Development of Plasmid Calibrators for Absolute Quantification of MicroRNAs by Real Time Quantitative PCR: **A new tool for personalized medicine**

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On translational and personalized Medecine
OMICS group
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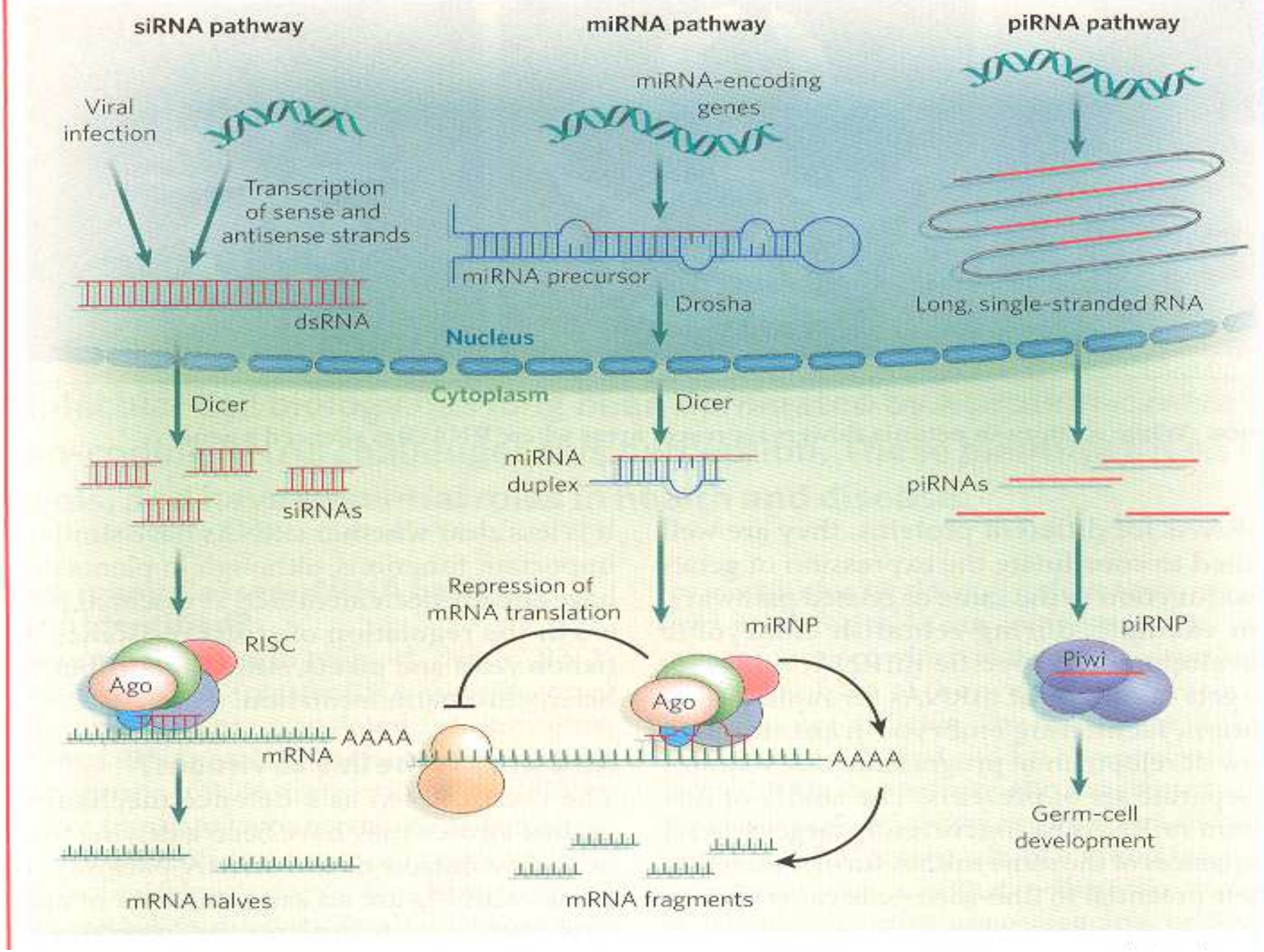
microRNAs and biology

- Discovery in 1990 in the plants,
- First described in humans in 2001 and more than 1 200 today by bio informatics (small RNA 21-25 nucleotides)
- Exponential publications
- Key roles in physiology & physiopathology
- Novel potential biomarkers +++
  - Classification of cancers much more easy (DNA arrays)
  - one microRNA controls 100 genes or more
  - Conserved between species +++
  - Very stable from harvesting to analysis
- New therapeutic weapons?
- And beyond medecine : food, plants, animals ….

Box 2 | Formation and function of small RNAs



Micro RNAs and plants
Since the 1990….

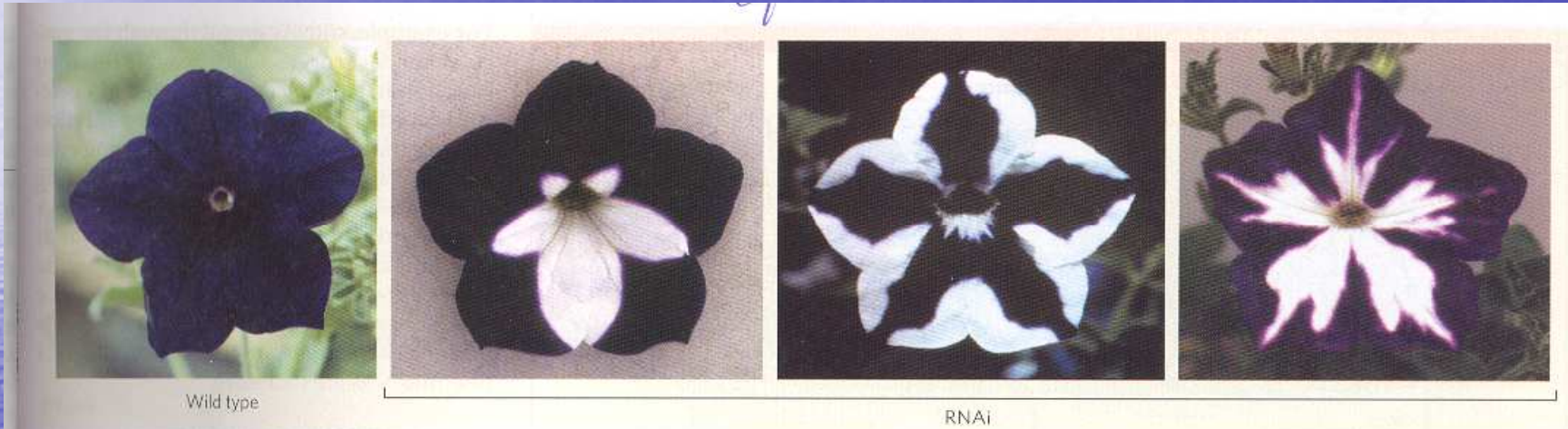


Figure 1 | First phenotypic description of RNA interference. White sections in petunia flowers represent areas where RNAi has silenced a gene involved in flower coloration.

Nature Journal

Micro RNAs and development

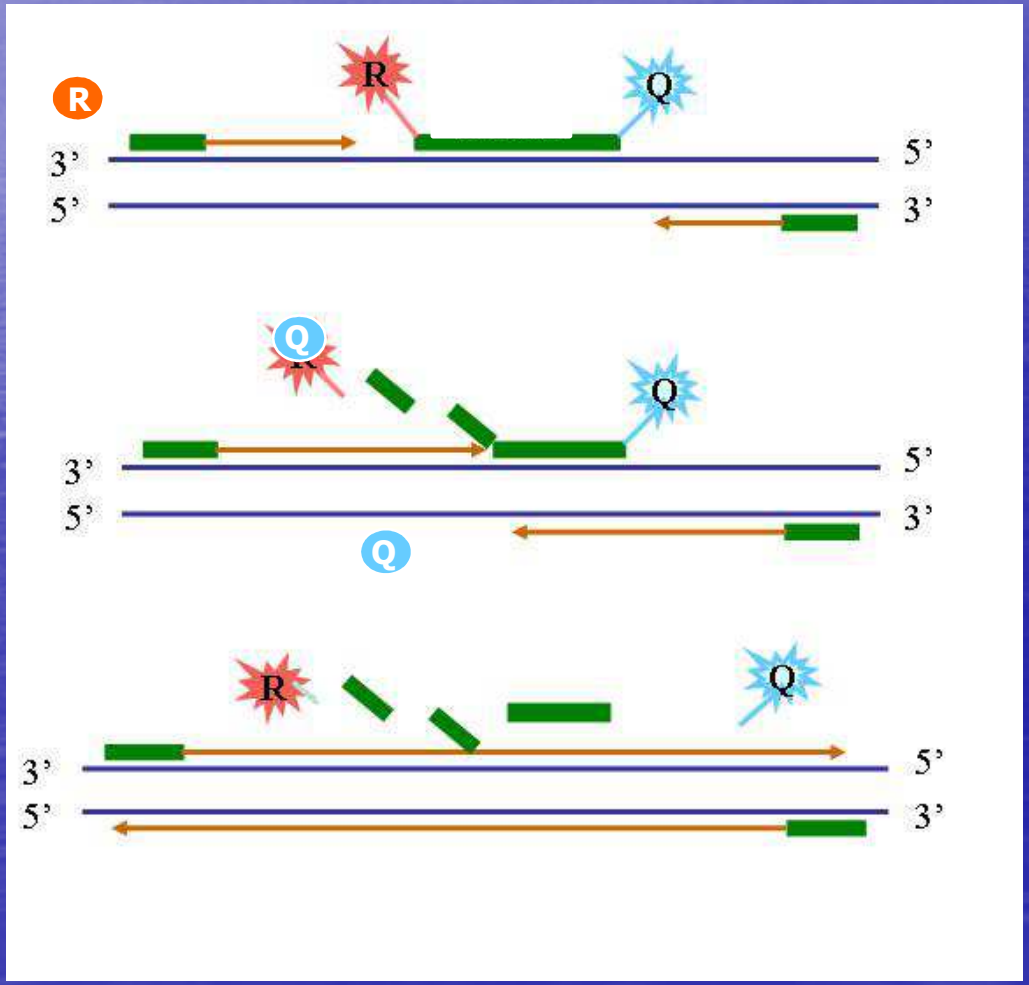
- Neuro development
  - Krichevsky 2003
  - Krol et al Cell 2010
- Heart
  - Thum et al Nature 2008 (heart failure)

- Hematopoïesis (MDS)
- Stem cells
- Cutaneous tissue
- Etc…(type 2 diabetis, inflammation).

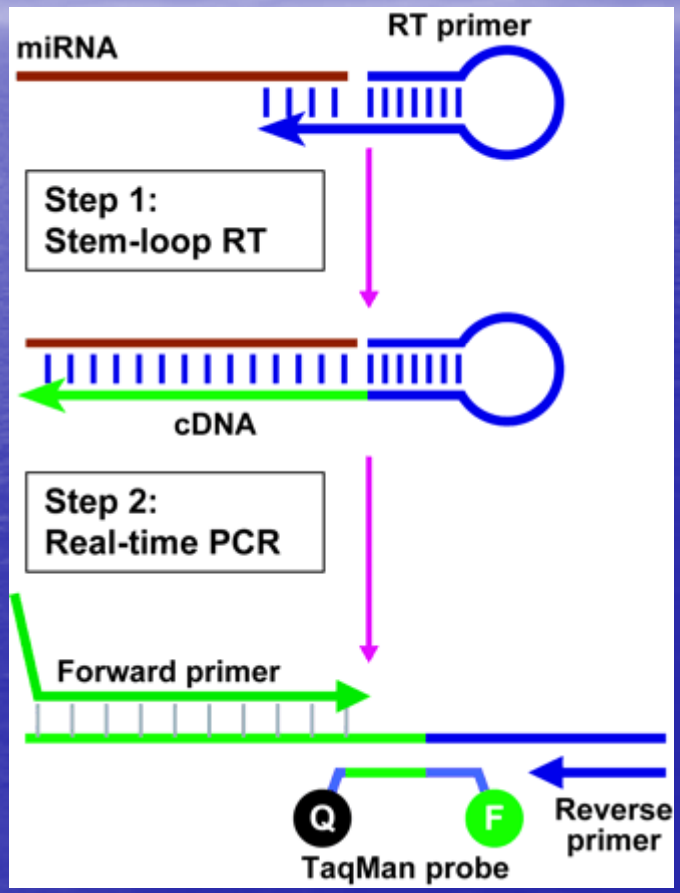
Micro RNAs and cancer

- chronic lymphoïd Leukemias (*Calin et al 2004)*
- Burkitt lymphomas (*Metzler 2004)*
- Colon adeno carcinomas (*Michael 2003)*
- Breast cancer: pronostic value (*Huang et al 2008* and Volinia & Croce PNAS 2013)
- Early Biomarker of pancreas cancer
  (Habbe N et al Cancer Biol. Ther. 2009)
- In Sera and or Plasma (prevention, diagnostic, follow up…)

TaqMan® technology: hydrolyse probe



Anchored RT PCR

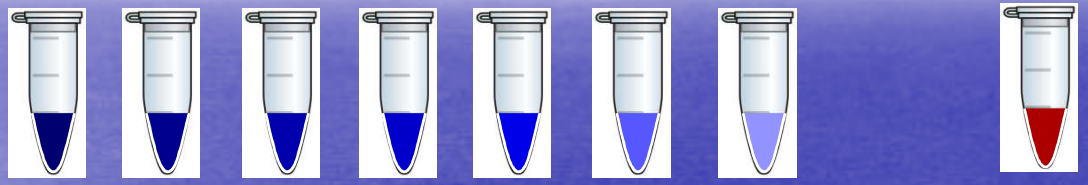


absolute quantification by qPCR

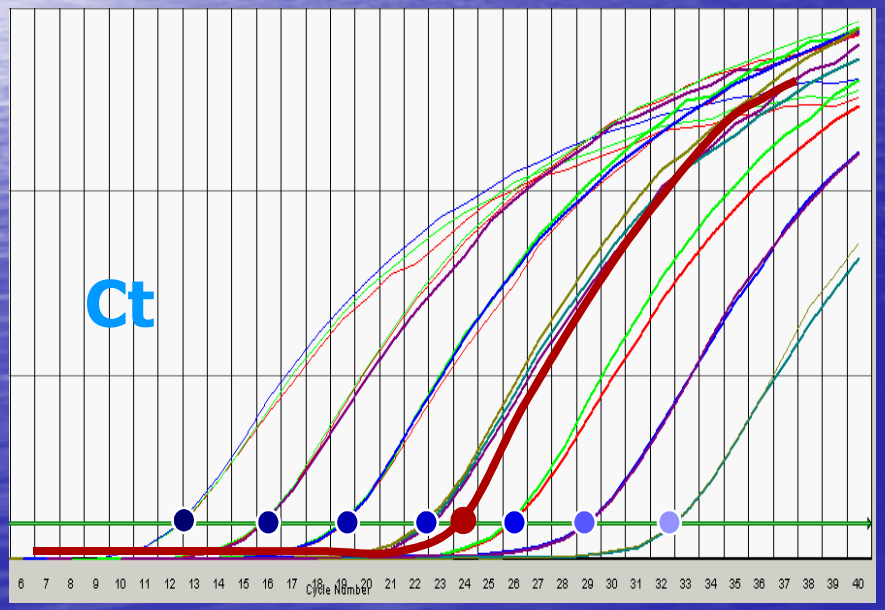
Principle

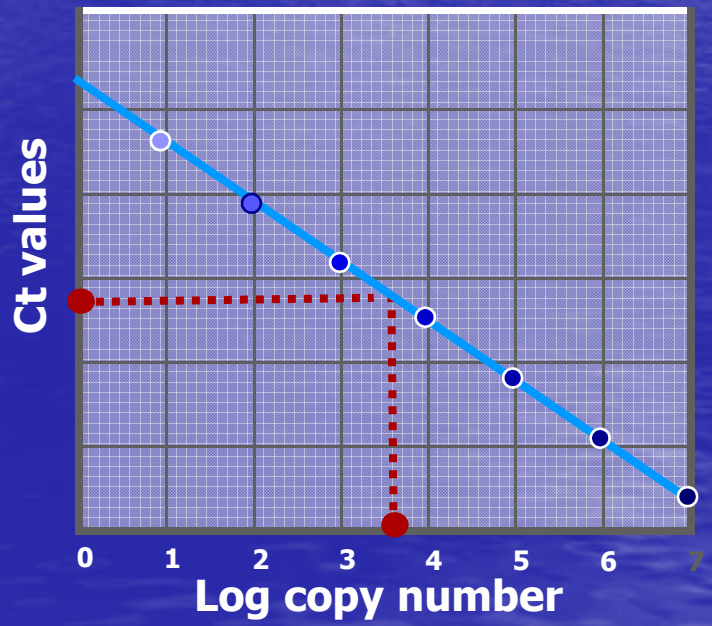
Calibration points

unknown



Copy number 10^7 10^6 10^5 10^4 10^3 10^2 10^1 ?





Absolute quantification by qPCR

Developpement of calibrators plasmidics calibrators

1) The classical way

Variable

Necessary time

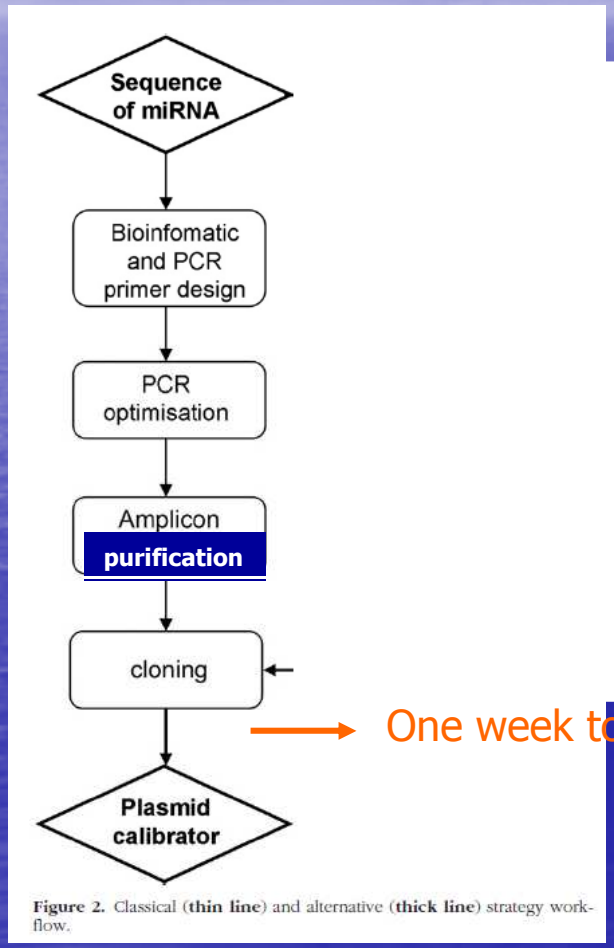


Figure 2. Classical (**thin line**) and alternative (**thick line**) strategy workflow.

→ One week to one month

Absolute quantification by qPCR

Developpement of plasmidics calibrators

2) Our way: simpler, quicker

1st step

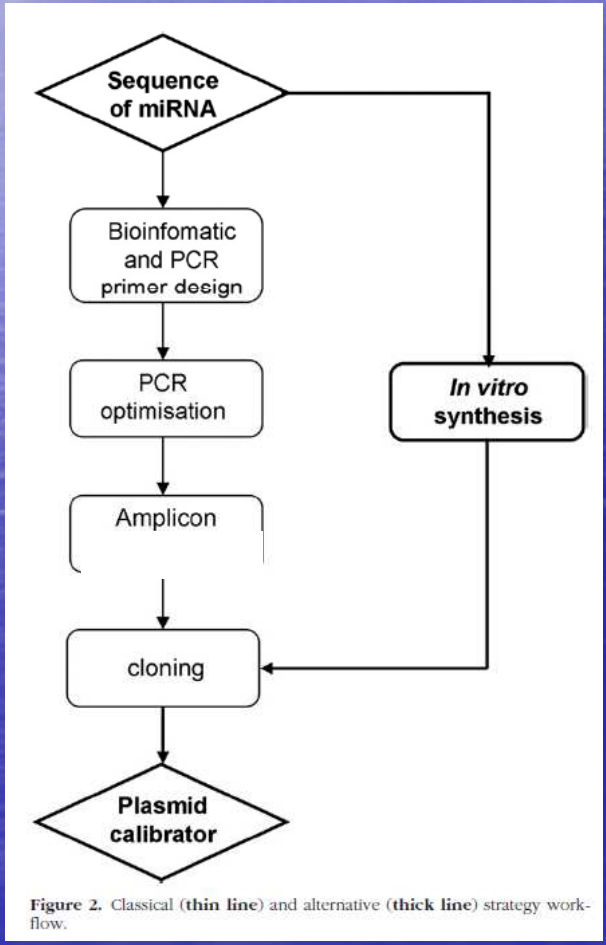


Figure 2. Classical (**thin line**) and alternative (**thick line**) strategy workflow.

2nd step

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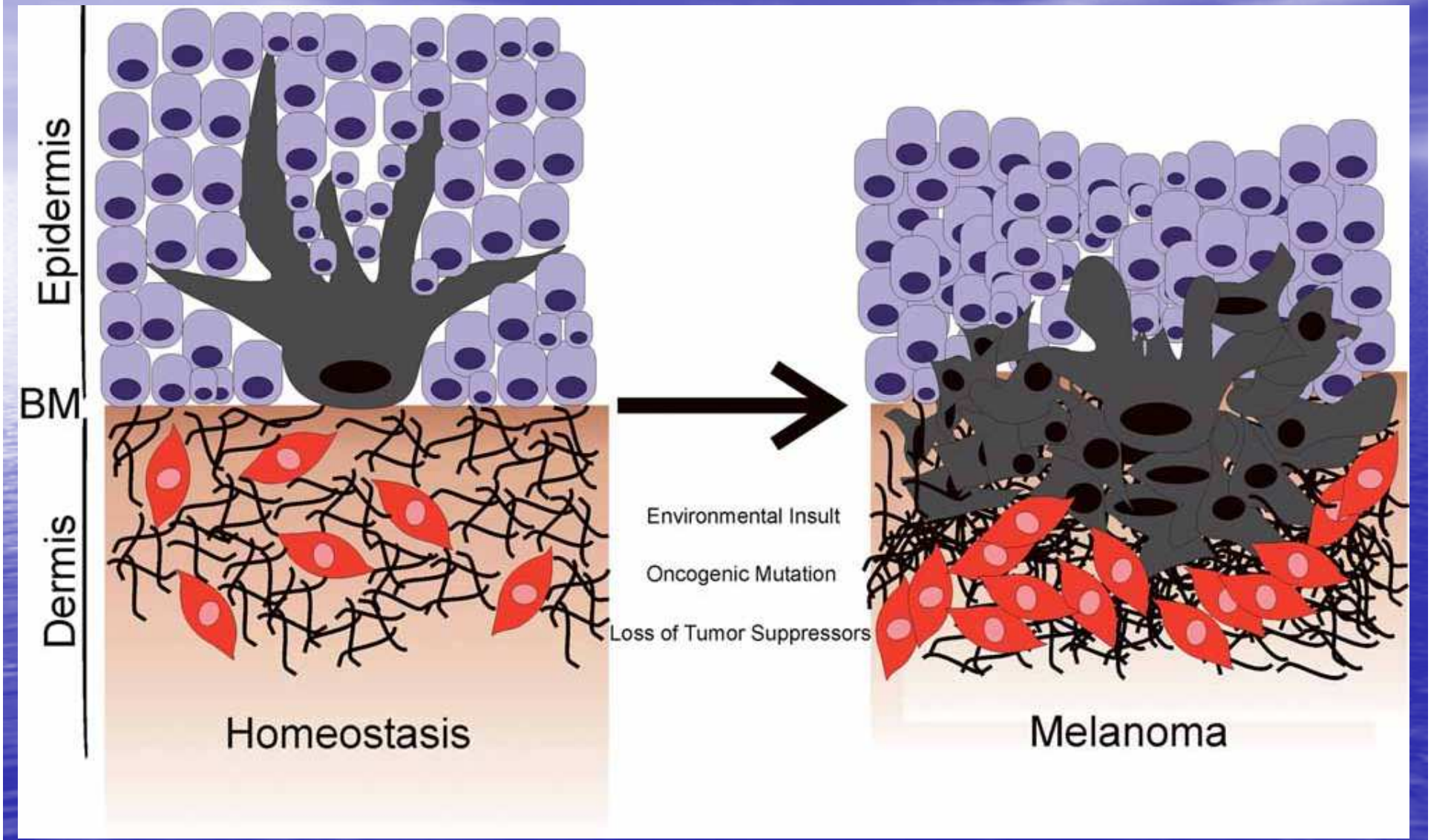
3rd step

One medical application

Melanomas

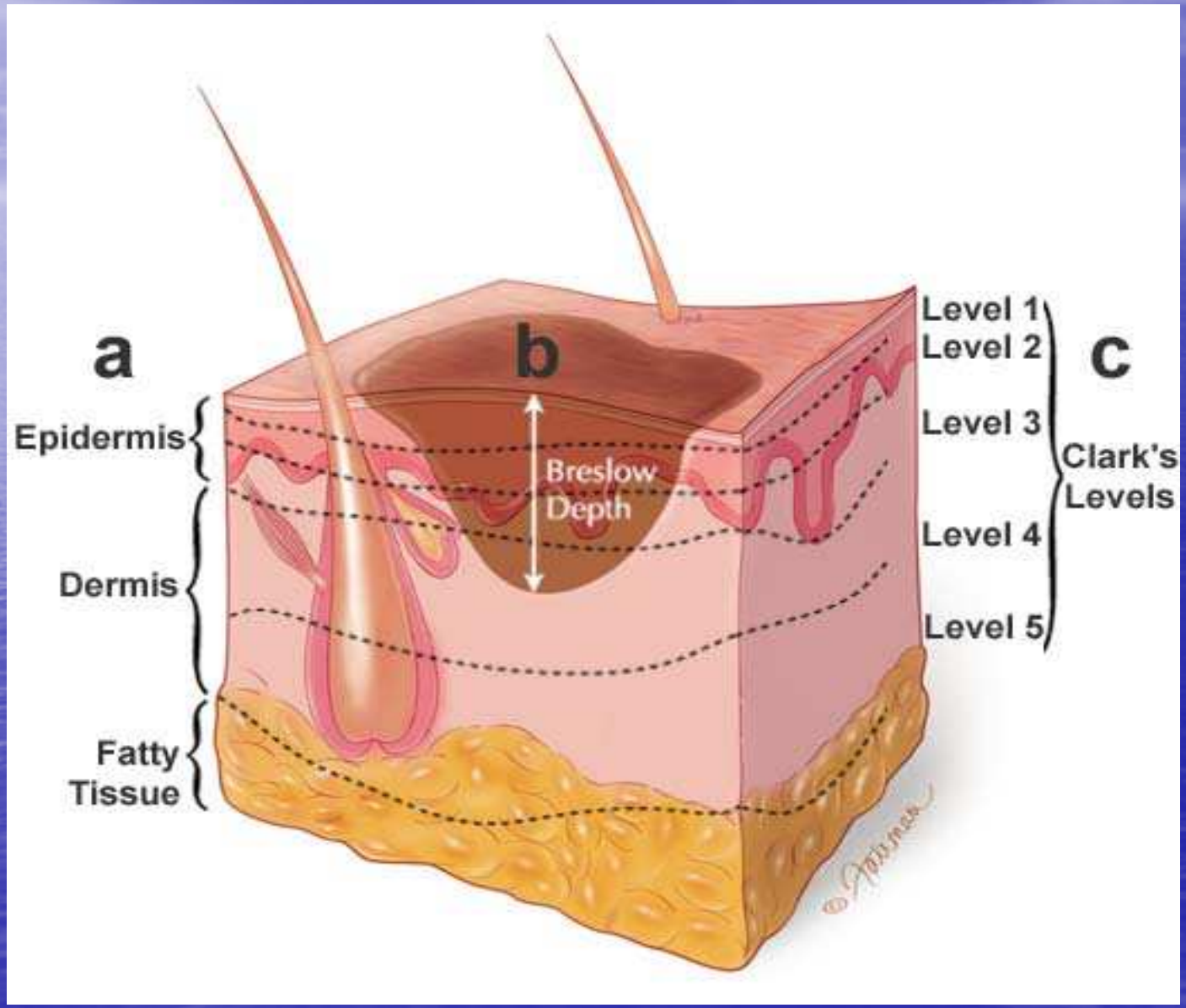

Epidermis
BM
Dermis
Homeostasis
Environmental Insult
Oncogenic Mutation
Loss of Tumor Suppressors
Melanoma

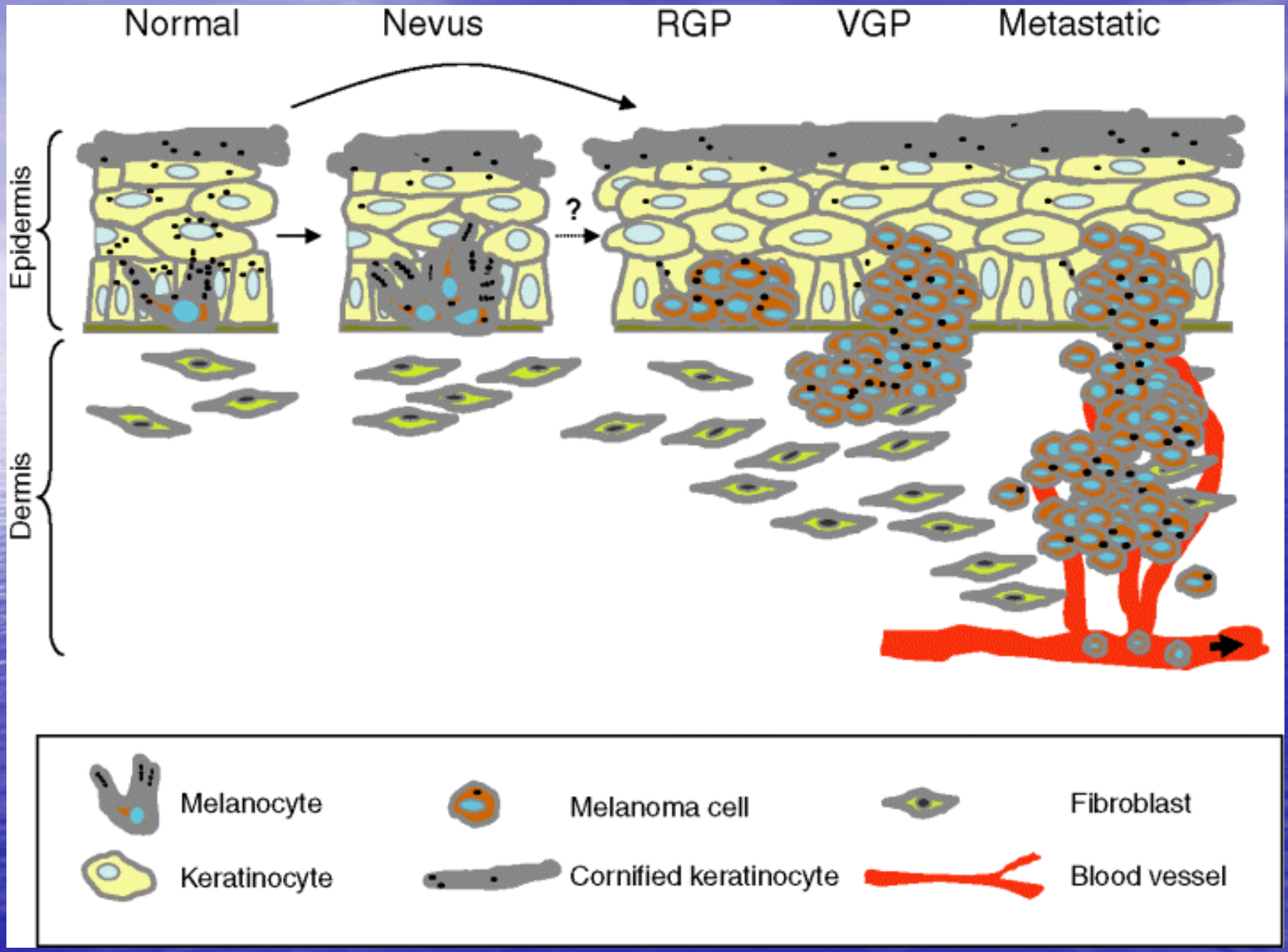
melanomas

- Coming from melanocytes in the epidermidis
- Public health problem:
  - 4% of skin cancer, but 74% of death by skin cancer
  - The incidence doubles every 5 years
  - Out of any therapy when there is a metastasis:

  Survival after 5 years is less than 5%

- quantification of Let7b and its role in melanomas
  - It works on paraffin tissues


a
b
c
Epidermis
Dermis
Fatty Tissue
Breslow Depth
Level 1
Level 2
Level 3
Level 4
Level 5
Clark's Levels


Normal
Nevus
RGP
VGP
Metastatic
Epidermis
Dermis
?
Melanocyte
Keratinocyte
Melanoma cell
Cornified keratinocyte
Fibroblast
Blood vessel

Let 7b and melanomas

- Physiopathology (research) and potential biomarker
- Need from the dermatology departments
- Let-7b as a biomarker:
  - Early diagnostic,
  - predictive marker: Loss of expression = bad pronostic
  - help for the therapeutic decisions and patient care

Quantification of MicroRNAs

- Notably in development and cancer
- On paraffine slides (done for Let7 in melanomas)
- On blood samples
- On sera or plasma ....

Technics
(univ. Patent n° 01/03551)

- Absolute calibration
- easy to use (done for BCR ABL for CML patients)
- Extrapolation for 1 copy -> identification of false positive
- very reproducible (multicentric studies)
- Can be integrated in kits on the market

Only available option allowing the stability and the robustness of the assay

necessary for

the standardisation of the RQ-PCR essays

multi-centric studies

clinical applications, such as minimal residual disease

For objective assement of (any) therapy effectivness…

conclusion

- Proof of principle: let7b for melanomas but what is THE good bio-marker?
  - For example: MicroRNA 200 is increased in melanomas Elson Schvab et al Plus One Octobre 2010
  - The solution? Screening at diagnosis then only ONE bio marker for the follow up?
- Absolute RQ PCR quantification: University patent pending (technology)
- Other diseases:
  - cancers (lung, breast, Acute myeloid leukemia ect…)
  - Metastasis (Su X et al Nature October 2010)
  - and others (cardio vascular, Injury of CNS, Neurodegenerative diseases like Parkinson or Alzheimer)
- To be solved: the normalization question
  - Specific for each tissue (tLDA)
  - Informatic tools available (geNorm, NormFinder etc…)
- Still in its infancy (standardization & external quality control)
  - The experience with BCR ABL of clinical application should be helpfull++

Thank you for your attention!