



# Selective enrichment of *Salmonella*



**Soraya Chaturongakul**

DEPARTMENT OF MICROBIOLOGY  
FACULTY OF SCIENCE, MAHIDOL UNIVERSITY  
E-MAIL: [SORAYA.CHA@MAHIDOL.AC.TH](mailto:SORAYA.CHA@MAHIDOL.AC.TH)

# *Salmonella* workflow



## First Phase

Sample  
collection

Sample  
processing

*Salmonella*  
Isolation

Confirmation

➔ Poultry (CS) and handlers (FNS, Stool)  
Human

## Second Phase

Transportation

*Salmonella*  
subtyping

➔ Multi-locus sequence type, serotype



# Isolation Workflow

Primary enrichment

Buffered Peptone Water

*invA* PCR

Secondary (selective)  
enrichment

Tetrathionate Broth

Rappaport-Vassiliadis Broth

Differential and  
selective isolation

XL Agar

SS Agar

*invA* PCR



# Identification of *Salmonella* using PCR



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# PCR



- DNA extraction ([GeneAid manual.pdf](#))
- Primers
  - Forward 5' GTG AAA TTA TCG CCA CGT TCG GGC AA 3'
  - Reverse 5' TCA TCG CAC CGT CAA AGG AAC C 3'
- PCR mix
  - F primer (10  $\mu$ M) 1  $\mu$ l
  - R primer (10  $\mu$ M) 1  $\mu$ l
  - 2x Master Mix 12.5  $\mu$ l
  - Sterile water 10.5  $\mu$ l
  - Total volume 25  $\mu$ l

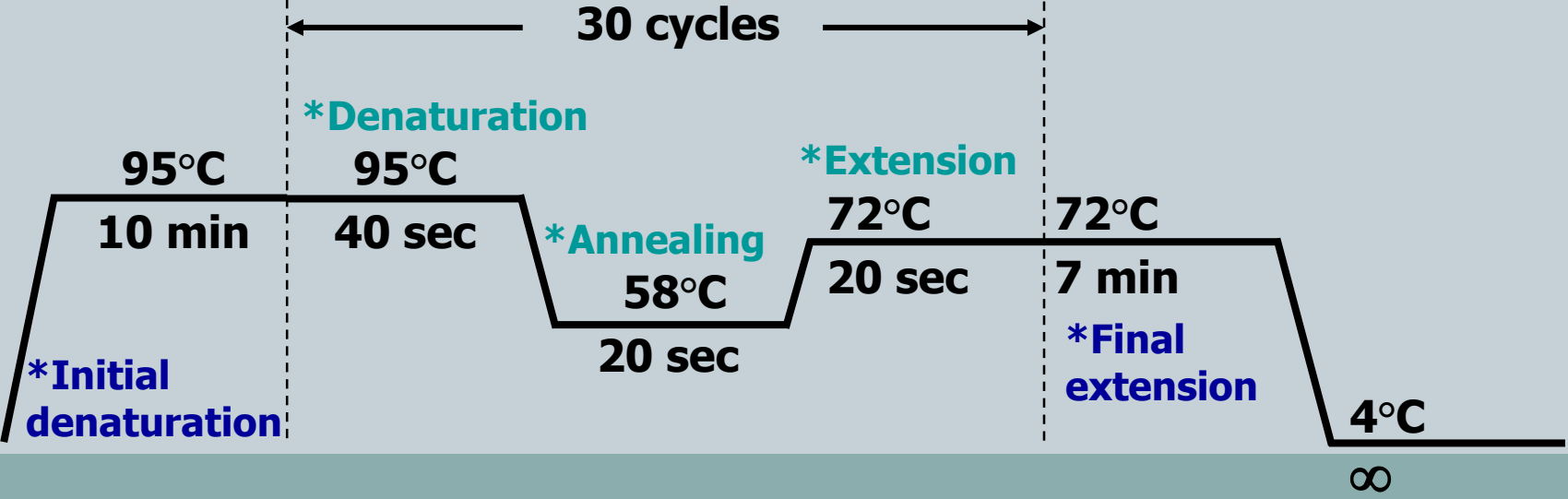


# PCR



- Samples
  - Positive control
  - Negative control
  - Cloacal swabs (dead chicken and dead duck)
  - Hand swab (Dr. Tran Huy Think's and Soraya's)

- PCR condition





# Gel electrophoresis



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# 1% agarose gel electrophoresis



- SYBR safe DNA dye (10,000x)
- Gel loading
  - Marker
  - Positive control
  - Negative control
  - Cloacal swab (dead chicken)
  - Hand swab (1)
  - Cloacal swab (dead duck)
  - Hand swab (2)
  - Marker
- Gel running
  - 80V, 60 min
- Imaging