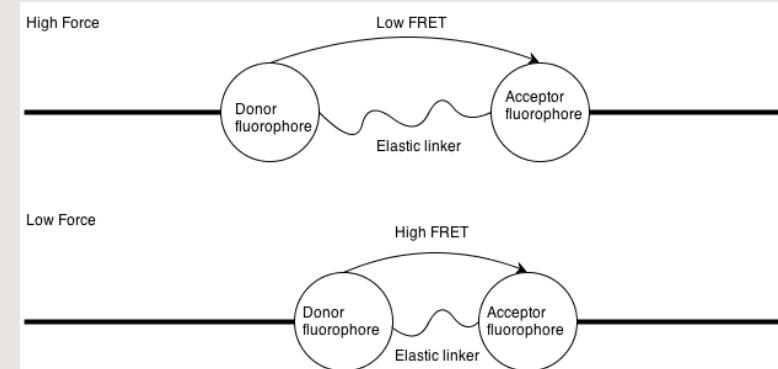


Genetic Validation of the *TsNcd* Construct

Kevin Do

Background

- Ncd is a kinesin motor protein
- Important for spindle assembly and female meiotic chromosome segregation
- Well-characterized *in vitro* but not *in vivo*
- Previous TS Module + Ncd = *TsNcd*
- Validation necessary to ensure *TsNcd* results are relevant to Ncd



Tests

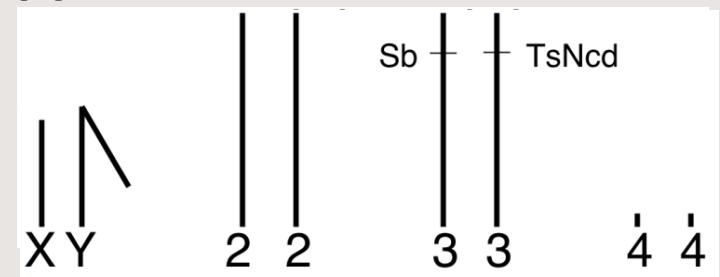
- Mapping test
- Dominant negative test
- Rescue test

Mapping Test Design

- $TsNcd$ injected into females
- Crossed with Sb males to get males below
 - Possible locations: X, 2, 3
- Test cross
 - w/w ; $+/+$; $+/+$ Female x Red Sb $TsNcd$ Males
- Markers
 - Red: $TsNcd$
 - Sb : 3rd chromosome

Mapping Test Results

- Test cross
 - $w/w; +/+; +/+$ Female x Red *TM3 Sb TsNcd* Males
- Or *Sb* female offspring → *TsNcd* not on 3rd
 - Red and *Sb* did not segregate from each other
- *TsNcd* sex-linked → *TsNcd* on X
 - All females orange ($n = 174$)
 - All males white ($n = 151$)
- χ^2 Test: $p < 0.0001$ that *TSNcd* is not sex-linked



Dominant Negative Test

- Interfere with wild-type Ncd function?
- Null mutant (ca^{nd}) causes missegregation
- $TsNcd$ Females x w/ $B^S Y$ Males
- No missegregants in 413 flies

Normal	X	Y	Missegre-gation	X	Y
X	XX Female (n = 200)	XY Male (n = 213)	Diplo-X	X/X/X (lethal)	X/X/Y Female (viable)
X	XX Female (n = 200)	XY Male (n = 213)	Nullo-X	X/0 Male (viable)	0/Y (lethal)



Rescue Test

- $TsNcd/TsNcd$; $+/+$; ca^{nd}/ca^{nd} Females x $w/B^S Y$; $+/+$; $+/+$ Males
- Similar to dominant negative test
- Confirm lack of Ncd
 - ca^{nd} is deletion mutant
 - Extract DNA, PCR with a primer in deleted region, run gel, check for Ncd band