# Development of an external standard for q-PCR
# Enforcement to the expression study of 3 homologous oligopeptide binding proteins in *Streptococcus thermophilus*

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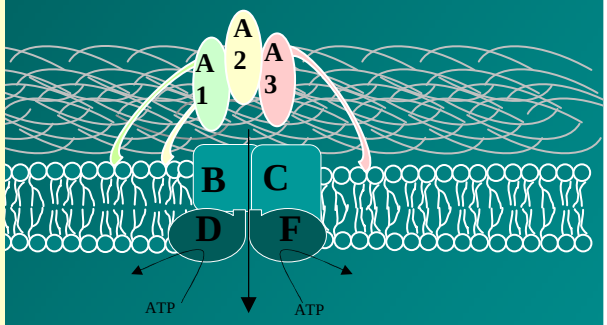
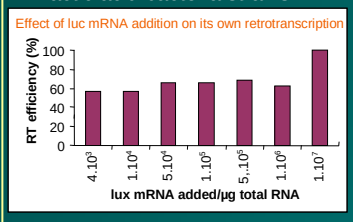
- Reliability of RT-qPCR methods strongly depends on an appropriate normalisation. In order to avoid the bias caused by the fluctuation of an housekeeping gene, and because the use of several housekeeping genes (Vandesompele et al., 2002) is laborious and expensive, we have chosen to develop an external standard added to the RNAs before the retrotranscription step.
- We have validated this external standard for bacterial gene expression measurement by RT-qPCR. Our model concerns the expression of oligopeptide binding proteins encoding genes during growth in milk. The oligopeptide transport system of *Streptococcus thermophilus* is fundamental for its optimal growth (Garault et al., 2002). It belongs to the ABC transporters family and is composed of 3 oligopeptide binding proteins (in our strain) encoded by the *amiA1 amiA2* and *amiA3* genes, 2 transmembrane proteins forming a channel and 2 ATPase providing energy to the system.




**Methods:** - **RNA purification**: Growth in milk, Rifampicine addition, citrate clarification of the medium and RNA purification using Trizol (Invitrogen)
- **Retrotranscritption**: DNAse treatment ( DNA-free, Ambion), addition of luciferase mRNA ( Luciferase Control RNA, Promega) and retrotranscription ( M-MLV Reverse Transcritase, Invitrogen)
- **q-PCR**: PE7700 instrument (TaqMan Universal PCR Master Mix, Applied Biosystem)

## Set up of an external standard

We selected a specific mRNA encoding the luciferase gene as external standard since it is commercialised by Promega and not found in the lactic acid bacteria strains.

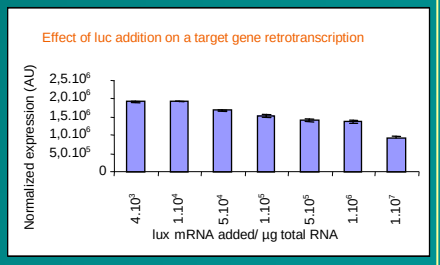



• **Calculation of a ρ factor**, influenced by the presence of polymerase inhibitors:

$$\rho = \frac{\text{luciferase cDNA}}{\text{luciferase mRNA}}$$

• **Expression results normalization**

$$= \frac{\text{cDNA target gene}}{\rho}$$




⇒The addition of $4.10^3$ to $10^7$ molecules of luciferase/µg of total RNA does not interfere with its own retrotranscription …

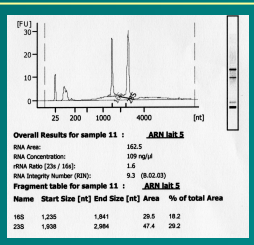
…nor with the target gene retrotranscription

⇒ **Addition of $10^6$ molecules of luciferase/µg total RNA for the study**

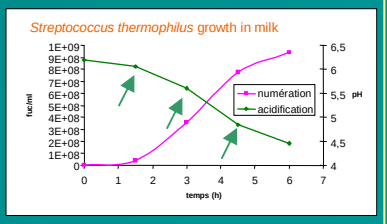
## Study of the amiA gene expression

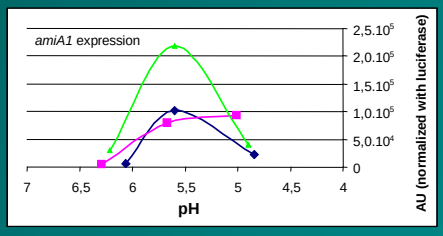
- **Quantitative RNA analysis**: with Nanodrop®(no sample dilution)

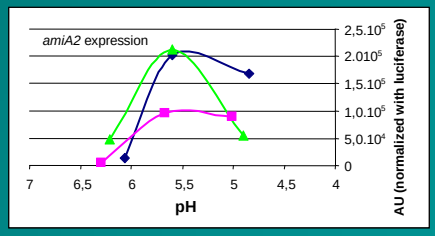
- **Qualitative RNA analysis**: Agilent®« procaroyte total RNA nano series», RNA Integrity Number(RIN)>9

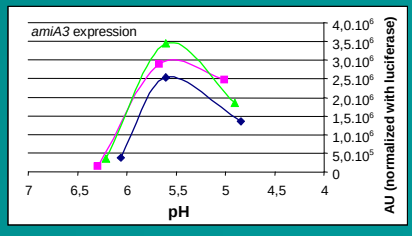



- **RNA extraction** at 3 different stages during growth in milk




-Similar expression profiles with an optimum expression in the middle of the exponential phase
- *amiA3* is significantly more expressed than *amiA1* and *amiA2*
-These results - and the fact that the growth of a Δ*amiA3* mutant stop at an early stage (data not shown) - suggest that this protein play a determinant part on bacterial growth in milk

**RT qPCR using luciferase as an external standard is effective for absolute quantification in bacteria lacking a suitable housekeeping gene.**

• Garault, P. et al (2002) J biol Chem.277:32-9
• Vandesompele, J. et al. (2002) Genome Biol. Jun 18;3(7):RESEARCH0034