

Conditions de fiabilité des mesures d'expression génique par RT-qPCR en cancérologie clinique

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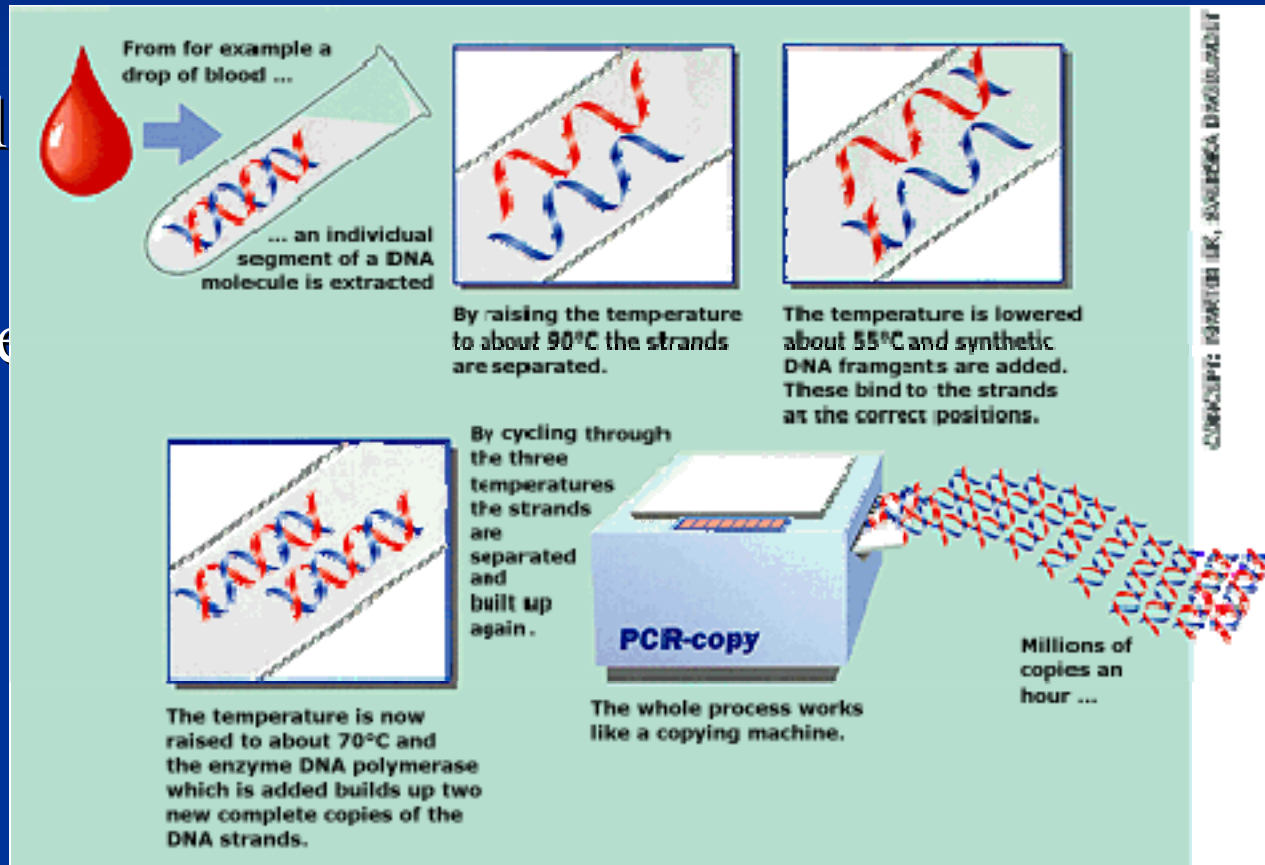
³ INSERM U590, Centre Léon Bérard, Lyon

PCR, qPCR, RT-qPCR...

□ Polymerase Chain Reaction

□ Real

□ Reve

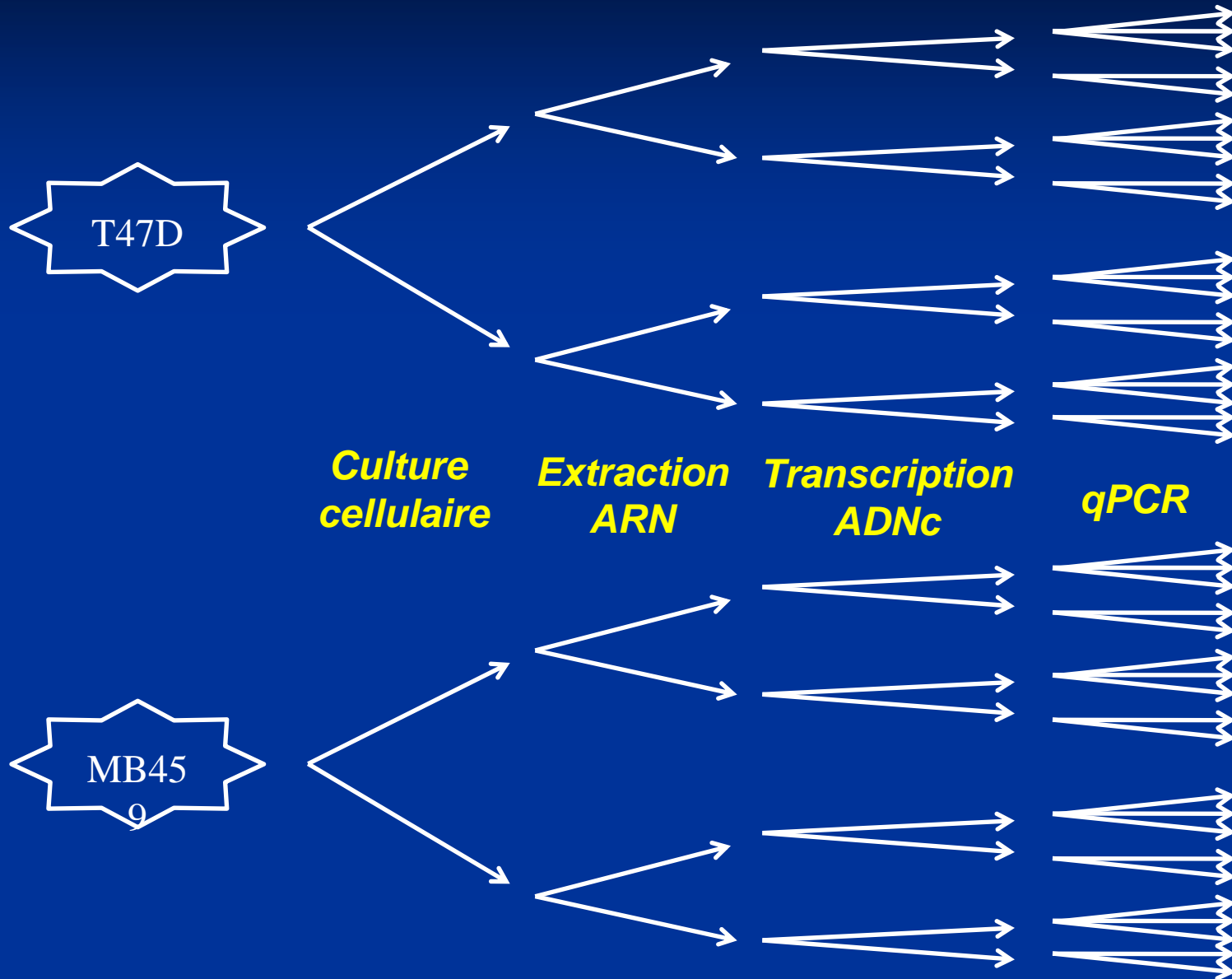


PCR

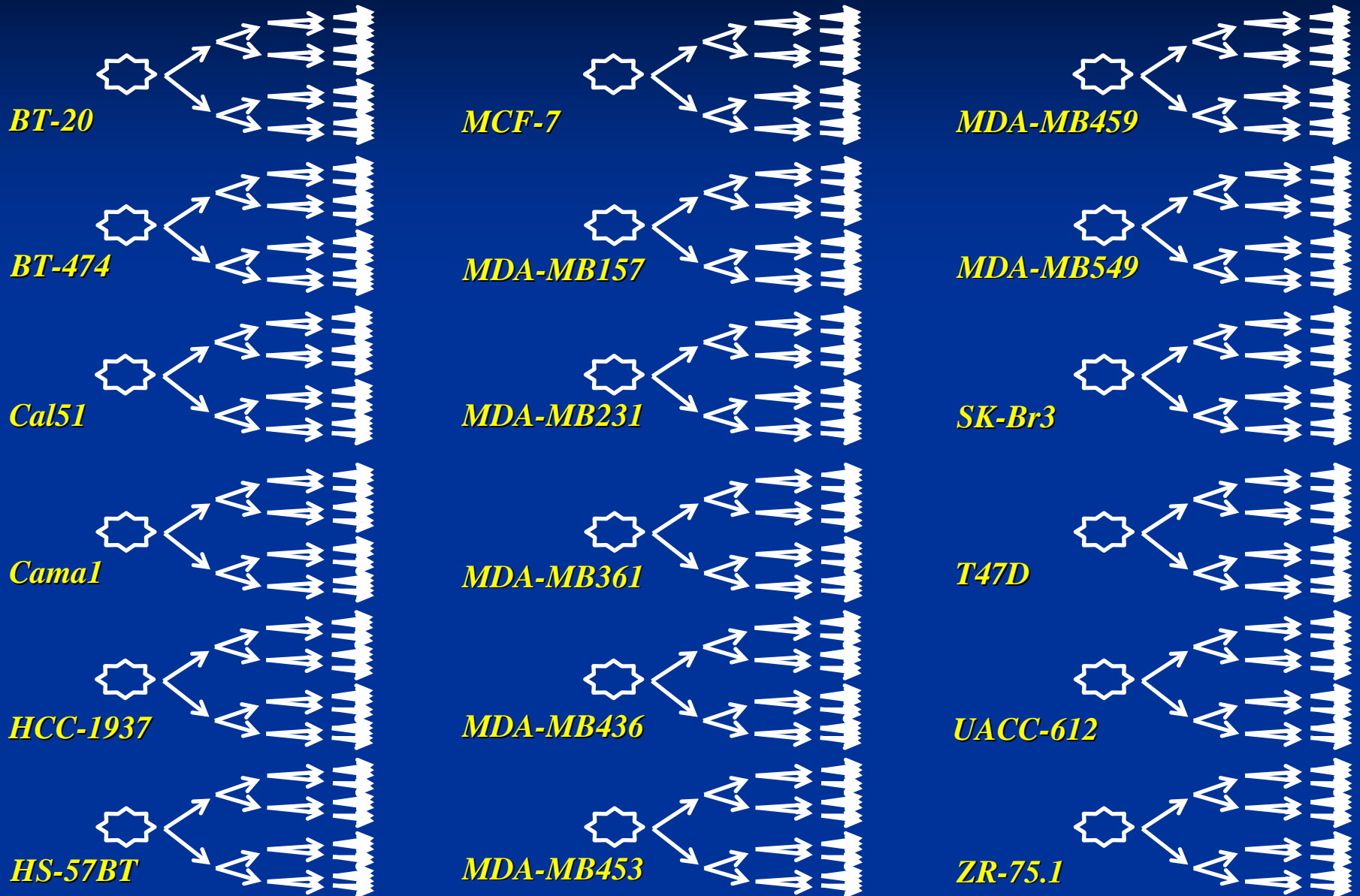
RT-qPCR en cancérologie



Plan expérimental

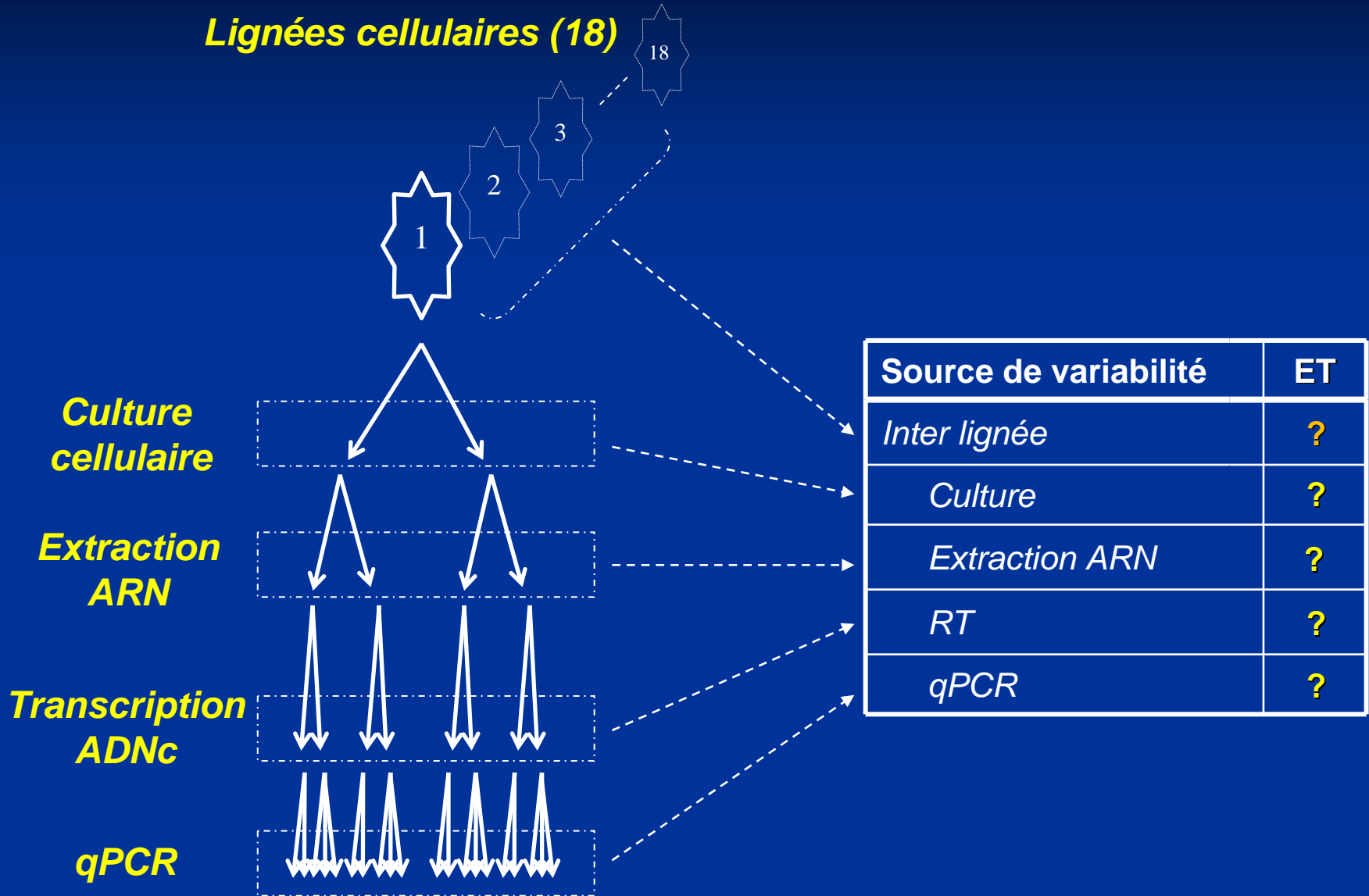


Plan expérimental



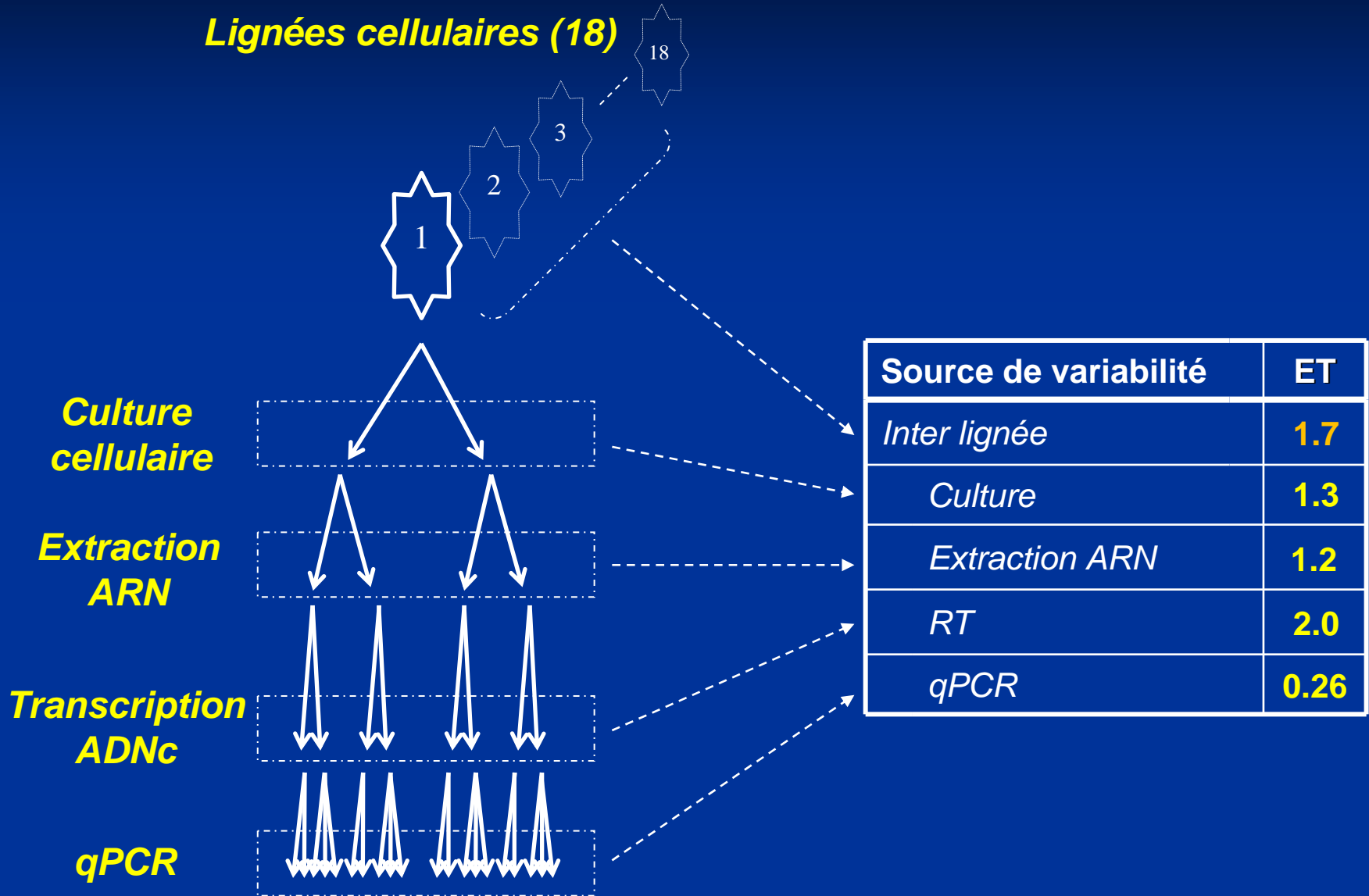
Estimation de l'erreur de mesure

Lignées cellulaires (18)



Estimation de l'erreur de mesure

Lignées cellulaires (18)



Conditions de fiabilité

Cell Culture → *RNA Extraction* → *RT* → *qPCR*

No replication
Misclassification rate=7.25%

Cell Culture → *RNA Extraction* → *RT 1* → *qPCR*
Cell Culture → *RNA Extraction* → *RT 2* → *qPCR*

RT replication
Misclassification rate=2.82%
→ Improvement=61.1%

Cell Culture → *RNA Extraction 1* → *RT* → *qPCR*
Cell Culture → *RNA Extraction 2* → *RT* → *qPCR*

RNA Extraction replication
Misclassification rate=2.84%
→ Improvement=60.9%

Cell Culture 1 → *RNA Extraction* → *RT* → *qPCR*

Cell Culture 2 → *RNA Extraction* → *RT* → *qPCR*

Cell Culture replication
Misclassification rate=0.73%
→ Improvement=90%

Conclusion

Murphy T, Rustin SA

Tichopad A, Kitchen R, Riedmaier I, Becker C, Ståhlberg A, Kubista M.

Design and optimization of reverse-transcription quantitative PCR experiments.

Clin Chem. 2009 Oct;55(10):1816-23. PubMed PMID: 19643838.

“We recommend the use of sample replicates preferentially to any other replicates when working with solid tissue, cell culture, and single cells, and we recommend the use of RT replicates when working with blood.”

measles, mumps and rubella vaccine/autism controversy.

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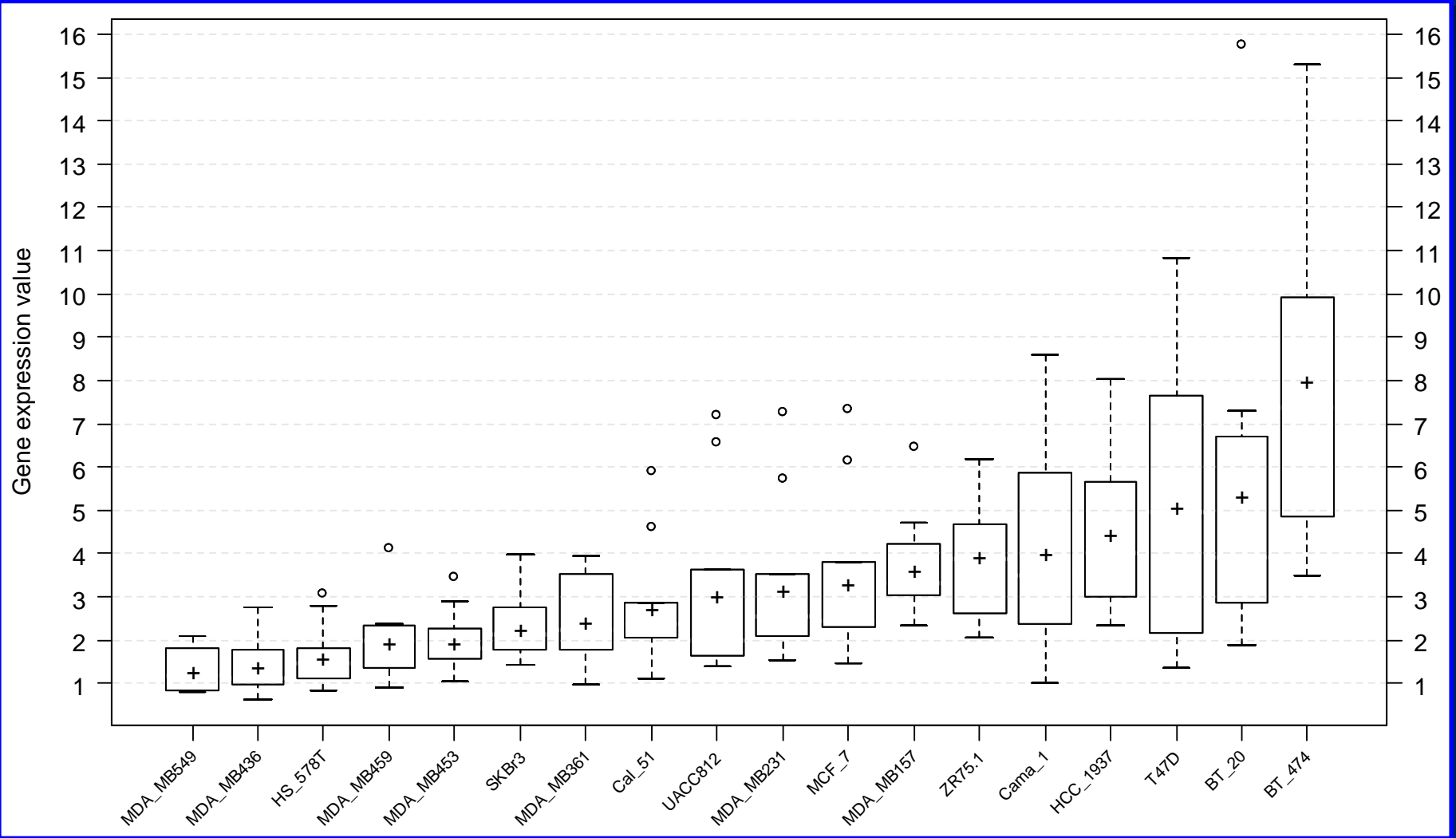
Biostatistiques HCL

René Ecochard
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Matthieu Laversanne
Pascal Roy
Fabien Subtil

Références

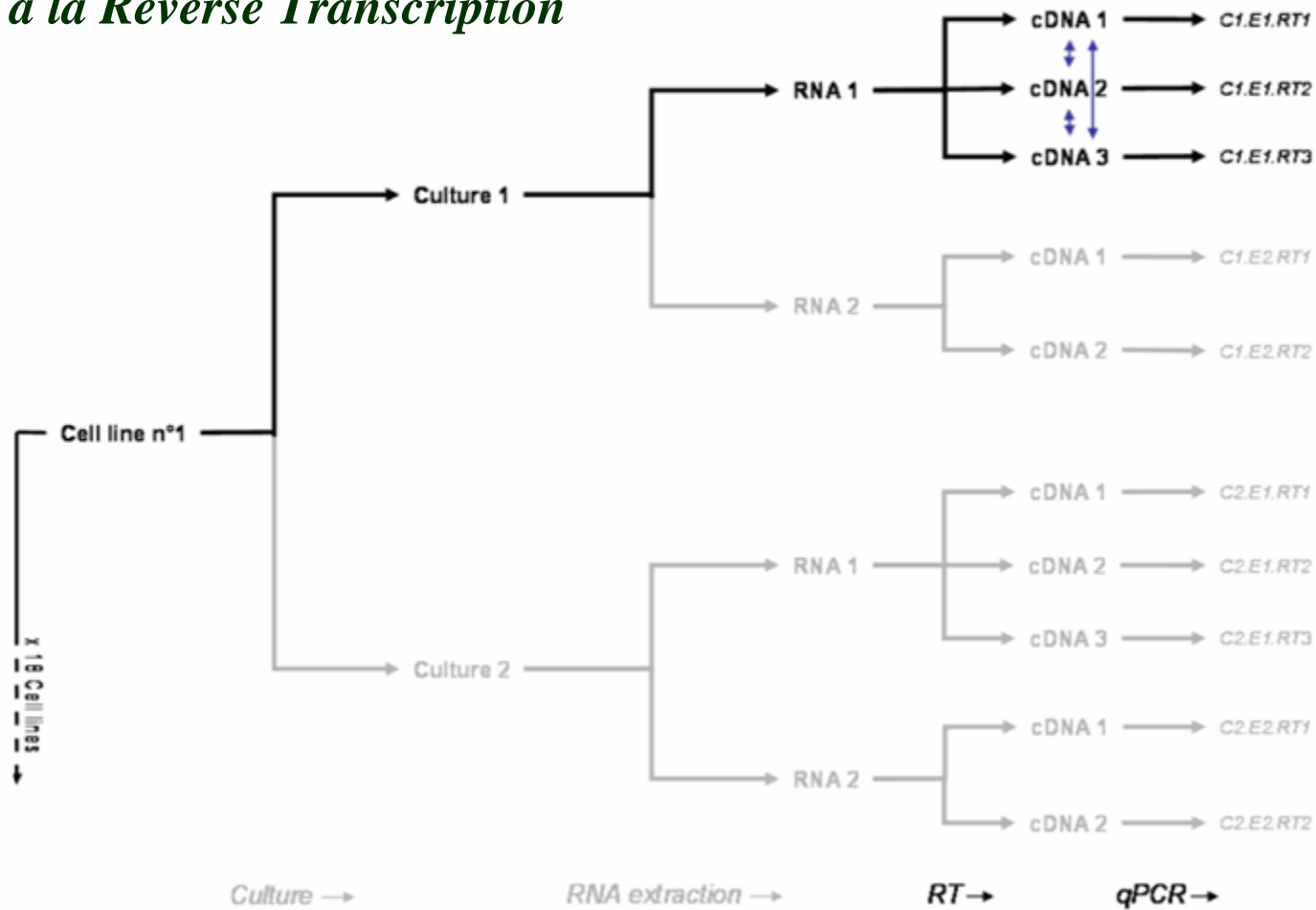
- 1: Combes JD, Grelier G, Laversanne M, Voirin N, Chabaud S, Ecochard R, Lasset C, Moyret-Lalle C. Contribution of cell culture, RNA extraction, and reverse transcription to the measurement error in quantitative reverse transcription polymerase chain reaction-based gene expression quantification. *Anal Biochem.* 2009 Oct 1;393(1):29-35. Epub 2009 Jun 13. PubMed PMID: 19527676.
- 2: Murphy J, Bustin SA. Reliability of real-time reverse-transcription PCR in clinical diagnostics: gold standard or substandard? *Expert Rev Mol Diagn.* 2009 Mar;9(2):187-97. Review. PubMed PMID: 19298142.
- 3: Pfaffl MW. The ongoing evolution of qPCR. *Methods.* 2010 Apr;50(4):215-6. PubMed PMID: 20215019.
- 4: Tichopad A, Kitchen R, Riedmaier I, Becker C, Ståhlberg A, Kubista M. Design and optimization of reverse-transcription quantitative PCR experiments. *Clin Chem.* 2009 Oct;55(10):1816-23. Epub 2009 Jul 30. PubMed PMID: 19643838.

Données



Estimation de l'erreur de mesure

Erreur de mesure due à la Reverse Transcription



Conditions de fiabilité

Expression génique estimée sur 2 mesures (réplication de la transcription)

