

# Interreg

## ITALIA-SLOVENIJA



### TRAIN

Progetto standard co-finanziato dal Fondo europeo di sviluppo regionale  
Standardni projekt sofinancira Evropski sklad za regionalni razvoj



UNIONE EUROPEA  
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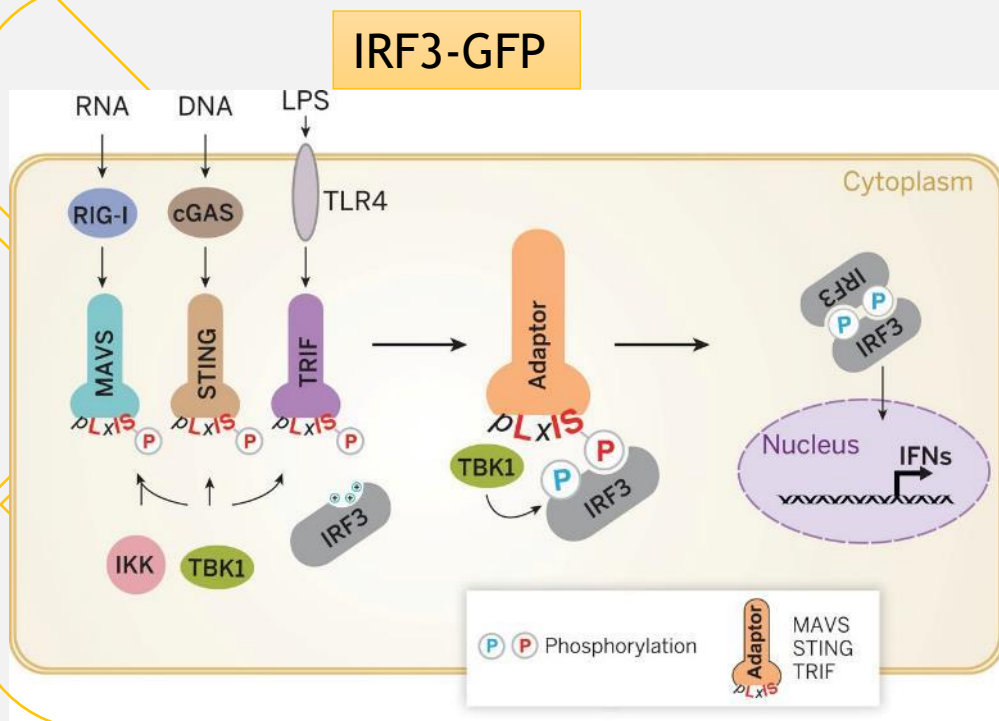
# REZULTATI EXPERTTEAM

*Speaker: Francesca Bruno*

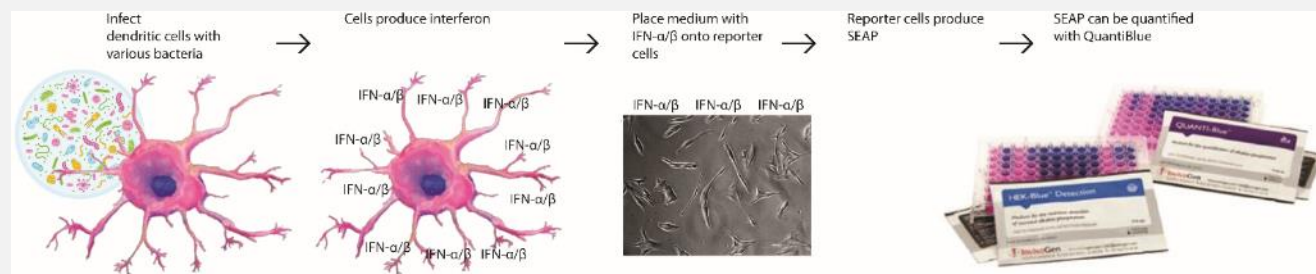


# Začetna ideja

Z molekularno metodo testirati prisotnost ali odsotnost enega ali več patogenov v hrani in preverjanje, ali je le-ta mrtev ali živ z HCA



## IFN reporter system



Tovrstni testi ne vedno razlikujejo med patogeni in nepatogeni in niso vedno dovolj občutljivi za naše potrebe


## Zavrnitev teh postopkov

# Detekcija patogenov v hrani

Bolezni, ki izhajajo iz uživanja hrane ali vode, onesnažene s patogeni in / ali njihovimi toksini, imajo širok razpon gospodarskih in javnozdravstvenih vplivov po vsem svetu

Običajne mikrobiološke metode

- Časovno potratne
- nezanesljive



Različni rezultati



Live pathogen

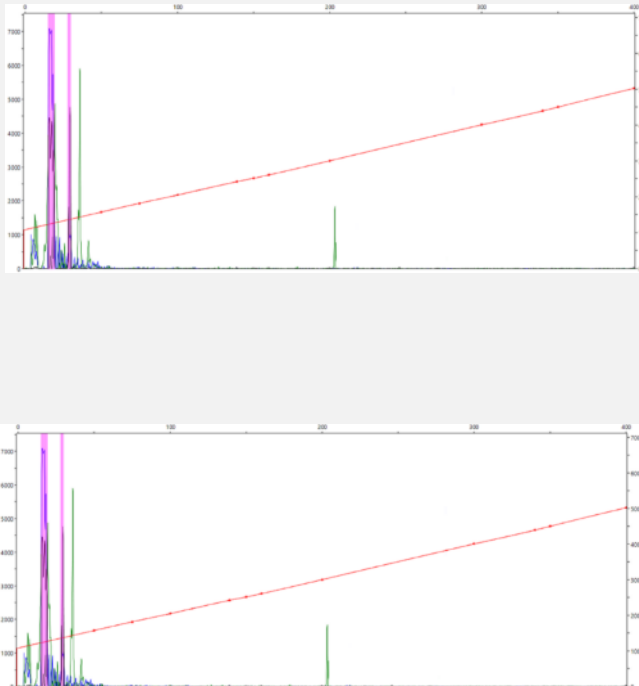


Dead pathogen



PCR metode

- Specifične
- Občutljive
- Hitrost detekcije



Isti rezultati

## Novi pristop detekcije mikroorganizmov z mRNA

mRNA ima razpolovni čas le nekaj minut (bakterijska DNA je bolj stabilna od RNA)

Detekcija mRNA bo dober indikator viabilnosti mikrobnih celic



Detekcija živih patogenih celic v hrani na bazi TaqMan- kvantitativne reverzne „Transcriptase Real-Time PCR“ (RT-qPCR)





## Salmonella enterica:

Kontaminacija s *Salmonella enterica* v raznih živilih je pomemben grožnja v javnem zdravstvu, na državni in internacionalni ravni.



invA gen preiskovan za prisotnost *Salmonella*

Color	Name	Type	Ct
	Salmonella A strain Live cDNA (1 Dnase treatment) qRT-PCR	Unknown	23,48
	Salmonella A strain Live RNA (1Dnase treatment) qPCR	Unknown	30,45



$$\Delta C_t(qRT-PCR)-(RT-PCR)>4$$

Color	Name	Type	Ct
	Salmonella A strain Dead cDNA (1 Dnase treatment) qRT-PCR	Unknown	23,53
	Salmonella A strain Dead RNA (1Dnase treatment) qPCR	Unknown	23,81

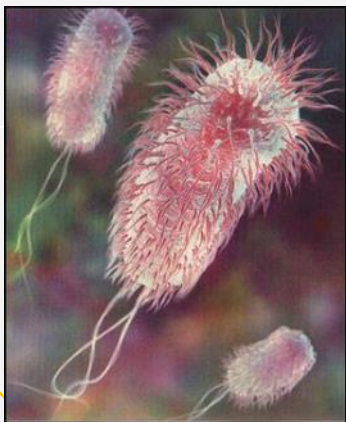
$$\Delta C_t(qRT-PCR)-(RT-PCR)<4 \longrightarrow \text{V vzorcu ni RNA}$$

Color	Name	Type	Ct
	Salmonella B strain Live cDNA (1 Dnase treatment) qRT-PCR	Unknown	23,41
	Salmonella B strain Live RNA (1Dnase treatment) qPCR	Unknown	30,05

$$\Delta C_t(qRT-PCR)-(RT-PCR)>4$$

Color	Name	Type	Ct
	Salmonella B strain Dead cDNA (1 Dnase treatment) qRT-PCR	Unknown	25,78
	Salmonella B strain Dead RNA (1Dnase treatment) qPCR	Unknown	26,61





$$\Delta C_t(qRT-PCR)-(RT-PCR)<4 \longrightarrow \text{V vzorcu je RNA}$$



## ESCHERICHIA COLI O157:H7

- E. coli ima veliko glavnih virulenčnih genov (stx1, stx2, fliC, eaeA, rfbE, uidA, hlyA)
- Vsi geni niso primerni za detekcijo viabilnih celic E.coli

**rfbE** gen preiskovan za prisotnost Escherichia, kodira O157 antigen










Color	Name	Type	Ct
	Escherichia coli Live cDNA (2 DNase treatment)	Unknown	27,84
	Escherichia coli Live RNA (non reverse-transcribed)	Unknown	32,12
	Escherichia coli Dead cDNA (2 DNase treatment)	Unknown	22,15
	Escherichia coli Dead RNA (non reverse-transcribed)	Unknown	23,43












$$\Delta C_t(qRT-PCR)-(RT-PCR)>4$$

$$\Delta C_t(qRT-PCR)-(RT-PCR)<4$$

→ V vzorcu ni RNA

## Multiplex detekcija obeh patogenih cDNA

Color	Name	Type	Ct	Given Conc (copies/reaction)	Calc Conc (copies/reaction)
	Multiplex Detection Mix (Escherichia coli 10 <sup>5</sup> copies/reaction)	Standard	24,52	1,00E+05	9,80E+04
	Multiplex Detection Mix (Escherichia coli 10 <sup>5</sup> copies/reaction)	Standard	24,35	1,00E+05	1,09E+05
	Multiplex Detection Mix (Escherichia coli 10 <sup>4</sup> copies/reaction)	Standard	28,17	1,00E+04	9,99E+03
	Multiplex Detection Mix (Escherichia coli 10 <sup>4</sup> copies/reaction)	Standard	28,14	1,00E+04	1,02E+04
	Multiplex Detection Mix (Escherichia coli 10 <sup>3</sup> copies/reaction)	Standard	31,97	1,00E+03	9,28E+02
	Multiplex Detection Mix (Escherichia coli 10 <sup>3</sup> copies/reaction)	Standard	32,09	1,00E+03	8,61E+02
	Multiplex Detection Mix (Escherichia coli 10 <sup>2</sup> copies/reaction)	Standard	34,85	1,00E+02	1,54E+02
	Multiplex Detection Mix (Escherichia coli 10 <sup>2</sup> copies/reaction)	Standard	35,99	1,00E+02	7,52E+01
	Multiplex Detection Mix (Escherichia coli Live cDNA+ Salmonella Live cDNA)	Unknown	28,83		6,63E+03

Color	Name	Type	Ct	Given Conc (copies/reaction)	Calc Conc (copies/reaction)
	Multiplex Detection Mix (Salmonella enterica 10 <sup>5</sup> copies/reaction)	Standard	24,74	1,00E+05	1,12E+05
	Multiplex Detection Mix (Salmonella enterica 10 <sup>5</sup> copies/reaction)	Standard	24,51	1,00E+05	1,31E+05
	Multiplex Detection Mix (Salmonella enterica 10 <sup>4</sup> copies/reaction)	Standard	28,40	1,00E+04	1,04E+04
	Multiplex Detection Mix (Salmonella enterica 10 <sup>4</sup> copies/reaction)	Standard	28,82	1,00E+04	7,90E+03
	Multiplex Detection Mix (Salmonella enterica 10 <sup>3</sup> copies/reaction)	Standard	32,26	1,00E+03	8,43E+02
	Multiplex Detection Mix (Salmonella enterica 10 <sup>3</sup> copies/reaction)	Standard	32,24	1,00E+03	8,50E+02
	Multiplex Detection Mix (Salmonella enterica 10 <sup>2</sup> copies/reaction)	Standard	36,31	1,00E+02	6,05E+01
	Multiplex Detection Mix (Salmonella enterica 10 <sup>2</sup> copies/reaction)	Standard	35,16	1,00E+02	1,27E+02
	Multiplex Detection Mix (Salmonella enterica 10 copies/reaction)	Standard	39,10	1,00E+01	9,80E+00
	Multiplex Detection Mix (Salmonella enterica 10 copies/reaction)	Standard	38,40	1,00E+01	1,54E+01
	Multiplex Detection Mix (Salmonella Live cDNA+ Escherichia coli Live cDNA)	Unknown	28,58		9,23E+03

## Zaključki:

- Odkrivanje povzročiteljev hrane, ki jih prenašajo živila, in ločevanje med živimi in mrtvimi patogeni, ki lahko preprečijo zavrževanje hrane
- Z istim ciklom q-PCR lahko zaznamo 2 (trenutno) različna povzročitelja hrane (salmonelo in E. coli) in razvijemo metodo, ki omogoča razlikovanje živih in mrtvih celic, ki pomnožujejo mRNA