

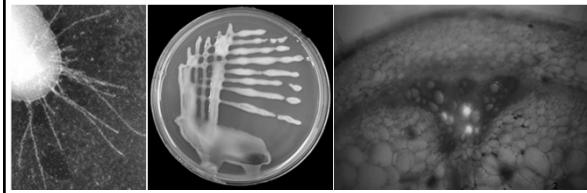
## An update on *Ralstonia solanacearum* and Bacterial Wilt disease

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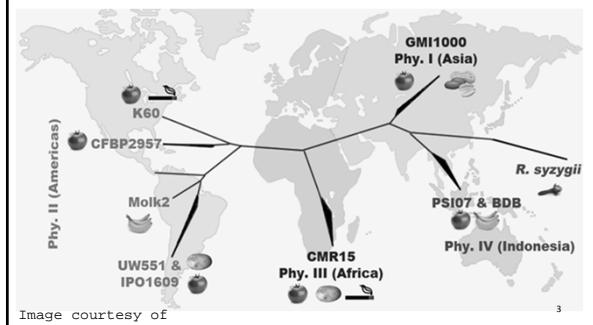
### *Ralstonia solanacearum*

- Gram-negative
- $\beta$ -proteobacterium
- Rod-shaped, xylem inhabiting
- Survive long-term in water, soil and infected plant materials

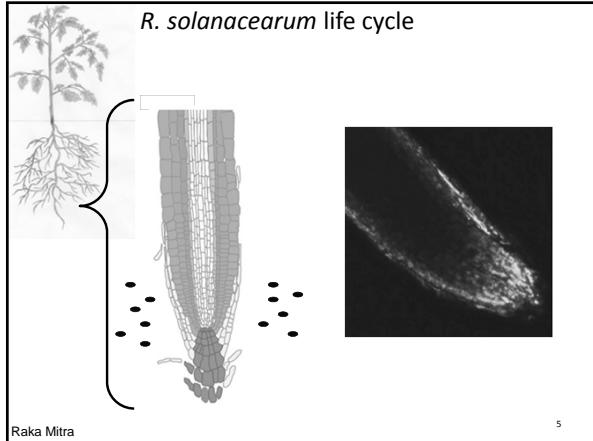


### *Ralstonia solanacearum*

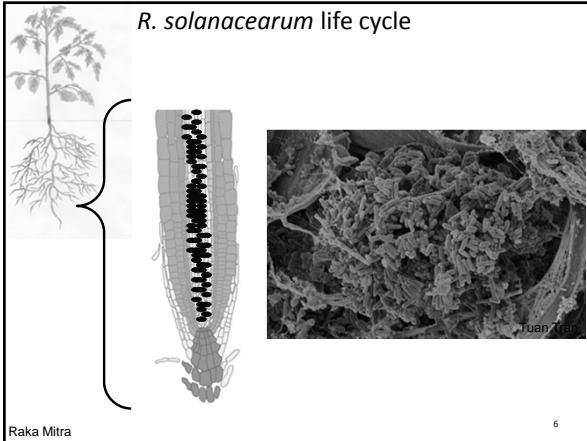
- Top 10 bacterial phytopathogens
- Widely distributed around the globe

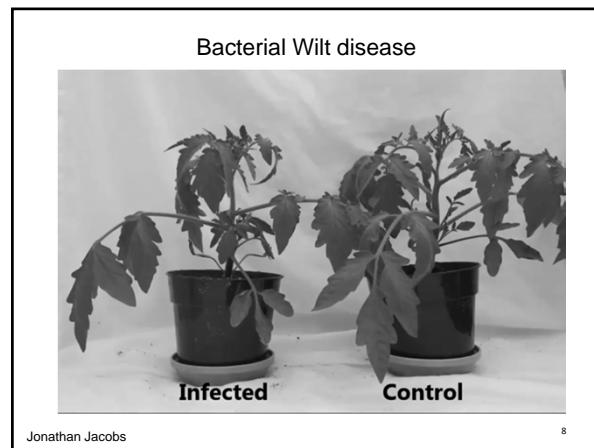
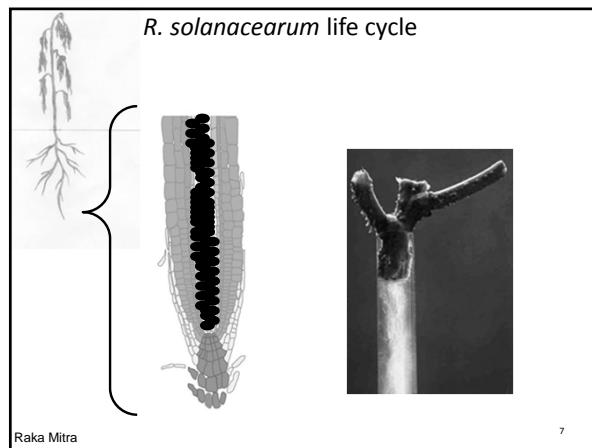


### *R. solanacearum* life cycle



### *R. solanacearum* life cycle





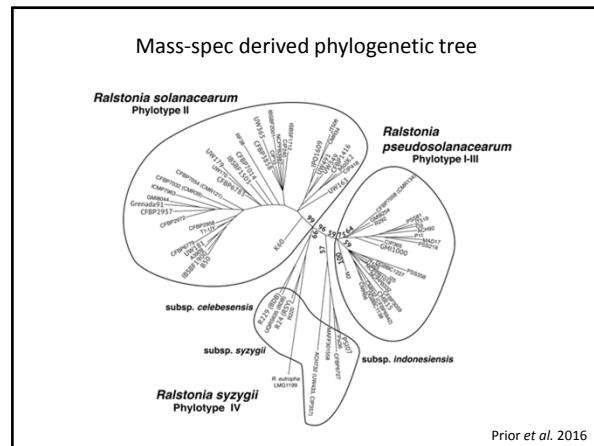
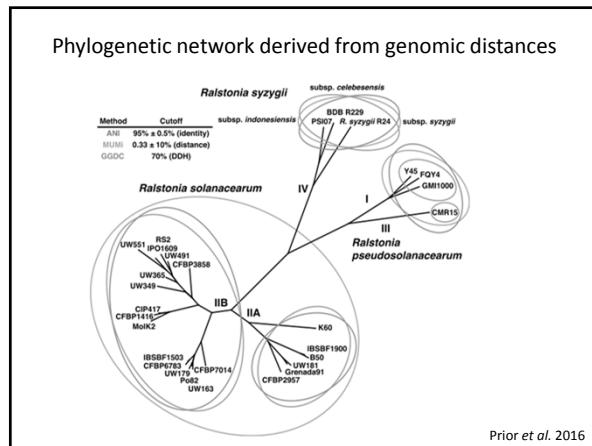
### Current classification systems

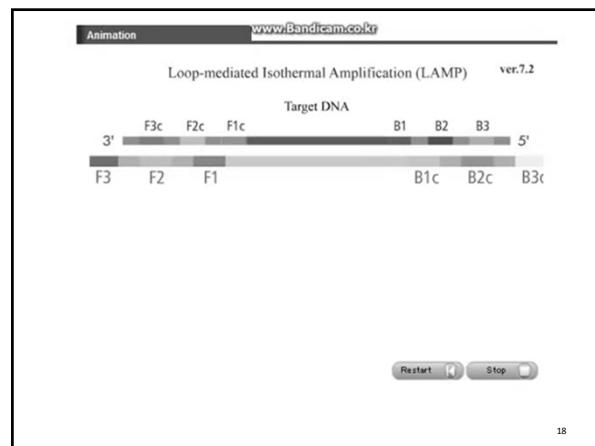
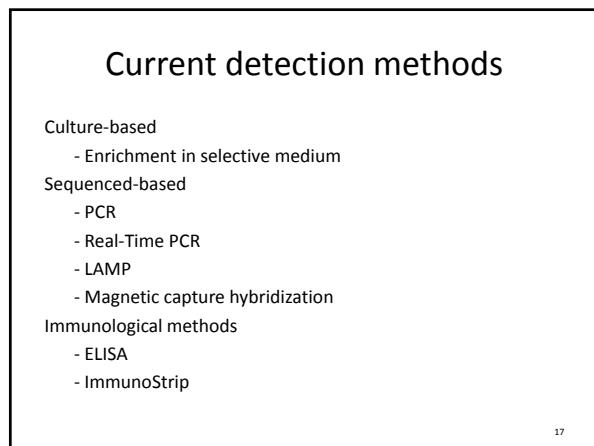
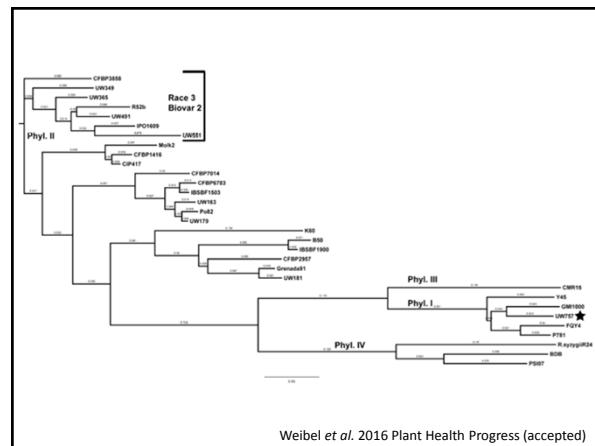
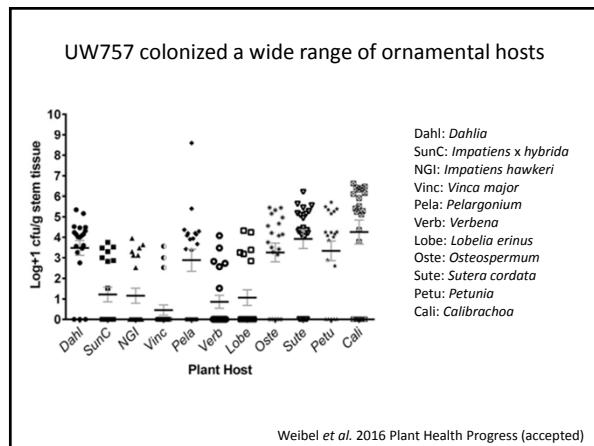
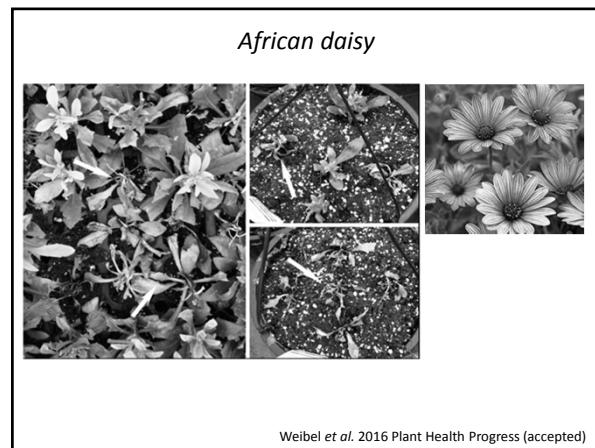
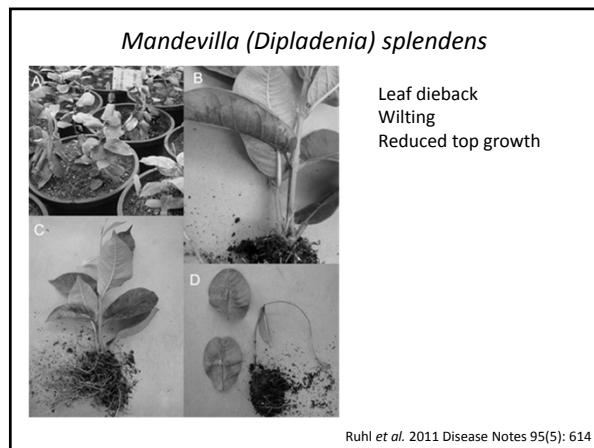
- Phylotype (sequence-based)
- Race (host)
- Biovar (carbon utilization)
- Sequevar (sequence-based)

### Race 3 biovar 2

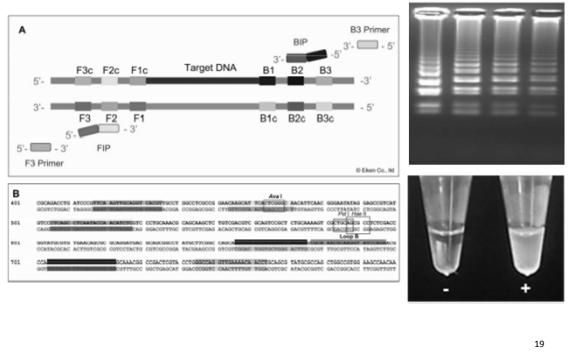
- A subgroup of *Ralstonia solanacearum*, R3bv2 is a select agent in the US (since 2002) and a quarantine pest in Europe and Canada.
- New regulations from USDA-APHIS consider all *R. solanacearum* to be R3bv2 until proven not to be

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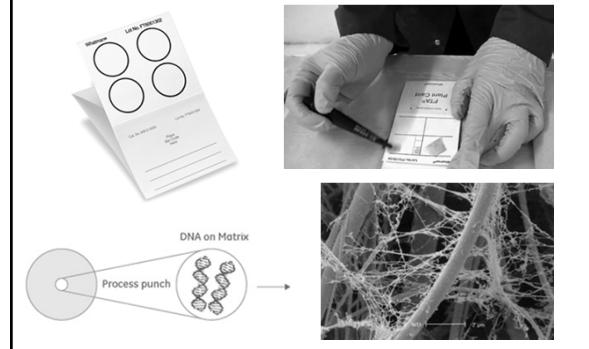




## Loop-mediated isothermal amplification (LAMP)



## FTA card for diagnostic of *R. solanacearum*

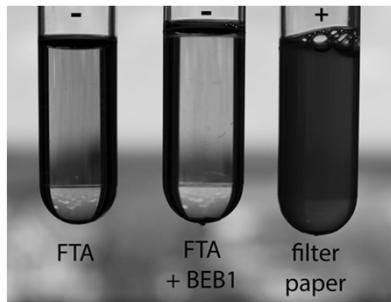


## FTA card for storage of microbial DNA

- Tested for >400 bacterial strains (61 genera)
  - DNA was stable for at least 3 years
  - Tested on many human pathogens:  
*Clostridium, H. influenzae, H. pylori,*  
*Salmonella, V. cholerae*, etc.

Rajendram et al. 2006. Journal of Microbiological Methods. 67: 582-592

## FTA card for diagnostic of *R. solanacearum*



Tran et al. 2016. Plant Disease

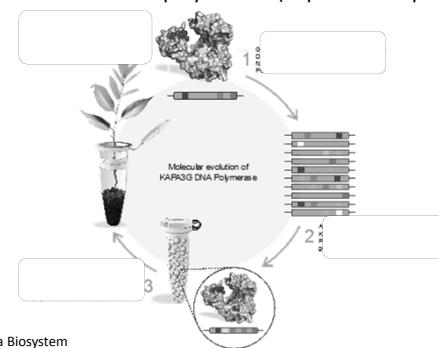
## Field tomato samples from Virginia

Sample	N <sup>a</sup>	PCR <sup>b</sup>	FTA+PCR <sup>c</sup>	BIO-PCR 48 <sup>d</sup>
<b>Asymptomatic plants</b>				
Population size unknown	18	94.4	94.4	88.2
Population size known ( $7.54 \times 10^6$ CFU/g)	9	100	100	100
<b>Symptomatic plants</b>				
Population size unknown	9	100	100	100
Population size known ( $6.59 \times 10^{10}$ CFU/g)	15	100	100	100

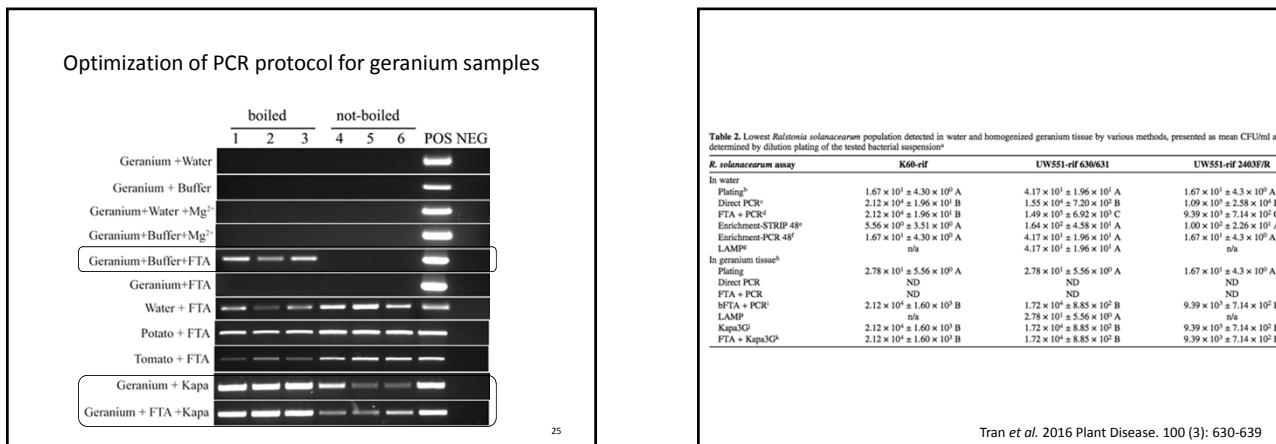


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Problem: PCR inhibitors from plant  
Solution: evolved DNA polymerase (Kapa 3G DNA pol)

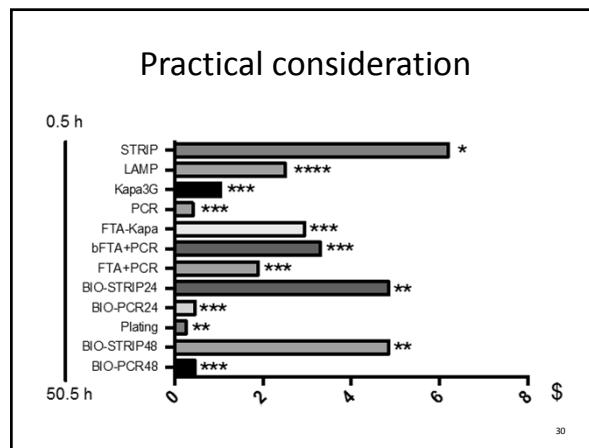
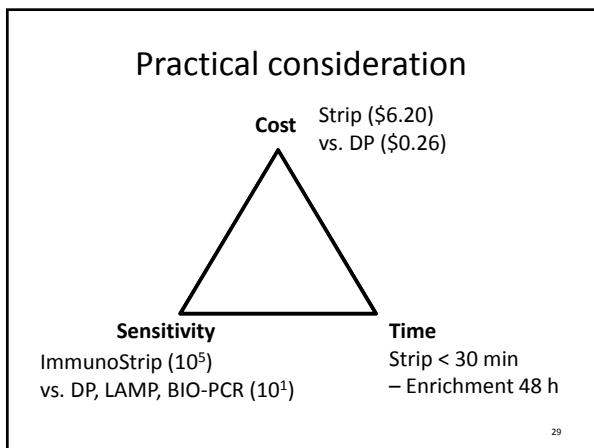
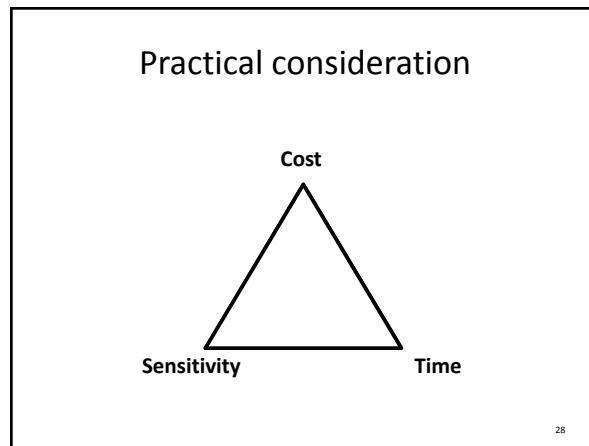
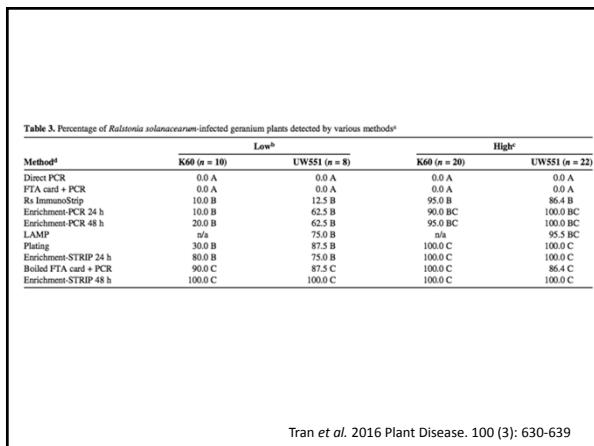


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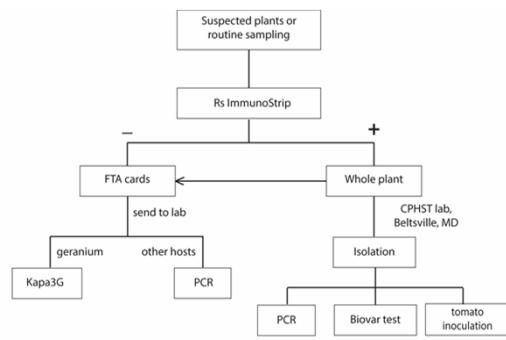


<b>Table 2. Lowest <i>Ralstonia solanacearum</i> population detected in water and homogenized geranium tissue by various methods, presented as mean CFU/ml as determined by dilution plating of the tested bacterial suspension<sup>a</sup></b>		
<i>R. solanacearum</i> assay	K60-rif	UW551-rif 630/631
In water	UW551-rif 2403F/R	
Plating <sup>b</sup>	1.67 × 10 <sup>1</sup> ± 4.30 × 10 <sup>0</sup> A	4.17 × 10 <sup>1</sup> ± 1.96 × 10 <sup>1</sup> A
Direct PCR <sup>c</sup>	2.12 × 10 <sup>2</sup> ± 1.96 × 10 <sup>2</sup> B	1.55 × 10 <sup>2</sup> ± 7.20 × 10 <sup>2</sup> B
FTA + PCR <sup>d</sup>	2.12 × 10 <sup>1</sup> ± 1.96 × 10 <sup>1</sup> B	1.49 × 10 <sup>2</sup> ± 6.92 × 10 <sup>2</sup> C
Enrichment-STRIP 48 <sup>e</sup>	5.56 × 10 <sup>0</sup> ± 3.51 × 10 <sup>0</sup> A	1.64 × 10 <sup>2</sup> ± 4.58 × 10 <sup>2</sup> A
Enrichment-PCR 48 <sup>f</sup>	1.67 × 10 <sup>1</sup> ± 4.30 × 10 <sup>0</sup> A	4.17 × 10 <sup>1</sup> ± 1.96 × 10 <sup>1</sup> A
LAMP <sup>g</sup>	n/a	4.17 × 10 <sup>1</sup> ± 1.96 × 10 <sup>1</sup> A
In geranium tissue <sup>h</sup>		
Plating	2.78 × 10 <sup>1</sup> ± 5.56 × 10 <sup>0</sup> A	2.78 × 10 <sup>1</sup> ± 5.56 × 10 <sup>0</sup> A
Direct PCR	ND	ND
FTA + PCR	ND	ND
FTA + PCR <sup>i</sup>	2.12 × 10 <sup>2</sup> ± 1.60 × 10 <sup>2</sup> B	1.72 × 10 <sup>2</sup> ± 8.85 × 10 <sup>2</sup> B
LAMP	n/a	2.78 × 10 <sup>1</sup> ± 5.56 × 10 <sup>0</sup> A
Kapa3G <sup>j</sup>	2.12 × 10 <sup>4</sup> ± 1.60 × 10 <sup>3</sup> B	1.72 × 10 <sup>2</sup> ± 8.85 × 10 <sup>2</sup> B
FTA + Kapa3G <sup>k</sup>	2.12 × 10 <sup>4</sup> ± 1.60 × 10 <sup>3</sup> B	1.72 × 10 <sup>2</sup> ± 8.85 × 10 <sup>2</sup> B

Tran et al. 2016 Plant Disease. 100 (3): 630-639



### Propose pipeline for detection of *Rs*



### Acknowledgement

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