

Interreg



UNIONE EUROPEA
EVROPSKA UNIJA

ITALIA-SLOVENIJA



TRAIN

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

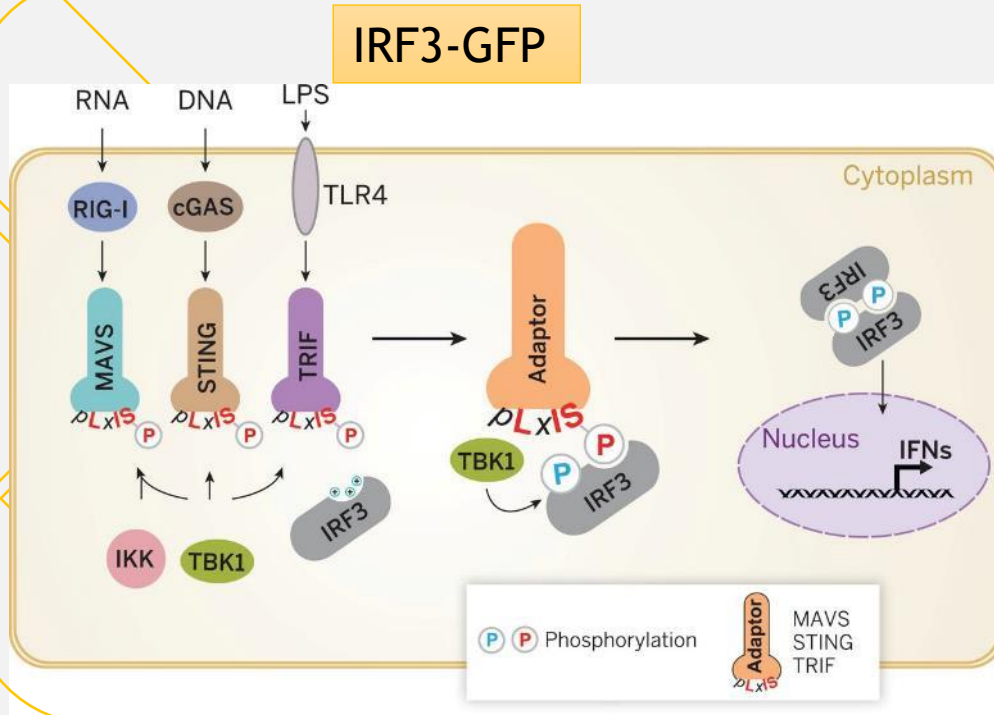
EXPERTEAM RESULTS

Speaker: Francesca Bruno

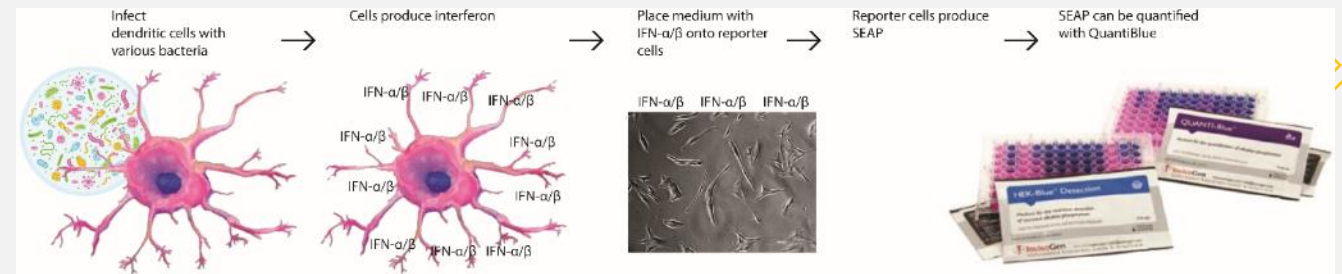


Initial idea:

Test, by a molecular method, the presence or absence of one or more pathogens in food and verify the pathogen is live or dead, by High content analysis (HCA)



IFN reporter system



Tested assays do not discriminate between pathogens and non-pathogens, and they are not sufficiently sensitive for our purpose

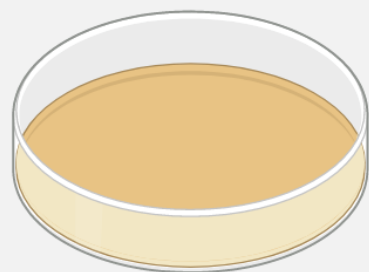
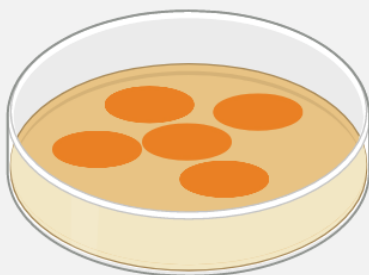
Quit these approaches

Foodborne pathogen detection

Illnesses resulting from the consumption of foods or water contaminated with pathogens and/or their toxins have a wide range of economic and public health impact worldwide

CONVENTIONAL MICROBIOLOGICAL METHODS

- Time-consuming
- Poor sensitive

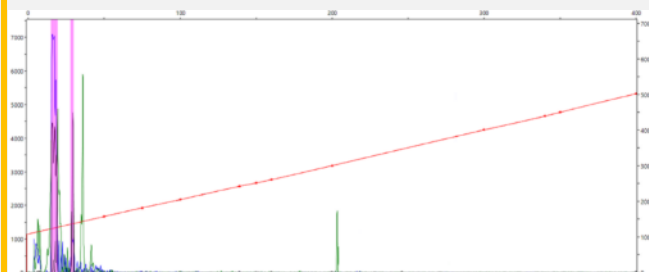
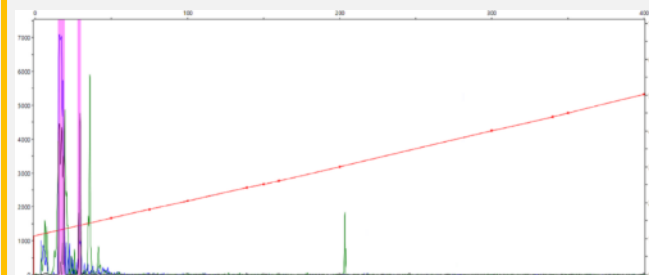


Different results



PCR METHODS

- Specific
- Sensitive
- Speed of detection



Same results

New approach based on detection of m.o. using **mRNA**

mRNA has half-life on only few minutes (bacterial DNA is more stable than bacterial RNA)

The detection of mRNA shall be a good indicator of viability of cells



Detection of LIVE pathogens cells in food by a TaqMan-based quantitative reverse Transcriptase Real-Time PCR (RT-qPCR)





Salmonella enterica:

Salmonella enterica contamination in various foods is a significant public health concern, domestically and internationally.

invA gene investigated for the presence of 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

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

→ No RNA in sample

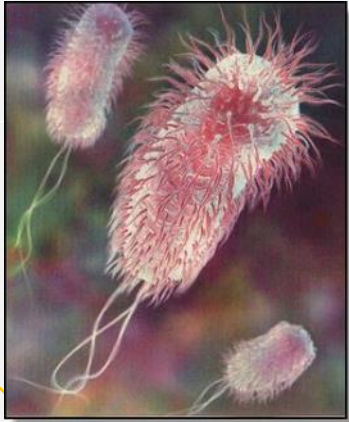
Color	Name	Type	Ct
	Salmonella B strain Live cDNA (1 Dnase treatment) qRT-PCR	Unknown	23,41
	Salmonella B strain Live RNA (1Dnase treatment) qPCR	Unknown	30,05

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

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

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





→ No RNA in sample



ESCHERICHIA COLI O157:H7

- E. coli has a lot of major virulence genes (stx1, stx2, fliC, eaeA, rfbE, uidA, hlyA)
- Not all genes are suitable targets for detecting viable E.coli

rfbE gene investigated for the presence of Escherichia, it encodes the 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












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

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

→ No RNA in sample

Multiplex detection of both pathogenes cDNA

Color	Name	Type	Ct	Given Conc (copies/reaction)	Calc Conc (copies/reaction)
	Multiplex Detection Mix (Escherichia coli 10 ⁵ copies/reaction)	Standard	24,52	1,00E+05	9,80E+04
	Multiplex Detection Mix (Escherichia coli 10 ⁵ copies/reaction)	Standard	24,35	1,00E+05	1,09E+05
	Multiplex Detection Mix (Escherichia coli 10 ⁴ copies/reaction)	Standard	28,17	1,00E+04	9,99E+03
	Multiplex Detection Mix (Escherichia coli 10 ⁴ copies/reaction)	Standard	28,14	1,00E+04	1,02E+04
	Multiplex Detection Mix (Escherichia coli 10 ³ copies/reaction)	Standard	31,97	1,00E+03	9,28E+02
	Multiplex Detection Mix (Escherichia coli 10 ³ copies/reaction)	Standard	32,09	1,00E+03	8,61E+02
	Multiplex Detection Mix (Escherichia coli 10 ² copies/reaction)	Standard	34,85	1,00E+02	1,54E+02
	Multiplex Detection Mix (Escherichia coli 10 ² 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 ⁵ copies/reaction)	Standard	24,74	1,00E+05	1,12E+05
	Multiplex Detection Mix (Salmonella enterica 10 ⁵ copies/reaction)	Standard	24,51	1,00E+05	1,31E+05
	Multiplex Detection Mix (Salmonella enterica 10 ⁴ copies/reaction)	Standard	28,40	1,00E+04	1,04E+04
	Multiplex Detection Mix (Salmonella enterica 10 ⁴ copies/reaction)	Standard	28,82	1,00E+04	7,90E+03
	Multiplex Detection Mix (Salmonella enterica 10 ³ copies/reaction)	Standard	32,26	1,00E+03	8,43E+02
	Multiplex Detection Mix (Salmonella enterica 10 ³ copies/reaction)	Standard	32,24	1,00E+03	8,50E+02
	Multiplex Detection Mix (Salmonella enterica 10 ² copies/reaction)	Standard	36,31	1,00E+02	6,05E+01
	Multiplex Detection Mix (Salmonella enterica 10 ² 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

Conclusions:

- Detect foodborne pathogens and discriminate between live and dead pathogen that can prevent to waste food
- With the same q-PCR cycle we can detect 2 (at moment) different foodborne pathogens (salmonella and E. coli), developing a method that can permit to distinguish live and dead cells amplifying mRNA of the m.o