

- Assessing the bias
  - Side effects
  - Off target effects
  - Affinity to methylated DNA
- DNA - TALEs interaction characterization
- Toxicity studies

Genome editing

TALEs expected applications

- TF
- Nucleases (TALENs)
- Recombinases
- Epigenetic-modifying enzymes

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	Device		
Criteria	TALENs	Zinc Finger	Meganucleases
Specificity	?	+	++
Toxicity	?	+	+
Activity	+	+	+
Size	-	+	-
Design/method	+	-	-

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