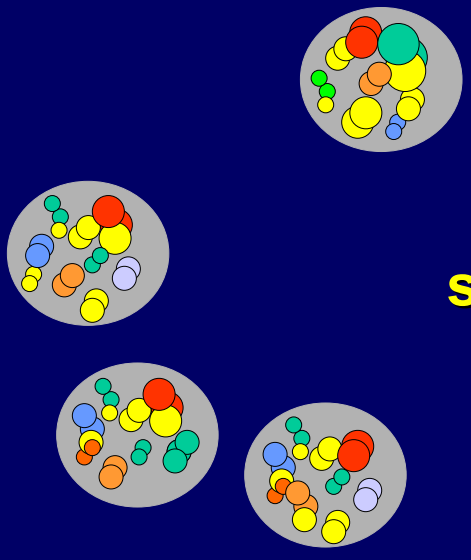
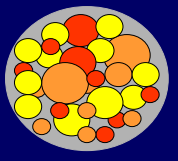
Antonio Peixoto
César Evaristo
Ivana Munitic
H.Veiga-Fernandes

Marta Monteiro

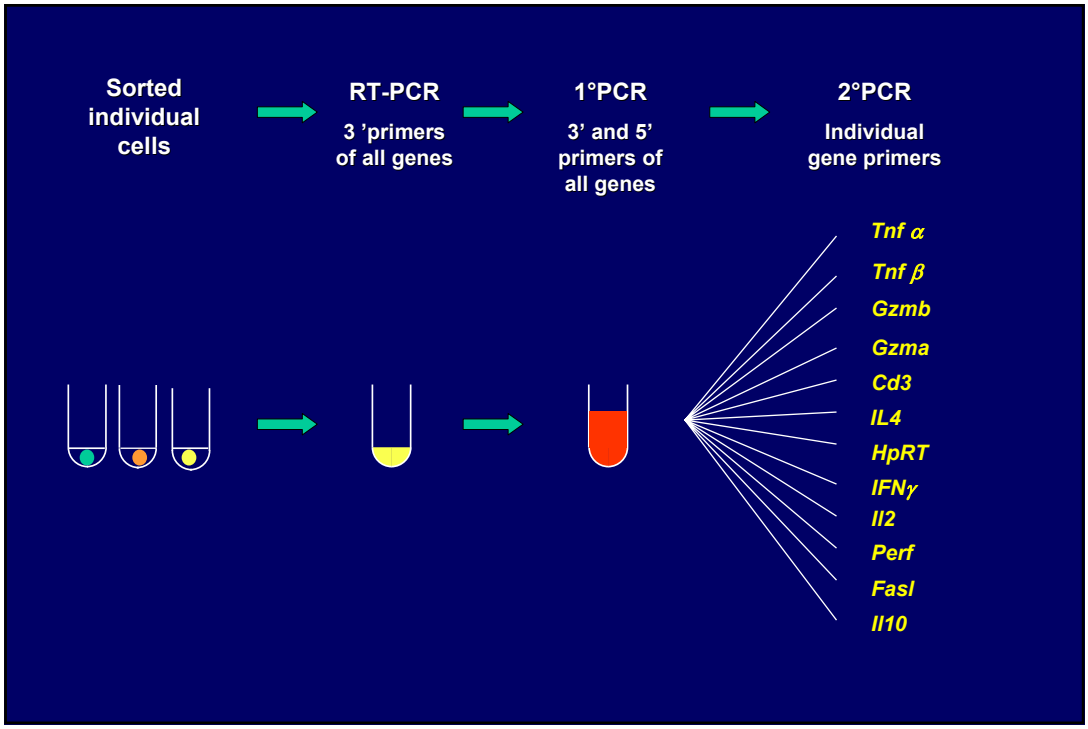


Quantify
simultaneously
20 ≠ mRNAs
in each cell

selected



Peixoto et al.
Genomic research 2004



Primer selection (I)

1° primers in different exons

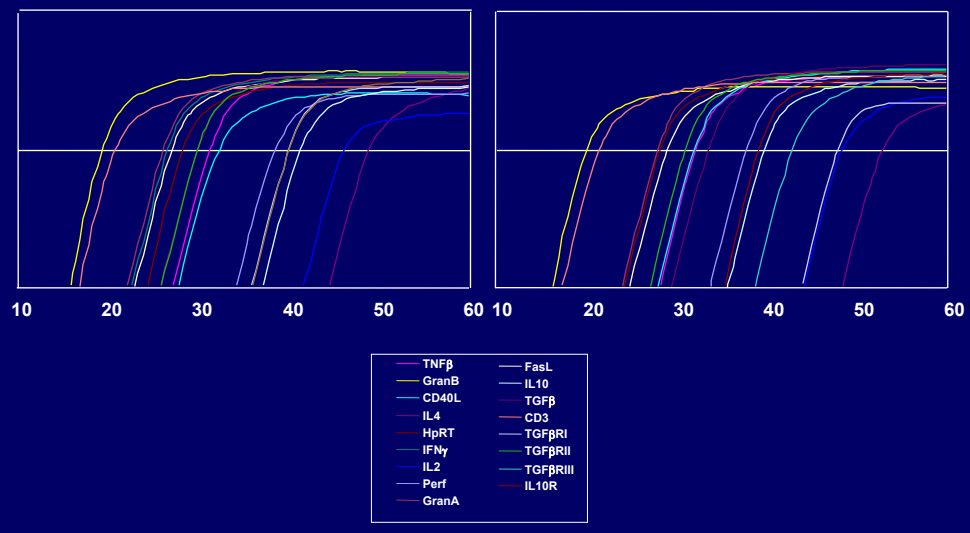
2° the same efficiency in all amplifications

2O bp primers targeting non-repetitive sequences
Similar melting temperatures
About 50% GC content

ALL PCRS HAVE THE SAME EFFICIENCY

1° PCR

2° PCR



RT-PCR

3' primers of all genes

1°PCR

3' and 5'primers of all genes

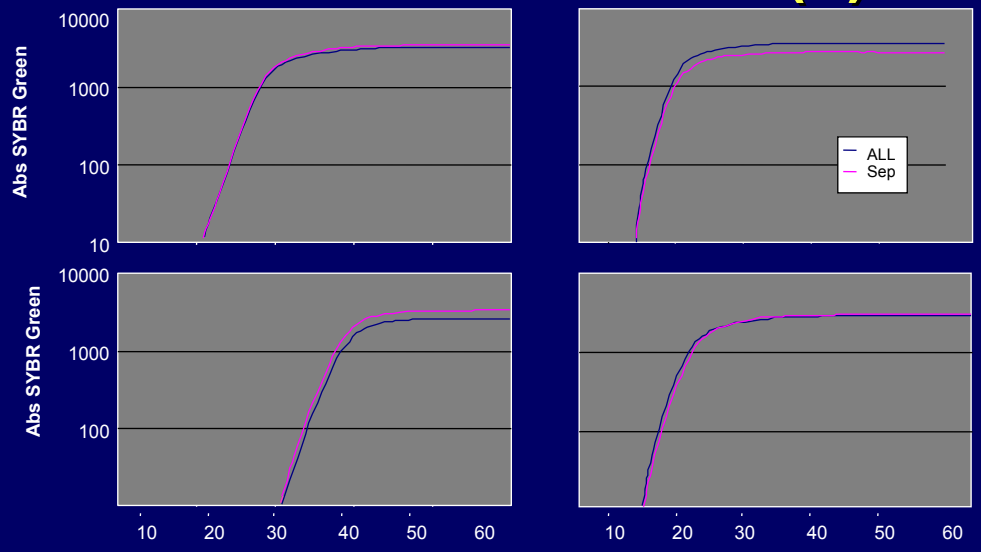
NO COMPETITION

between

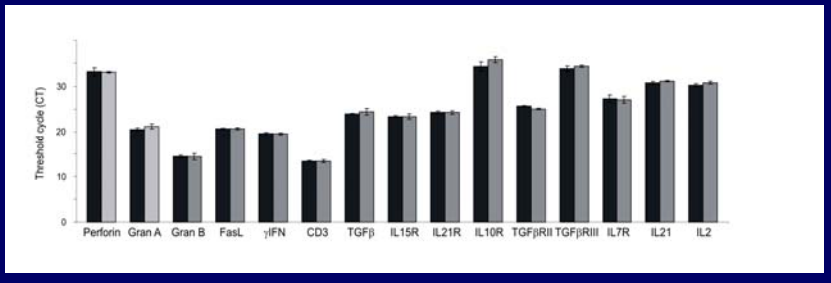
40 primers

40 amplicons

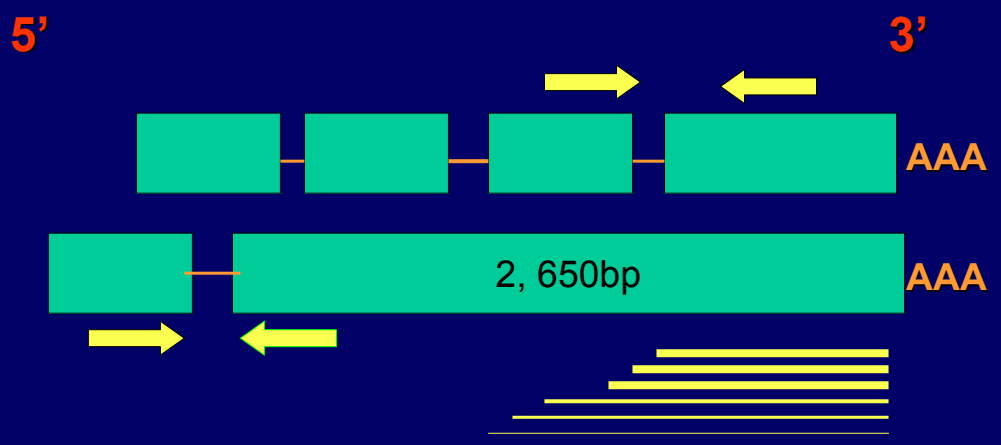
Primer selection (II)



Absence of competition between primers and amplicons



Primers may be located at different distances from poly AAA



HOW TO AVOID RT BIAS???

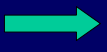
RT-PCR

3’ primers
of all genes

1°PCR

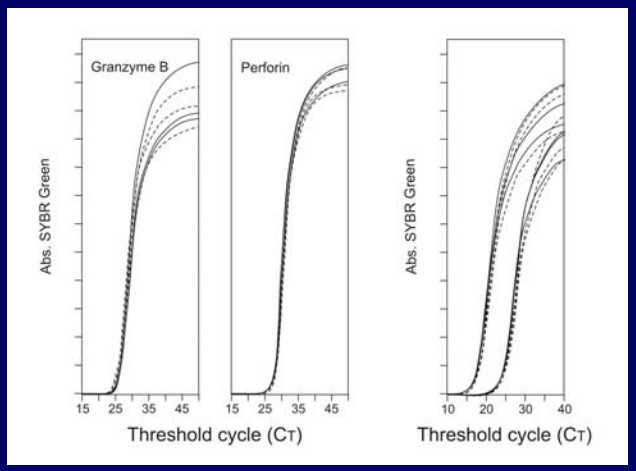
3’ and 5’primers
of all genes

SPECIFIC
RT-PC

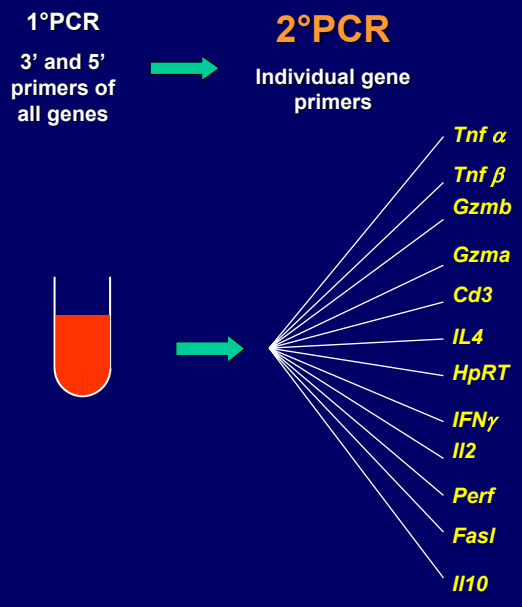


NO COMPETITION

between
40 primers
40 amplicons



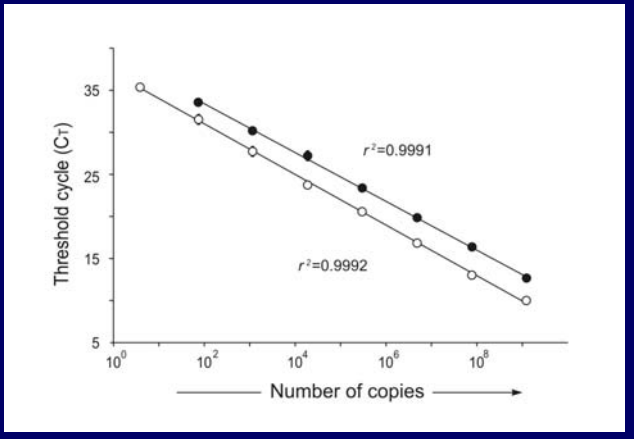
100% Efficient RT



From 1° to 2° PCR

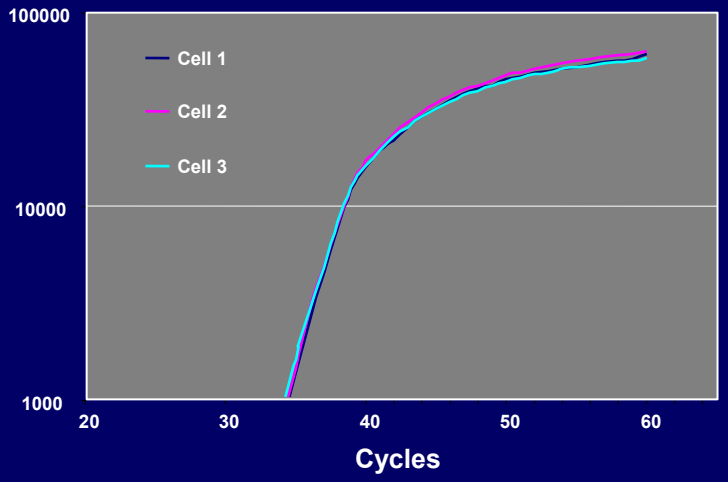
- Maintainance of abundance relationships

Abundance relationships



10^9 to 4 copies

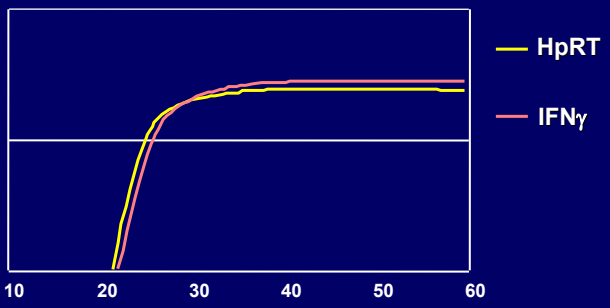
sensitivity-reproductibility



2 gene Copies can be quantified

Single cell versus population analysis??

Quantitative RT-PCR is misleading



A mRNA expressed by all cells (at low level)
may give the same signal
as a mRNA expressed by rare cells (at high level)

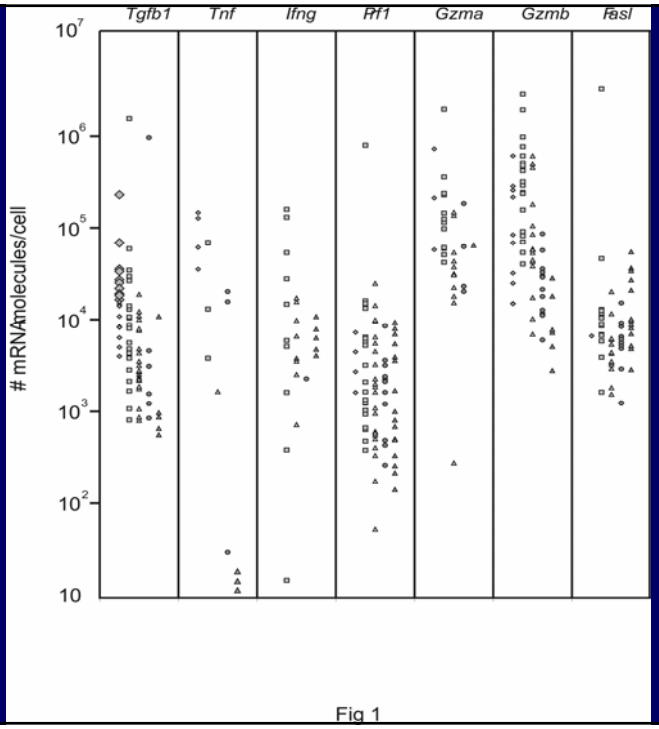
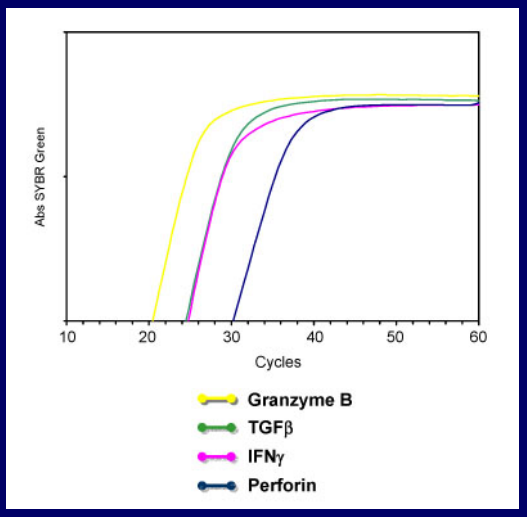
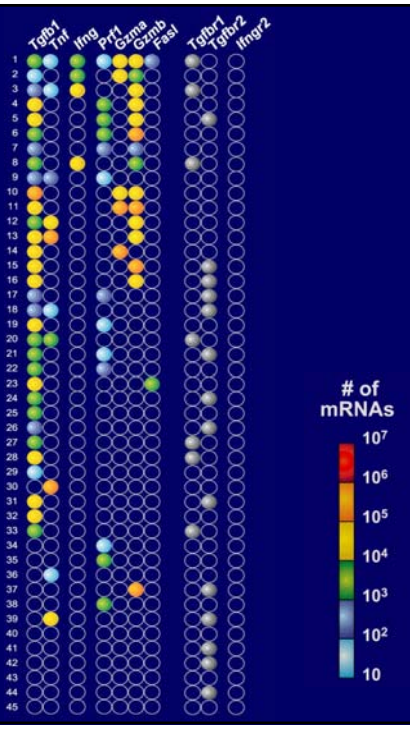


Fig 1





POPULATION VS SINGLE-CELL

- The most expressed gene is Granzyme B
- Cells ARE CYTOTOXIC
- Cells are
- ANTI-INFLAMMATORY

- The most expressed gene is TGF-β
- Cells ARE NOT CYTOTOXIC
- Cells are
- PRO-INFLAMMATORY

Analysis of gene expression in populations
is
good to « catch »genes

IS INSUFFICIENT and

Highly misleading

Single cell analysis is fundamental
to
gasp cell heterogeneiety
and
understand cell properties