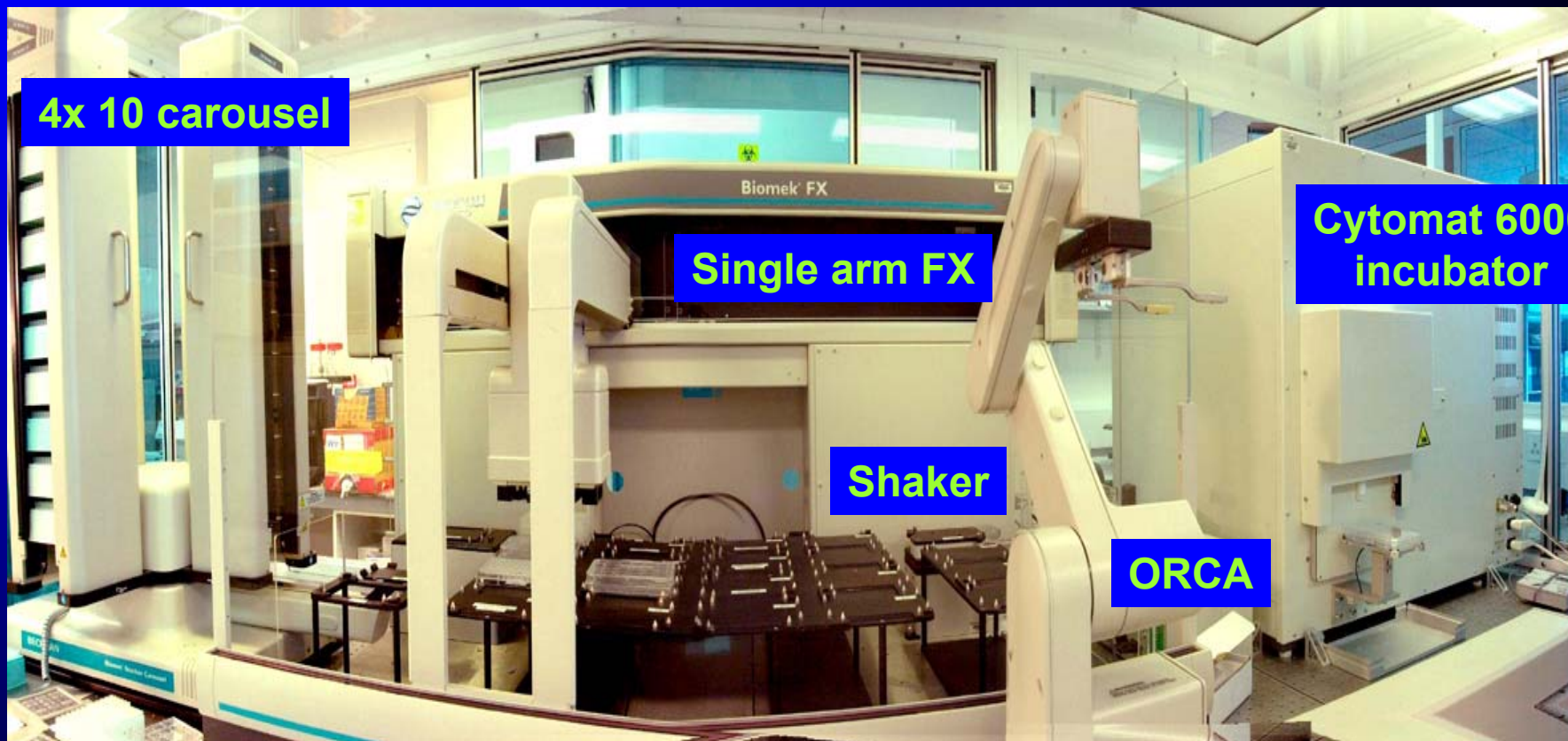




A small core system in a small-scale cell culture facility

**Simon Q.J. Rice
(GEPB)**

Components of our Baby-Core System (BCS)



4x 10 carousel

Single arm FX

Cytomat 6000 incubator

Shaker

ORCA

Components of our Baby-Core System (BCS) contd.



Resolving challenges

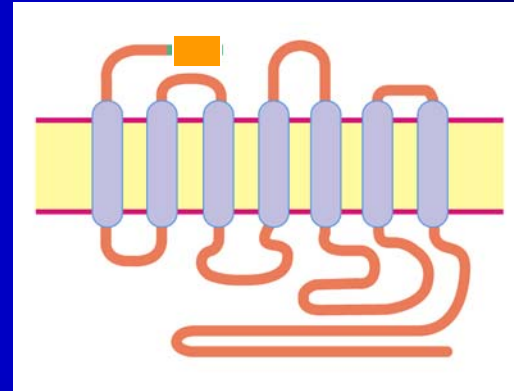
- Living cells ∴ pH, temperature must be controlled.
- Sterile environment required throughout.
 - HEPA filter cabinet fitted around BCS.
 - Installed in clean room.
 - Sterile barrier tips to prevent contamination of work & culture.
 - Cost implications.
- Shear forces on cells
 - integrity must be maintained
- Non-availability of some sterile plasticware e.g. Reservoirs.
 - With few exceptions no BC plasticware available.
 - Commonly produced w/o lids.
 - Generic plasticware manufacturers still catching up.

Orphan Cell Line Generation Process

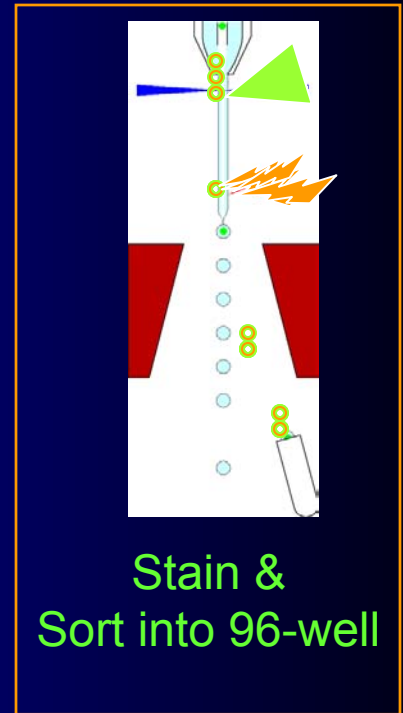
Orphan-
Create expression vector
HA-epitope tag



Transient expression



Mass Culture

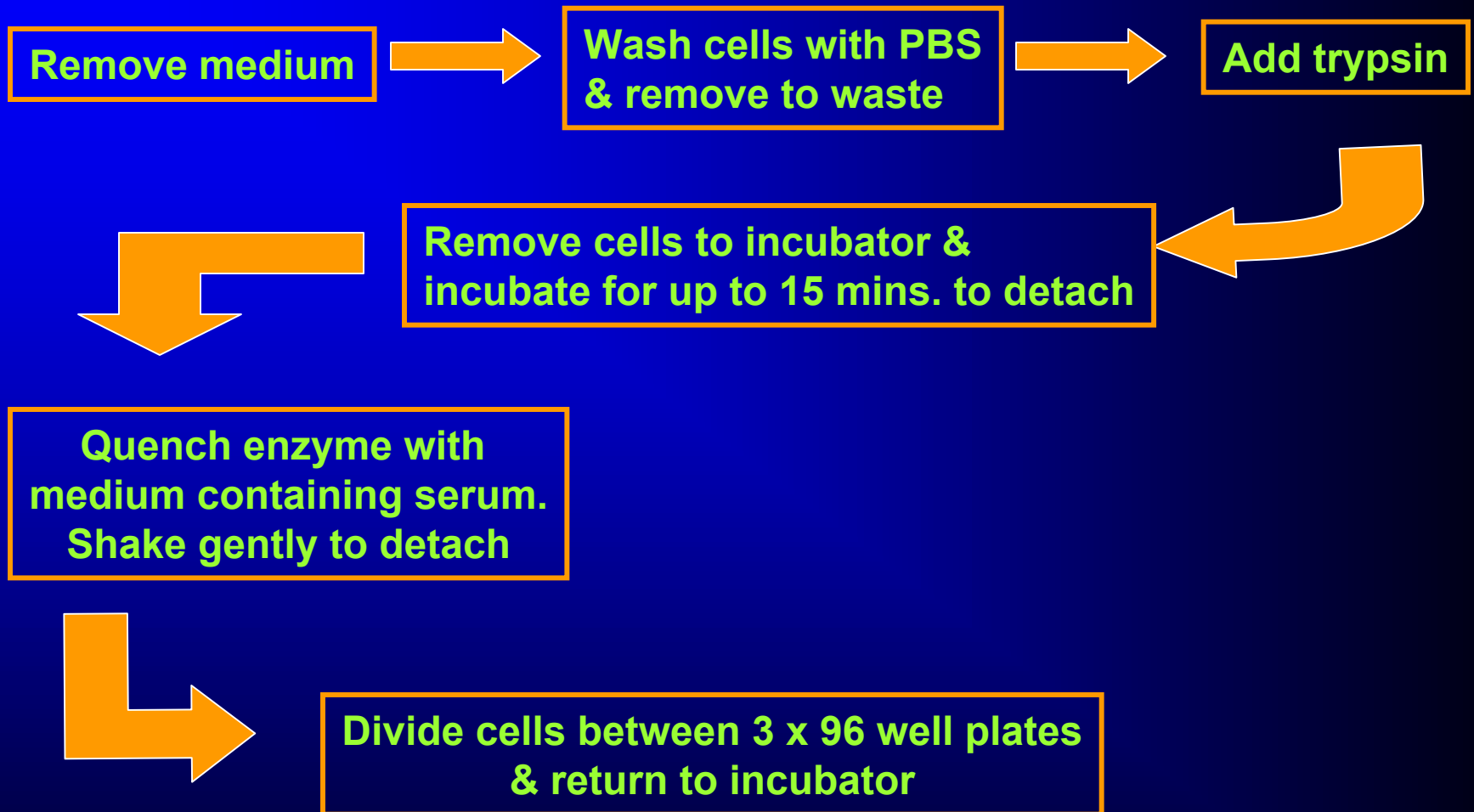


Stain &
Sort into 96-well

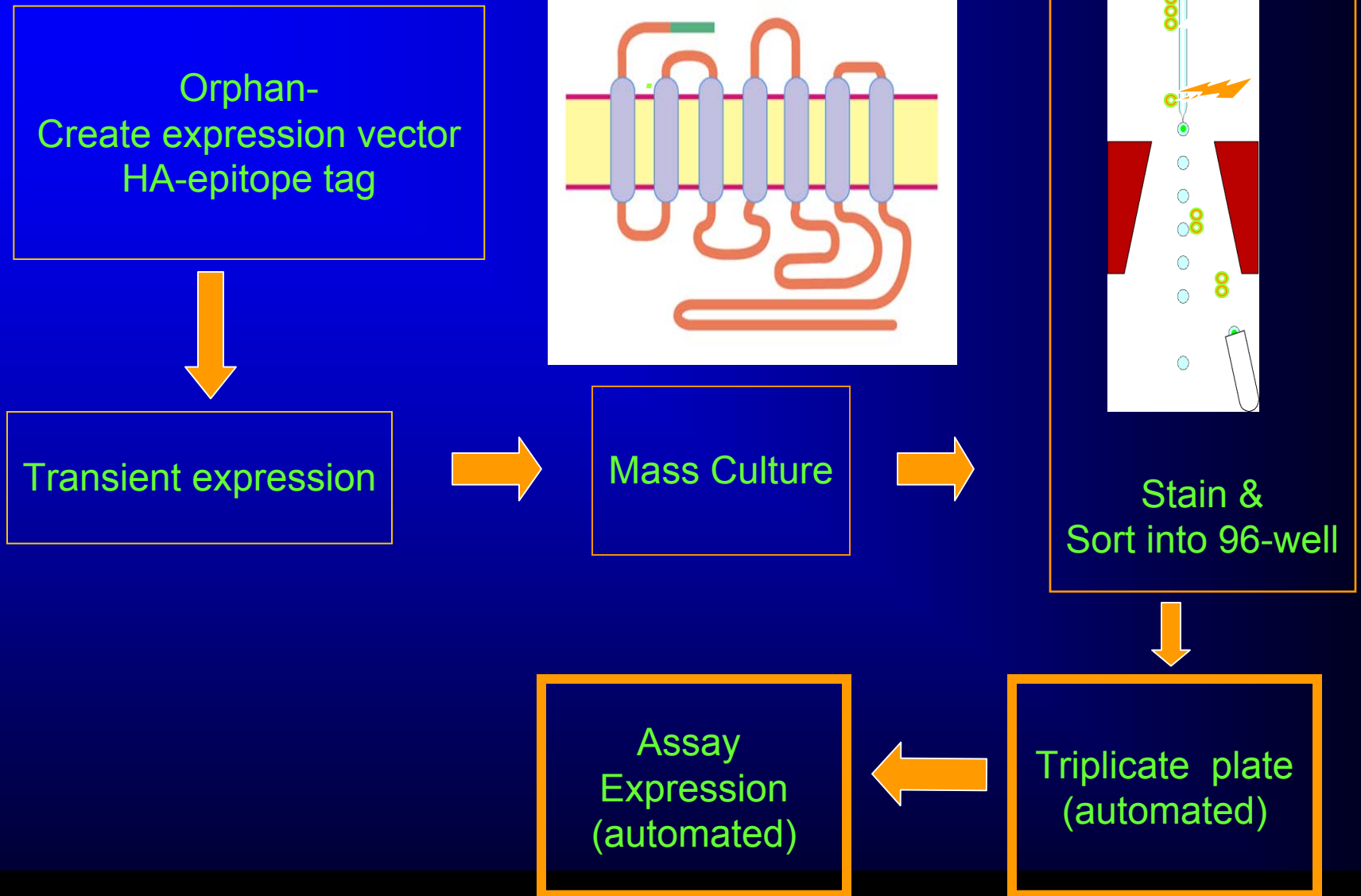


Triplicate plate
(automated)

FX method



Orphan Cell Line Generation Process



Automated Fluorescent Cell Surface Receptor Assay

Cells seeded into Corning black 96-well plate

Cell monolayer rinsed in PBS

Wash

1°Ab
60 mins @ 37° C

Wash

Cells fixed in 3.7% formaldehyde
15 mins @ 37° C

2° Ab Alexa Fluor 594
rabbit anti mouse 30-60 mins @ 37° C

Wash

3° Ab Alexa 594
goat anti rabbit 30 - 60 mins @ 37° C

Resuspend in wash buffer
& read on plate reader

Wash

Robotic (hands-free) 96-well RNA preparation procedure:

96 clones growing in culture medium in 96-well plate

Each cell monolayer washed 2x with PBS

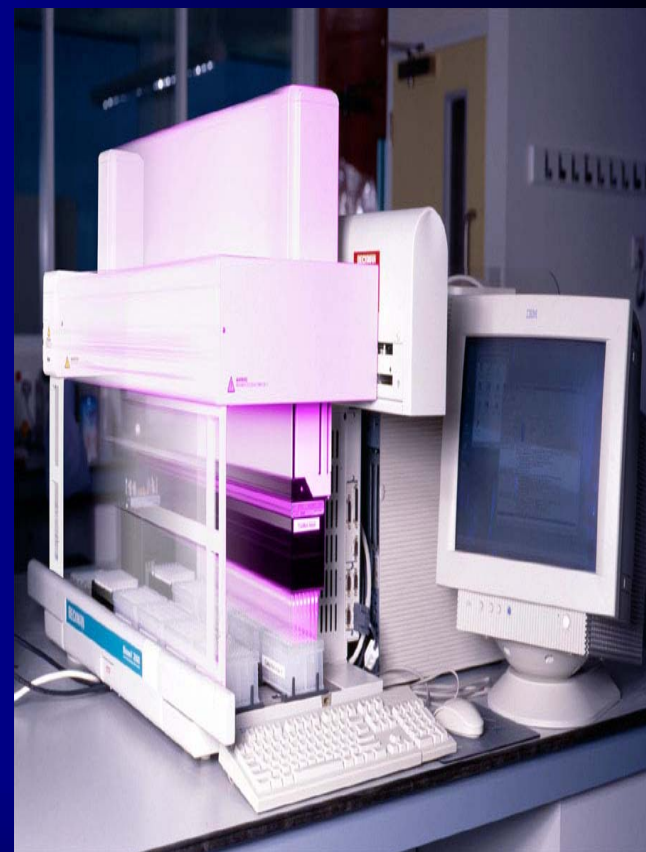
Cells lysed and added to RNA columns

RNA washed on column

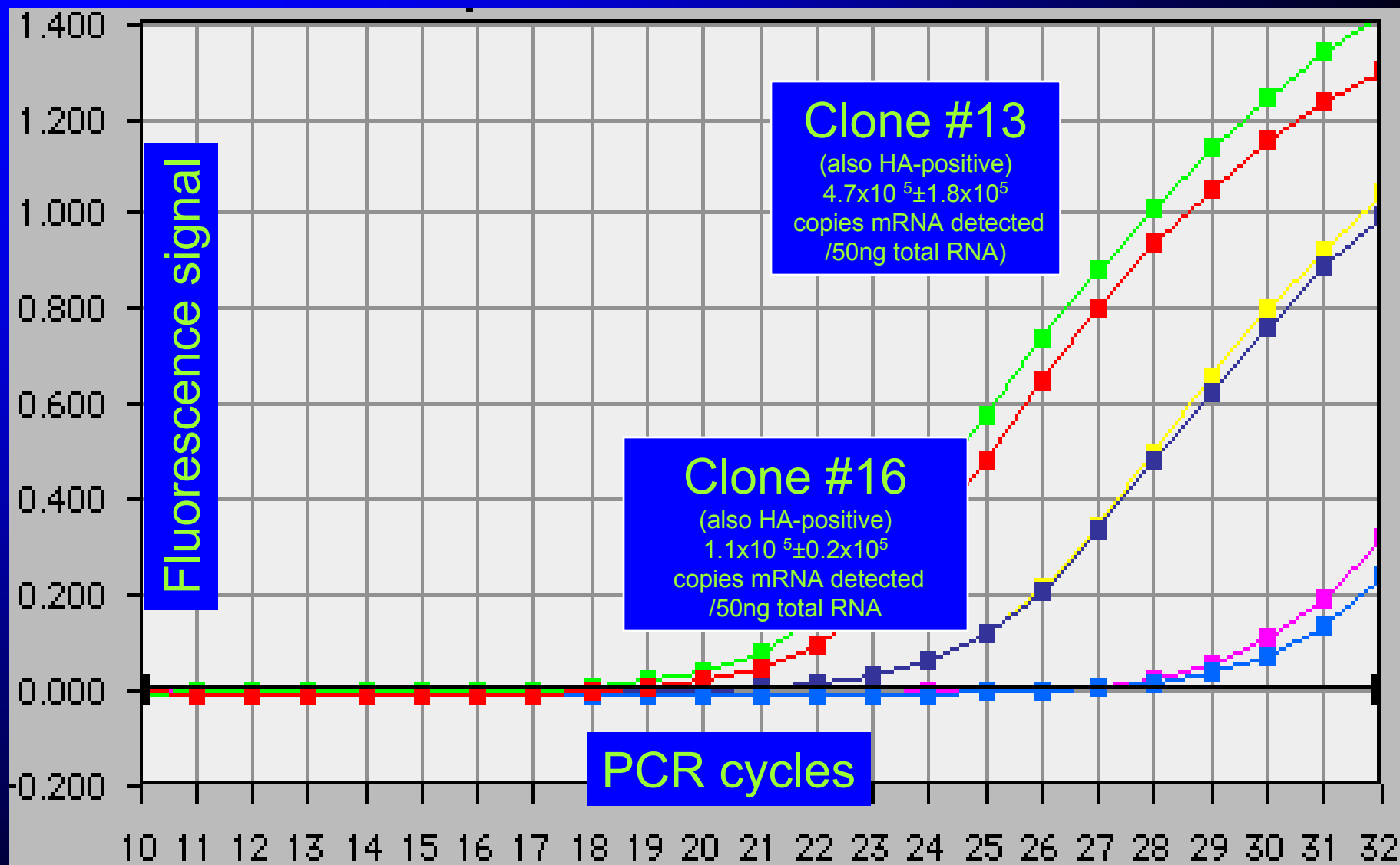
RNA DNase-treated on each column

DNase inhibited and washed away

RNA eluted in nuclease-free water



Taqman Multiplex PCR



measurements in duplicate

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