FUM

In the Name of God

Modulation of Sox2 Gene Expression in Cattle Fibroblasts by using Transcription Activator Like Effector-Transcription Factors (TALE-TFs)

Mojtaba Tahmoorespur, Ph.D.

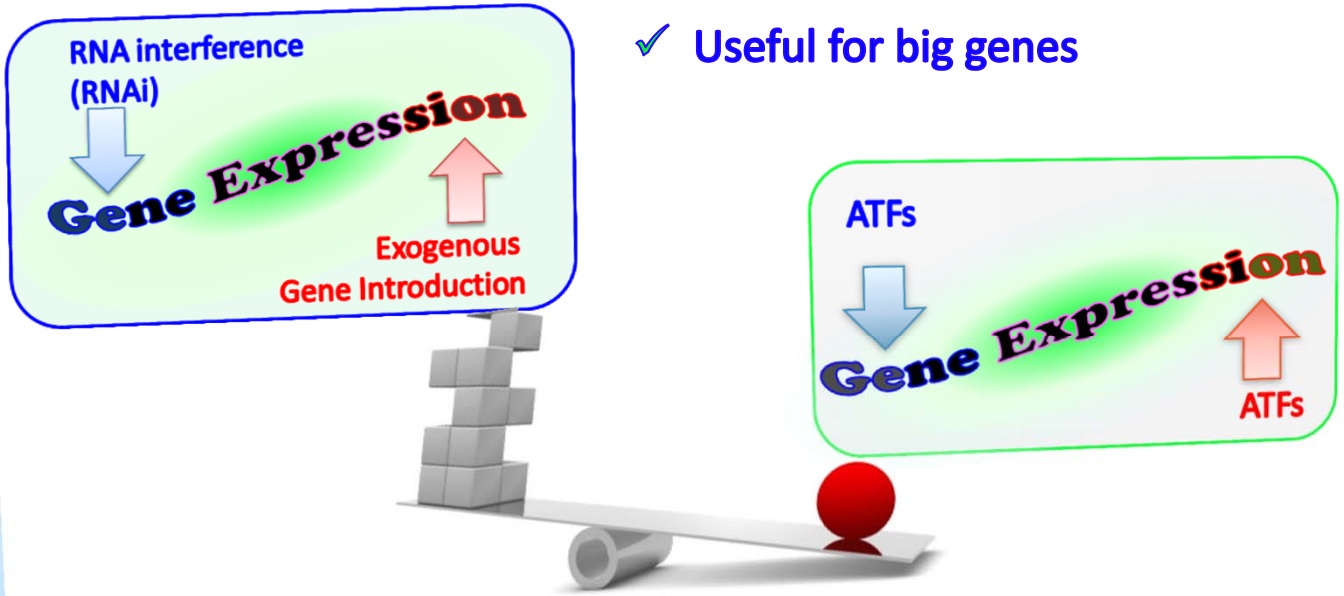
Gene Expression and Cell Fate

RNA interference (RNAi)

Exogenous Gene Introduction

Artificial Transcription Factors (ATFs)

- ✓ Expressing correct spliced version of mRNAs
- ✓ All gene products will be produced
- ✓ Useful for big genes



Artificial Transcription Factors (ATFs)

- **Introduction**
- Literature Review
- Research Objectives
- Material & Methods
- Results & Discussion

TALE-TFs

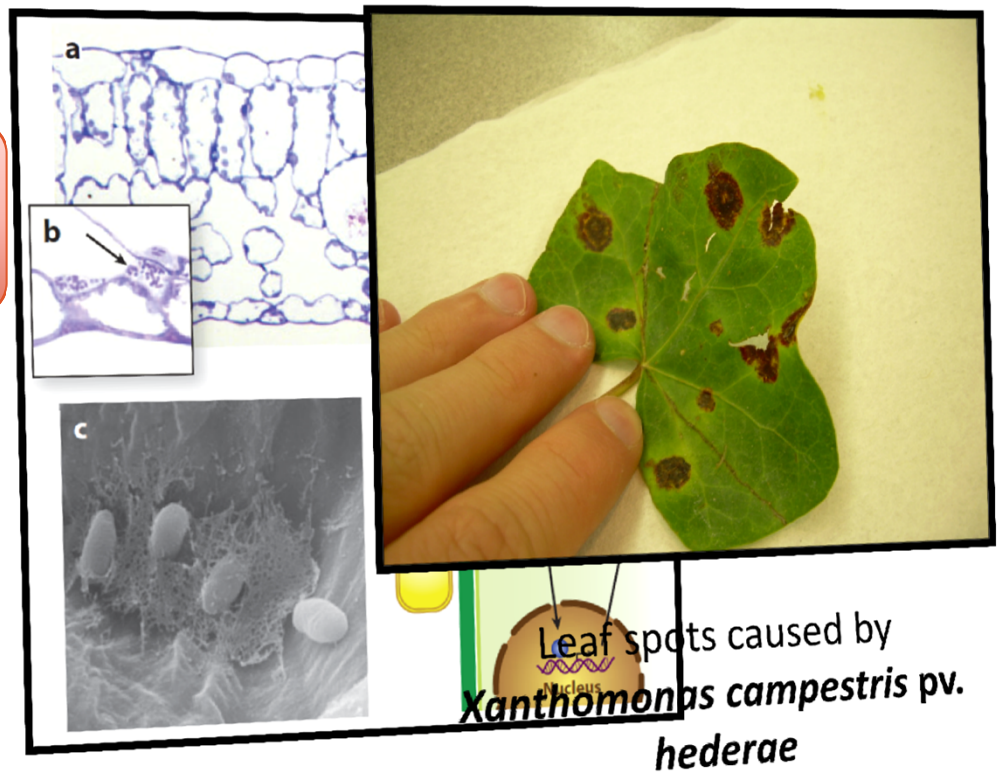
Transcription Activator Like Effector Transcription Factors

ZF-TFs

Zinc Finger-Transcription Factors

TAL Effectors

FROM *XANTHOMONAS*



Leaf spots caused by ***Xanthomonas campestris* pv. *hederae***

Boch et al. (2009) Science

TAL Effectors

PROTEIN STRUCTURE

- Introduction
- **Literature Review**
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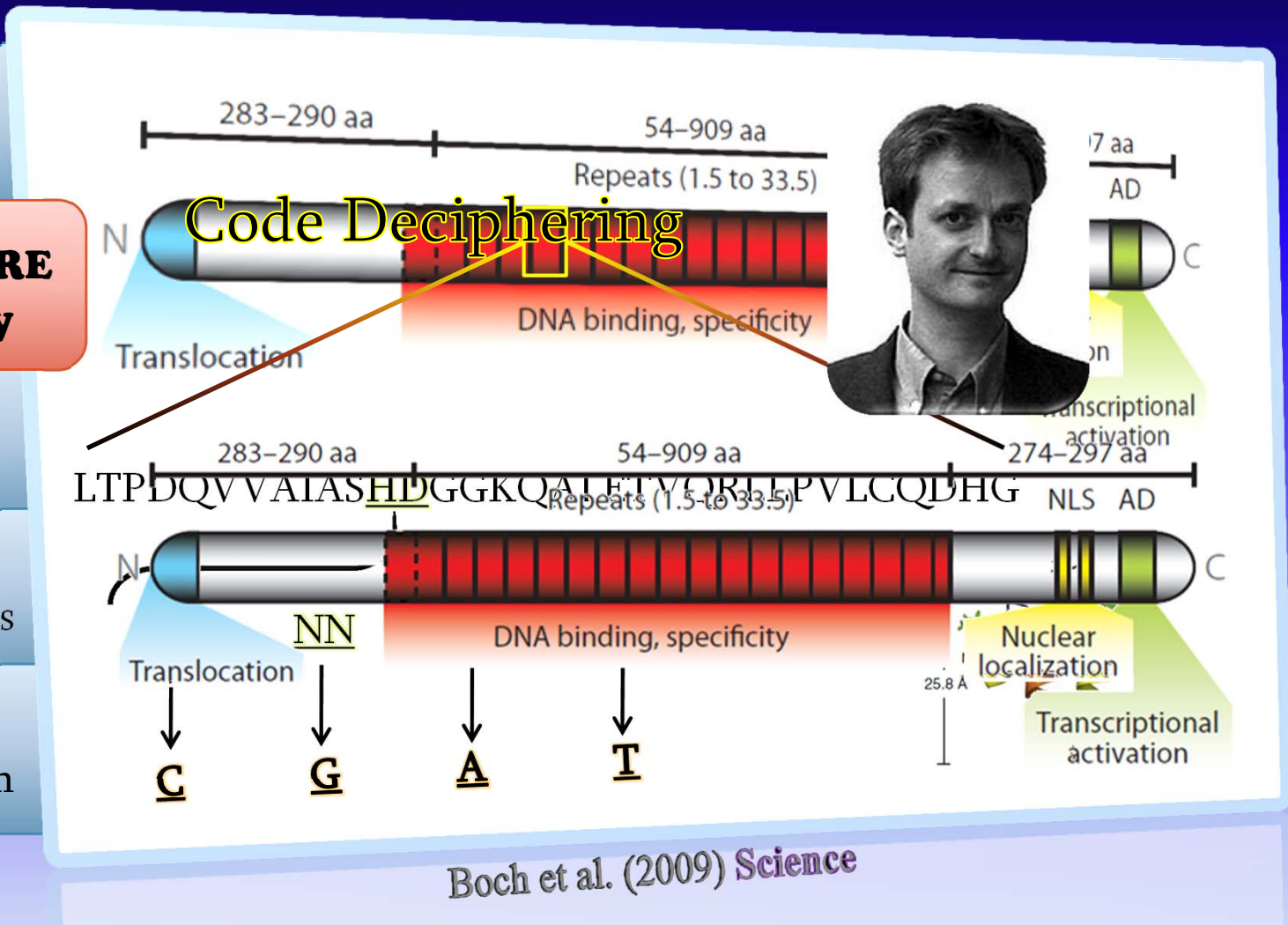

Boch et al. (2009) Science

TAL Effectors

DNA INTERACTION

- Introduction
- **LITERATURE REVIEW**
- Research Objectives
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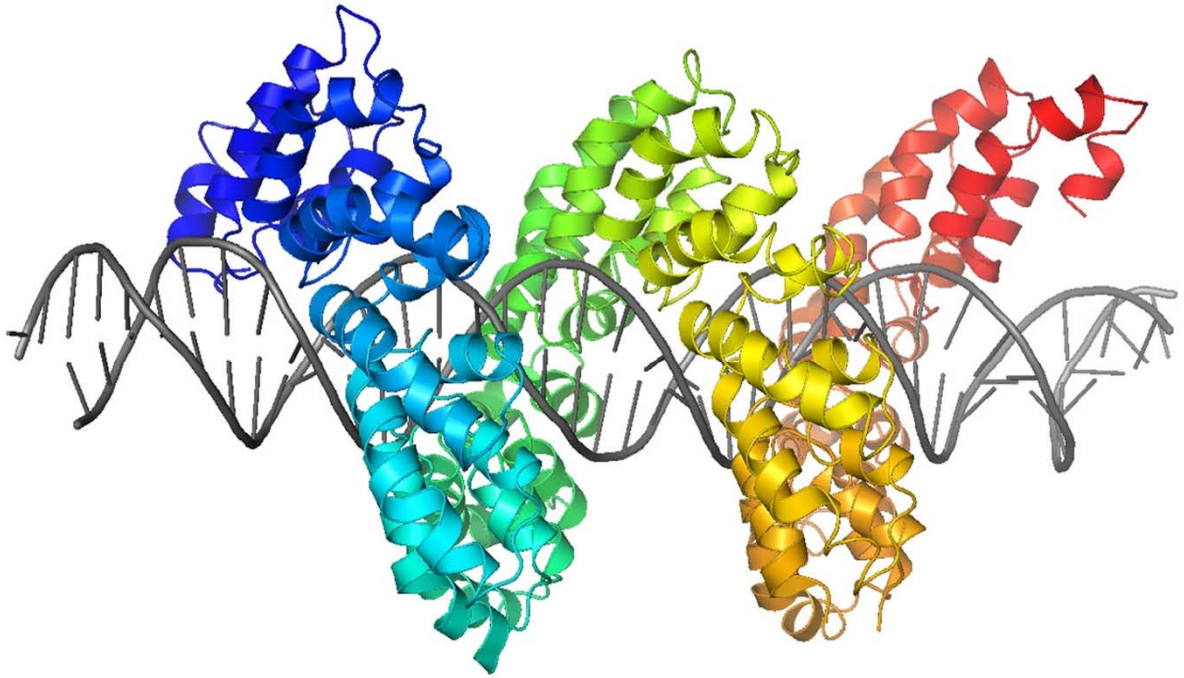

TAL Effectors

ENABLED CONTROL OF THE GENOME

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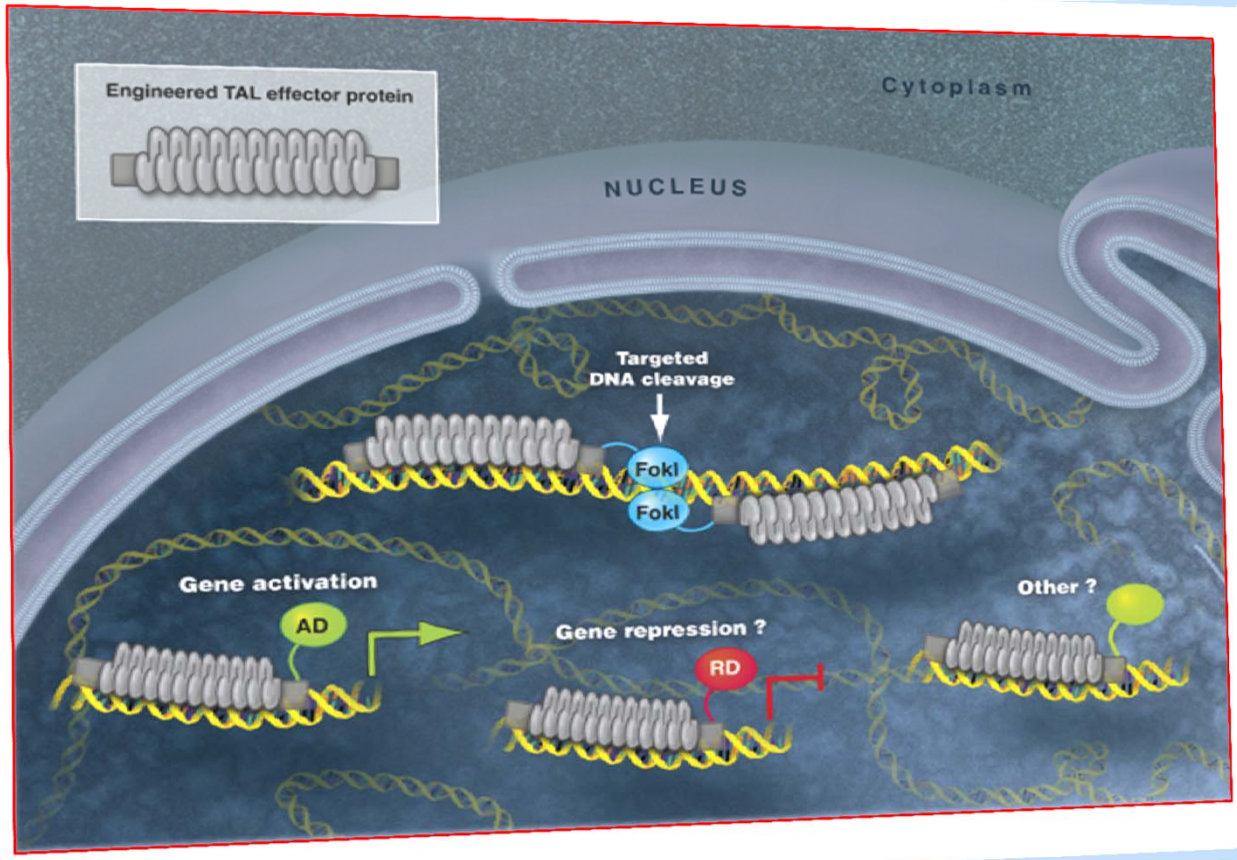

Bogdanove A. and Voytas D. (2011) Science

SOX2! Swich ON!

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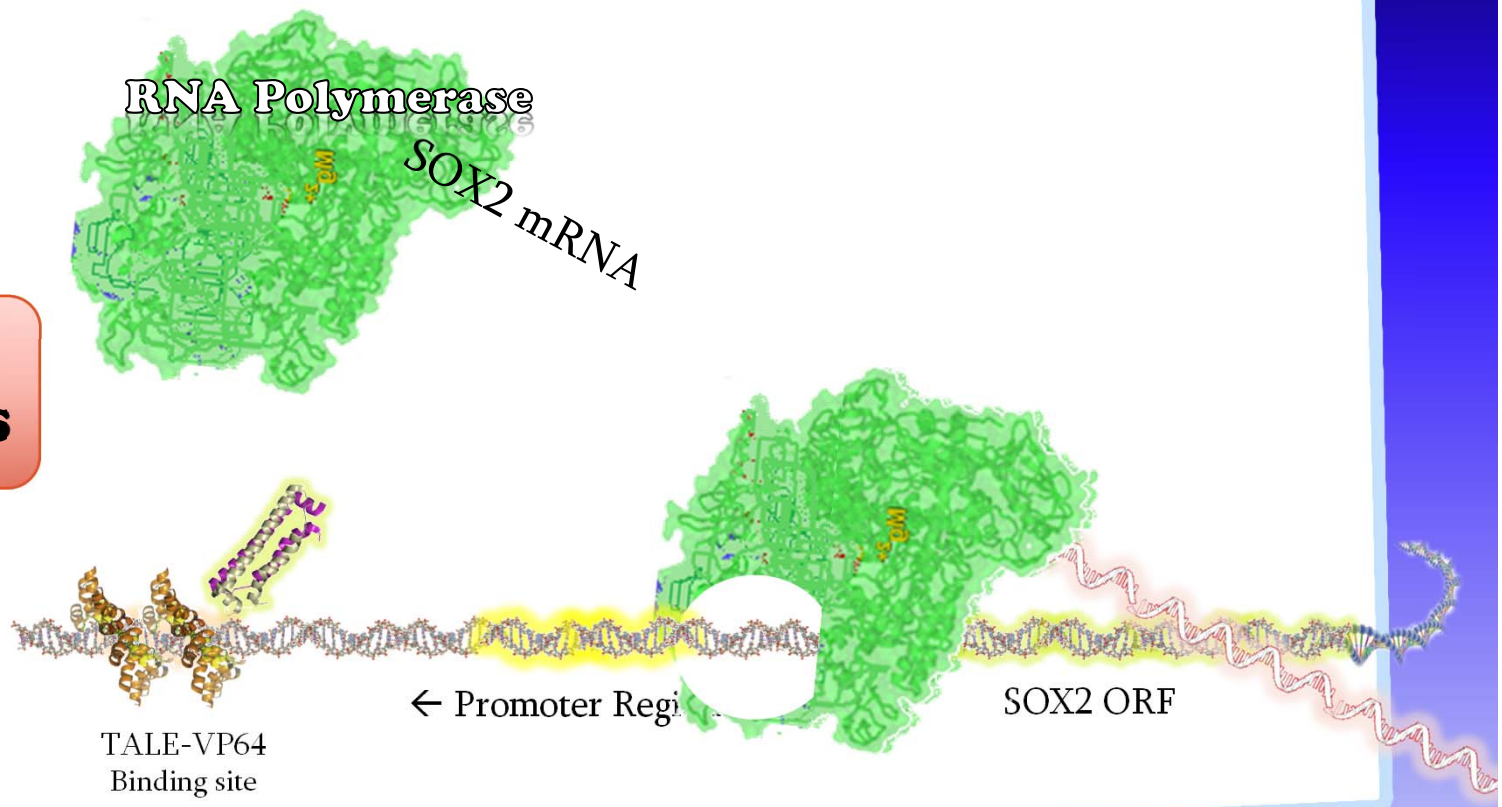


1st Experiment

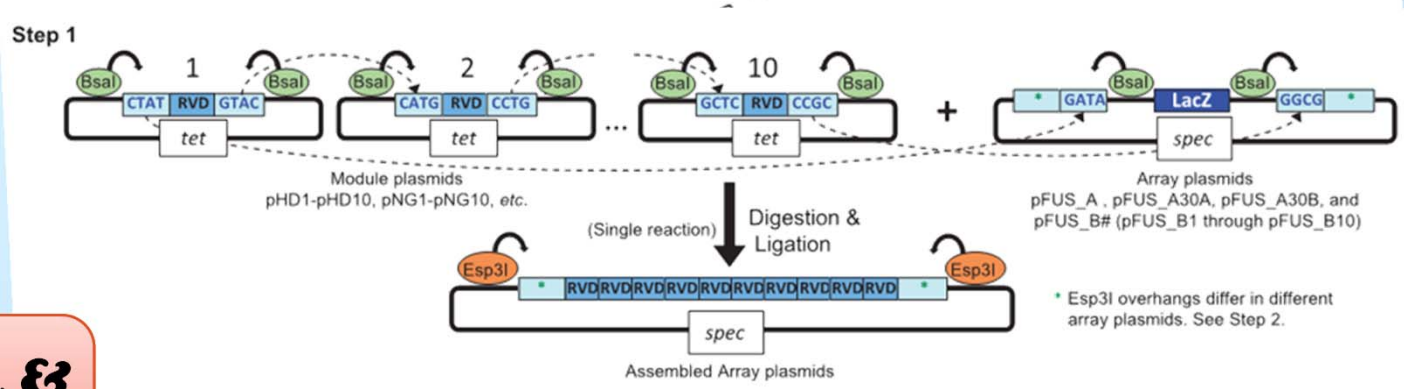
- Introduction
- Literature Review
- Research Objectives
- **Material & Methods**
- Results & Discussion

(i) Developing a method for generating TALE-TFs

(ii) Evaluating synergetic effects of multiple TALE-TFs

Golden-Gate Cloning

1st – Developing a method for generating TALE-TFs



Cermak et al. (2011) Nuc Acids Res

Golden-Gate Cloning

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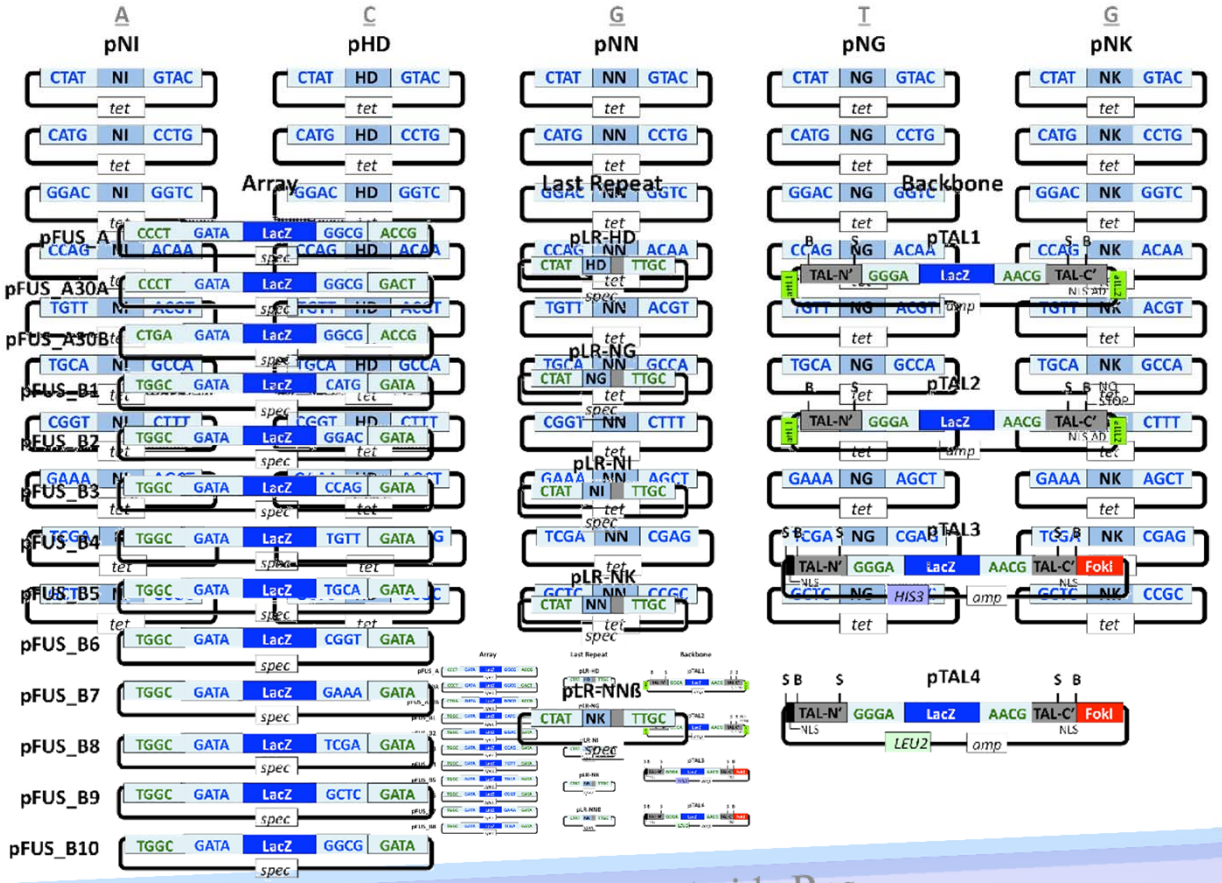

Cermak et al. (2011) Nuc Acids Res

Constructing new TALE-TF backbone

Compatible with Golden-Gate Cloning Method

1st – Developing a method for generating TALE-TFs

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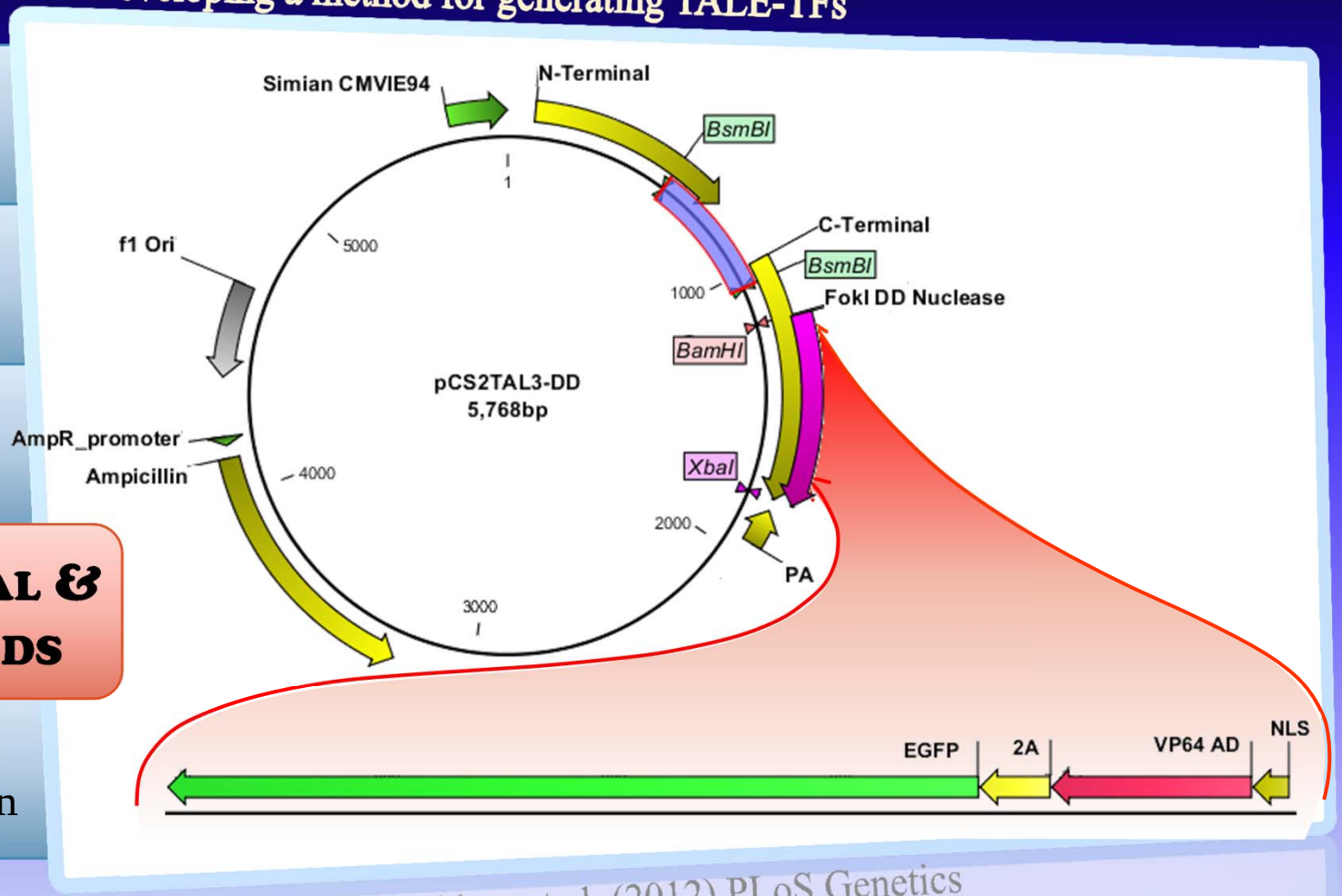

Dahlem et al. (2012) PLoS Genetics

Generating Sox2-TALE-TFs

1st – Developing a method for generating TALE-TFs

- Introduction
- Literature Review
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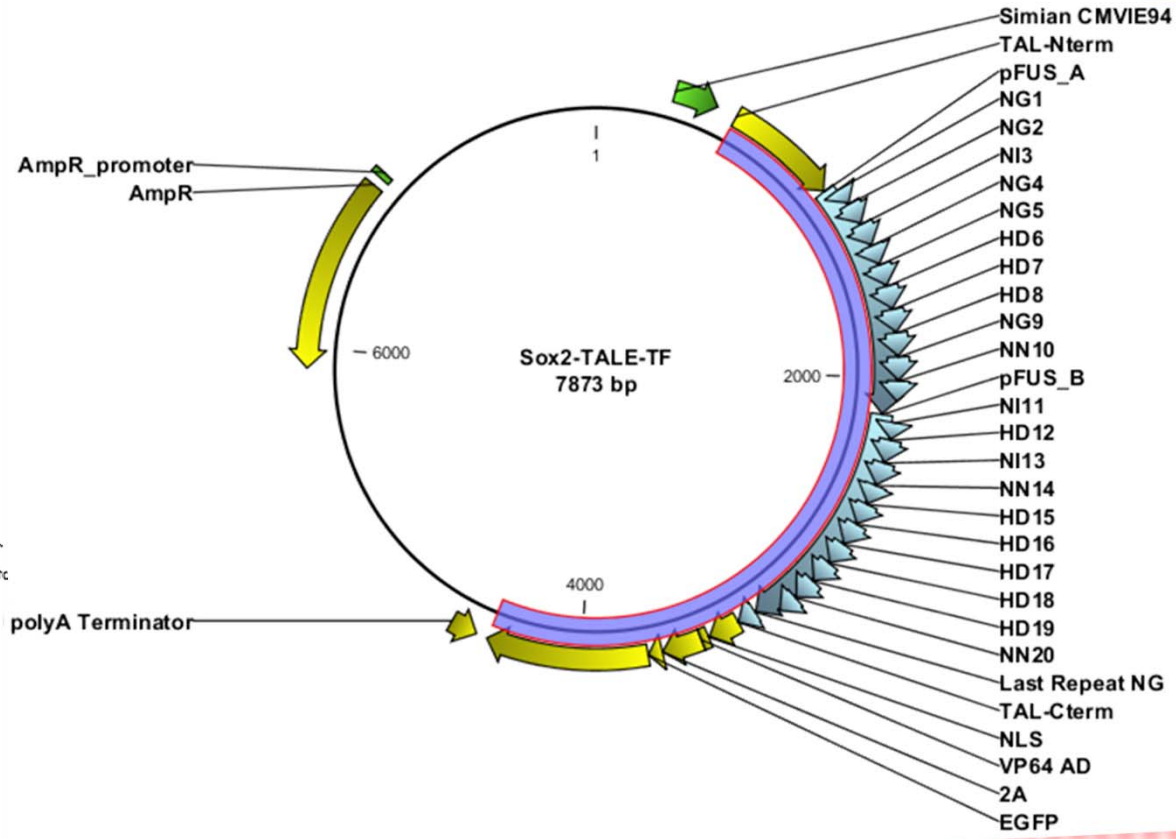

Generating Sox2-TALE-TFs

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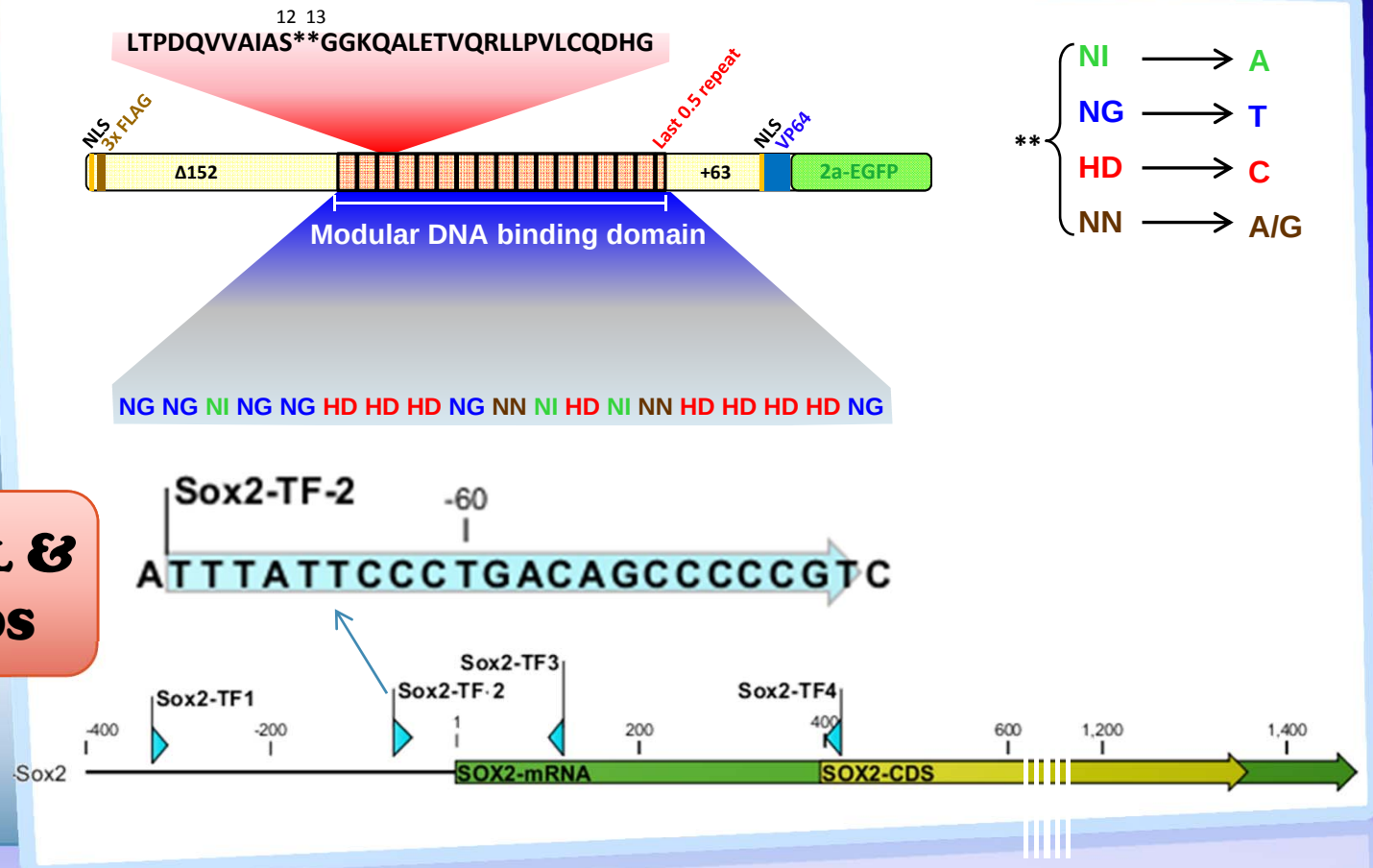

Golden-Gate Cloning

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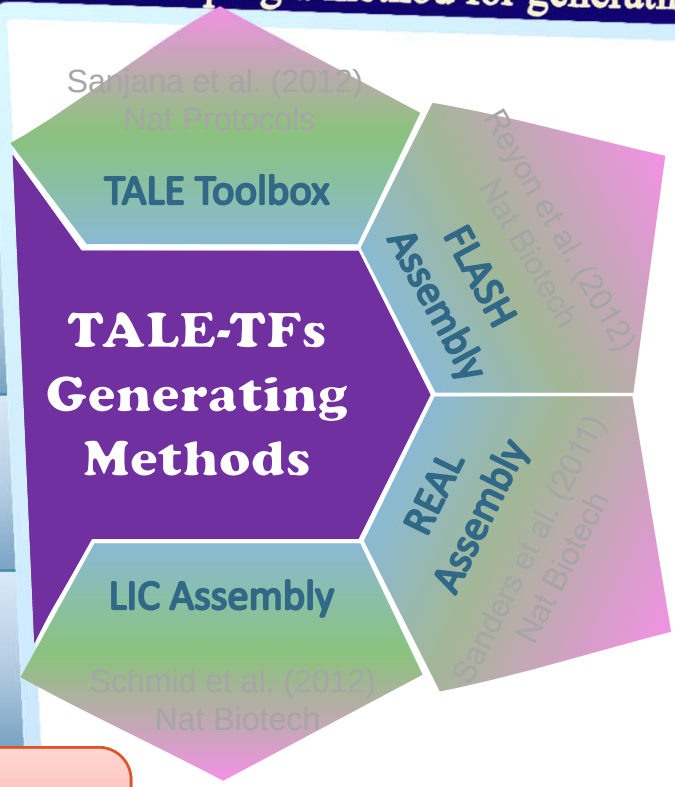
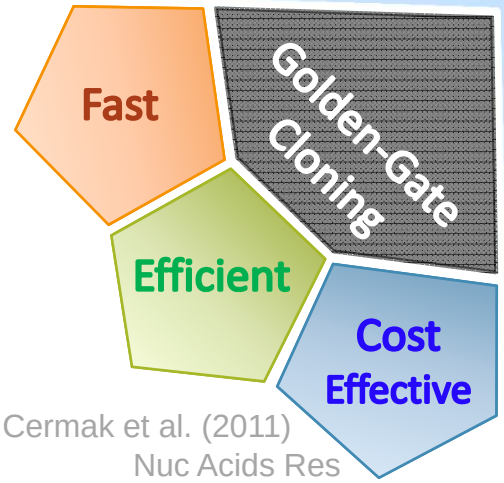




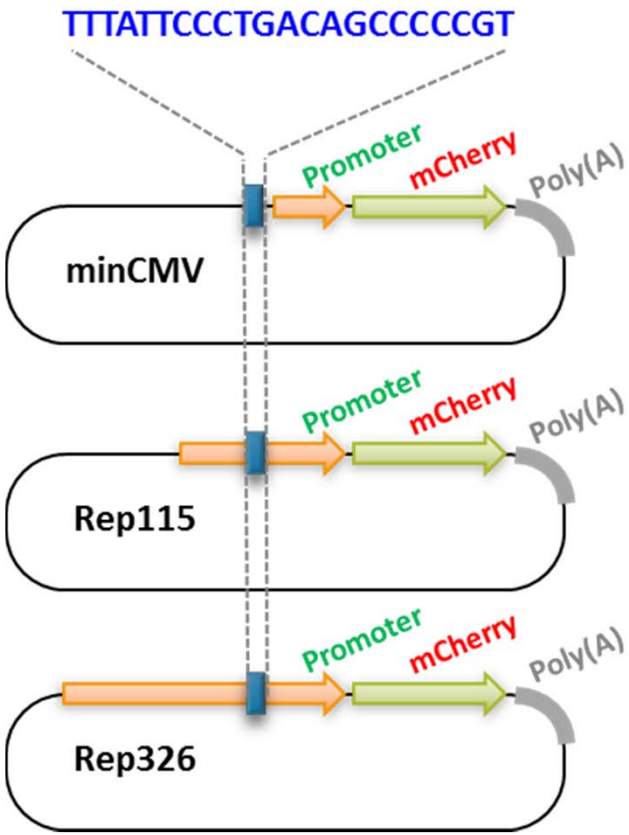
Cermak et al. (2011) Nuc Acids Res

Perez-Pinera et al. (2013) Nat Meth

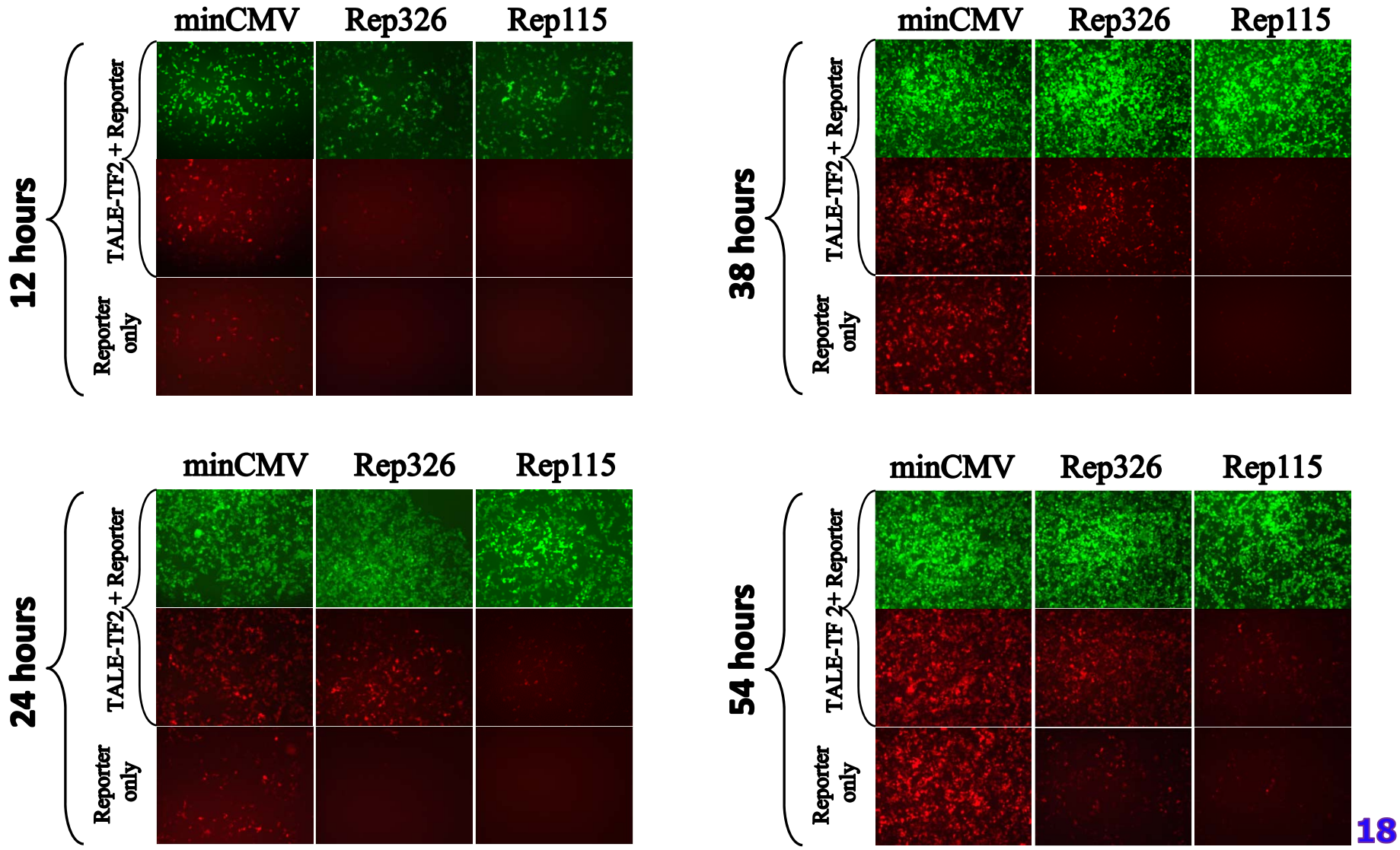
TALE-TF Destination Backbone Compatible with Golden-Gate Cloning Method

Constructing Reporters

1st – Developing a method for generating TALE-TFs



Proof of Concept…



2nd Experiment

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Sequencing Sox2 Promoter

2nd – Evaluating the synergistic effects of multiple TALE-TFs

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- Literature Review
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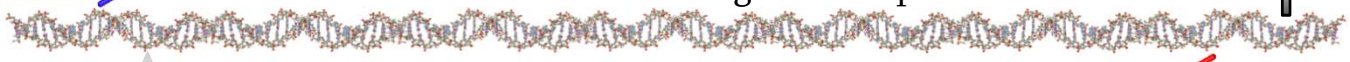
TCCGAAAAGGCGTGTGGTGTGAC
bSox2-Promoter-F

TSS

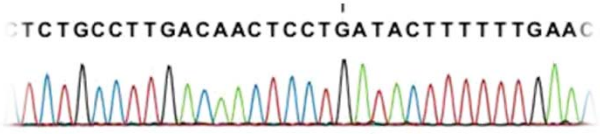
← SOX2 Promoter Region 320 bp →



PCR

Cloning into pGEMt

Sequencing



Sox2 TALE-TFs Binding Sites

2nd – Evaluating the synergistic effects of multiple TALE-TFs

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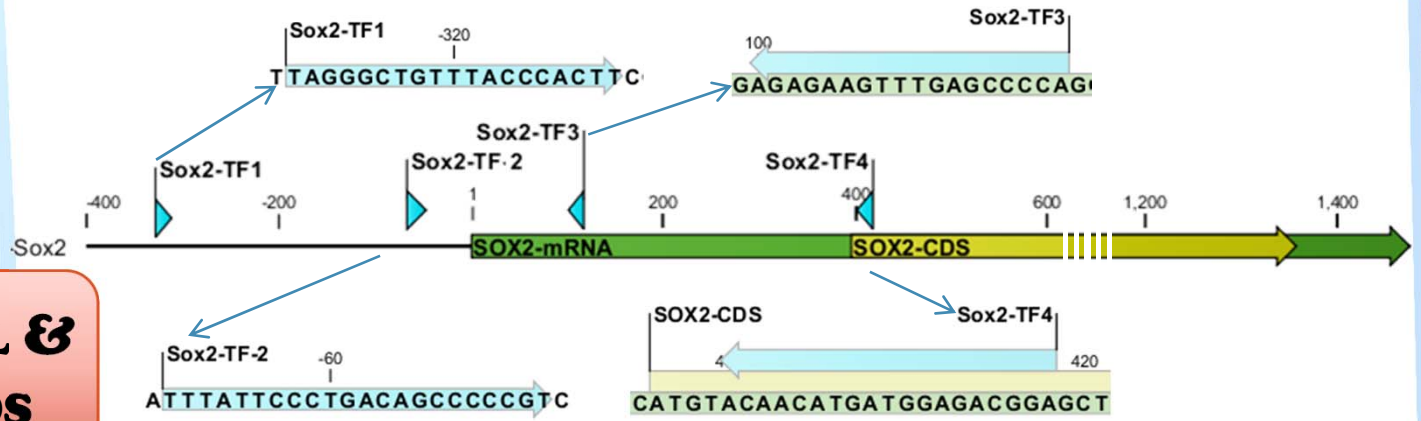
TAL Effector Nucleotide Targeter 2.0



Constructing pMXs-TALE-TFs

2nd – Evaluating the synergistic effects of multiple TALE-TFs

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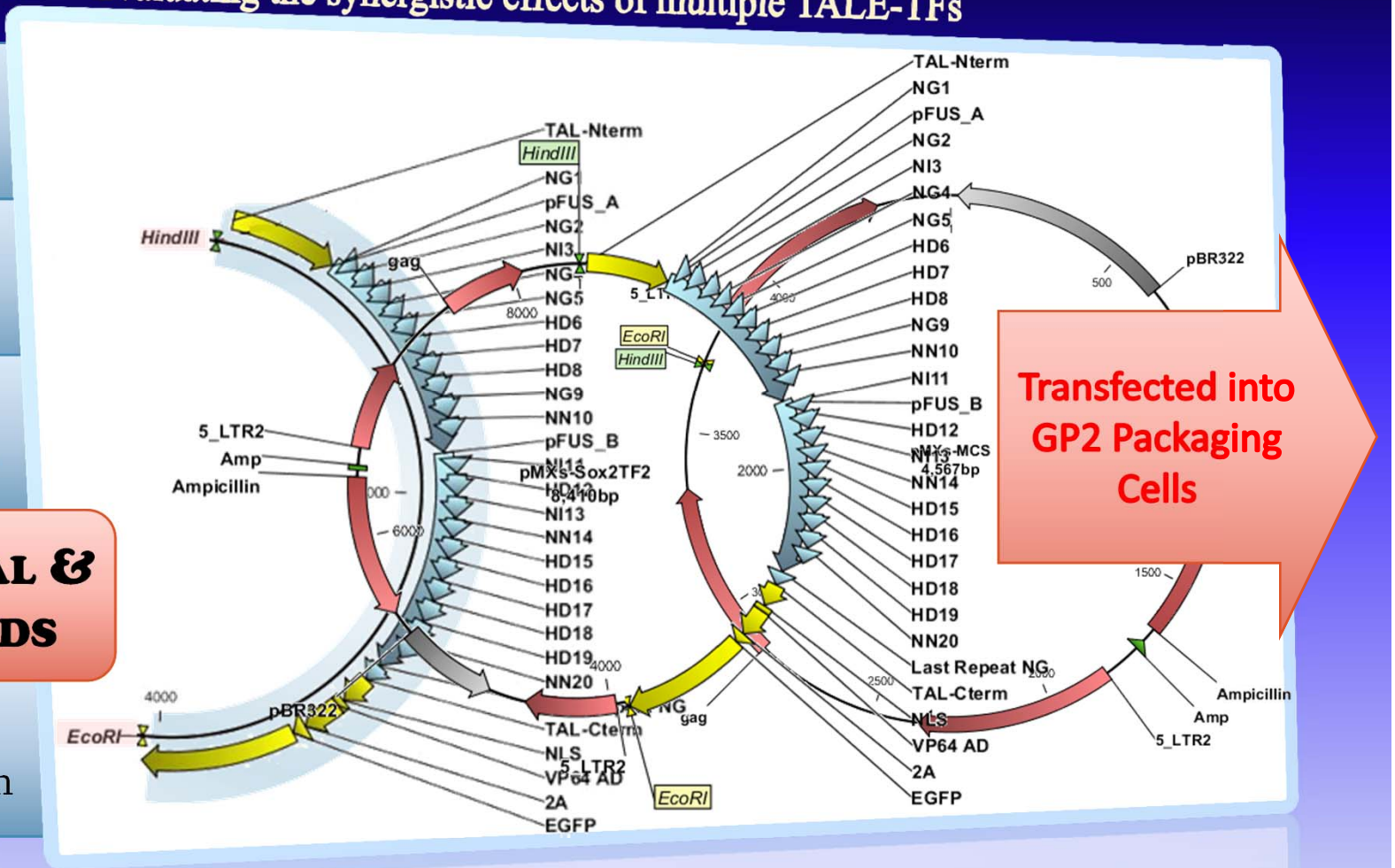
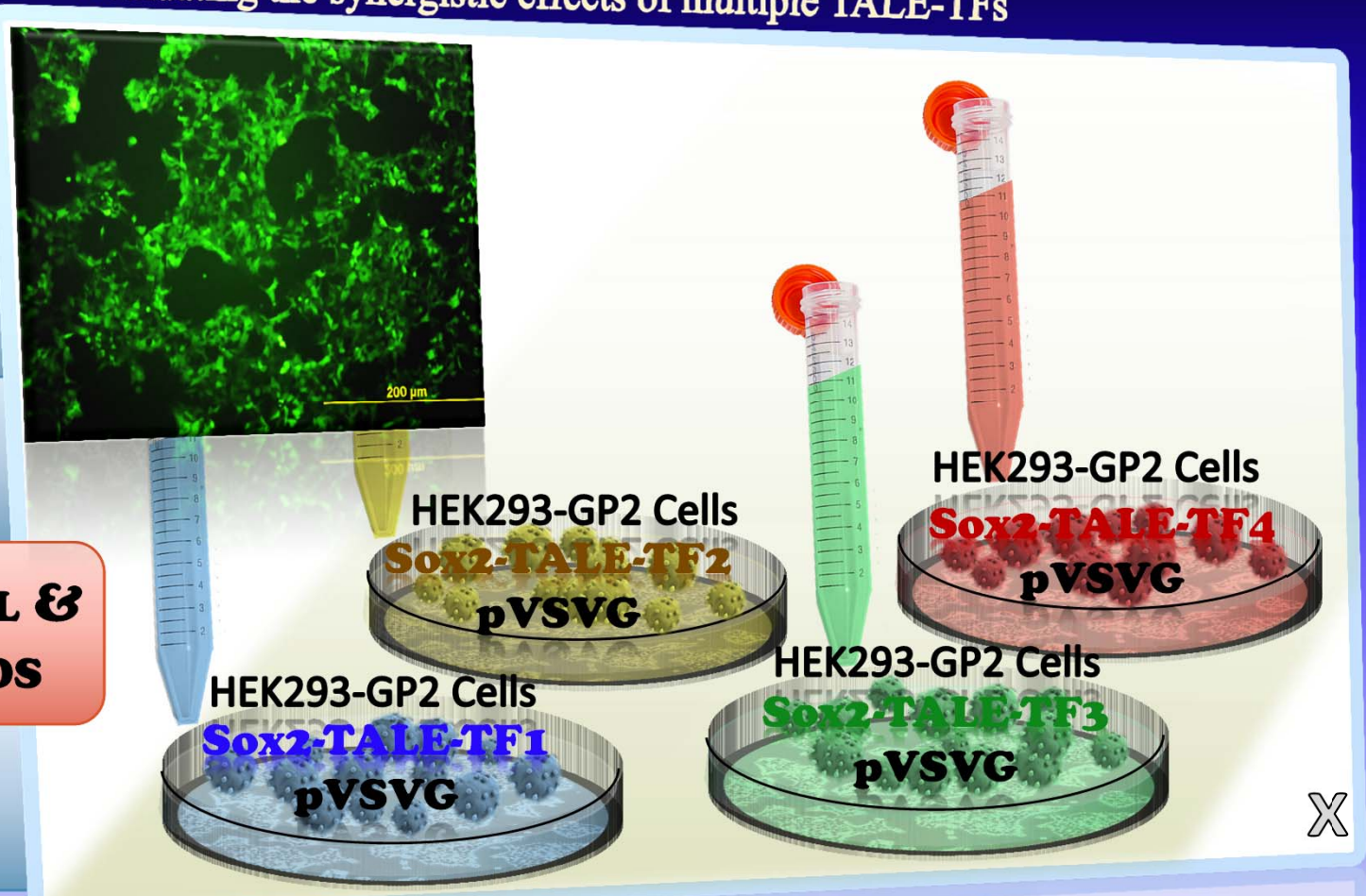


Virus Production

2nd – Evaluating the synergistic effects of multiple TALE-TFs



Virus Production

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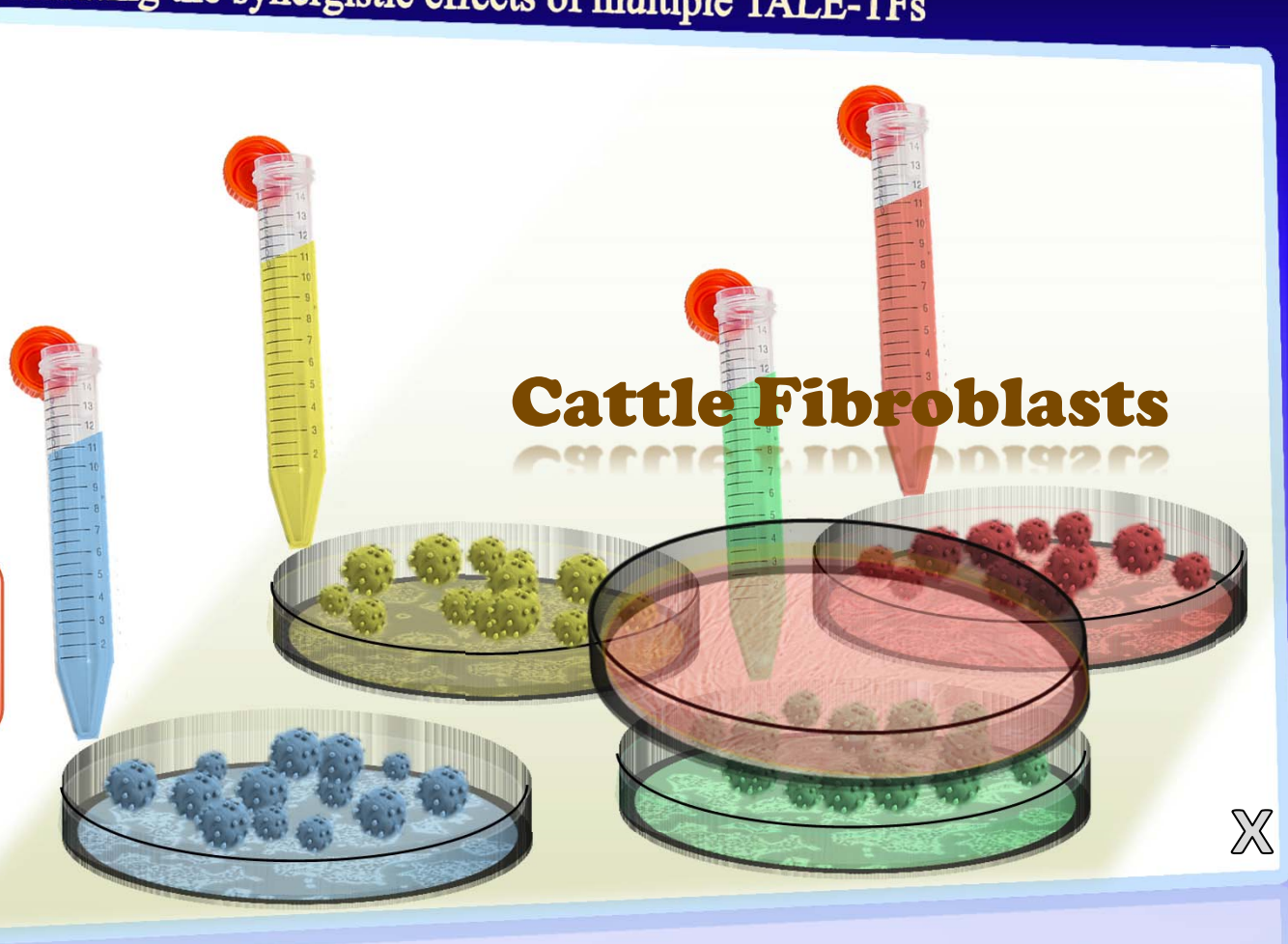
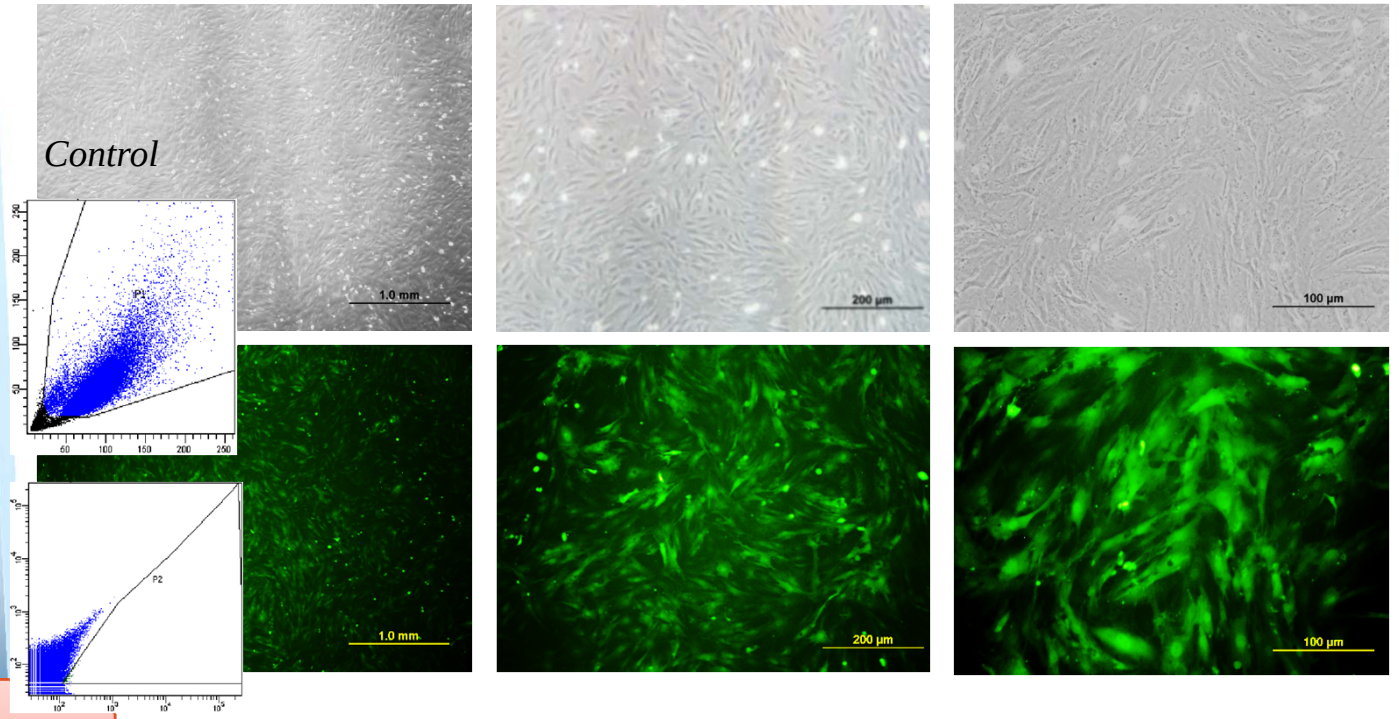


Retroviral Transduction of TALE-TFs into Cattle Fibroblasts

2nd – Evaluating the synergistic effects of multiple TALE-TFs

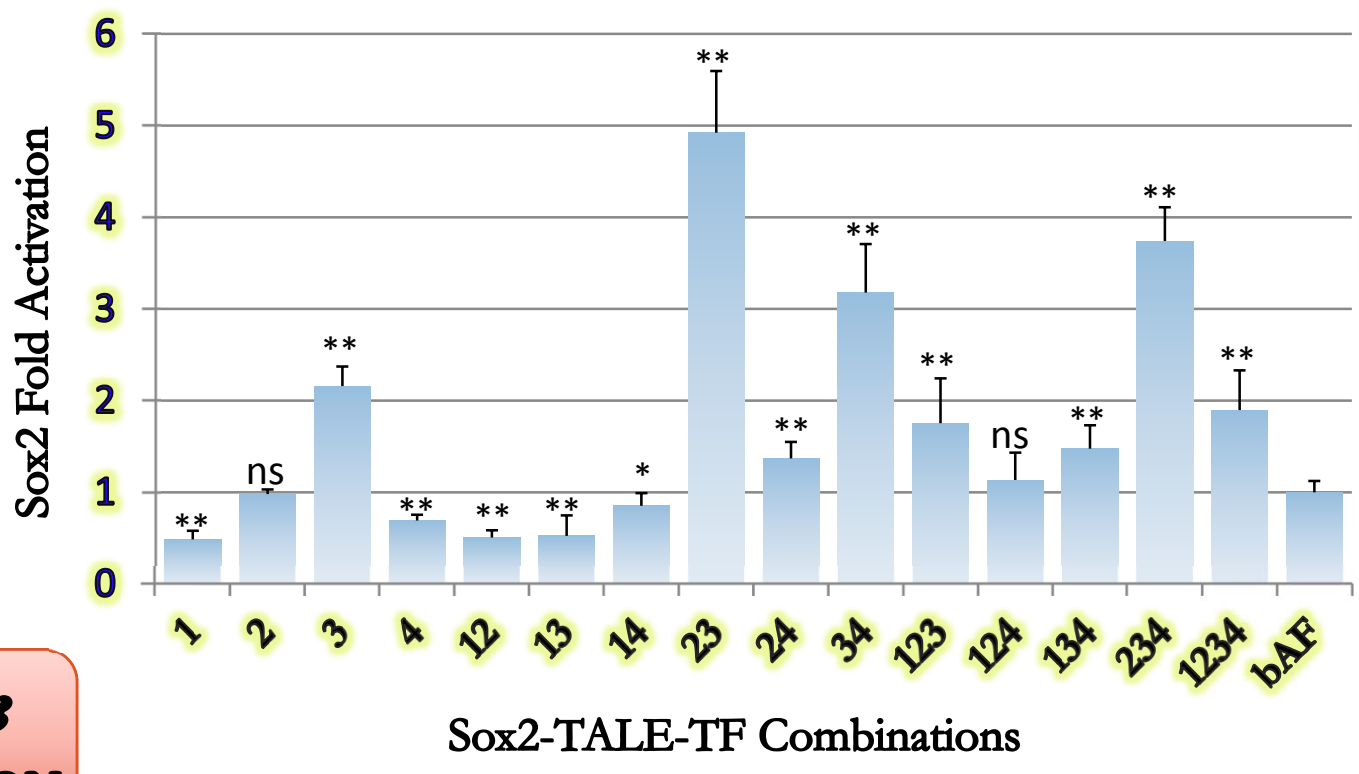
Transduction Efficiencies

2nd – Evaluating the synergistic effects of multiple TALE-TFs



The Cooperative Activity of Sox2-TALE-TFs

2nd – Evaluating the synergistic effects of multiple TALE-TFs



t test

Comparing to other studies…

2nd – Evaluating the synergistic effects of multiple TALE-TFs

Reference	Cell type	Gene targeted	AD	Fold Change	TALE length
Morbitzer et al., *PNAS* (2010)	Plants	Egl3, Bs4, Bs3 Knat1, UPA20	Native	**n.d.**	??.5
Zhang et al., *Nat Biotech* (2011)	HEK 293FT	**Sox2**	VP64	**5.5**	12.5
	HEK 293FT	Klf4	VP64	**2.2**	12.5
	HEK 293FT	cMyc	VP64	**n.a.**	12.5
	HEK 293FT	Oct4	VP64	**n.a.**	12.5
Miller et al., *Nat Biotech* (2011)	HEK293	NTF3	VP16	**30**	17.5
Geissler et al., *PLoS One* (2011)	HEK293-Rex	PUMA	VP16	**1.5**	17.5
	HEK293-Rex	IFN a1	VP16	**3**	19.5
	HEK293-Rex	IFN b1	VP16	**3.5**	17.5
Cermak et al., *Nucleic Acids Res* (2011)	Plants	Bs3	Native	**n.d.**	13.5
Bultmann et al., *Nucleic Acids Res* (2012)	Mouse ES cells	Oct4	VP16	**4**	17.5
	Mouse neural SC	Oct4	VP16	**30***	17.5
Cong et al., *Nat Commun* (2012)	Human 293FT	CACNA1C	VP64	**3-5**	16.5
Tremblay et al., *Hum Gene Ther* (2012)	Human 293FT	FXN	VP64	**3.1**	13.5
Garg et al., *Nucleic Acids Res* (2012)	Human U-2OS	OSGIN2	VP64	**4.8**	18.5
	Human U-2OS	ZC3H10	VP64	**1.3**	18.5
Wang et al., *Angew Chem Int Ed Engl* (2012)	Hela	ROCK1	VP64	**n.d**	16.5

* In the presence of epigenetic modifiers, n.a. ; Not Applicable, n.d. ; Not Determined

Predicting TALE-TFs Activity

2nd – Evaluating the synergistic effects of multiple TALE-TFs

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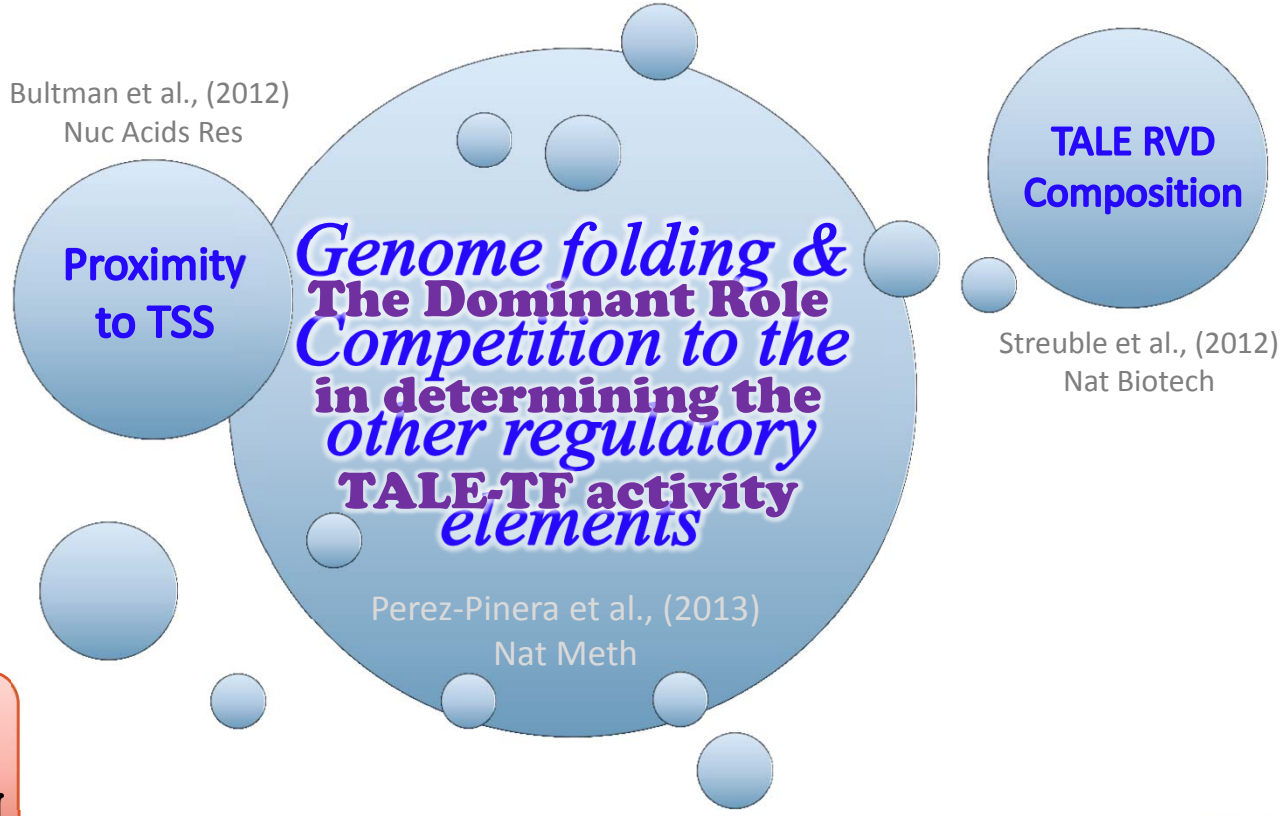

Conclusion

Our results provide proof of principle that artificial transcription factors can be used for modulating endogenous gene expression in livestock cells.

Also the possibility to generate and delivering a combination of artificial transcription activators opens up a window for fine tuning gene expression in mammalian cells and should enable many applications of these tools for biological research.

Acknowledgement

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Prof. Paul Verma

Dr. Huseyin Sumer

Dr. Jun Liu