

Maîtriser l'impact de l'ADN génomique contaminant dans la RT-qPCR avec ValidPrime

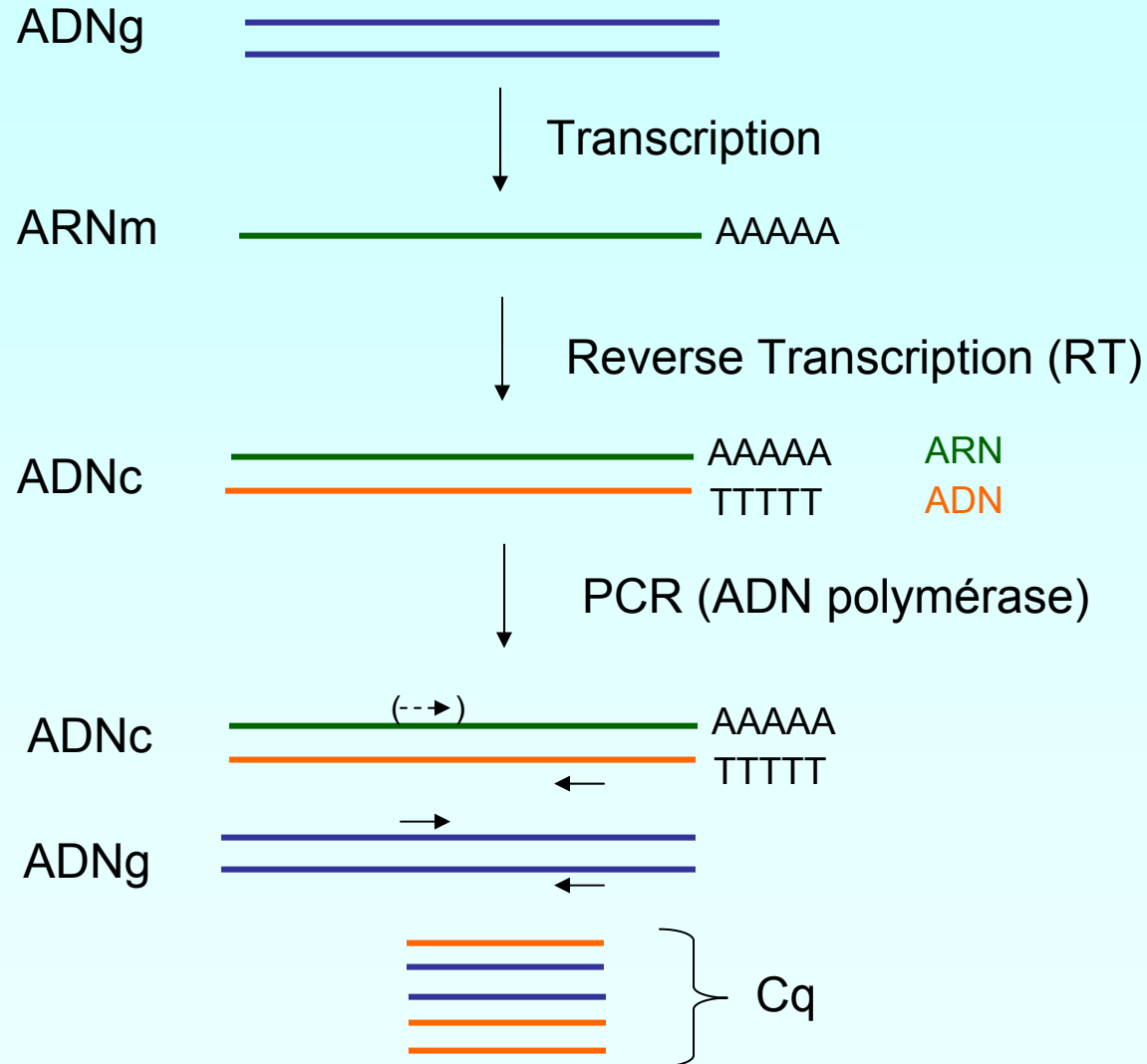
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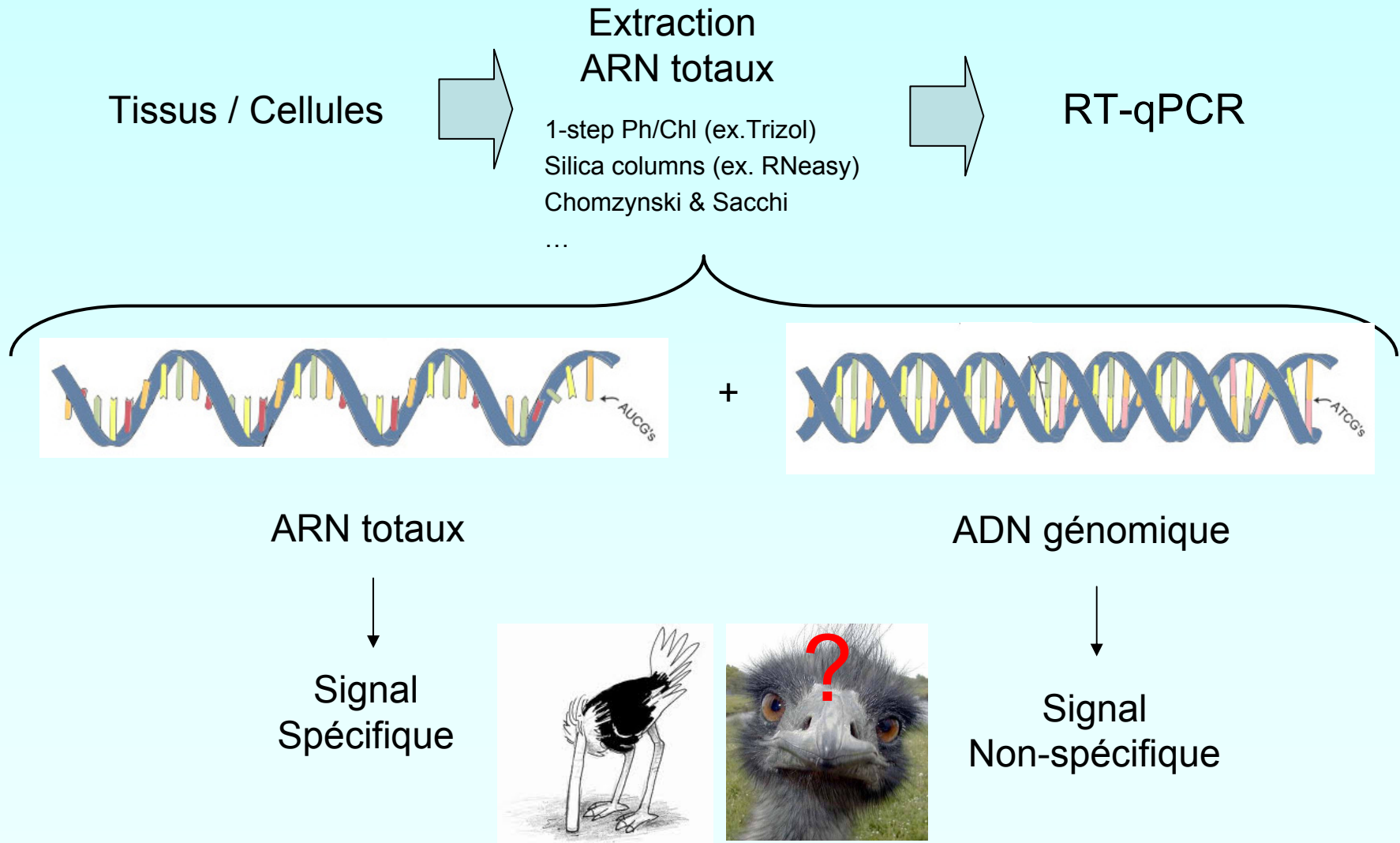


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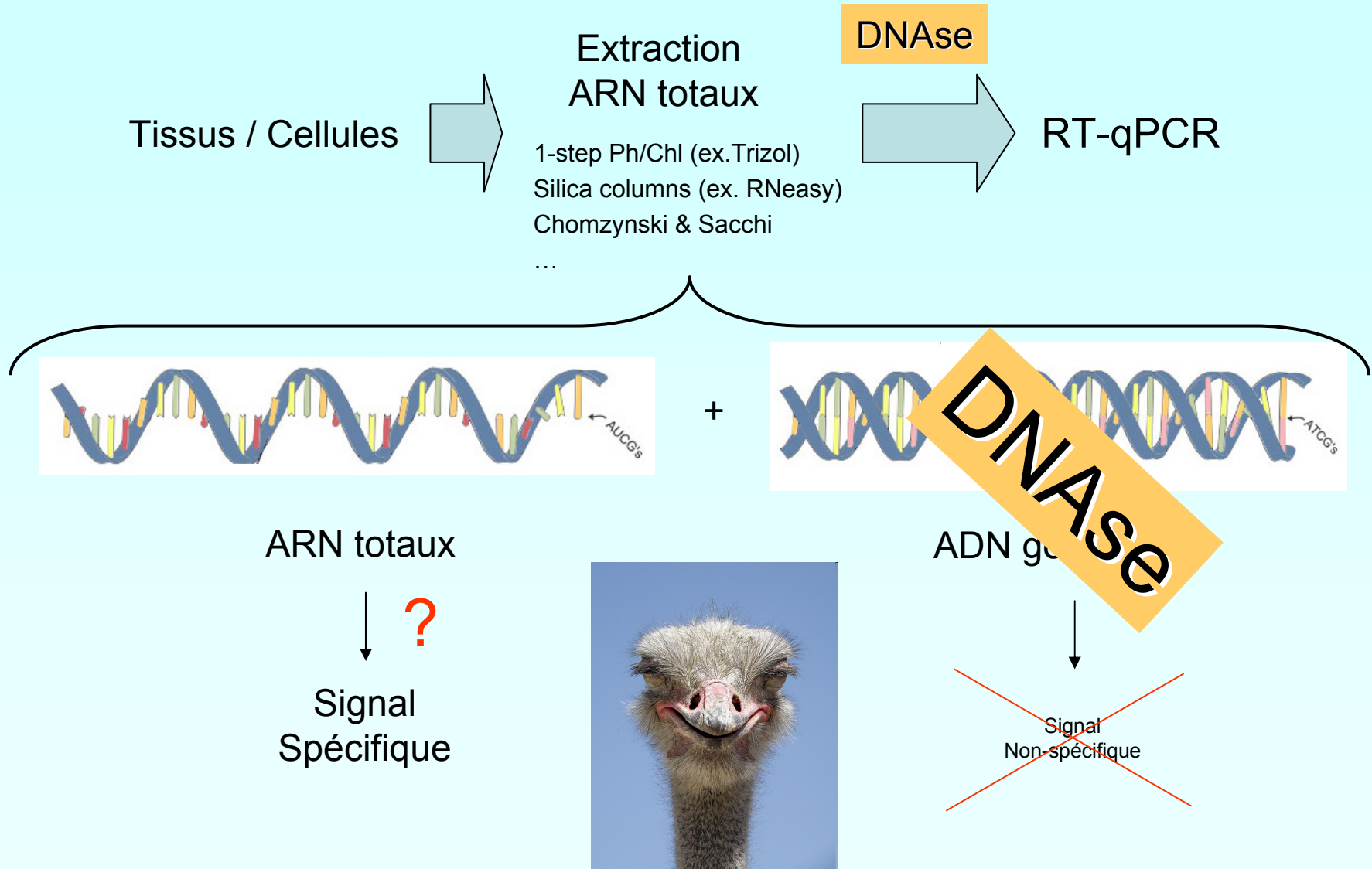
RT-qPCR : **La** méthode pour l'étude de l'expression génique



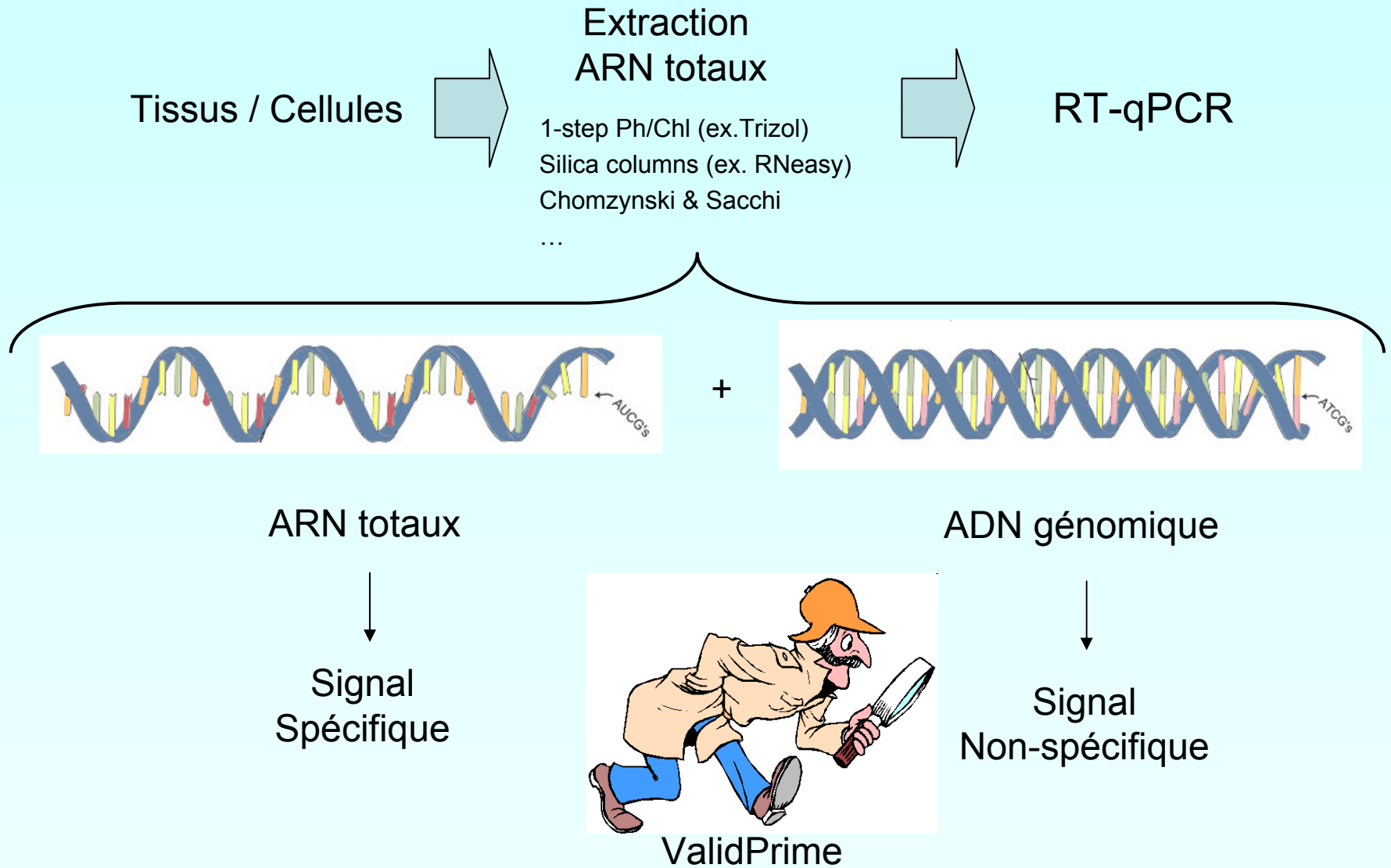
La contamination par l'ADNg - un problème (in)connu..?!



Solution courante : « Traiter le mal par le mal... »



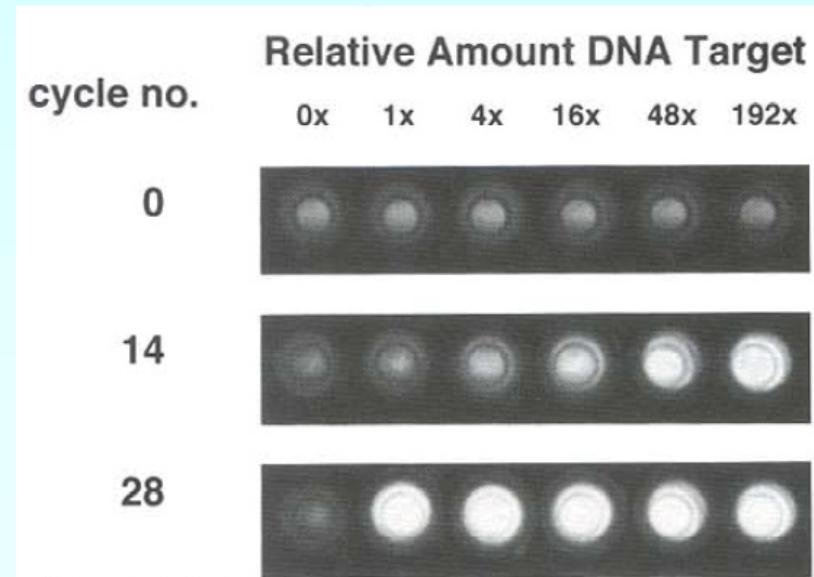
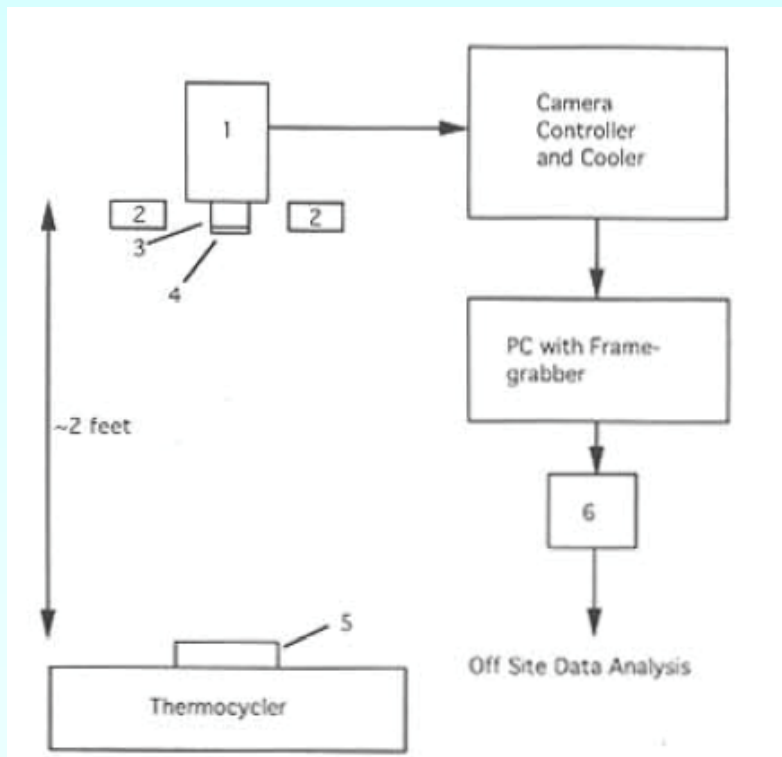
ValidPrime : évaluer l'impact de l'ADN génomique



La PCR vs la qPCR – une petite rétrospective

PCR (Mullis, 1986) – « endpoint analysis » : résultat OUI/NON

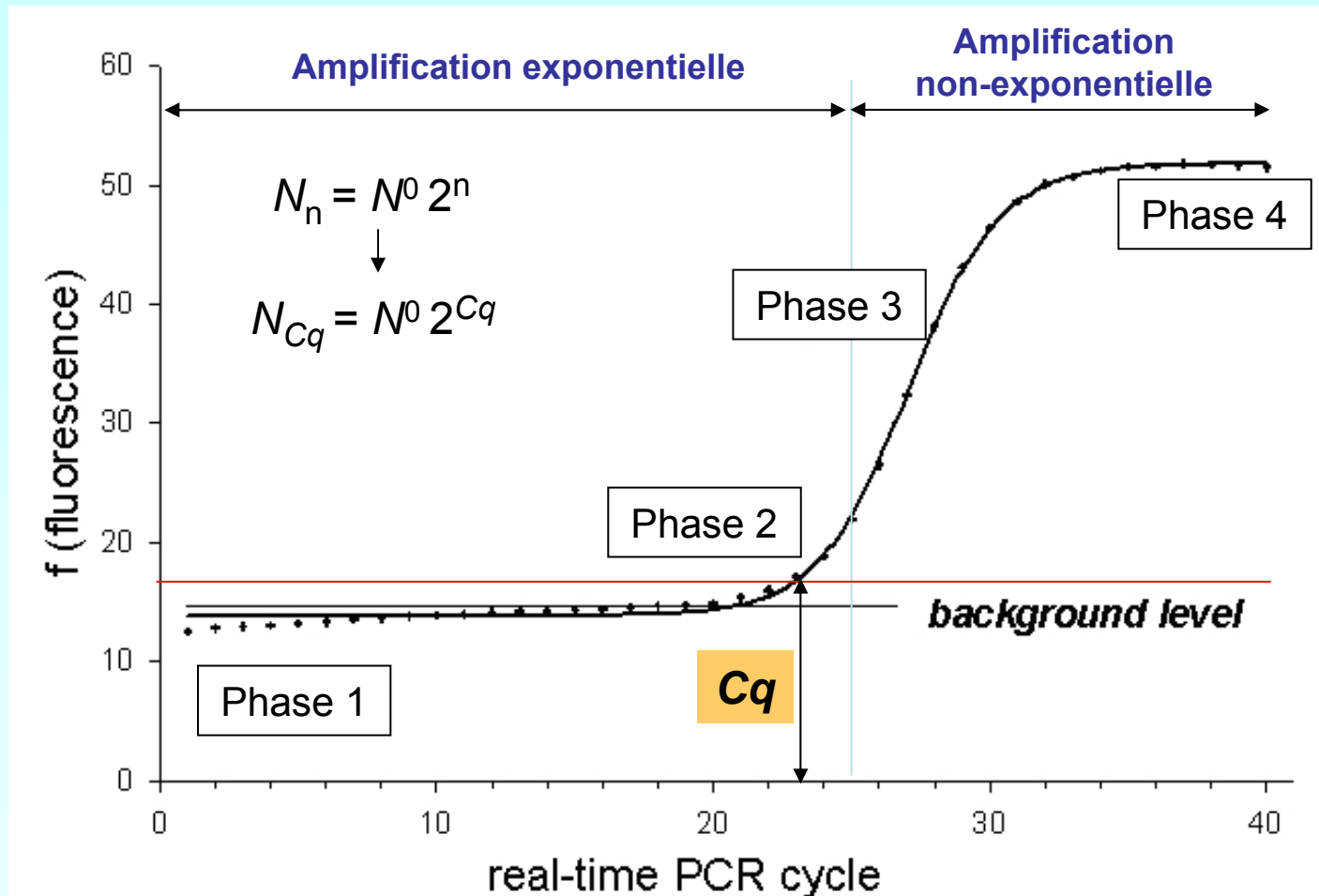
qPCR (Higushi, 1993) – « Kinetic PCR » la détection en temps réel d'un rapporteur ayant une affinité pour l'ADN double brin



(Higushi et al., 1993)

PCR : Amplification exponentielle...une question de timing !

Echelle linéaire !!



Phase 1 : Signal en dessous de la limite de détection

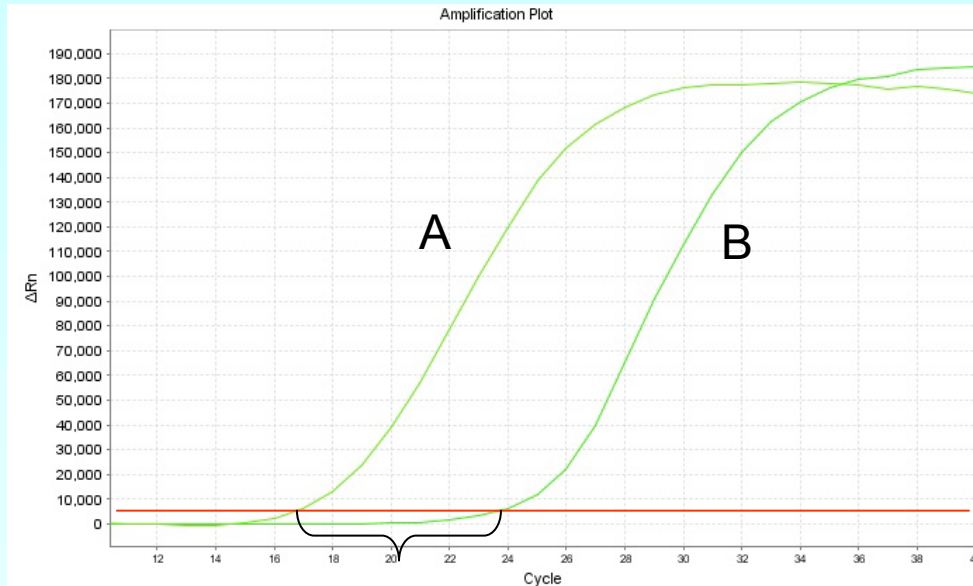
Phase 2 : Amplification exponentielle détectable

Phase 3 : Phase linéaire

Phase 4 : Phase plateau

Les « pièges intellectuelles » de la qPCR...

- Les C_q sont en échelle logarithmique



$$\Delta Cq: 23.7 - 16.8 = 6.9$$

$$\frac{A}{B} = 2^{6.9} = 119$$

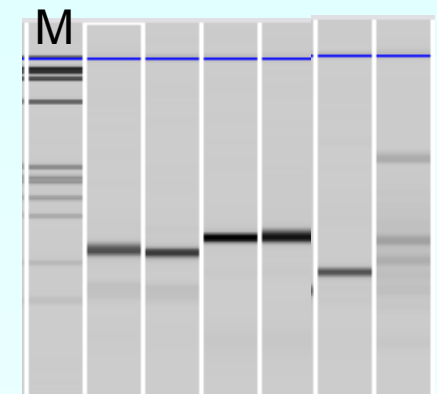
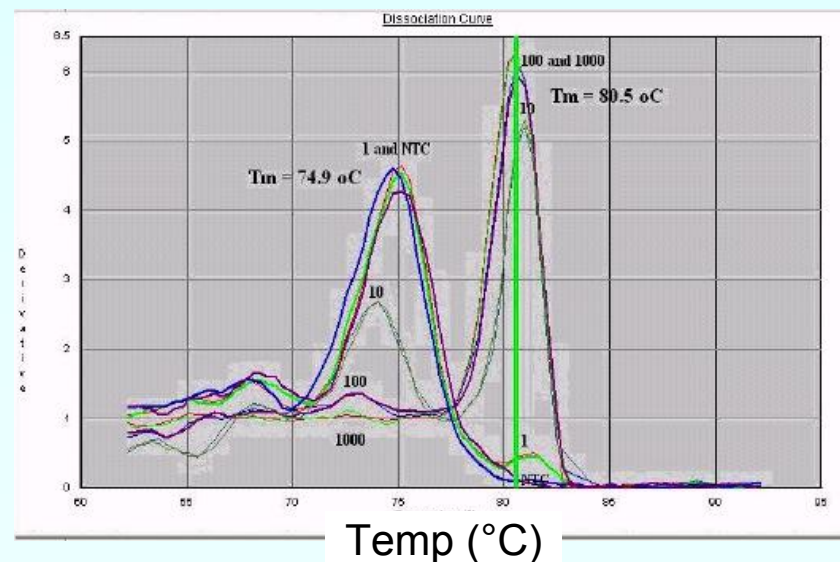
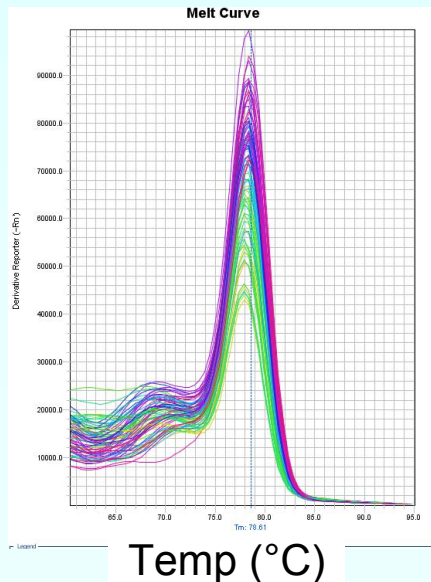
$$\Delta Cq_{GOIn}^{B-A}$$

- Les C_q sont inversement corrélé à la quantité N^0

Quand $N^0 \uparrow : C_q \downarrow$

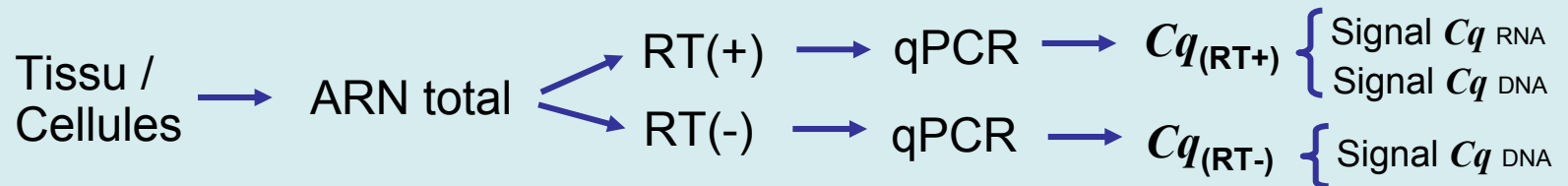
Comment s'assurer d'une bonne spécificité ?

- Nouveaux Design : Structure de gène / BLAST / Logiciel performant
- Primers publiés : BLAST / ***In-silico* PCR** (UCSC)
- Contrôles :
 - NTC « No-Target-Control » (primer-dimers)
 - RT(-) : (Réaction RT sans RTase)
(présence de l'ADN génomique)
- Post-manip :
 - Courbe de dissociation (T_m de l'amplicon)
 - Analyse des produits PCR



Electrophorèse capillaire
(MultiNA)

La RT(-) : le contrôle classique



$\Delta Cq [RT_{(-)} - RT_{(+)}] > 5$: signal dérivé de l'ADN < 3 % $\Rightarrow$ Contamination négligeable

ΔCq	RNA (%)	DNA (%)
0	0.0	100.0
1	50.0	50.0
2	75.0	25.0
3	87.5	12.5
4	93.8	6.25
5	96.9	3.1



$\Delta Cq [RT_{(-)} - RT_{(+)}] < 5$: signal dérivé de l'ADN > 3 % $\Rightarrow$ Pas de solution proposée...!!

...et pourquoi pas simplement traiter à la DNase...?

- Risque de dégradation de l'ARN (lors de l'inactivation ou la manipulation)
- Inactivation partielle $\Rightarrow$ dégradation de l'ADNc
- Composants des tampons/agent d'inactivation inhibent la RT et la PCR (max volume dans RT)
- Augmentation du coût + temps de manipulation
- Est-ce que la DNase a été efficace ? (vérification nécessaire...)

Le traitement à la DNase « classique »
n'est pas une solution optimale !



Amélioration...
Nouvelle génération de DNase : « dsDNase »
Spécifique pour l'ADN double brin
(Articzymes/Tataa Biocenter)
(Cependant >1€/point...)



Approche différente :

Quel est vraiment l'impact d'ADNg sur mon signal total ?

Gènes multi-exons:



Solution : Bon design !

Primers dans différents exons
(séparés d'un grand intron !)

Ou

Primer chevauchant sur 2 exons

Signal ADNg : **0**

► Mais il faut le valider expérimentalement !!

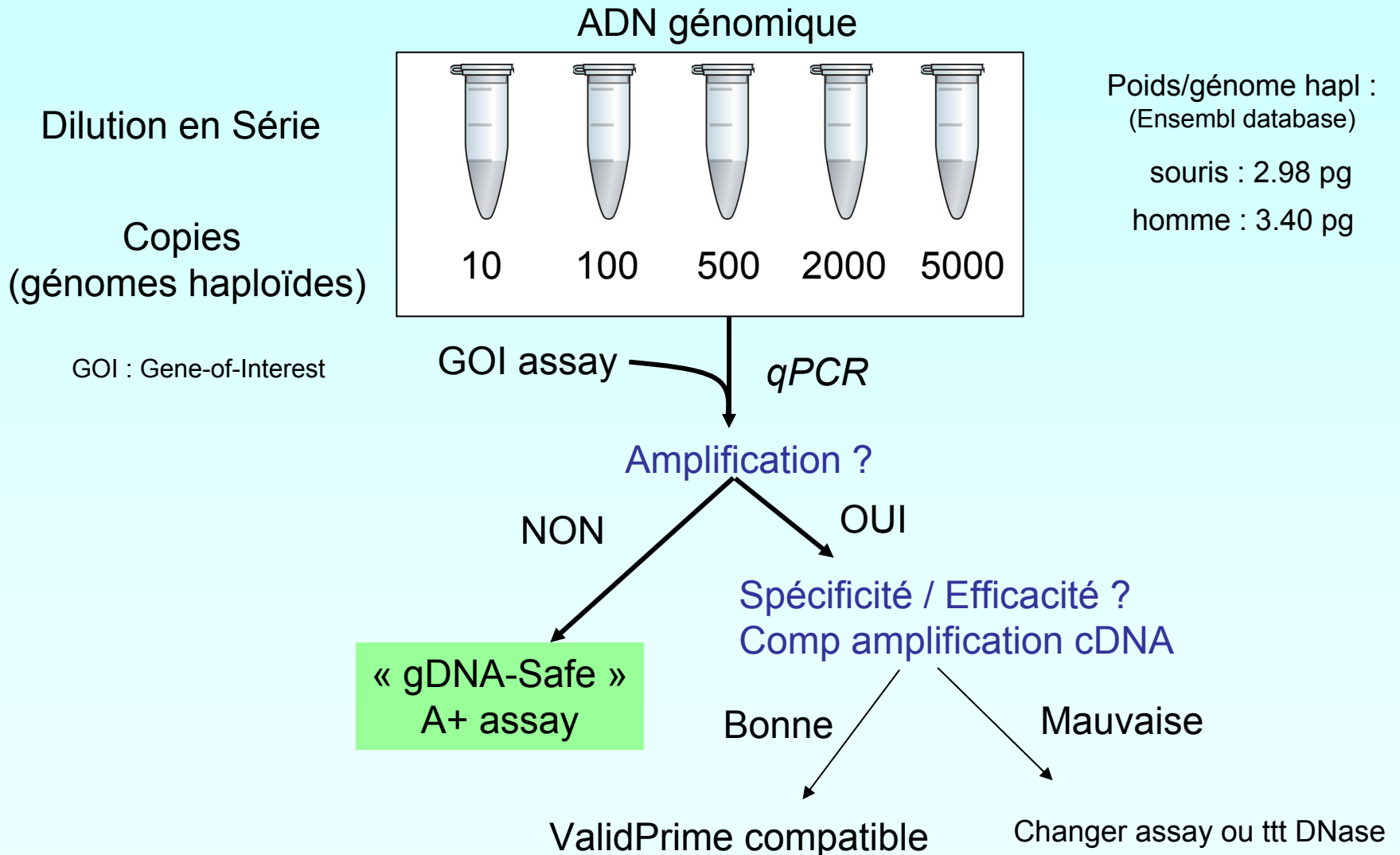
Gènes mono-exon:

Gènes avec
Pseudogènes :

► Moyen de quantifier le signal Cq_{DNA}
et de pouvoir le soustraire du signal total

ValidPrime permet de faire les deux !

Validation de qPCR assays avec ValidPrime



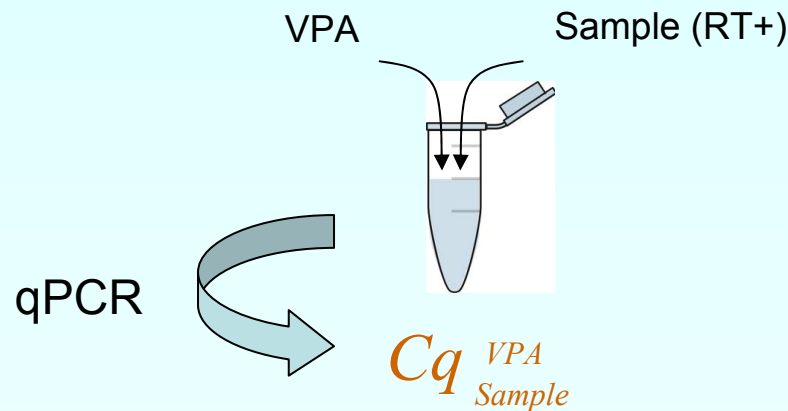
Détermination du Cq_{DNA} avec ValidPrime

Des amorces qui ciblent de l'ADNg spécifiquement



ValidPrime Assay (VPA)

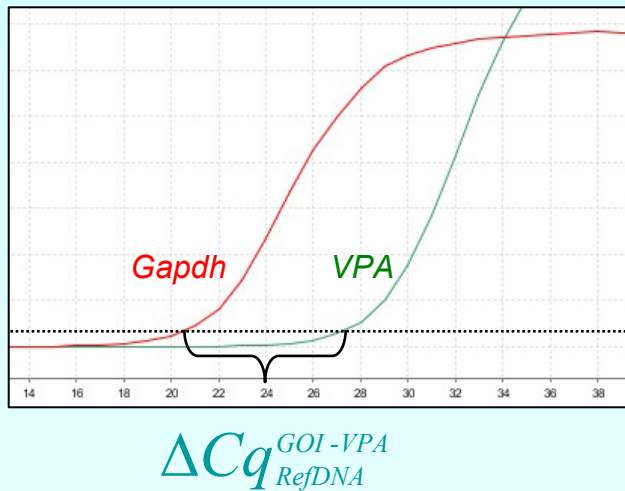
qPCR assay ciblant une région intergénique sans évidence de transcription.
1 copie par génome haploïde
(UCSC Genome browser)



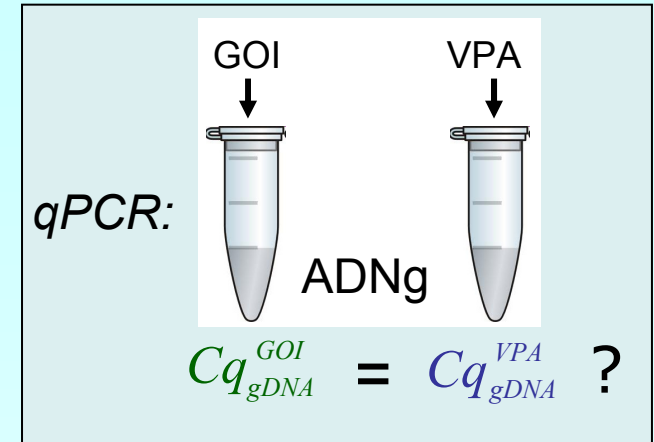
= mesure de la contamination de l'ADNg dans échantillon

Détermination du Cq_{DNA} avec ValidPrime

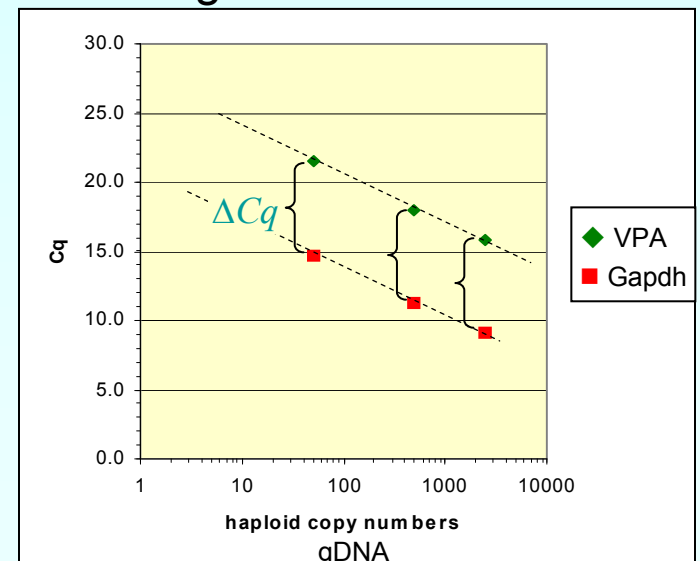
$$Cq_{DNA}^{GOI}_{Sample} = Cq_{Sample}^{VPA} + \underbrace{Cq_{gDNA}^{GOI} - Cq_{gDNA}^{VPA}}_{\Delta Cq_{gDNA}^{GOI-VPA}}$$



L'ADNg sert comme référence
« calibrateur »



Si >1 gDNA concentrations



ΔCq Constant !

Comment corriger le signal total (Cq_{NA}) par rapport au Cq_{DNA} ?

Tissus / Cellules $\rightarrow$ ARN totale $\rightarrow$ RT(+) $\rightarrow$ qPCR $\rightarrow$ $Cq_{(RT+)}$ $\left\{ \begin{array}{l} \text{Signal } Cq_{RNA} \\ \text{Signal } Cq_{DNA} \end{array} \right\} Cq_{NA}$
 NA : Nucl. Acids

$$\text{Quantité totale : } 2^{-Cq_{NA}} = 2^{-Cq_{RNA}} + 2^{-Cq_{DNA}}$$

(= Somme des « quantités relatives ARN et ADN »)

$$2^{-Cq_{RNA}} = 2^{-Cq_{NA}} - 2^{-Cq_{DNA}}$$

ValidPrime :

$$Cq_{RNA} = -\log_2 (2^{-Cq_{NA}} - 2^{-Cq_{DNA}})$$

$$Cq_{DNA} = Cq_{Sample}^{VPA} + Cq_{gDNA}^{GOI} - Cq_{gDNA}^{VPA}$$

Alternative :

$$Cq_{RNA} = -\log_2 (2^{-Cq_{RT+}} - 2^{-Cq_{RT-}})$$

ValidPrime : Plan expérimental

- ✓ Rajouter le VPA parmi les assays
- ✓ Rajouter l'ADNg parmi les échantillons

Cq_{NA}	GOI 1	GOI 2	GOI 3	VPA
Sample 1	27.22	25.78	28.67	29.02
Sample 2	26.73	25.54	28.02	26.97
Sample 3	26.42	25.31	27.68	26.34
gDNA	29.62	29.41	30.60	28.61

Cq_{DNA}

$$Cq_{DNA} = Cq_{Sample}^{VPA} + Cq_{gDNA}^{GOI} - Cq_{gDNA}^{VPA}$$

$$Cq_{DNA_{SI}}^{GOI1} = 29.02 + 29.62 - 28.61 = 30.03$$

Signal provenant de l'ADNg

Cq_{RNA}

$$Cq_{RNA} = -\log_2(2^{-Cq_{NA}} - 2^{-Cq_{DNA}})$$

$$Cq_{RNA_{SI}}^{GOI1} = -\log_2(2^{-27.22} - 2^{-30.03}) = 27.44$$

Signal provenant de l'ARN (ADNc)

$\%DNA$

$$\%DNA = (2^{-Cq_{DNA}} / 2^{-Cq_{NA}}) * 100$$

$$\%DNA_{SI}^{GOI1} = (2^{-30.03} / 2^{-27.22}) * 100 = 14.3 \%$$

Fraction du signal total
provenant de l'ADNg

Pourquoi pas
simplement faire des
contrôles RT(-)...?!



La RT(-) : un contrôle imparfait....(1)

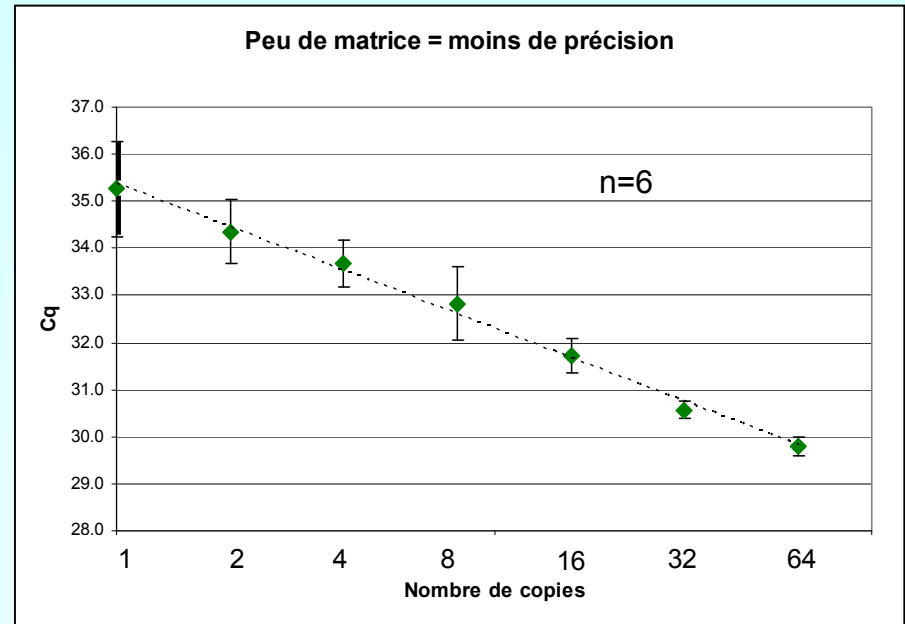
1/ Les amorces « GOI » sont conçues pour amplifier de l'ADNc, pas de l'ADNg

2/ RT(-) : souvent Cq élevés : précision de la mesure diminue (effets stochastiques)

Table 1. True target copy number*			
95% Confidence interval			
Target copy Number	Range low	Range high	Deviation
8	3	13	±62%
40	28	52	±30%
200	173	228	±14%
1,000	938	1,062	±6%

*Poisson-distribution-based estimates of the true target copy number in an aliquot pipetted from a highly diluted solution of known concentration.

Stenman & Orpana (Nat. Biotechnol. 2001)



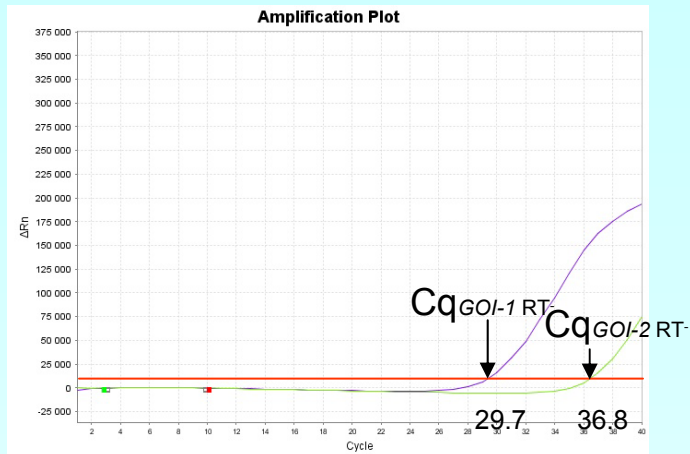
Moyennes +/-SD

Cq > 30 : SD 0.3-1.0

Cq < 30 : SD 0.1-0.3

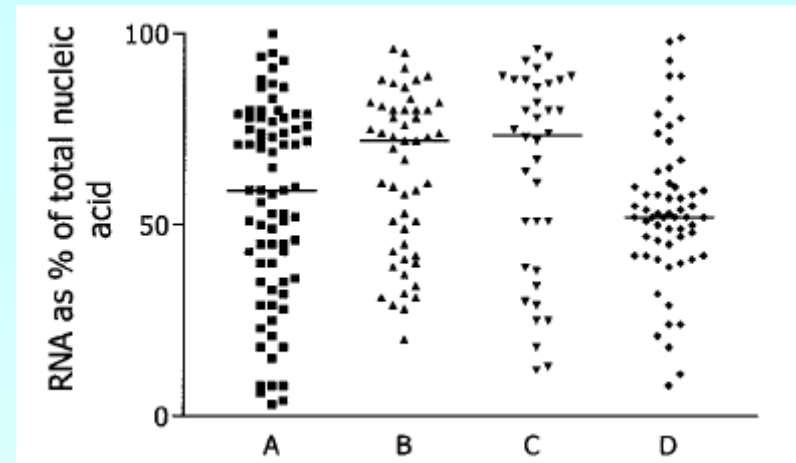
La RT(-) : un contrôle imparfait....(2)

$Cq_{RT(-)}$: Gène (GOI) -dépendent !!



GOI : Gene-of-Interest

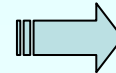
$Cq_{RT(-)}$: Echantillon -dépendent !!



Purification par RNase-easy
Ratio conc avant et après traitement DNase
(Bustin 2002)

Le signal RT(-) dépend des « assays » **ET** des échantillons

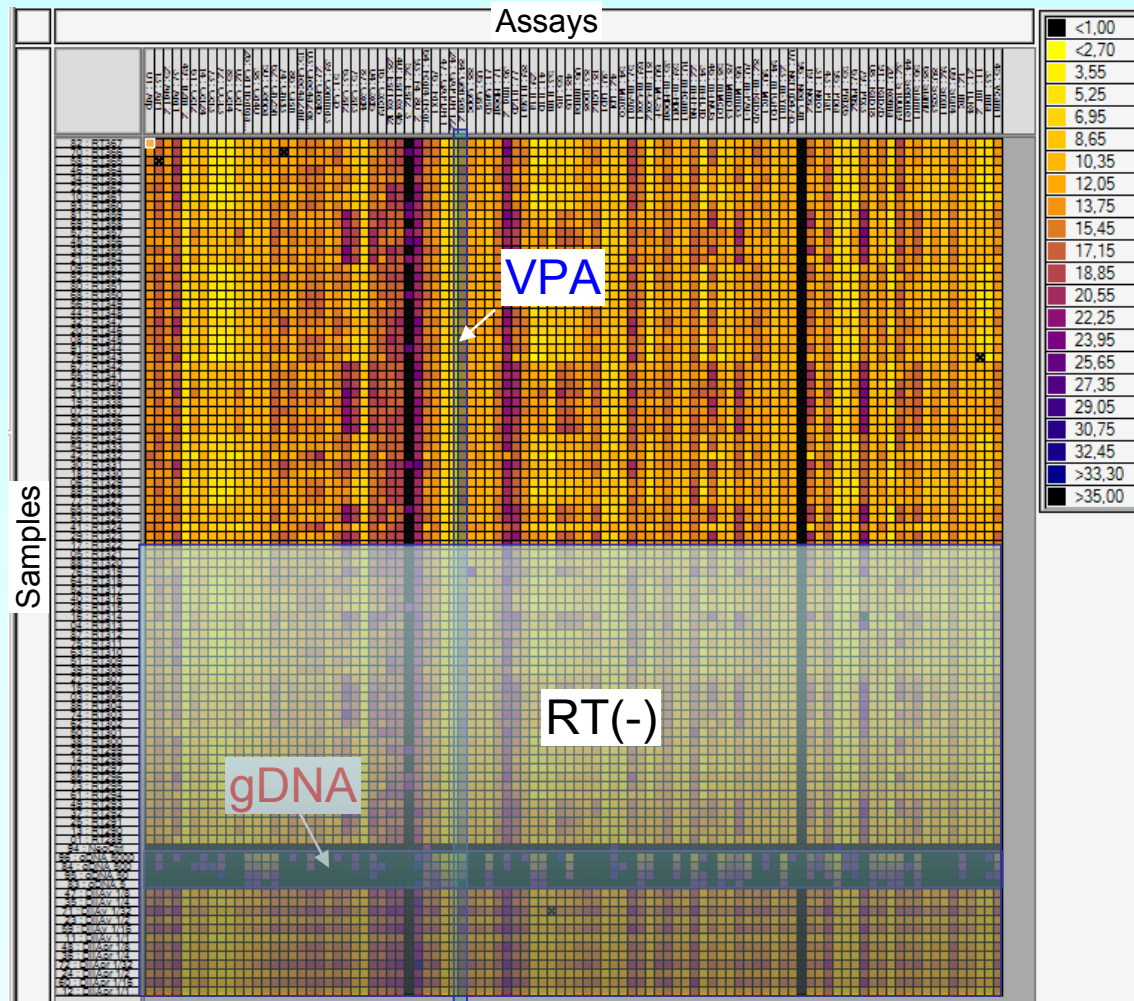
Nombre de contrôles requis : $m + m \times n$
 m : nombre d'échantillons
 n : nombre d'assays
RT(-) qPCR



>50% des réactions = Contrôles !!

« ValidPrime » réduit considérablement le nombre de contrôles

Ex. technologie Fluidigm/BioMark



VPA : ValidPrime Assay

gDNA : ADN génomique

RT(-) approach :

$m \text{ samples} * n \text{ assays}$

50 % utilisé pour des ctrl nég !!

$48 * 96 = 4608 \text{ qPCR} + 48 \text{ RT(-)} = \mathbf{4656}$

« ValidPrime »

$m + n + 1$

$48 + 48 + 1 = 97$

98 % diminution

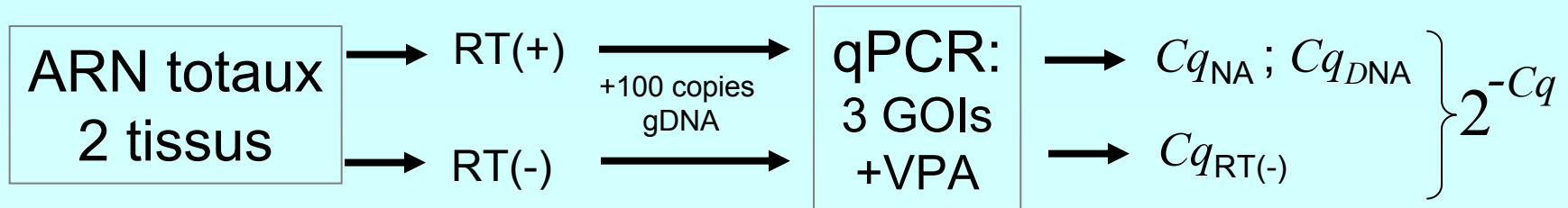
Si >1 conc (p) gDNA

$m + p \times n + p$

Si 4 conc gDNA

$48 + 192 + 4 = 246$

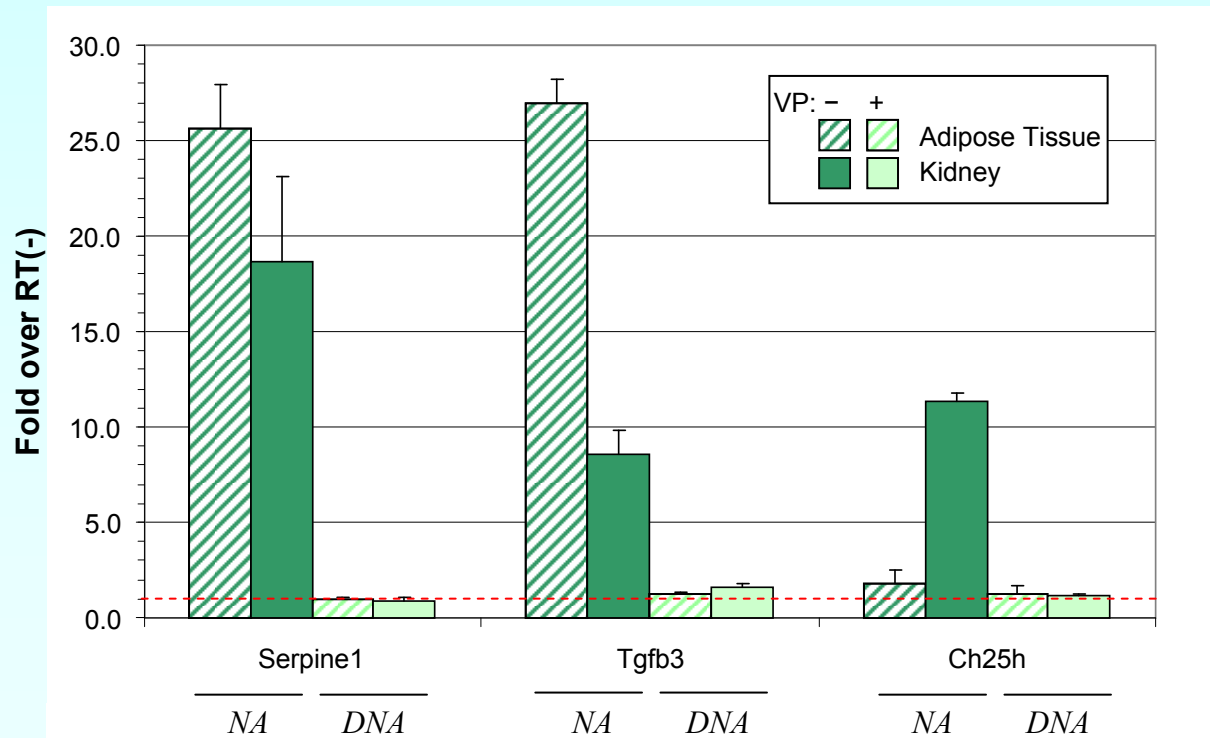
Est-ce que $Cq_{DNA} = Cq_{RT(-)}$?



$$RQ = 2^{-Cq}$$

$RQ_{NA} \text{ (ou } RQ_{DNA})$

$RQ_{RT(-)}$

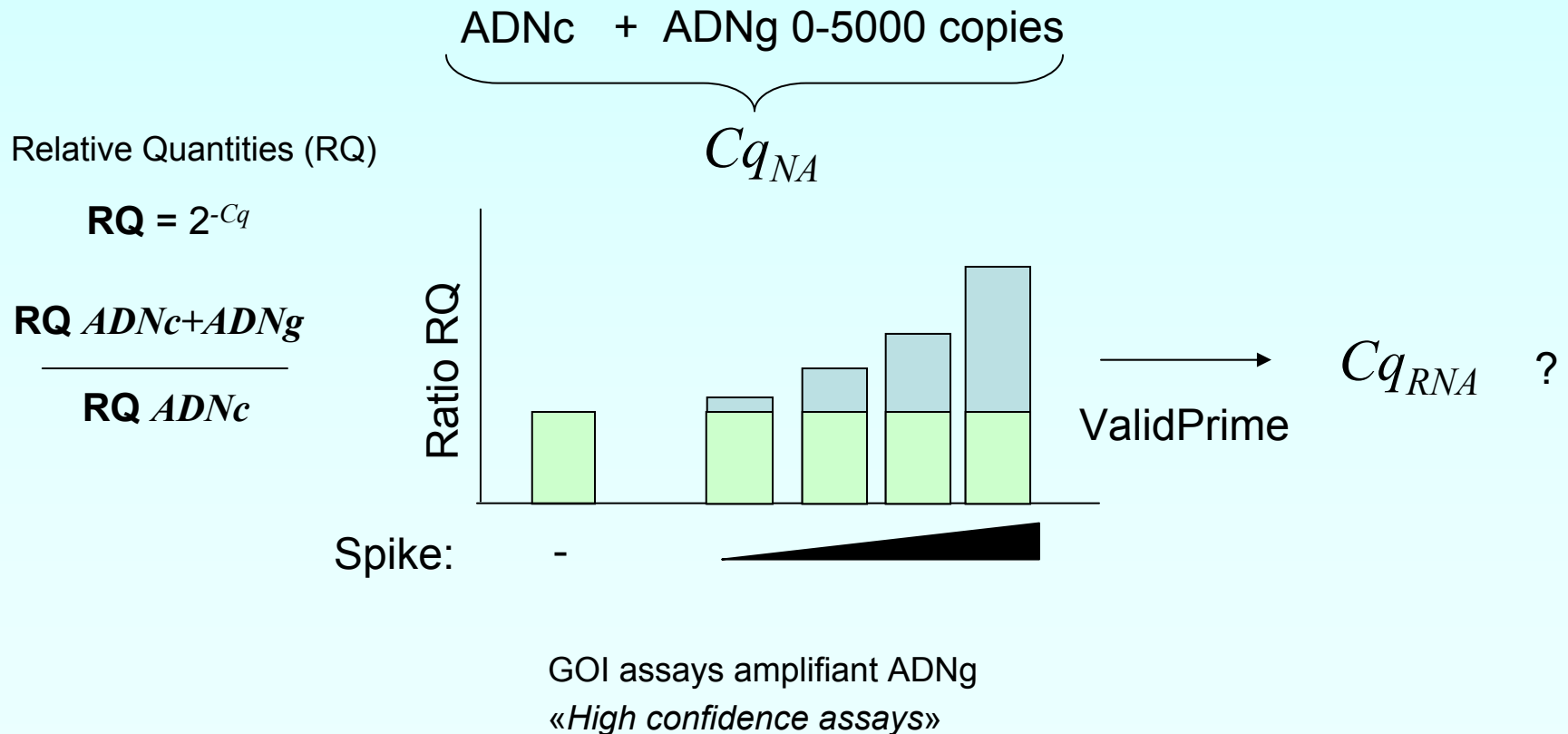


$$Cq_{DNA} \cong Cq_{RT(-)} \quad 1.20 \pm 0.29$$

Quelle est la justesse de l'estimation de Cq_{RNA} par ValidPrime...?

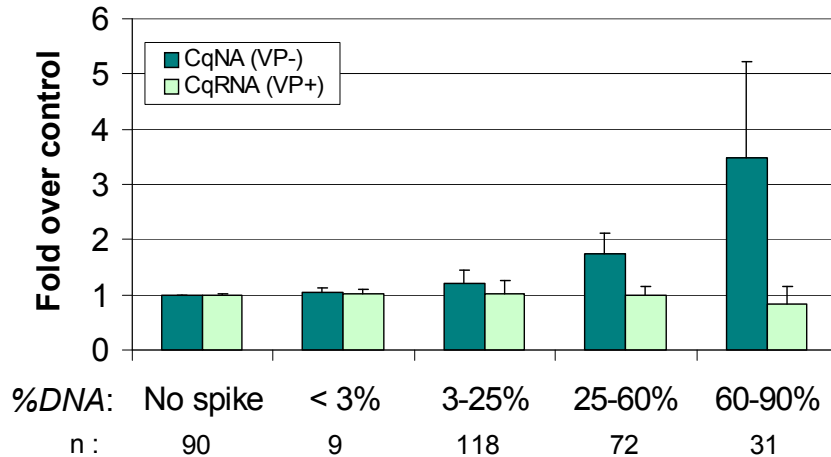
$$Cq_{RNA} = -\log_2(2^{-Cq_{NA}} - 2^{-Cq_{DNA}}) \quad = \text{correct ?}$$

Correction du signal provenant de l'ADNg exogène « spiking »



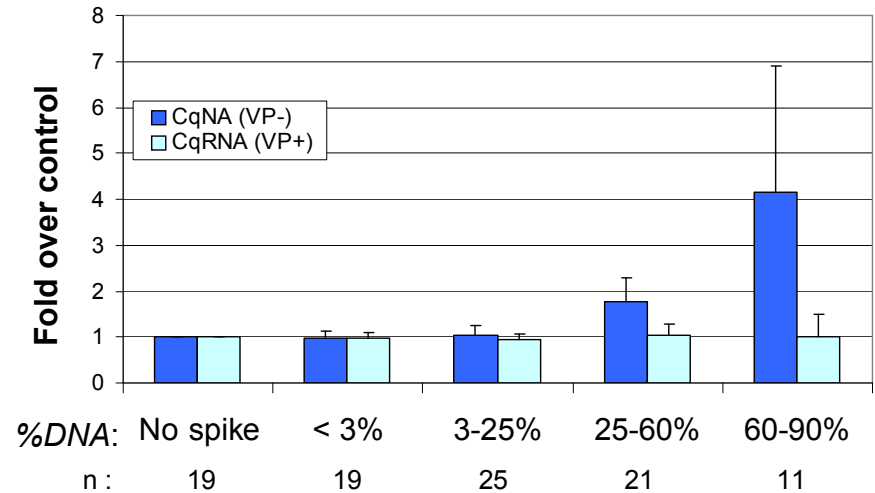
Proof-of-Principle (1) : ADNg exogène

StepOnePlus



(17 GOI assays)

BioMark



(19 GOI assays)

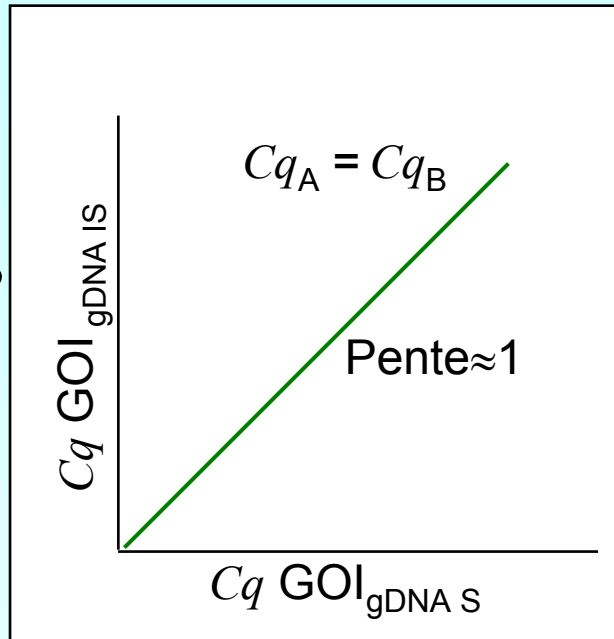
$$\%DNA = (2^{-Cq_{DNA}} / 2^{-Cq_{NA}}) * 100$$

La correction du signal de l'ADN exogène :
Très bonne à <60 %DNA

ValidPrime - Proof of principle (2) : ADNg endogène

2 assays (A & B) ciblant le même GOI

Sans ADNg



3 GOIs :
Il1b
Serpine1
Chi3l3

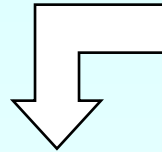
Efficacité: A = B

A: gDNA-sensitive
B: gDNA-insensitive

>50 RT(+)

qPCR

Cq_A & Cq_B

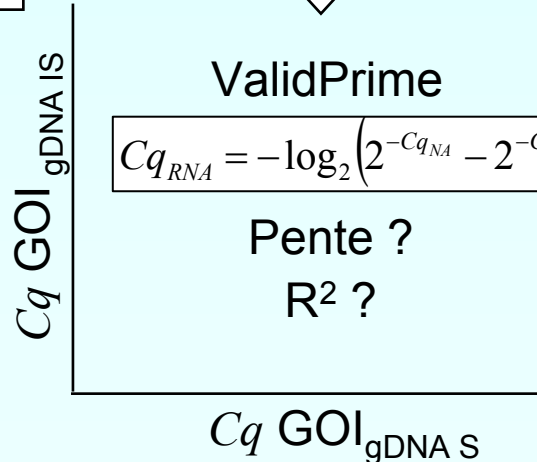


ValidPrime

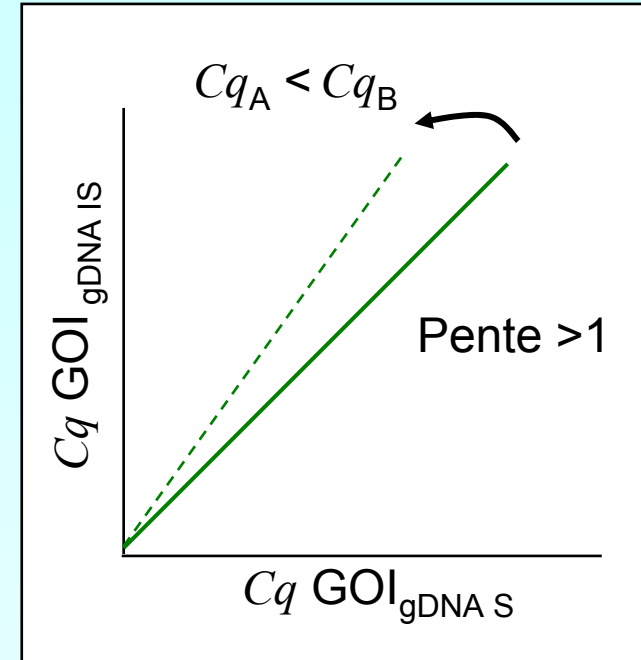
$$Cq_{RNA} = -\log_2 \left(2^{-Cq_{NA}} - 2^{-Cq_{DNA}} \right)$$

Pente ?

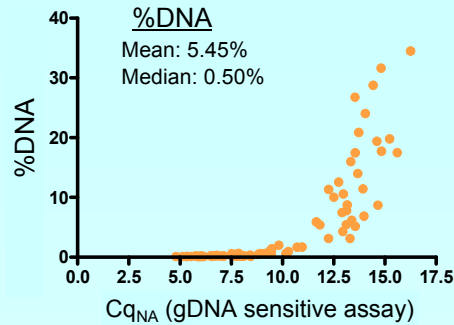
R^2 ?



Avec ADNg



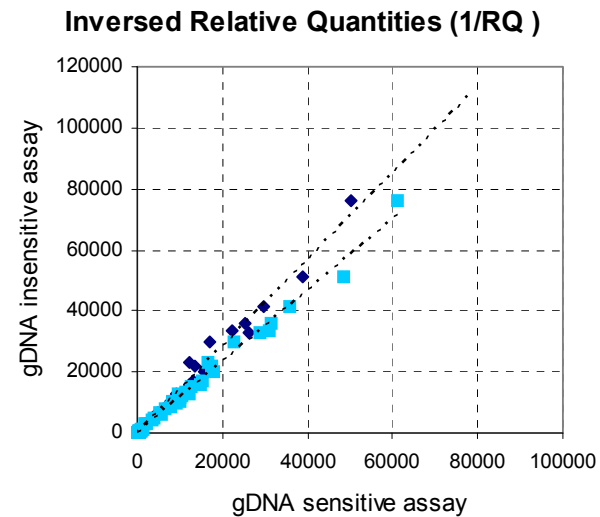
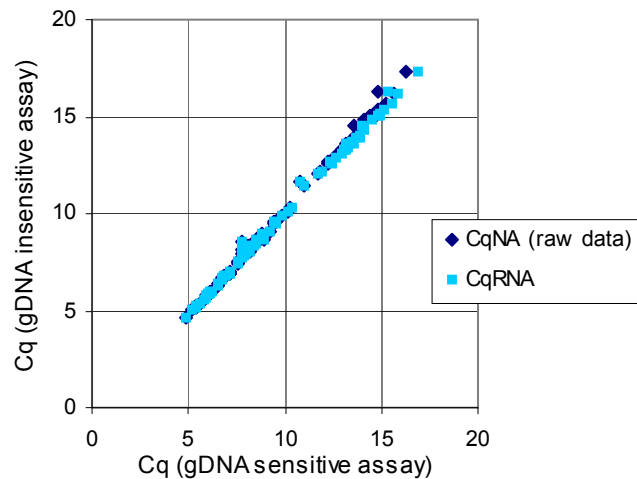
Correction des signaux provenant de l'ADNg endogène (GOI : *Il1b*)



Moyenne %DNA : 5.45%

Faible expression : %DNA $\uparrow$

Forte expression : %DNA $\downarrow$



Data type	Correction	Slope	R ²	n
CqNA	No	1.082	0.996	81
CqRNA	Yes	1.047	0.997	81

Pente : 1.08 $\Rightarrow$ 1.05

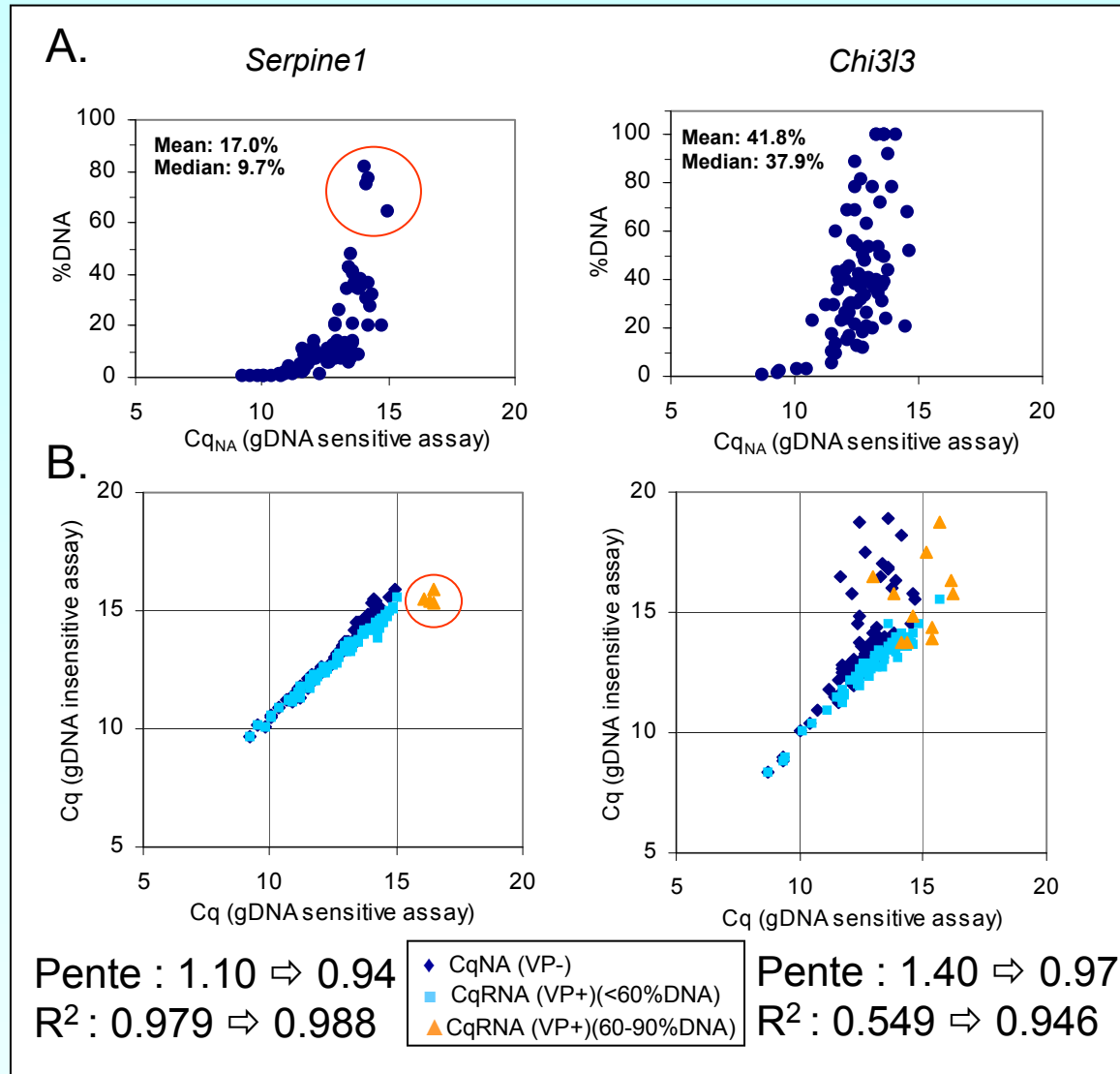
R² : 0.996 $\Rightarrow$ 0.997

Data type	Correction	Slope	R ²
1/RQ [CqNA]	No	1.420	0.987
1/RQ [CqRNA]	Yes	1.170	0.992

Pente : 1.42 $\Rightarrow$ 1.17

R² : 0.987 $\Rightarrow$ 0.992

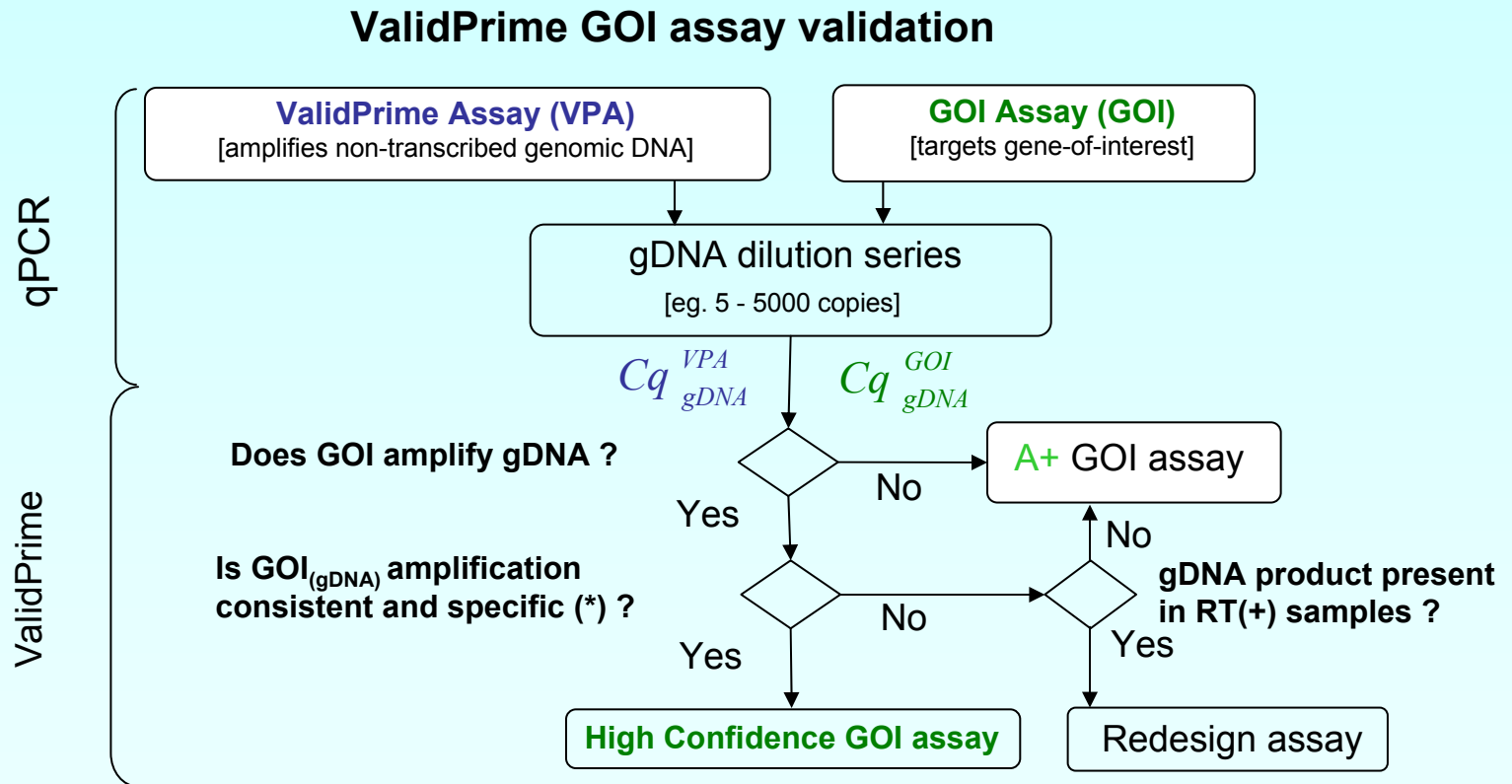
Correction des signaux provenant de l'ADNg endogène (*GOI : Serpine1 & Chi3l3*)



ValidPrime améliore la linéarité et la dispersion !

ValidPrime en pratique....

1. Vérifier la sensibilité de vos qPCR assays vis-à-vis de l'ADNg.

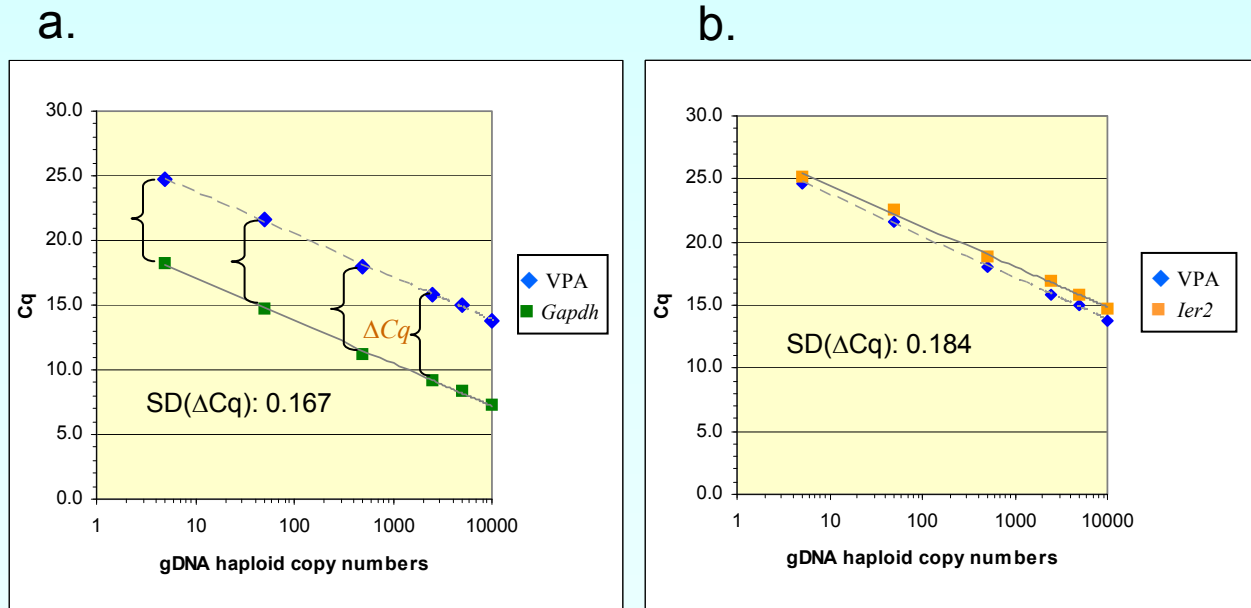


(*) Efficiency evaluation and melting curve / electrophoresis-based analysis

GOI qPCR assay : “High confidence test”

$$Cq_{DNA\ Sample}^{GOI} = Cq_{Sample}^{VPA} + \underbrace{Cq_{gDNA}^{GOI} - Cq_{gDNA}^{VPA}}_{\Delta Cq_{gDNA}^{GOI-VPA}}$$

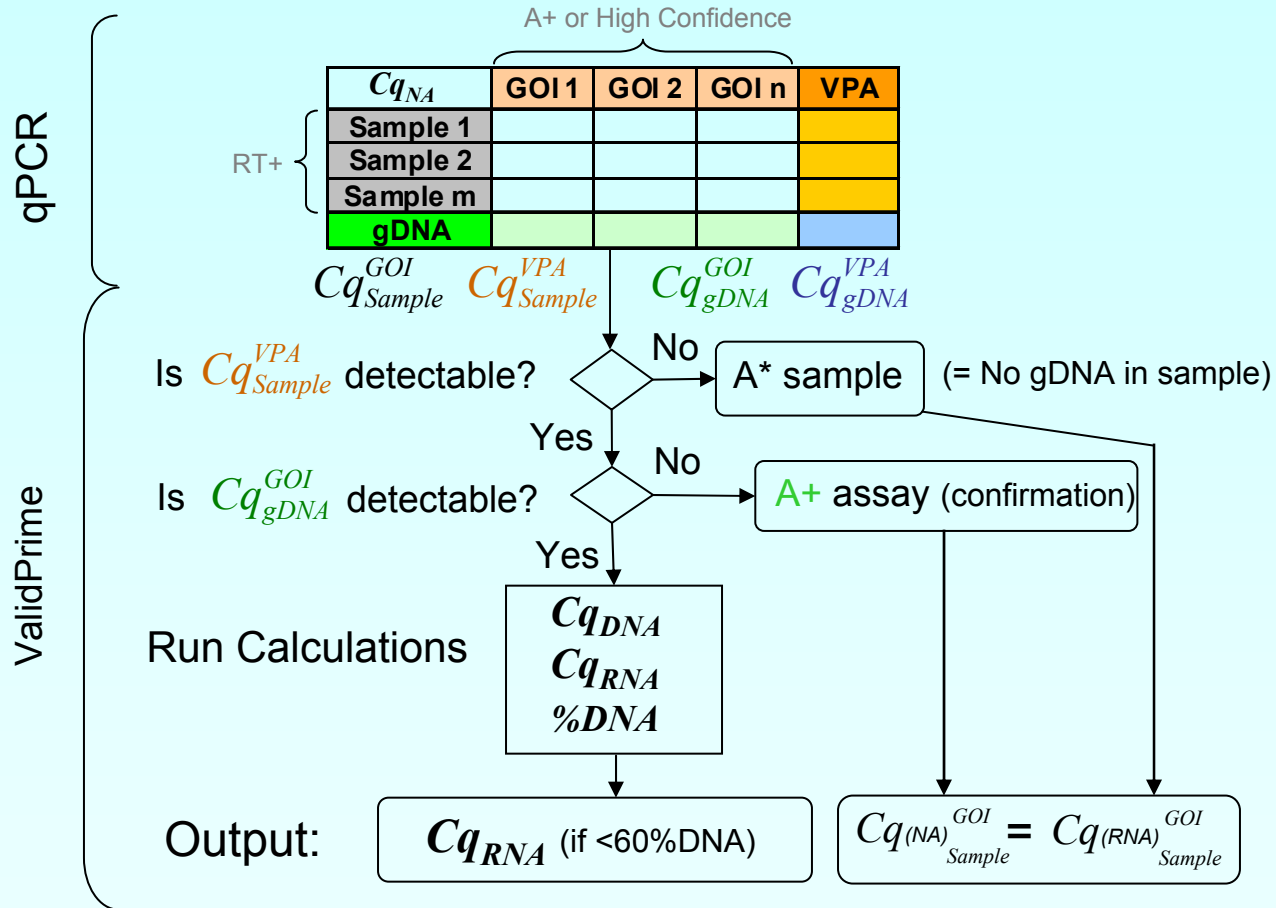
Analogie avec le méthode « $\Delta\Delta C_t$ » : GOI et VPA : Efficacités équivalentes !



High Confidence Assay : $SD(\Delta Cq) < 0.3$

ValidPrime en pratique....

Cq_{RNA} calculation with ValidPrime-validated GOI assays



Output (Cq_{RNA}) ready for downstream preprocessing analysis

ValidPrime en pratique....le gh-validprime software

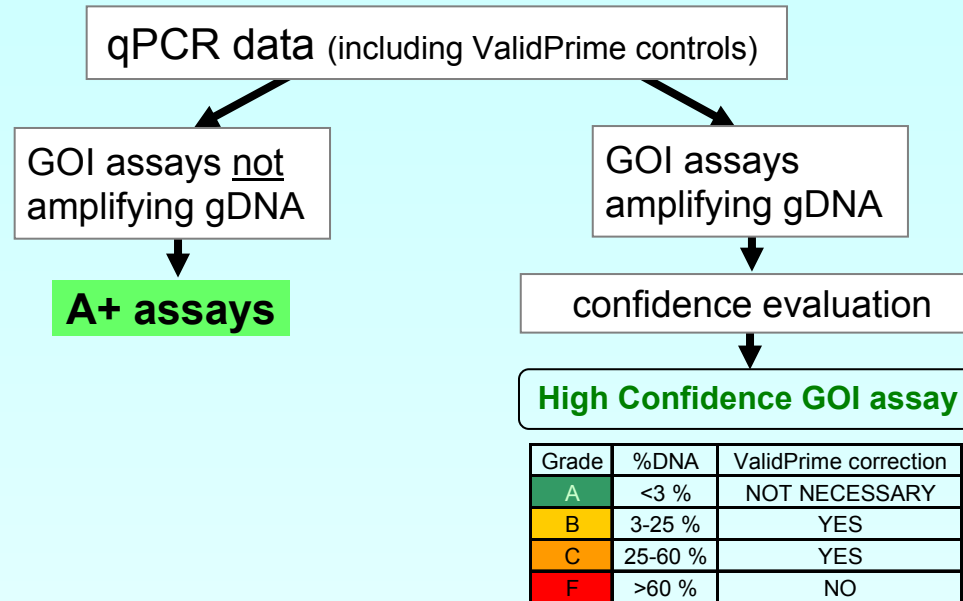
The screenshot displays the ValidPrime software interface. On the left is a control panel with sections for Load, Check, and Run. The Load section includes buttons for 'Small Demo' and 'BioMark Demo', an 'Input File' dropdown set to ':/demo2.txt', a 'Format' dropdown set to 'BioMark', a checked 'Auto find VPA/gDNA' checkbox, and a 'Configure StepOne' button. The Check section has a checked 'Validate Assays? (Is a gDNA dilution series present?)' checkbox, input fields for 'Cq cutoff' (38), 'User-flag for EXPFAIL' (FAIL), and 'Flag for NOAMP' (NOAMP), a 'Validprime Assay' dropdown set to 'VPA' with a 'Browse' button, a 'gDNA Sample(s)' input field containing '(83),gDNA 50 (#95),gDNA 500 (#84),gDNA 5000 (#96)' with a 'Browse' button, a checked 'Parse gDNA concentrations from row labels?' checkbox, a 'Check/Set [gDNA]' button, 'Ignore Assays' and 'Ignore Samples' input fields with 'Browse' buttons, and input fields for 'Min-Count for SD' (3), 'High All-SD Cutoff' (0.3), and 'High LOO-SD Cutoff' (0.2). The Run section has input fields for 'Grade A max' (3), 'Grade B max' (25), and 'Grade C max' (60), and a checked 'Correct A Samples? (not recommended)' checkbox. On the right is a data table with 8 columns: an identifier column, VPA, GO11, GO12, GO13, GO14, GO15, and GO16. The table contains 28 rows of data, each starting with an identifier like 'RT289 (#1)'.

	VPA	GO11	GO12	GO13	GO14	GO15	GO16
RT289 (#1)	17.45637786	13.56573182	9.319156403	13.51328835	15.7494485	11.23220535	11.48455453
RT297 (#2)	17.41717364	13.44317224	9.748955857	15.79384051	15.69217714	11.46584871	10.09459996
RT305 (#3)	16.4734894	14.76262853	13.57789744	14.36516659	19.70502195	12.17271618	14.84454836
RT313 (#4)	17.92815415	13.77664061	15.78810023	14.99541135	19.73703371	11.99792566	16.7592379
RT321 (#5)	17.0931535	14.00842252	10.27639343	14.81324785	18.20338261	12.20497312	12.82650908
RT329 (#6)	17.30755251	13.80243758	8.923687001	15.37558354	16.08764212	12.03915687	10.31059493
RT337 (#7)	16.15645145	14.34413414	13.00274143	12.92861597	16.8457717	11.86473039	14.11916596
RT345 (#8)	16.95502297	14.80266562	7.710306954	17.12705116	15.09251714	12.86406696	9.794595527
RT353 (#9)	15.46884438	13.73698347	8.458928188	14.93295027	14.82980038	12.0389579	9.318182286
RT361 (#10)	17.6873438	13.37398772	8.357732466	15.47499464	16.04683704	11.53462218	11.37949852
RT290 (#13)	17.45814033	14.39081855	14.78450212	14.55525195	19.43298538	11.49489126	14.37590873
RT298 (#14)	17.87040922	12.9988065	11.72794107	14.95965264	14.76882935	11.09960576	12.10951209
RT306 (#15)	17.35941899	14.4208659	13.8587892	14.21546154	19.25511087	11.62078505	14.61843364
RT314 (#16)	16.42533836	14.03572324	14.39121881	15.19064655	19.76392827	12.11441595	17.2587765
RT322 (#17)	15.29915864	13.94156595	13.19693356	12.82451117	18.37815283	11.04416427	14.71190239
RT330 (#18)	15.38112247	13.28592298	10.75566933	12.85218359	15.91051325	10.36763929	12.0372588
RT338 (#19)	16.80208608	14.35758118	13.13841799	14.97434967	18.37625592	12.26687295	13.53180488
RT346 (#20)	15.63009576	14.61484156	9.903178765	14.45577112	14.99922008	12.61280339	9.953064833
RT354 (#21)	17.3605004	13.88459482	14.45176385	13.62897101	19.82287796	11.2085229	15.87662472
RT362 (#22)	16.26400467	14.71156448	7.873578439	15.42608233	15.76067706	12.43494843	9.410246589
RT291 (#25)	17.5580902	14.45314007	14.55307503	14.85424775	20.36105277	11.37985883	14.63628504
RT299 (#26)	15.60858624	13.96630566	11.22580096	14.71629747	15.99488275	11.58874557	12.32969183
RT307 (#27)	15.64468348	14.51783959	13.12249431	13.39713185	18.24161607	11.79051107	13.24999434
RT315 (#28)	16.70419443	12.57001357	10.85270628	16.08711727	14.0837328	10.39542938	11.20675045

Jason Iacovoni (plateau bio-info; I2MC)

ValidPrime GOI assay validation and grading

A. ValidPrime GOI assay validation : ≥ 4 gDNA conc



B. ValidPrime-validated GOI assays : 1 - 3 gDNA conc

		VP correction	OutPut Data/Flags		
Grade	%DNA	High Conf Assay	A+ assays	High Conf. assays	
A+	ND	-	C _{qNA}	-	Sample independent
A	<3 %	NO	-	C _{qNA}	
B	3-25 %	YES	-	C _{qRNA}	Sample dependent
C	25-60 %	YES	-	C _{qRNA}	
F	>60 %	NO	-	HIGHDNA	

Envie de savoir plus...

Nucleic Acids Research Advance Access published January 6, 2012

Nucleic Acids Research, 2012, **1–10**
doi:10.1093/nar/gkr1259

Correction of RT–qPCR data for genomic DNA-derived signals with ValidPrime

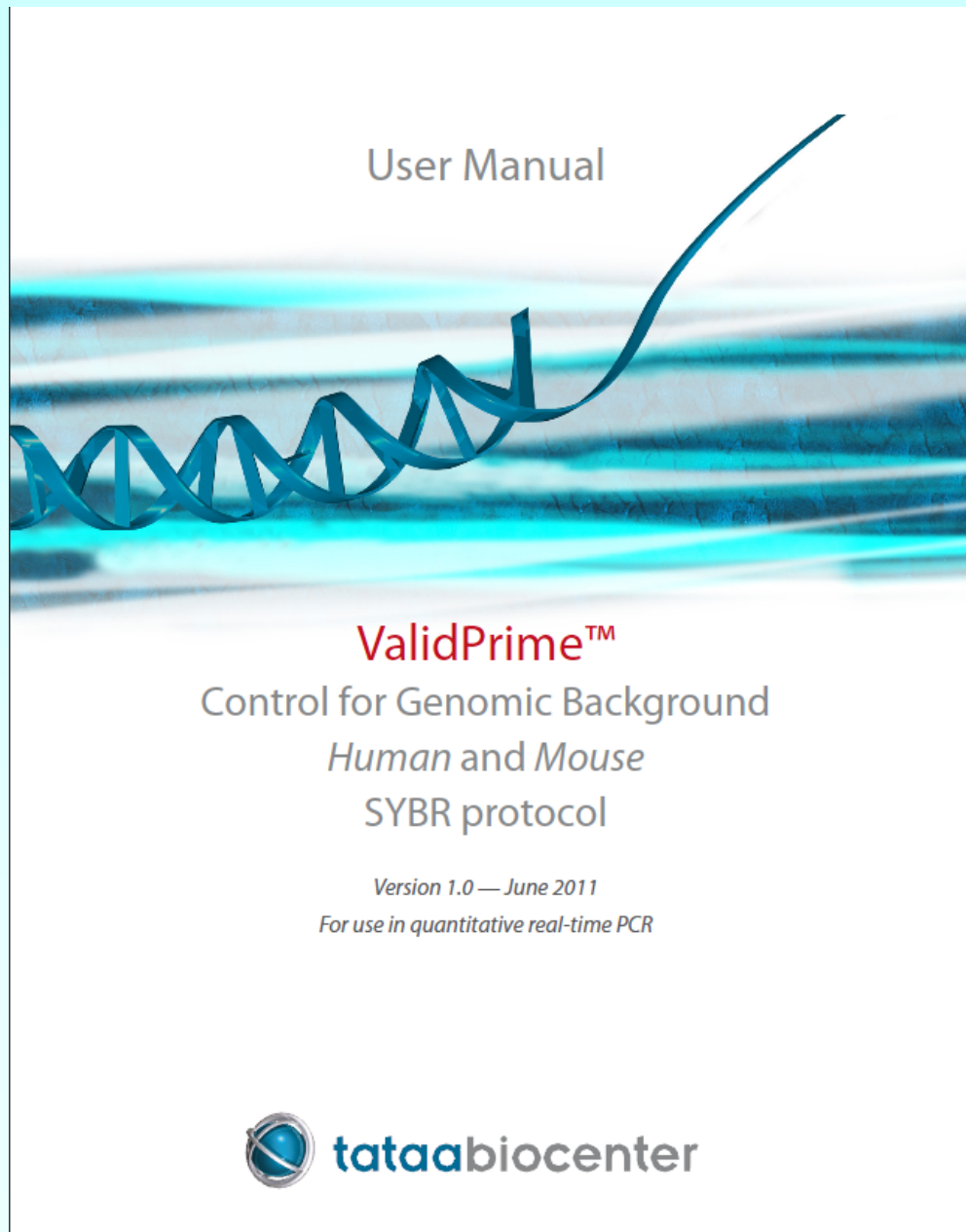
Henrik Laurell^{1,*}, Jason S. Iacovoni¹, Anne Abot¹, David Svec^{2,3}, Jean-José Maoret^{1,4}, Jean-François Arnal^{1,5} and Mikael Kubista^{2,3}

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Received September 17, 2011; Revised November 21, 2011; Accepted December 5, 2011

henrik.laurell@inserm.fr

Envie d'essayer ValidPrime...



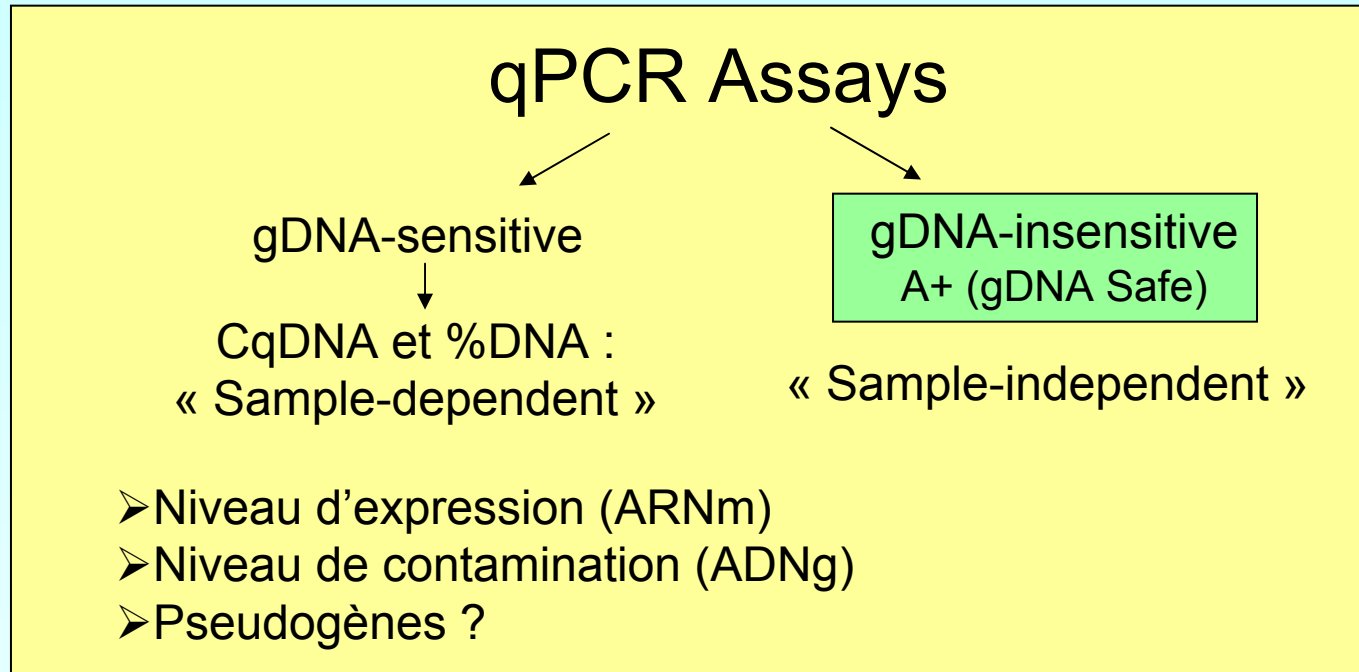
- ▶ Human
- ▶ Mouse
- ▶ “Universal”
vertebrate

“SYBR”
ou
TaqMan

Distributeur
France :



Quelques notions à retenir

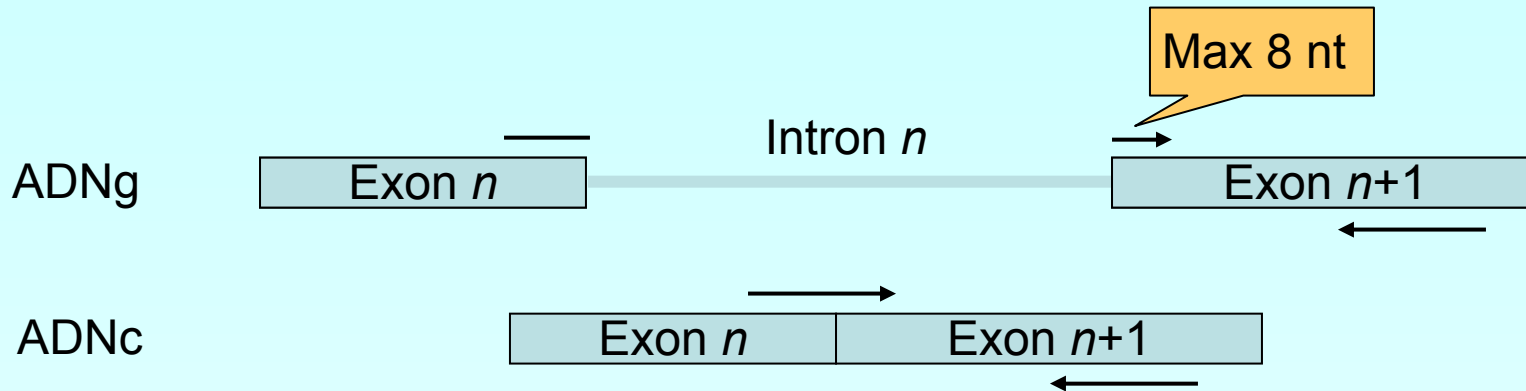


Prenez soin du design de vos « assays »
et
Vérifier *in-silico* ceux des autres avant de vous en servir...

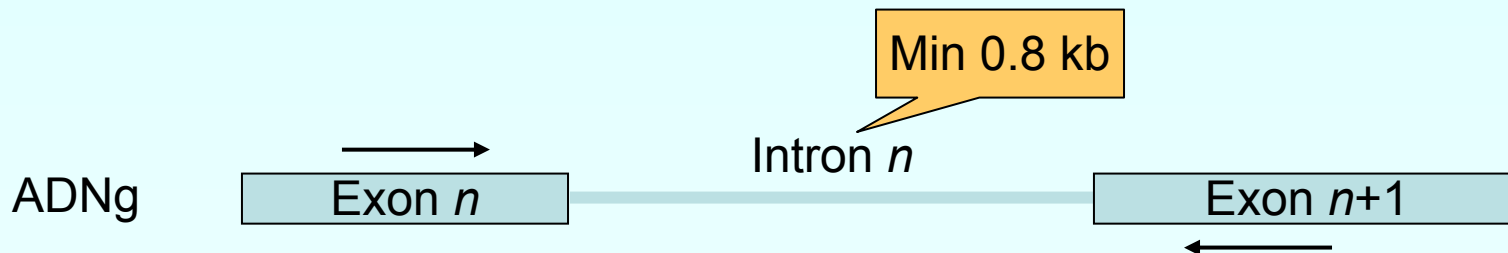


Comment désigner « **A+** (gDNA Safe) » assays

1) Un primer chevauchant entre deux exons



2) Primers dans deux exons séparés par un grand intron



Primer Blast (NCBI) / In-silico PCR _Genome browser (UCSC)

http://genome.ucsc.edu/

UCSC Genome Bioinformatics

Genomes **Blat** Tables Gene Sorter **PCR** VisiGene Proteome Session FAQ Help

About the UCSC Genome Bioinformatics Site

Welcome to the UCSC Genome Browser website. This site contains the reference sequence and working draft assemblies for a large collection of genomes. It also provides portals to the [ENCODE](#) and [Neandertal](#) projects.

We encourage you to explore these sequences with our tools. The [Genome Browser](#) zooms and scrolls over chromosomes, showing the work of annotators worldwide. The [Gene Sorter](#) shows expression, homology and other information on groups of genes that can be related in many ways. [Blat](#) quickly maps your sequence to the genome. The [Table Browser](#) provides convenient access to the underlying database. [VisiGene](#) lets you browse through a large collection of *in situ* mouse and frog images to examine expression patterns. [Genome Graphs](#) allows you to upload and display genome-wide data sets.

The UCSC Genome Browser is developed and maintained by the Genome Bioinformatics Group, a cross-departmental team within the Center for Biomolecular Science and Engineering ([CBSE](#)) at the University of California Santa Cruz ([UCSC](#)). If you have feedback or questions concerning the tools or data on this website, feel free to contact us on our [public mailing list](#).

News

News Archives ►

To receive announcements of new genome assembly releases, new software features, updates and training seminars by email, subscribe to the [genome-announce](#) mailing list.

07 March 2012 - New Mouse Browser Available

We have released the latest Genome Browser for the December 2011 Mouse genome assembly produced by the [Mouse Genome Reference Consortium](#) (Genome Reference Consortium GRCh38, UCSC version mm10). This version, which includes approximately 2.6 Gb of sequence, is considered to be "essentially complete". The assembly includes chromosomes 1-19, X, Y, M (mitochondrial DNA) and chr*_random (unlocalized) and chrUn_* (unplaced clone contigs). Note that the UCSC mm10 database contains only the reference strain C57BL/6J.

Bulk downloads of the sequence and annotation data are available via the Genome Browser [FTP server](#) or the [Downloads](#) page. The Mouse browser annotation tracks were generated by UCSC and collaborators worldwide. See the [Credits](#) page for a detailed list of the organizations and individuals who contributed to the success of this release.

29 February 2012 - RGD Genes available for rn4

We are pleased to announce the release of RGD Genes for rat (Baylor 3.4/rn4). This track shows protein-coding gene annotations curated by [RGD](#). This track replaces UCSC Known Genes as the main gene track for this assembly.

More details about this track can be found on the [RGD Genes track description page](#).

We'd like to thank RGD for providing the base annotations for this track. The RGD Genes track was produced by Fan Hsu, Mary Goldman and Hiram Clawson.

10 February 2012 - dbSNP 135 Available for hg19: We are pleased to announce the release of four tracks derived from dbSNP build 135, available on the human assembly (GRCh37/hg19). [Read more.](#)

2 February 2012 - New UCSC Genes Track Released for GRCh37/hg19: We're happy to announce the release of an updated UCSC Genes track for the GRCh37/hg19 human Genome Browser. [Read more.](#)

==> [News Archives](#)

Genome
Browser

ENCODE

Neandertal

Blat

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Browser

Gene Sorter

In Silico PCR

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In-silico PCR : un outil très utile pour la qPCR

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UCSC In-Silico PCR

Genome:

Assembly:

Target:

Forward Primer:

Reverse Primer:

Max Product Size:

Min Perfect Match:

Min Good Match:

Flip Reverse Primer: ☐

About In-Silico PCR

In-Silico PCR searches a sequence database with a pair of PCR primers, using an indexing strategy for fast performance.

Configuration Options

Genome and Assembly - The sequence database to search.
Target - If available, choose to query transcribed sequences.
Forward Primer - Must be at least 15 bases in length.
Reverse Primer - On the opposite strand from the forward primer. Minimum length of 15 bases.
Max Product Size - Maximum size of amplified region.
Min Perfect Match - Number of bases that match exactly on 3' end of primers. Minimum match size is 15.
Min Good Match - Number of bases on 3' end of primers where at least 2 out of 3 bases match.
Flip Reverse Primer - Invert the sequence order of the reverse primer and complement it.



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UCSC In-Silico PCR

The sequences and coordinates shown below are from UCSC Genes, not from the genome assembly. The links lead to the Genome Browser at the position of the entire target sequence.

>[uc008gzz.1](#) [Klf9:933+1016](#) 84bp GGAAACACGCCTCCGAAA GGGCTTTAAGATGGGAGGATT
GGAAACACGCCTCCGAAAagaggcacaaagtgcacctacagtggctgtggg
aaagtctatggaAAATCCTCCCATCTTAAAGCCC

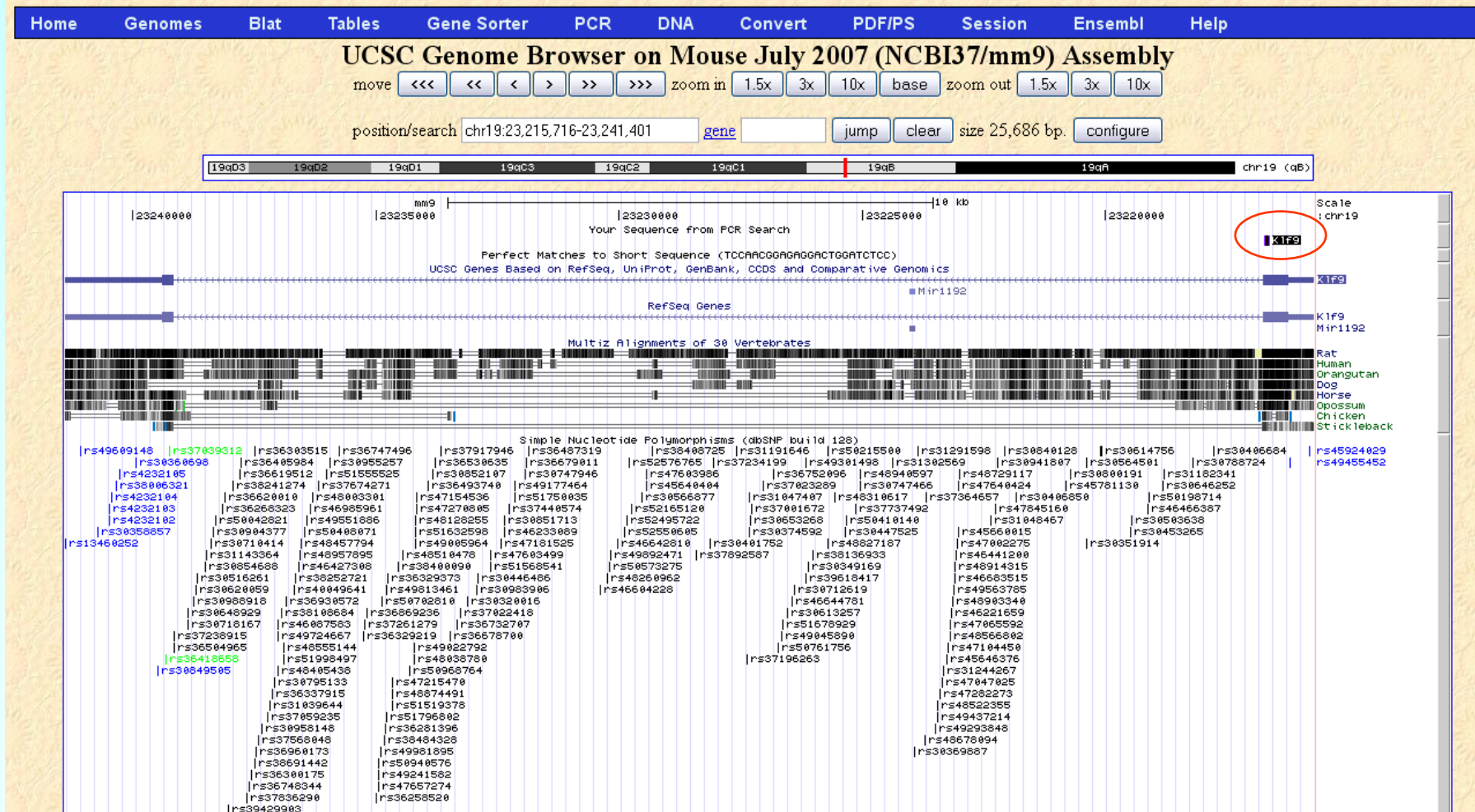
Primer Melting Temperatures

Forward: 62.1 C ggaaacacgcctccgaaa
Reverse: 61.7 C gggctttaagatgggaggattt

The temperature calculations are done assuming 50 mM salt and 50 nM annealing oligo concentration. The code to calculate the melting temp comes from [Primer3](#).

In-silico PCR/ Genome Browser

- Vue instantanée de la structure du gène
- L'amplicon ciblé par les amorces indiquée



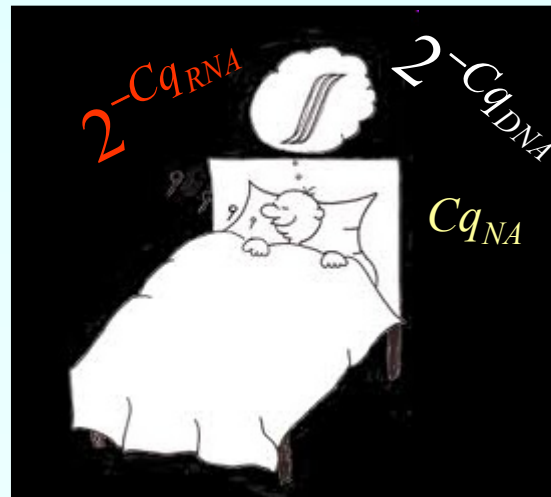
Un grand merci à



Jason Iacovoni,
Jean-José Maoret,
Mikael Kubista

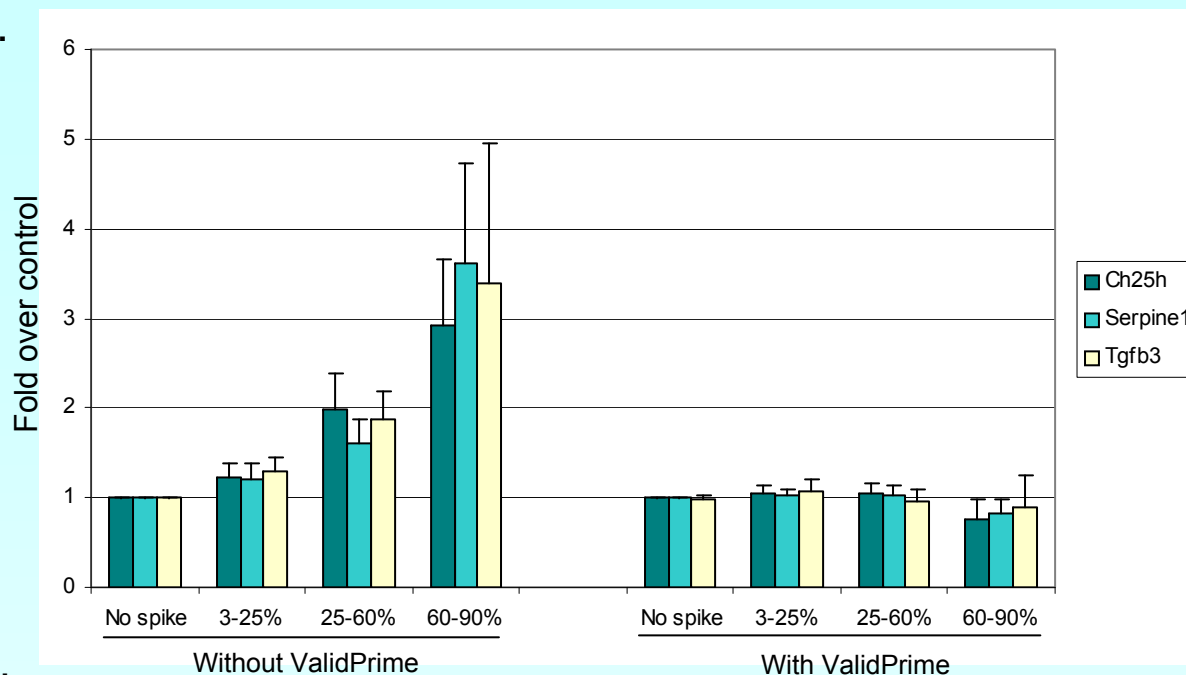


...et à vous !!



ValidPrime applied on targets with one or multiple genomic loci.

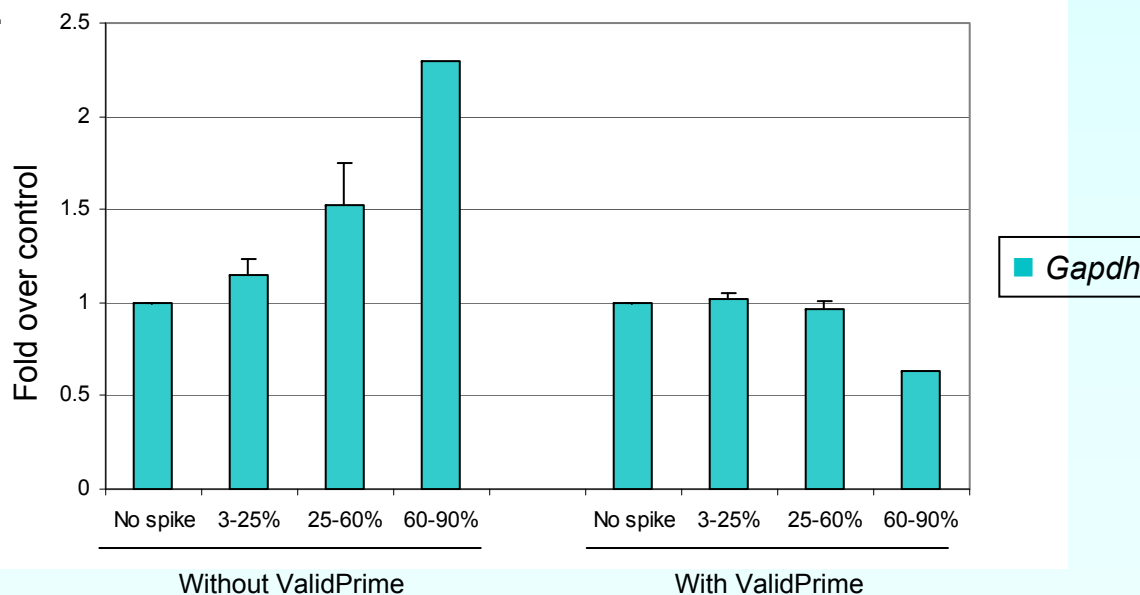
a.



n=88

mean +/-SD

b.



n=25

Derivation of Cq_{RNA}

$$(S1) \quad N_{Cq_{DNA}} = N_{DNA}^0 2^{Cq_{DNA}}$$

$$(S2) \quad N_{Cq_{RNA}} = N_{RNA}^0 2^{Cq_{RNA}-1}$$

$$(S3) \quad N_{NA} = N_{DNA} + N_{RNA}$$

$$(S4) \quad N_{Cq_{NA}} = N_{DNA}^0 2^{Cq_{NA}} + N_{RNA}^0 2^{Cq_{NA}-1}$$

$$(S5) \quad N_{Cq_{NA}} = N_{Cq_{DNA}} = N_{Cq_{RNA}}$$

$$(S6) \quad N_{DNA}^0 2^{Cq_{DNA}} = N_{DNA}^0 2^{Cq_{NA}} + N_{RNA}^0 2^{Cq_{NA}-1}$$

$$(S7) \quad N_{RNA}^0 2^{Cq_{RNA}-1} = N_{DNA}^0 2^{Cq_{NA}} + N_{RNA}^0 2^{Cq_{NA}-1}$$

$$(S8) \quad N_{RNA}^0 2^{Cq_{NA}-1} = N_{DNA}^0 (2^{Cq_{DNA}} - 2^{Cq_{NA}})$$

$$(S9) \quad N_{RNA}^0 (2^{Cq_{RNA}-1} - 2^{Cq_{NA}-1}) = N_{DNA}^0 2^{Cq_{NA}}$$

$$(S10) \quad \frac{2^{Cq_{RNA}-1} - 2^{Cq_{NA}-1}}{2^{Cq_{NA}-1}} = \frac{2^{Cq_{NA}}}{2^{Cq_{DNA}} - 2^{Cq_{NA}}}$$

$$(S11) \quad 2^{Cq_{RNA}-1} = \frac{2^{2Cq_{NA}-1}}{2^{Cq_{DNA}} - 2^{Cq_{NA}}} + 2^{Cq_{NA}-1}$$

$$(S12) \quad Cq_{RNA} = 1 + \log_2 \left(\frac{2^{2Cq_{NA}-1}}{2^{Cq_{DNA}} - 2^{Cq_{NA}}} + 2^{Cq_{NA}-1} \right)$$

$$(S13) \quad Cq_{RNA} = 1 + \log_2 \left(\frac{2^{Cq_{DNA}+Cq_{NA}-1}}{2^{Cq_{DNA}} - 2^{Cq_{NA}}} \right)$$

$$(S14) \quad Cq_{RNA} = Cq_{DNA} + Cq_{NA} - \log_2 (2^{Cq_{DNA}} - 2^{Cq_{NA}})$$

$$(S15) \quad Cq_{RNA} = -\log_2 (2^{-Cq_{NA}} - 2^{-Cq_{DNA}})$$