

# Knockout mice created by TALEN-mediated gene targeting

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Presented by Jessica

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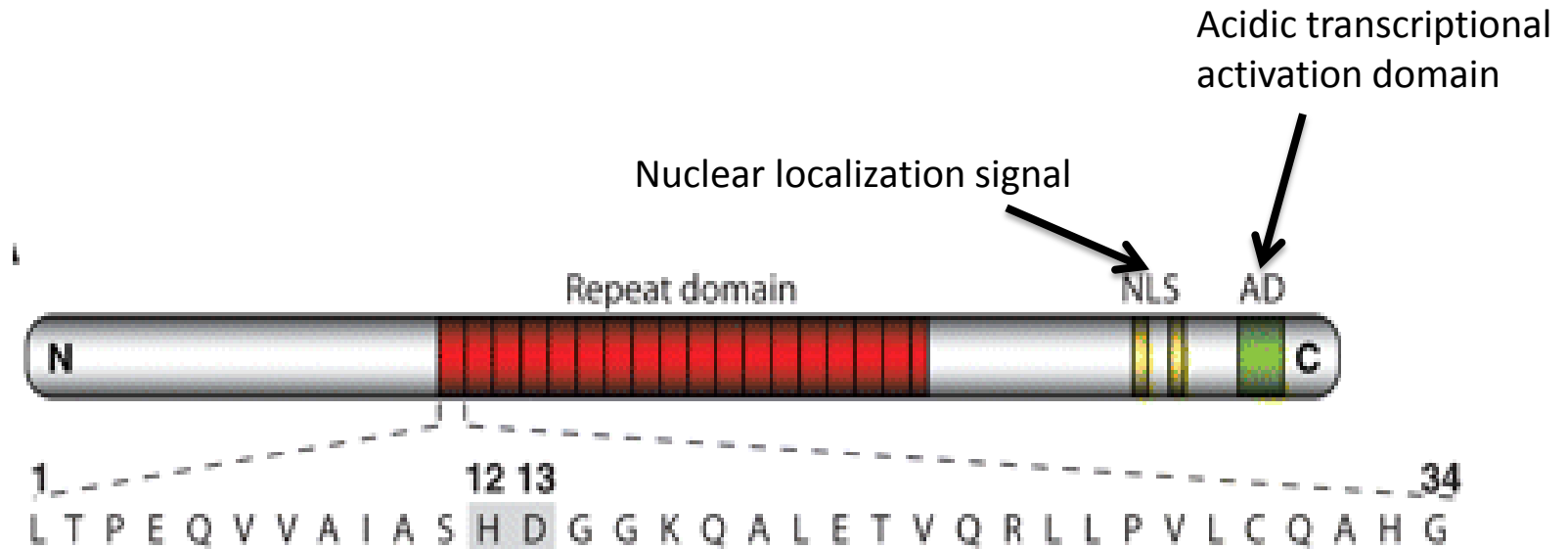
Sung, Y. H., et al. (2013). Knockout mice created by TALEN-mediated gene targeting. *Nature biotechnology*, 31(1), 23–4. doi:10.1038/nbt.2477

# Gene-specific knockout mice

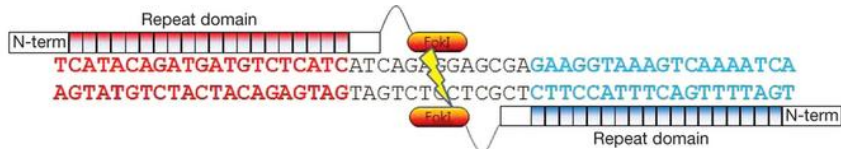
- Knocked out gene – existing gene is replaced or disrupted
- Causes change in mouse phenotype
- One of the most powerful ways to study gene function in living animals



# Review on TALENs

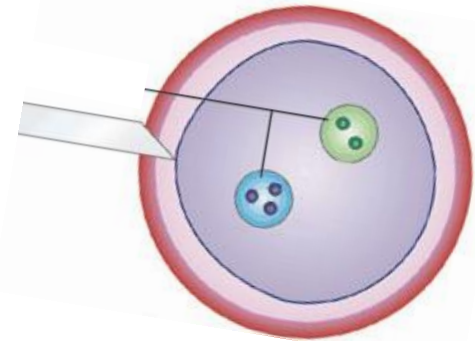


# Overview of how to generate knockout mice with TALENs

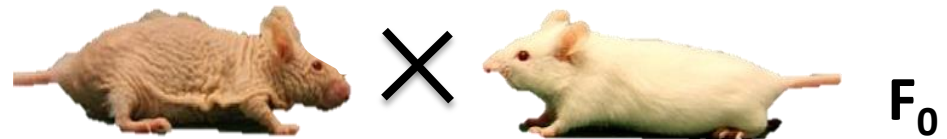


Design specific TALENs

Inject TALEN mRNA into mice embryo



Generate mutant mice

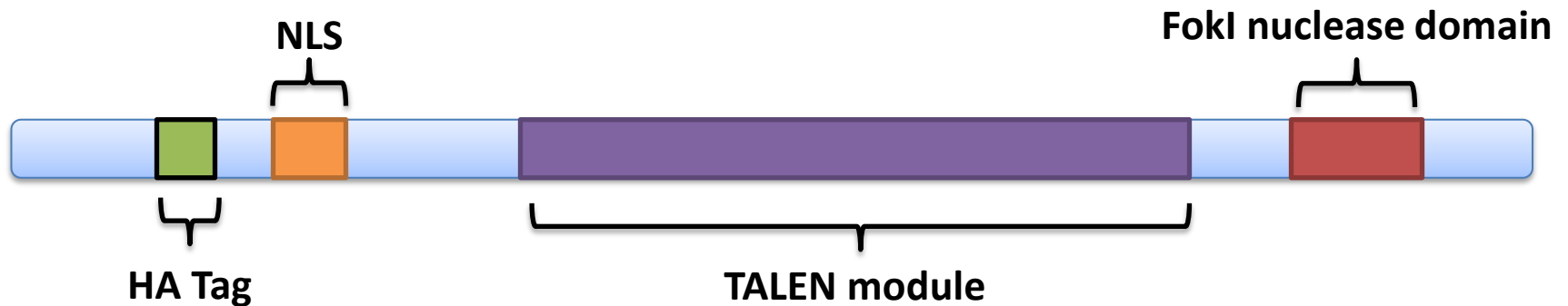


F<sub>0</sub>



F<sub>1</sub>

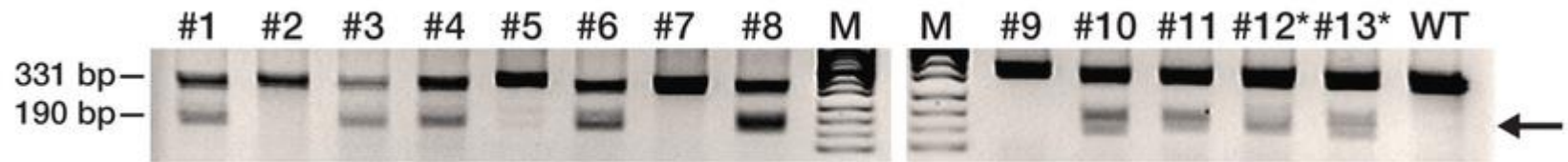
# Step 1: Design and synthesize highly active TALENs



- HA Tag – for antibody tagging
- NLS – nuclear localization signal
- TALEN module – target either Pibf1 or Sepw1
- FokI nuclease domain – creates non-specific cleavage

# Step 2: Identify mutant mice

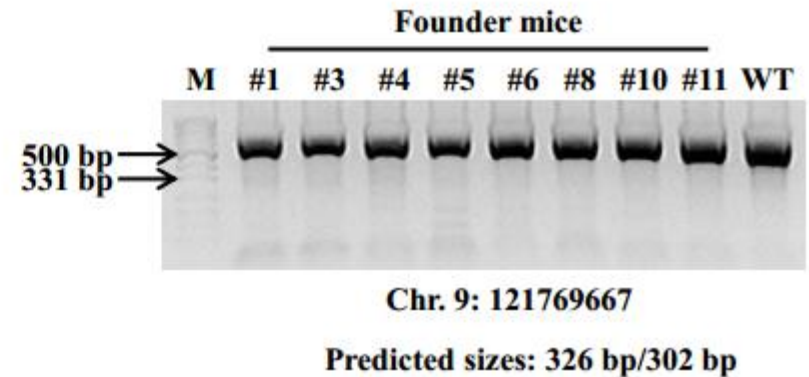
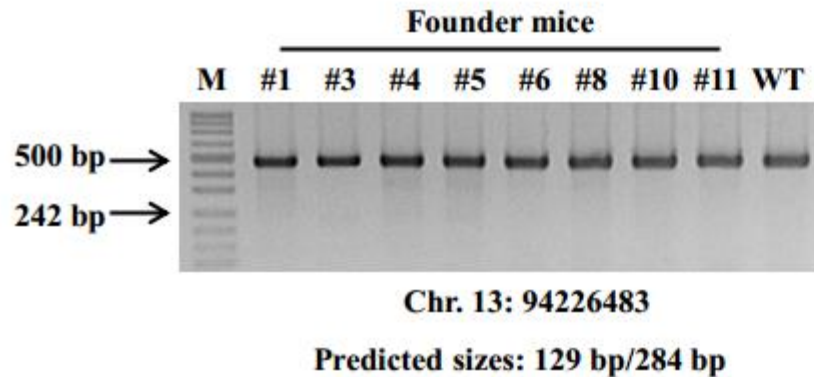
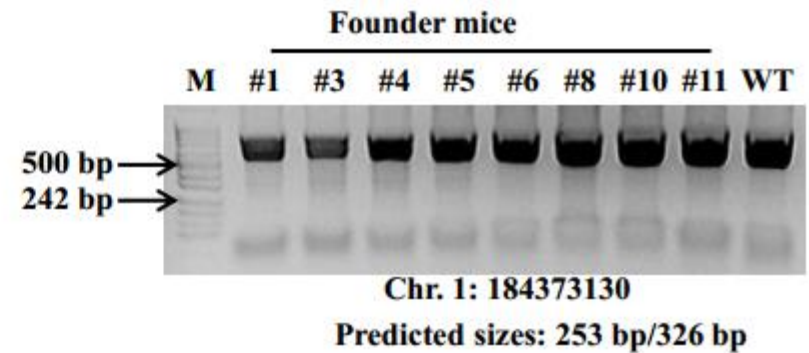
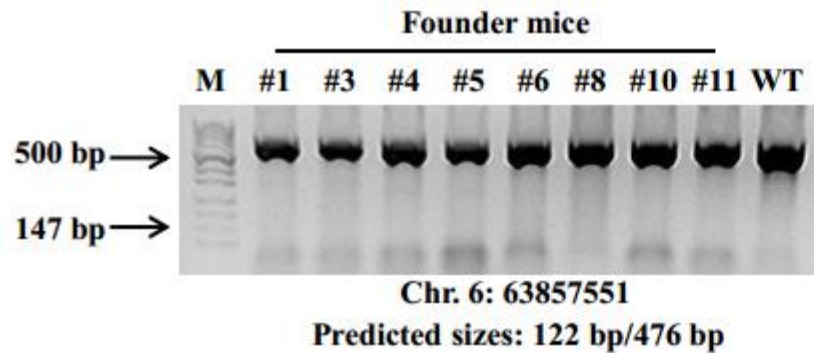
## Pibf1-TALEN:



|     | H | T   | D   | D           | V          | S           | S          | S          | E           | E   | R   | E   | G   | K   | V   | K   | I   | Amino acid sequence |     |                     |   |        |
|-----|---|-----|-----|-------------|------------|-------------|------------|------------|-------------|-----|-----|-----|-----|-----|-----|-----|-----|---------------------|-----|---------------------|---|--------|
|     | T | CAT | ACA | GAT         | GAT        | GTC         | TCA        | TCA        | TCA         | GAG | GAG | CGA | GAA | GGT | AAA | GTC | AAA | ATC                 | A   | Wild-type sequence  |   |        |
| #1  | T | CAT | ACA | GAT         | GAT        | <u>ac</u> - | ---        | ---        | ---         | --- | --- | --- | --A | GGT | AAA | GTC | AAA | ATC                 | A   | Δ21 bp + aa change  |   |        |
|     | T | CAT | ACA | <u>Gg</u> T | GAT        | G--         | ---        | ---        | ---         | --- | --- | --- | -AA | GGT | AAA | GTC | AAA | ATC                 | A   | Δ21 bp + aa change  |   |        |
| #3  | T | CAT | ACA | GAT         | GAT        | GTC         | TCA        | TCA        | TCA         | GAG | --- | CGA | GAA | GGT | AAA | GTC | AAA | ATC                 | A   | Δ3 bp               |   |        |
|     | T | CAT | ACA | GAT         | GAT        | GTC         | TCA        | TCA        | TCA         | G-- | --- | CGA | GAA | GGT | AAA | GTC | AAA | ATC                 | A   | Δ5 bp (frameshift)  |   |        |
| #4  | T | CAT | ACA | GAT         | GAT        | GTC         | TCA        | TCA        | TCA         | G-G | GAG | CGA | GAA | GGT | AAA | GTC | AAA | ATC                 | A   | Δ1 bp (frameshift)  |   |        |
|     | T | CAT | ACA | GAT         | GAT        | GTC         | TCA        | TCA        | TCA         | GAG | GAG | CGA | GAA | GGT | AAA | GTC | AAA | ATC                 | A   | WT                  |   |        |
| #5  | T | CAT | ACA | GAT         | GAT        | GTC         | TCA        | TCA        | TC-         | --- | -AG | CGA | GAA | GGT | AAA | GTC | AAA | ATC                 | A   | Δ5 bp (frameshift)  |   |        |
|     | T | CAT | ACA | GAT         | GAT        | GTC         | TCA        | TCA        | TCA         | GAG | GAG | CGA | GAA | GGT | AAA | GTC | AAA | ATC                 | A   | WT                  |   |        |
| #6  | T | CAT | ACA | GAT         | GAT        | GTC         | TC-        | ---        | ---         | --- | --- | --- | --G | CGA | GAA | GGT | AAA | GTC                 | AAA | ATC                 | A | Δ12 bp |
|     | T | CAT | ACA | GAT         | GAT        | G--         | ---        | ---        | ---         | --- | --- | --- | -AA | GGT | AAA | GTC | AAA | ATC                 | A   | Δ21 bp              |   |        |
| #8  | T | CAT | ACA | GAT         | GA-        | ---         | ---        | ---        | ---         | --- | --- | --- | --A | GGT | AAA | GTC | AAA | ATC                 | A   | Δ24 bp              |   |        |
|     | T | CAT | ACA | GAT         | GAT        | GTC         | <u>Tac</u> | <u>aga</u> | <u>t</u> -- | --- | --- | --- | GAA | GGT | AAA | GTC | AAA | ATC                 | A   | Δ11 bp (frameshift) |   |        |
| #10 | T | CAT | ACA | GAT         | GAT        | GTC         | TCA        | TCA        | TCA         | G-G | GAG | CGA | GAA | GGT | AAA | GTC | AAA | ATC                 | A   | Δ1 bp (frameshift)  |   |        |
|     | T | CAT | ACA | GAT         | <u>GAa</u> | ---         | ---        | ---        | ---         | --- | --- | --- | --- | --- | AAA | GTC | AAA | ATC                 | A   | Δ27 bp + aa change  |   |        |
| #11 | T | CAT | ACA | GAT         | GAT        | GTC         | TCA        | TCA        | TCA         | G-- | GAG | CGA | GAA | GGT | AAA | GTC | AAA | ATC                 | A   | Δ2 bp (frameshift)  |   |        |
|     | T | CAT | ACA | GAT         | GA-        | ---         | ---        | ---        | ---         | --- | --- | --- | --A | GGT | AAA | GTC | AAA | ATC                 | A   | Δ24 bp              |   |        |

# Pibf1 shows no off-target cleavage

## T7E1 test of off-target sites



# Step 3: Investigate Dose dependency for Pibf1-TALEN

Table 1: TALEN-mediated *Pibf1* gene targeting in C57BL/6J mice

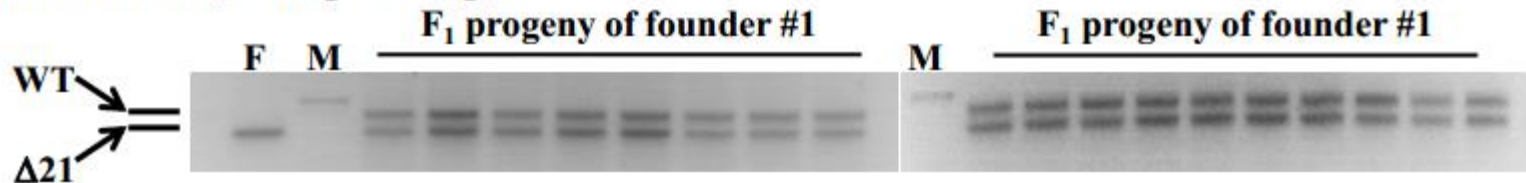
| Dose of TALEN- <i>Pibf1</i> mRNA (ng/ $\mu$ l) | Number of injected zygotes | Number of surviving zygotes | Two-cell embryos | Transferred embryos | Newborns                | Founders <sup>*</sup>    |
|--|----------------------------|-----------------------------|------------------|---------------------|-------------------------|--------------------------|
| 50   | 276                        | 263 (95.3%)                 | 262 (99.6%)      | 243                 | 13 (5.3%) <sup>†</sup>  | 10 (76.9%) <sup>††</sup> |
| 20   | 183                        | 176 (96.2%)                 | 176 (100%)       | 176                 | 39 (22.2%) <sup>†</sup> | 19 (48.7%)               |

- Mutation rate approximately proportional to injection dose of Pibf1-TALEN mRNA
- More frequent bi-allelic mutations in high dose
- Relatively large deletions more frequent with high-dose
- Higher number of mutant mice produced by low-dose injections

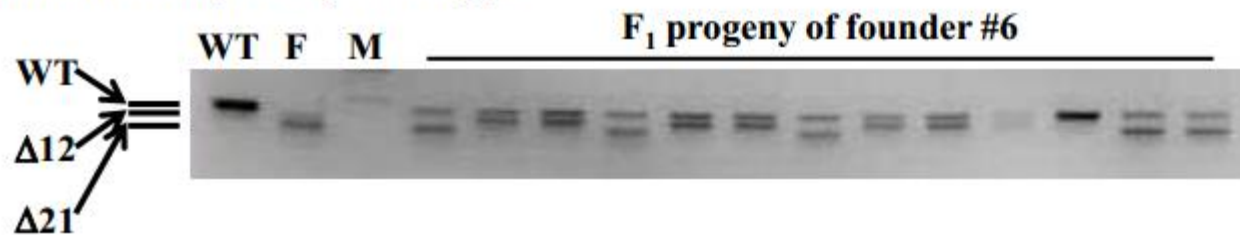


# Step 4: Produce F<sub>1</sub> offsprings and determine genotypes

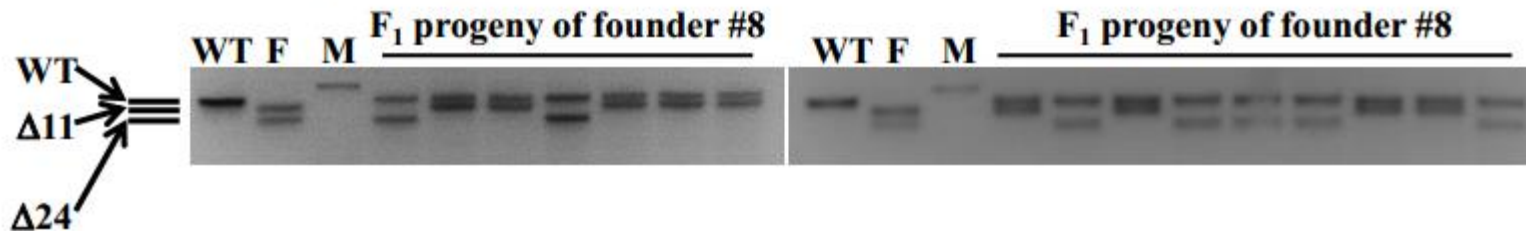
Founder #1 ( $\Delta 21$  bp/ $\Delta 21$  bp)



Founder #6 ( $\Delta 12$  bp/ $\Delta 21$  bp)



Founder #8 ( $\Delta 11$  bp/ $\Delta 24$  bp)



# Conclusion

- TALEN activity in one-cell embryos is sufficient to induce mutations
- TALEN activity not likely to be maintained after the first cleavage of one-cell embryos
- TALEN-mediated gene targeting is an efficient method for creating heritable null mutations in a specific locus of the mouse genome

# Limitations

- 15% gene knockouts are developmentally lethal, limiting ability to study development into adult mice
- Same gene has different functions in humans
- Limited control over base pair deletion
- Off-target binding
- Dose-dependent activity

# Significance and Future Developments

- Target specific sequences in the genome
- Easy to manipulate TALENs to target any sequence
- Can target other genes
- Find a more specific method for cleavage

# Discussion Questions

- Is there a way to make the DNA cleavage more predictable?
- Is this method really more efficient than current methods for creating knockout mice?
- What other methods may this be combined with to create more control?