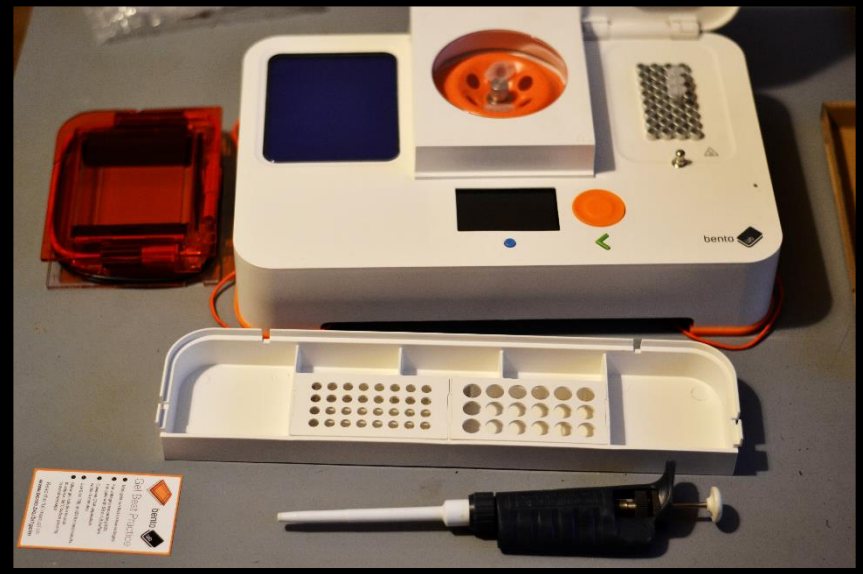
TP moléculaire :
Extraction ADN, PCR, électrophorèse



Stage biospéléo 16-17 octobre 2021

Week-end en salle, conférence et TP

Marina FERRAND

Fédération Française de Spéléologie

COMITE DEPARTEMENTAL DE SPELEOLOGIE.

CDS 94

BIOCAF

INVENTAIRE BIOSPELEOLOGIQUE DES CARRIERES SOUTERRAINES FRANCILIENNES

eesc

Travaux pratiques

TP1 : EXTRACTION DE L'ADN

Espèce collectée
à identifier

Extraction de
Son ADN

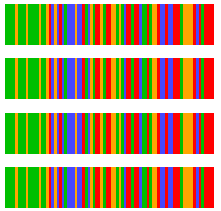
Ethanol 90%

Tampon

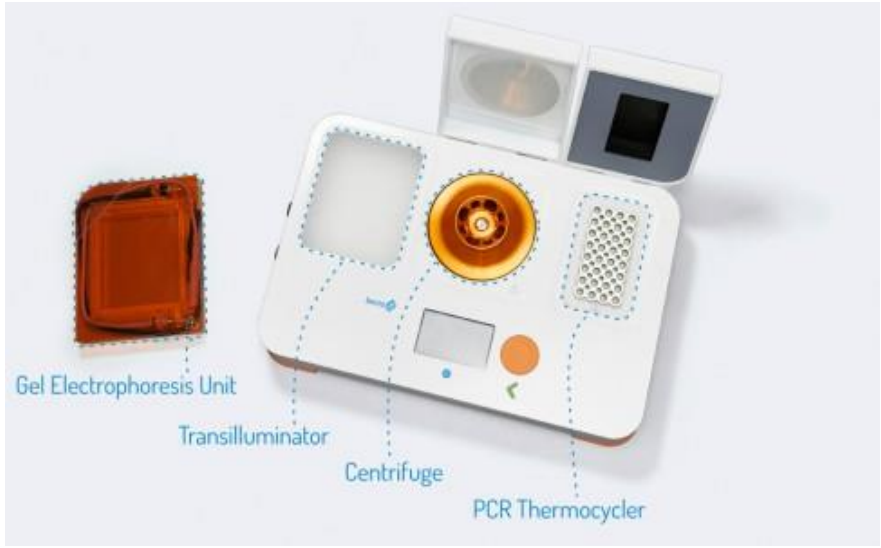
TP2 : PCR

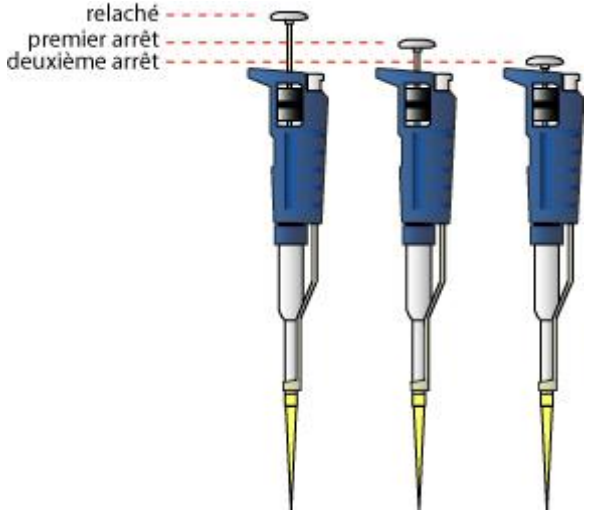
Amplifier le gène
Standard de barcoding

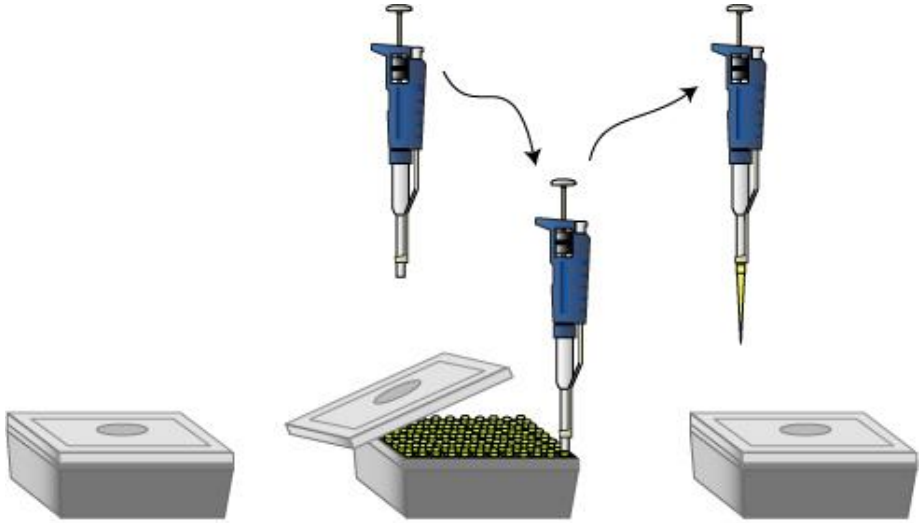
COI



PCR





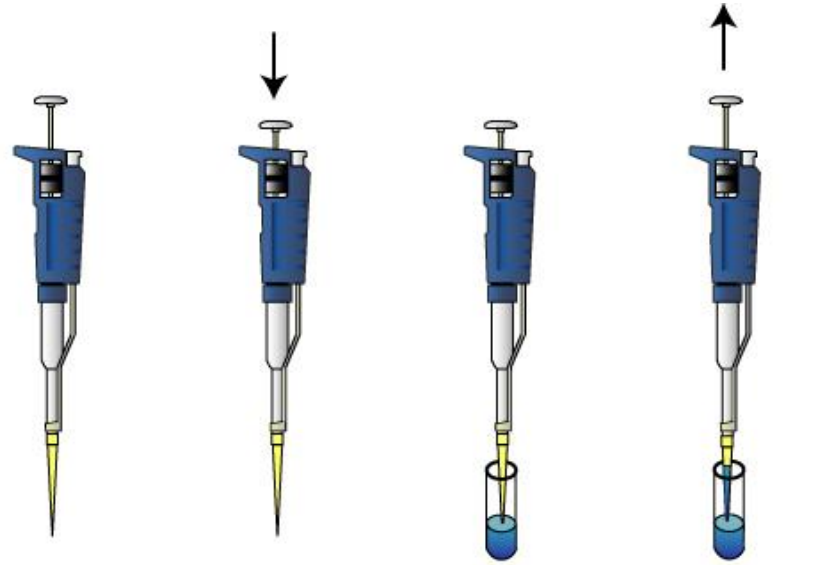


1. Ouvrir la boîte de pointes.

2. Prendre une pointe à l'aide de la pipette

3. Refermer la boite

ATTENTION: Ne jamais pipeter de liquide sans pointe

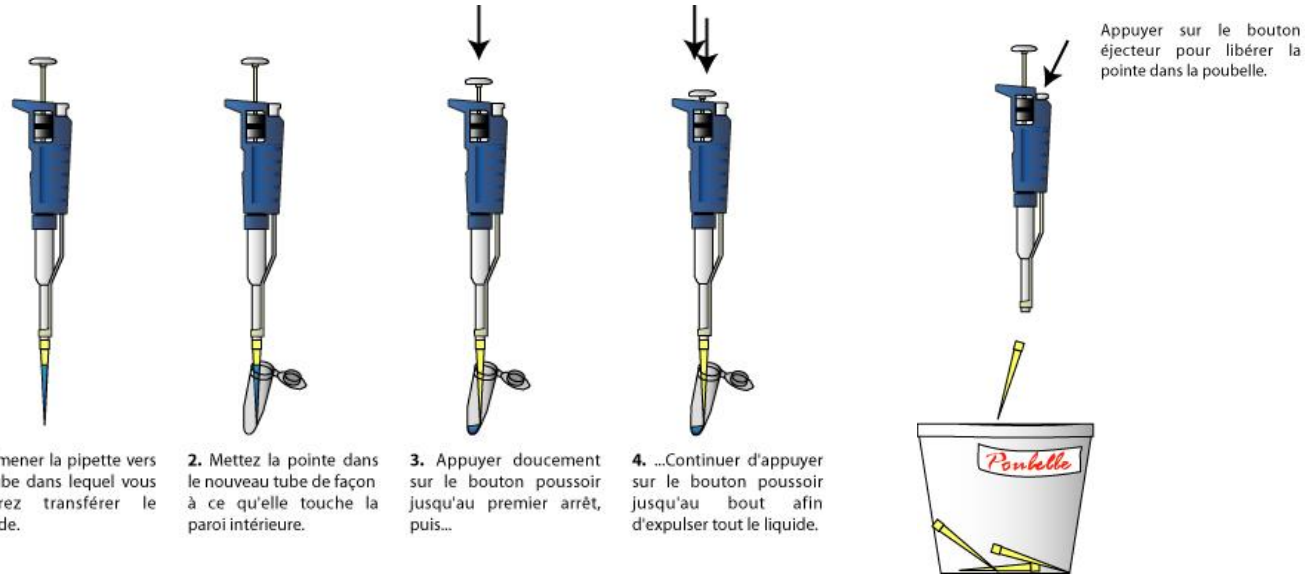


1. Régler la pipette et mettre une pointe.

2. Appuyer jusqu'au premier arrêt.

3. Mettre l'extémité de la pointe dans la solution à prélever.

4. Relacher le piston doucement.



1. Amener la pipette vers le tube dans lequel vous désirez transférer le liquide.

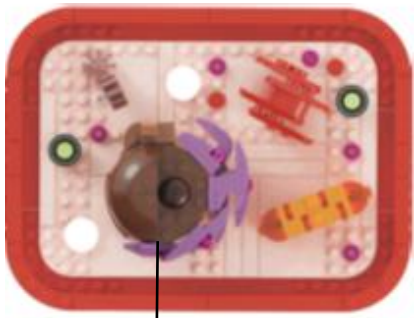
2. Mettez la pointe dans le nouveau tube de façon à ce qu'elle touche la paroi intérieure.

3. Appuyer doucement sur le bouton poussoir jusqu'au premier arrêt, puis...

4. ...Continuer d'appuyer sur le bouton poussoir jusqu'au bout afin d'expulser tout le liquide.

Appuyer sur le bouton éjecteur pour libérer la pointe dans la poubelle.

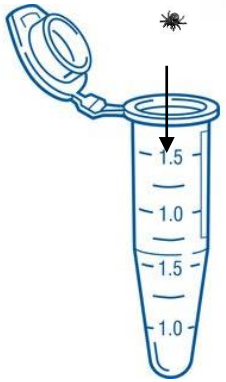
TP1 : Extraction ADN



ECHANTILLON

1er tube

échantillon



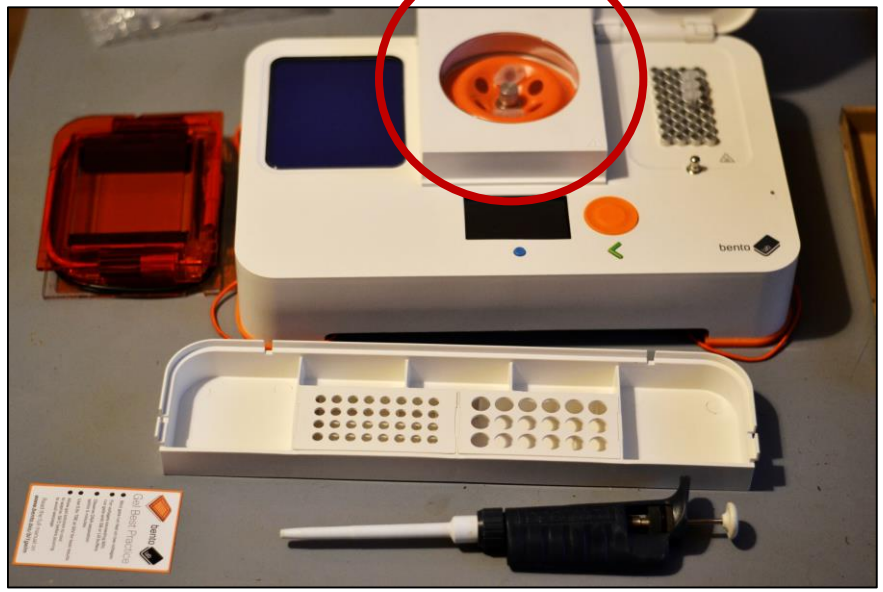
sable de Fontainebleau +200µL tampon d'extraction

BROYAGE

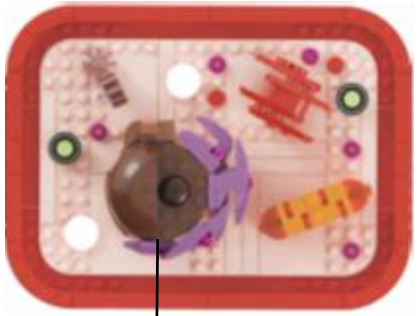
Centrifugation

Séparation des débris

Centrifugeuse



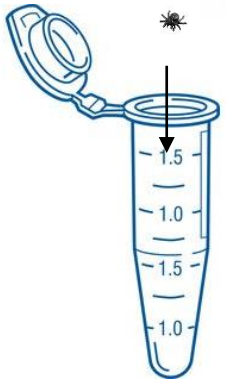
TP1 : Extraction ADN



ECHANTILLON

1er tube

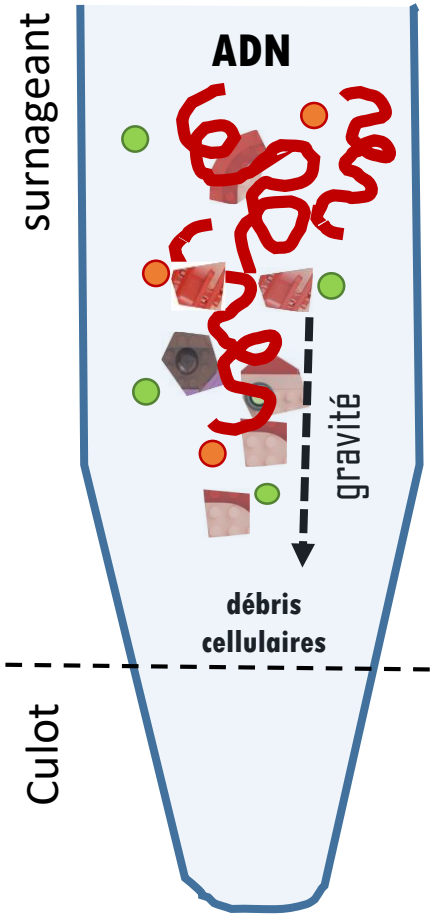
échantillon



sable de Fontainebleau +200µL tampon d'extraction

BROYAGE

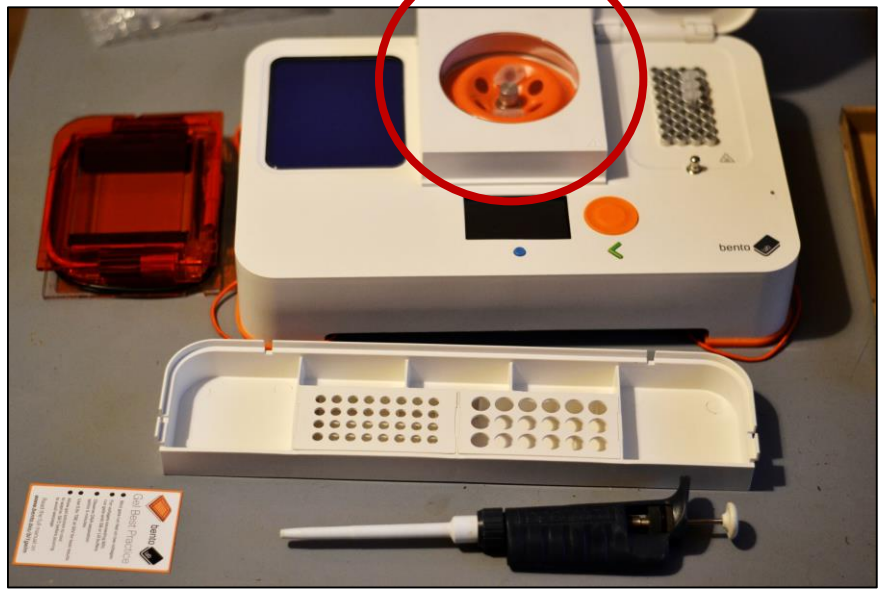
10minutes de centrifugation

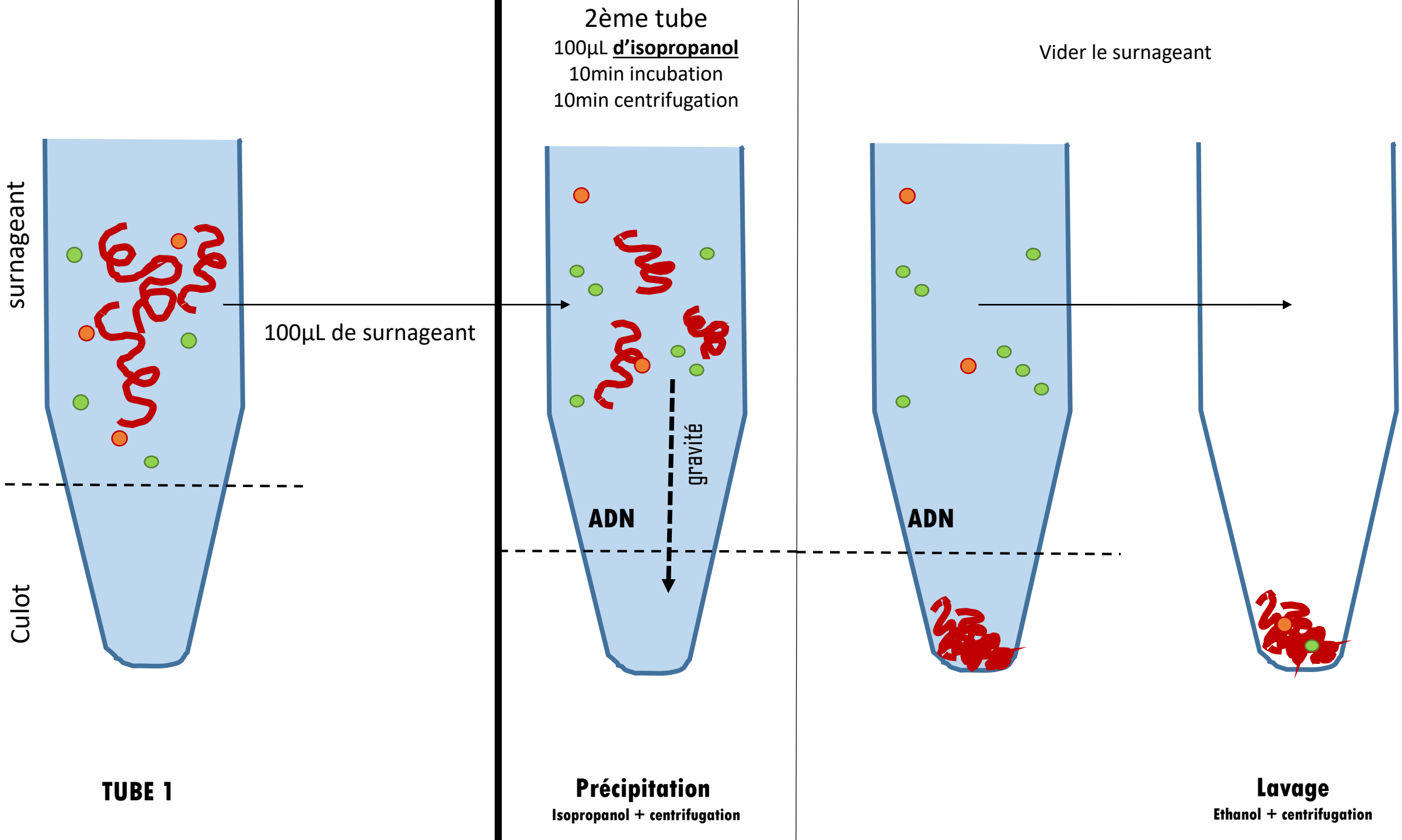


Centrifugation

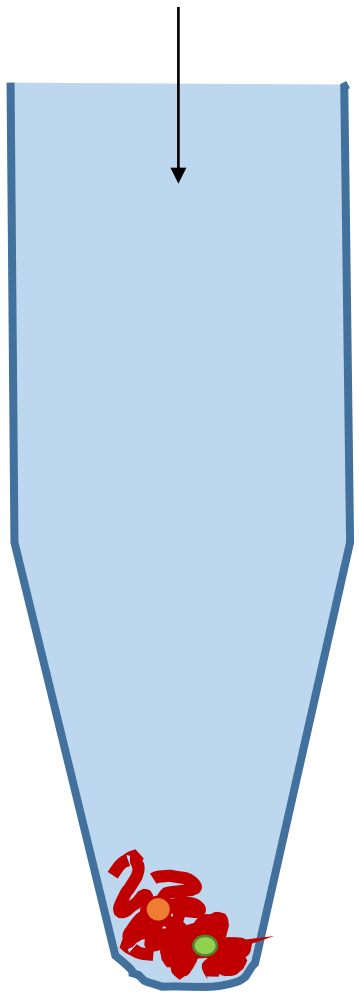
Séparation des débris

Centrifugeuse



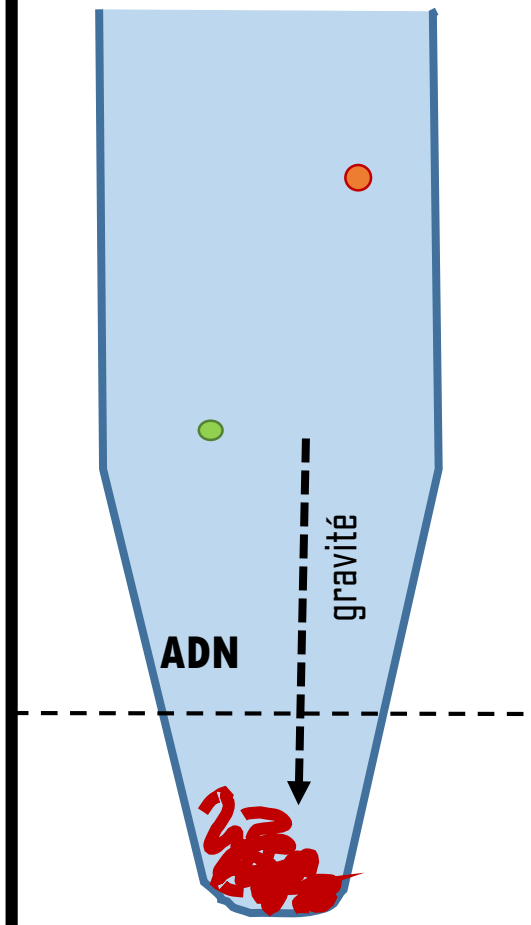

2ème tube
100µL d'isopropanol
10min incubation
10min centrifugation
Vider le surnageant
surnageant
100µL de surnageant
gravité
ADN
ADN
Culot
TUBE 1
Précipitation
Isopropanol + centrifugation
Lavage
Ethanol + centrifugation

+50µL d'Ethanol 70%



TUBE 2

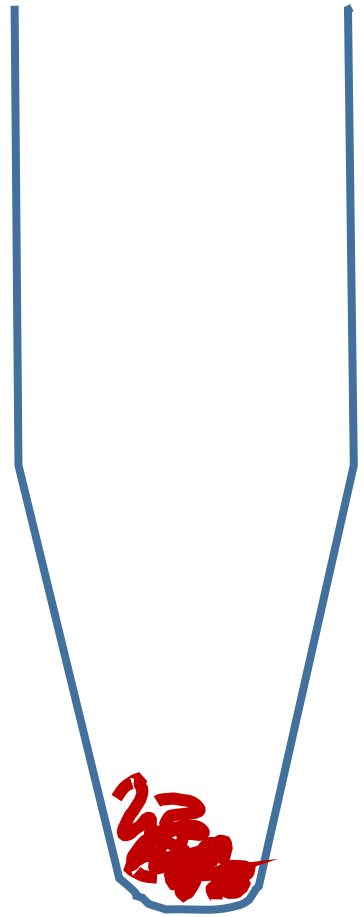
5min centrifugation



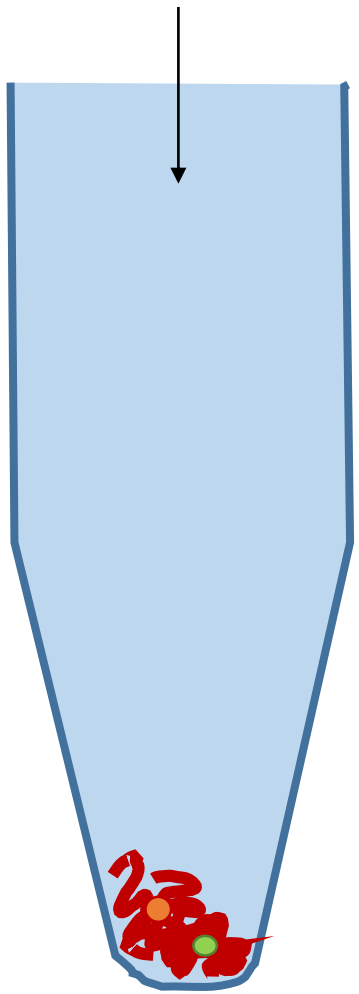
Lavage

Ethanol + centrifugation

Vider le surnageant

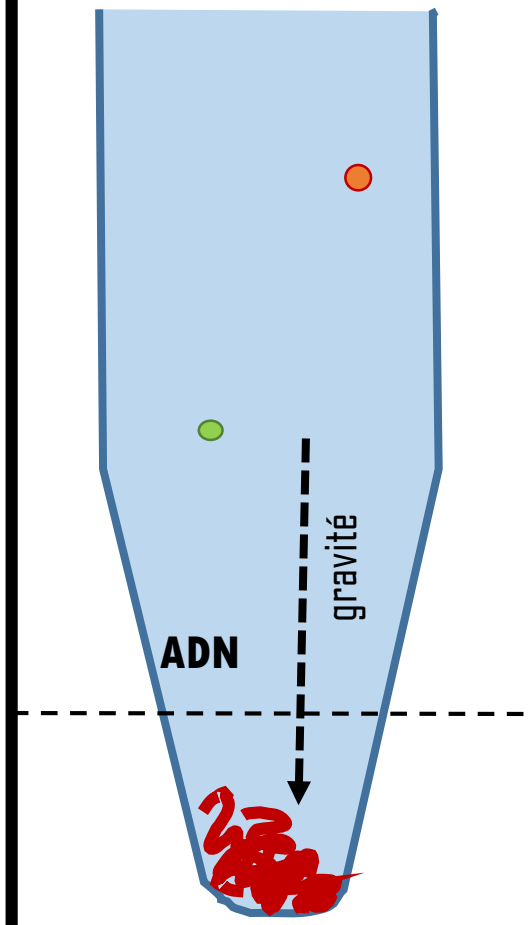


+50µL d'Ethanol 70%



TUBE 2

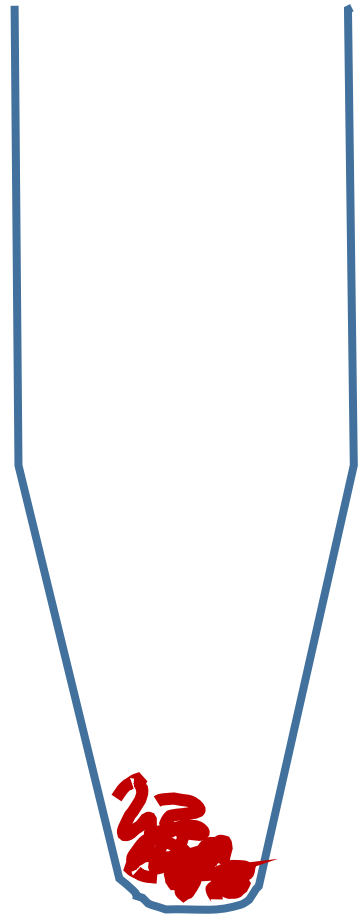
5min centrifugation



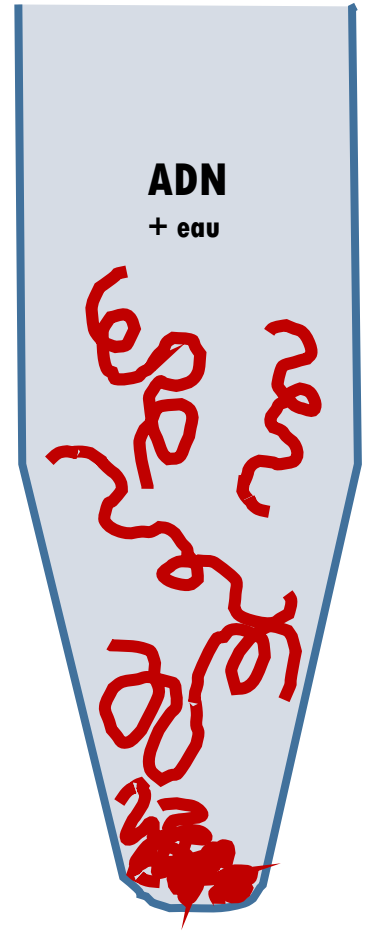
Lavage

Ethanol + centrifugation

Vider le surnageant



Vider le surnageant
Ajouter 50µL d'eau distillée



Suspension

Dans l'eau

PCR

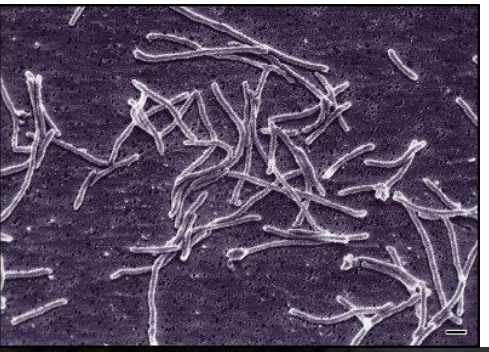
Polymérase Chain Reaction

ADN polymérase (Taq)

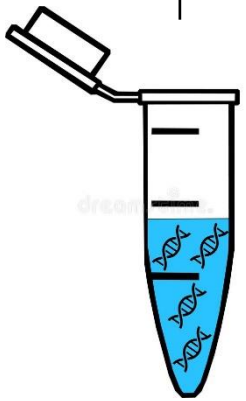
ADN total

Amorces (primers)

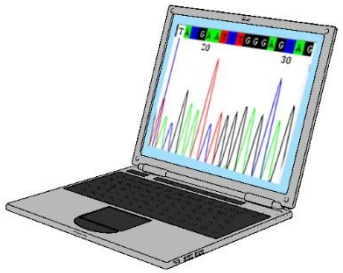
Nucléotide (dntp)

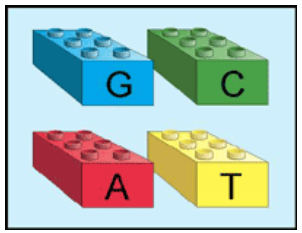


Thermus aquaticus



ATTCGGGATTTCGGATTCA





PCR

Polymérase Chain Reaction

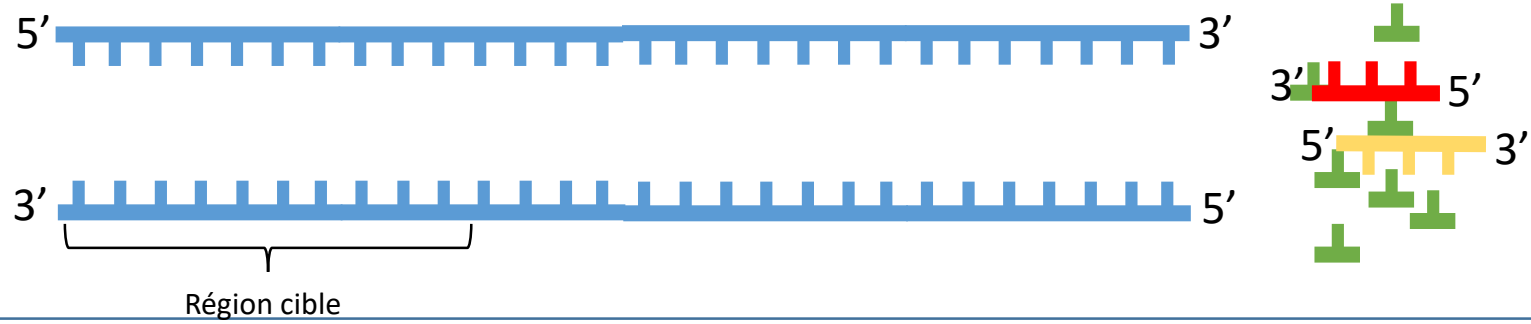
X 30

Dénaturation :

98°C

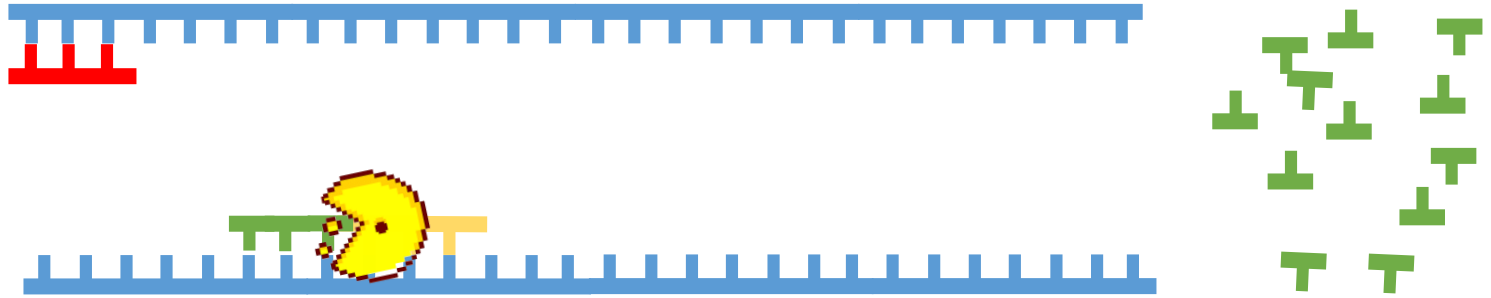
Hybridation :

45°C



Elongation :

72°C



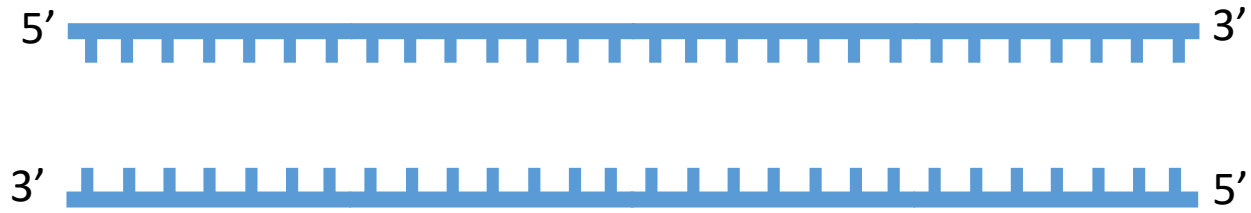
PCR

Polymérase Chain Reaction

X 30

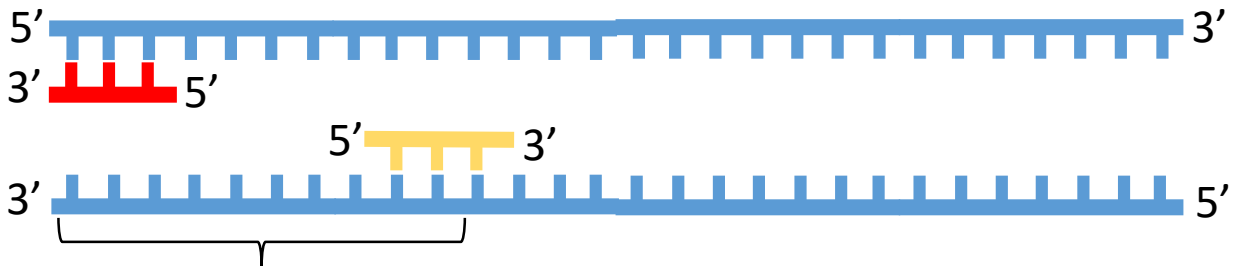
Dénaturation :

98°C



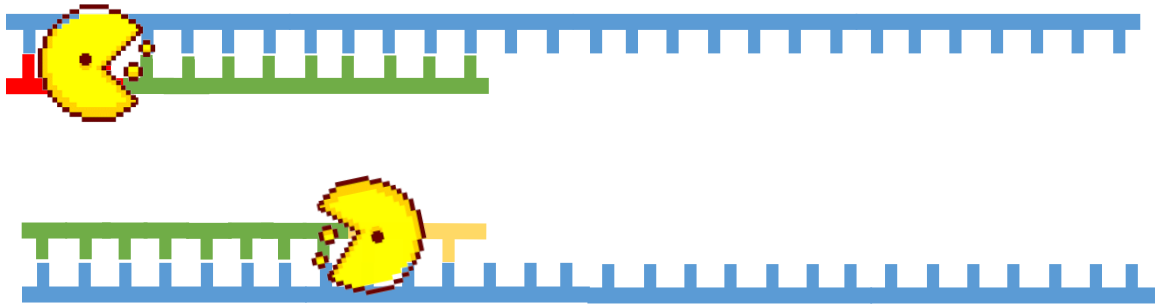
Hybridation :

40-60°C



Elongation :

72°C



PCR

Polymérase Chain Reaction

X 30

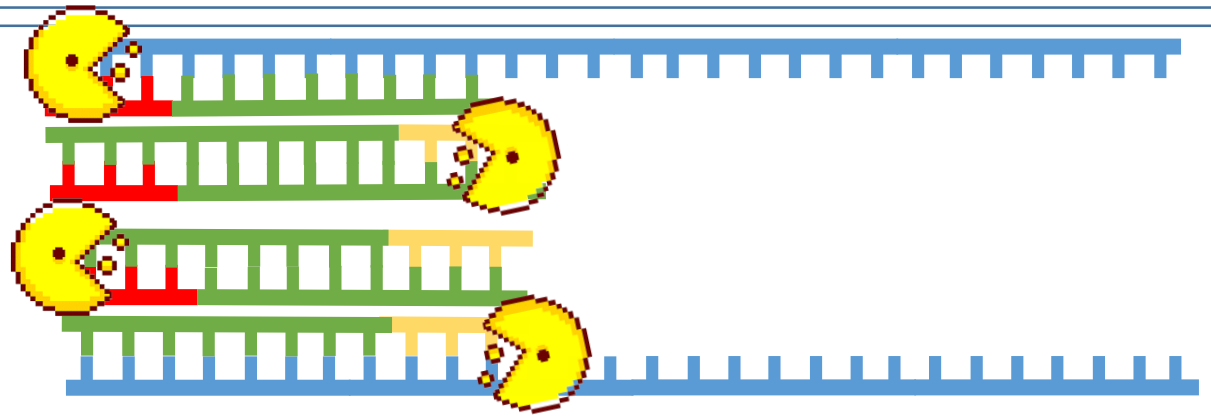
Dénaturation : 98°C

5’ 3’

3’ 5’

Hybridation : 40-60°C

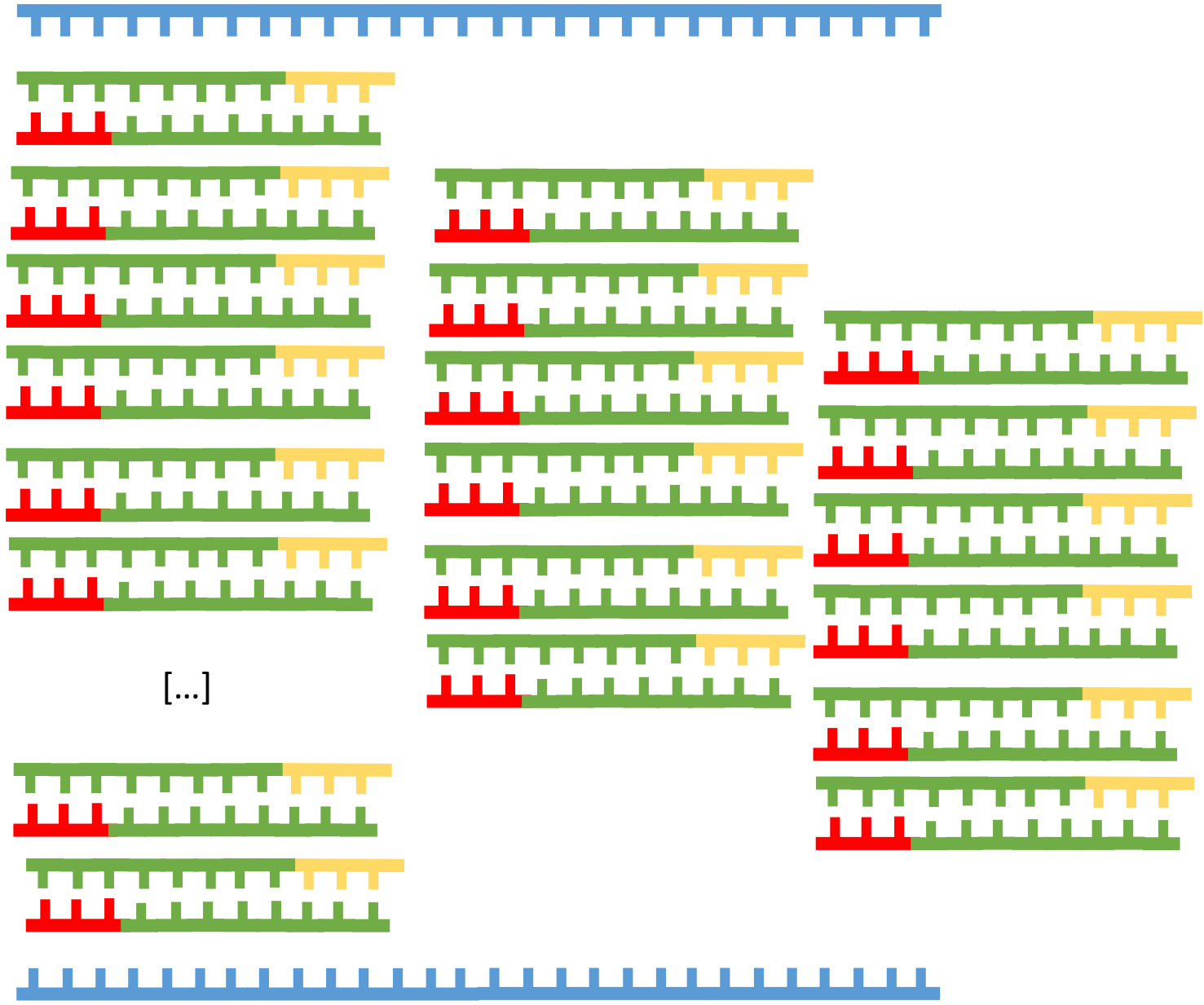
Elongation : 72°C



Le nombre de copies double à chaque fin de cycle.

PCR

Fin du cycle 30



Le nombre de copies double à chaque fin de cycle.

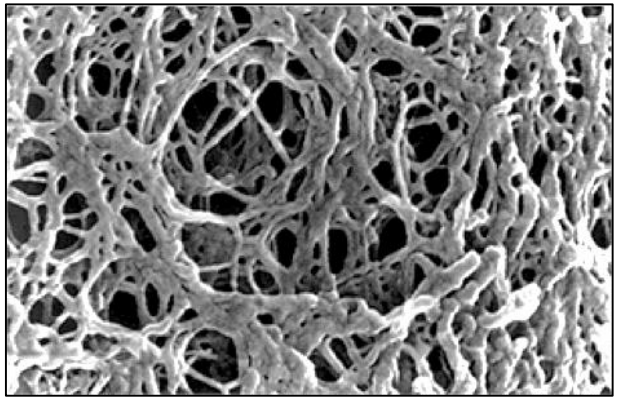
Vérifier que la PCR a fonctionné :

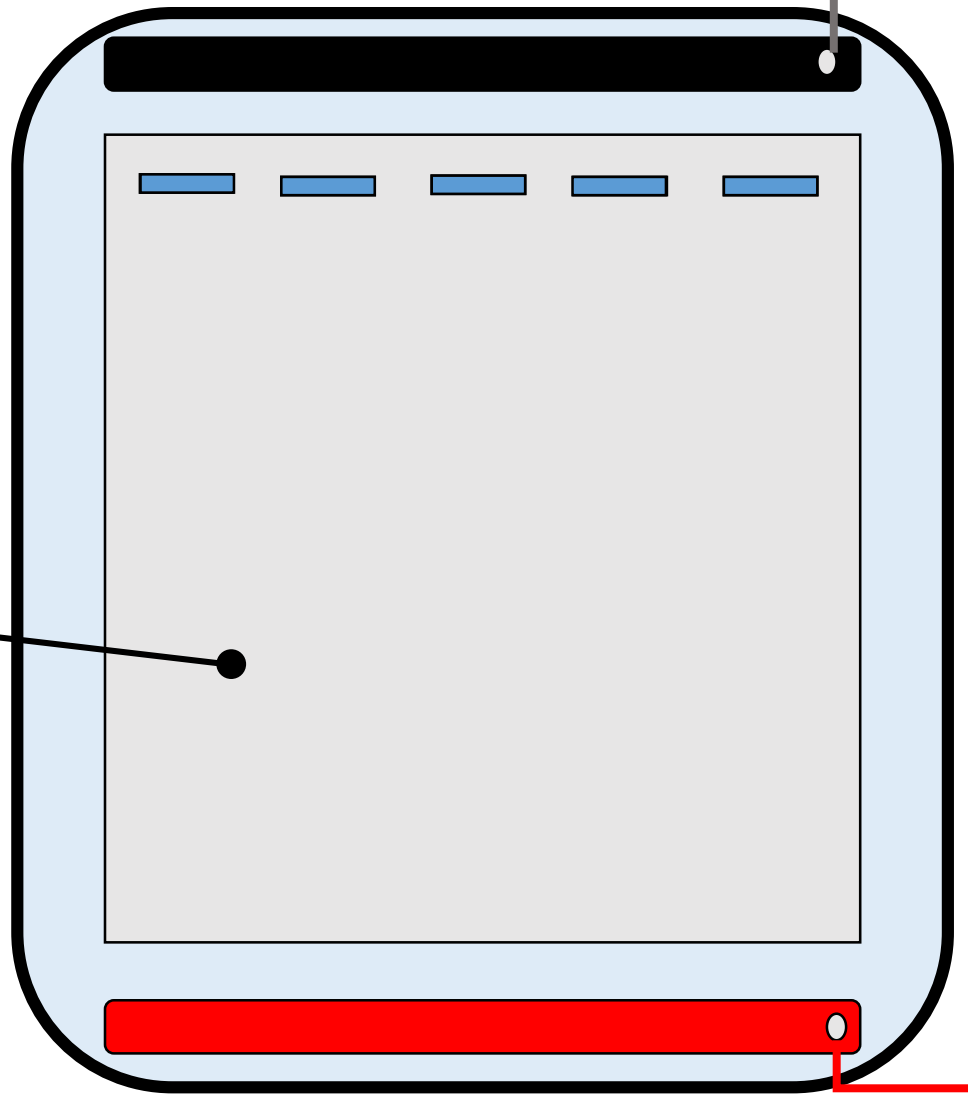
Est-ce que l'ADN est amplifié ?

Est-ce que c'est la bonne région ?

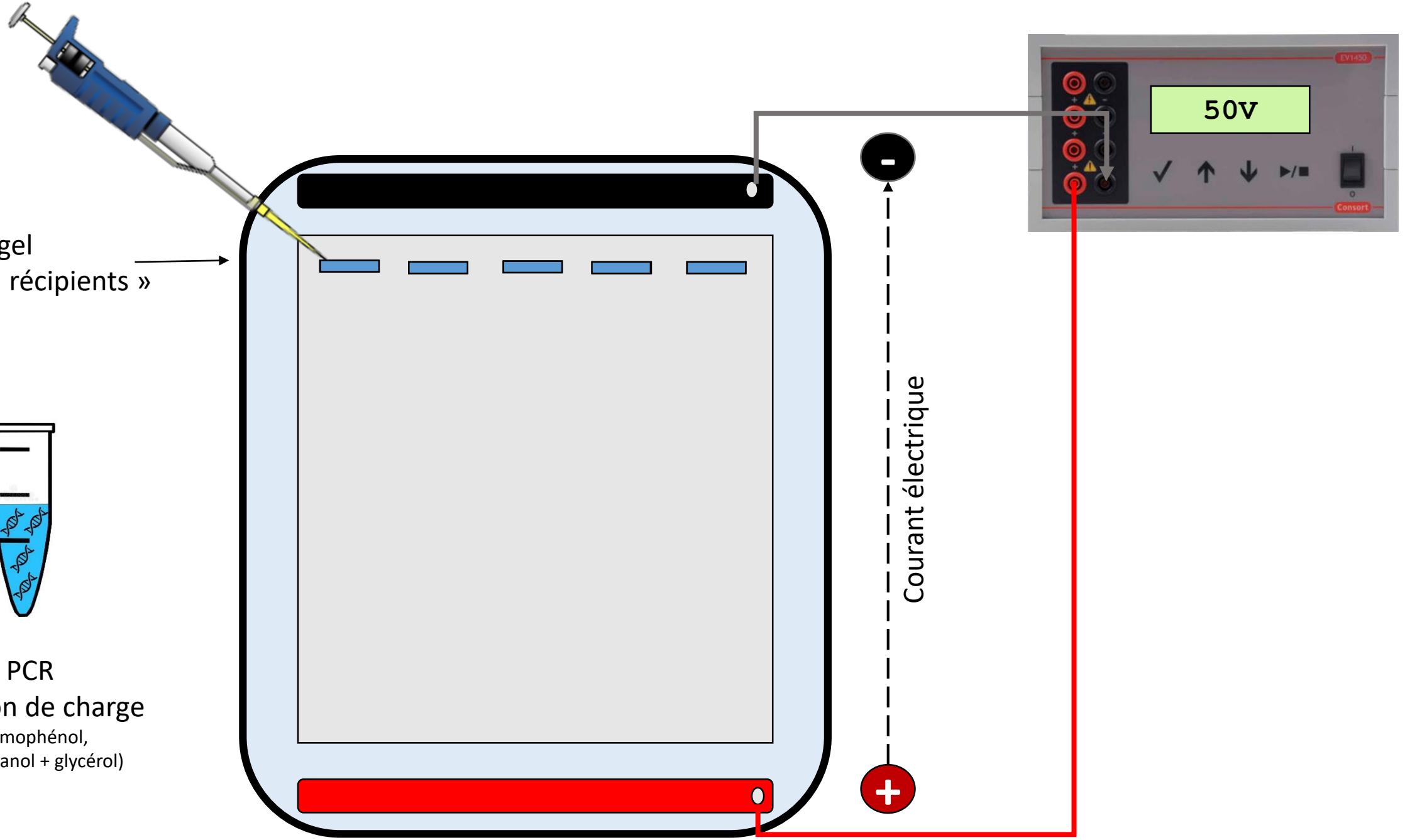
Electrophorèse sur gel d'agarose

Agarose 2%
Tampon TAE
(pour Tris, Acétate, EDTA, pH 8)
Fluorochrome
(GreenGel : Lumière LED)

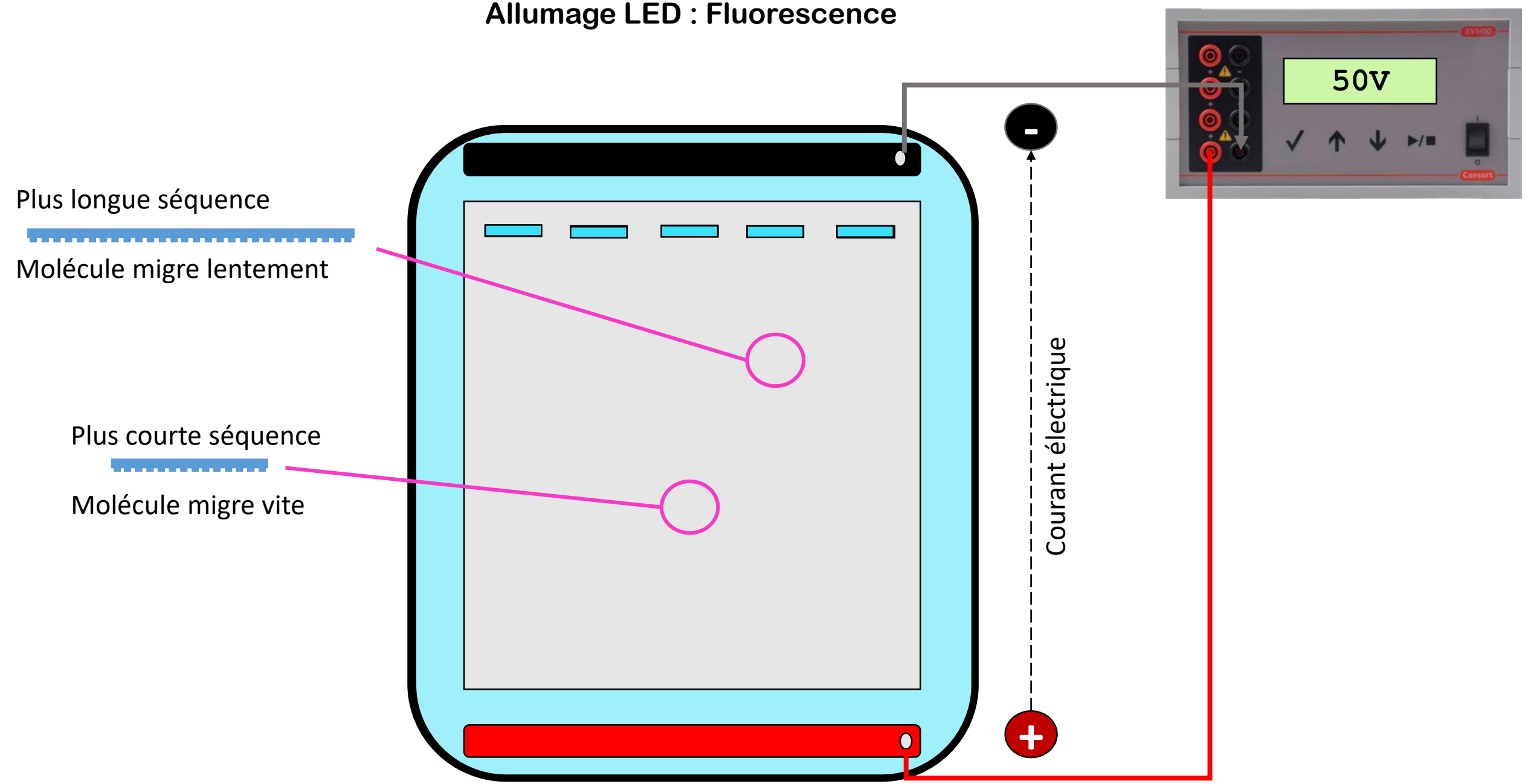




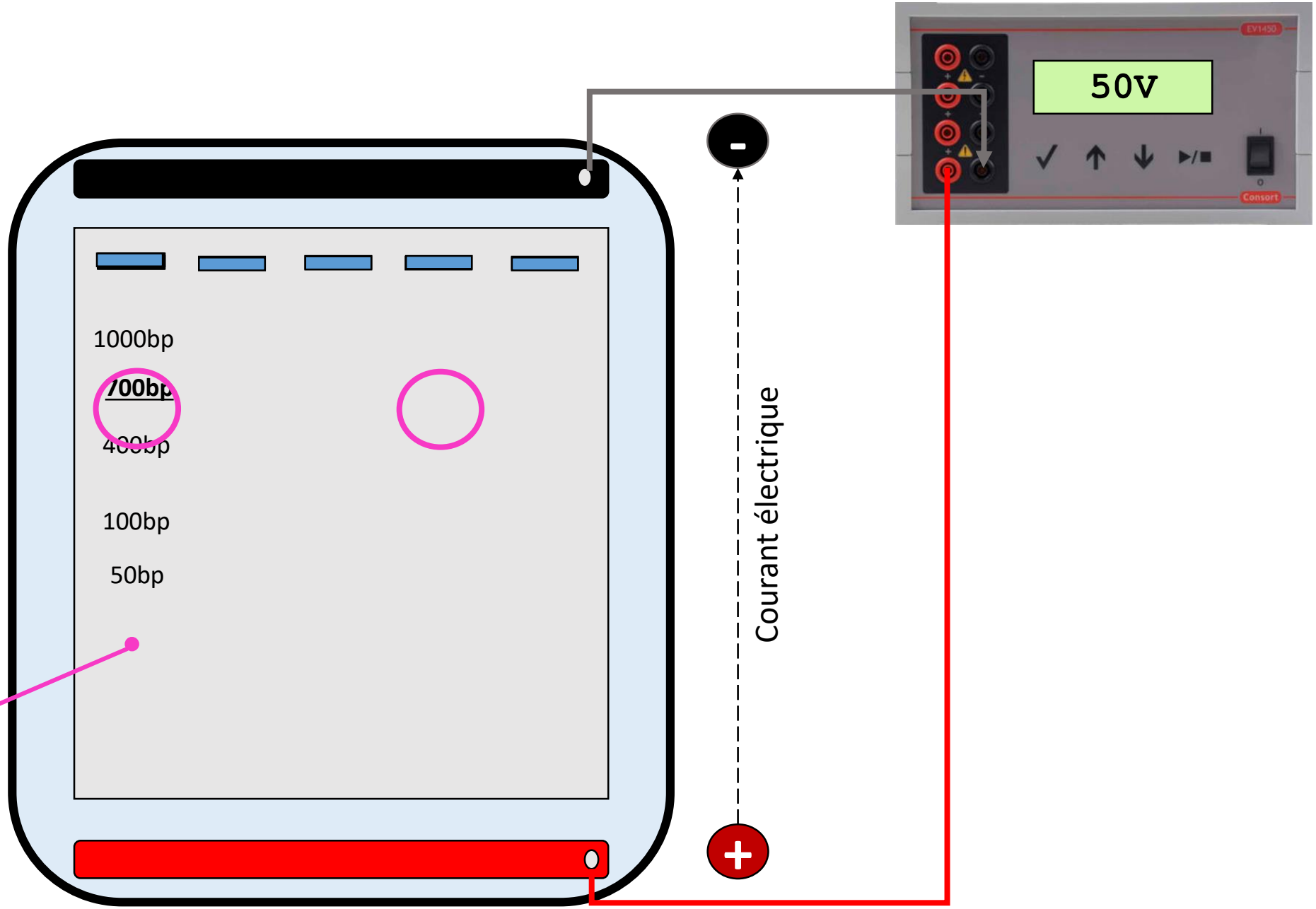
Courant électrique


Les puits du gel
= des trous « récipients »
Résultat PCR
+ tampon de charge
(bleu de bromophénol,
de xylène cyanol + glycérol)
50v
Courant électrique

Allumage LED : Fluorescence

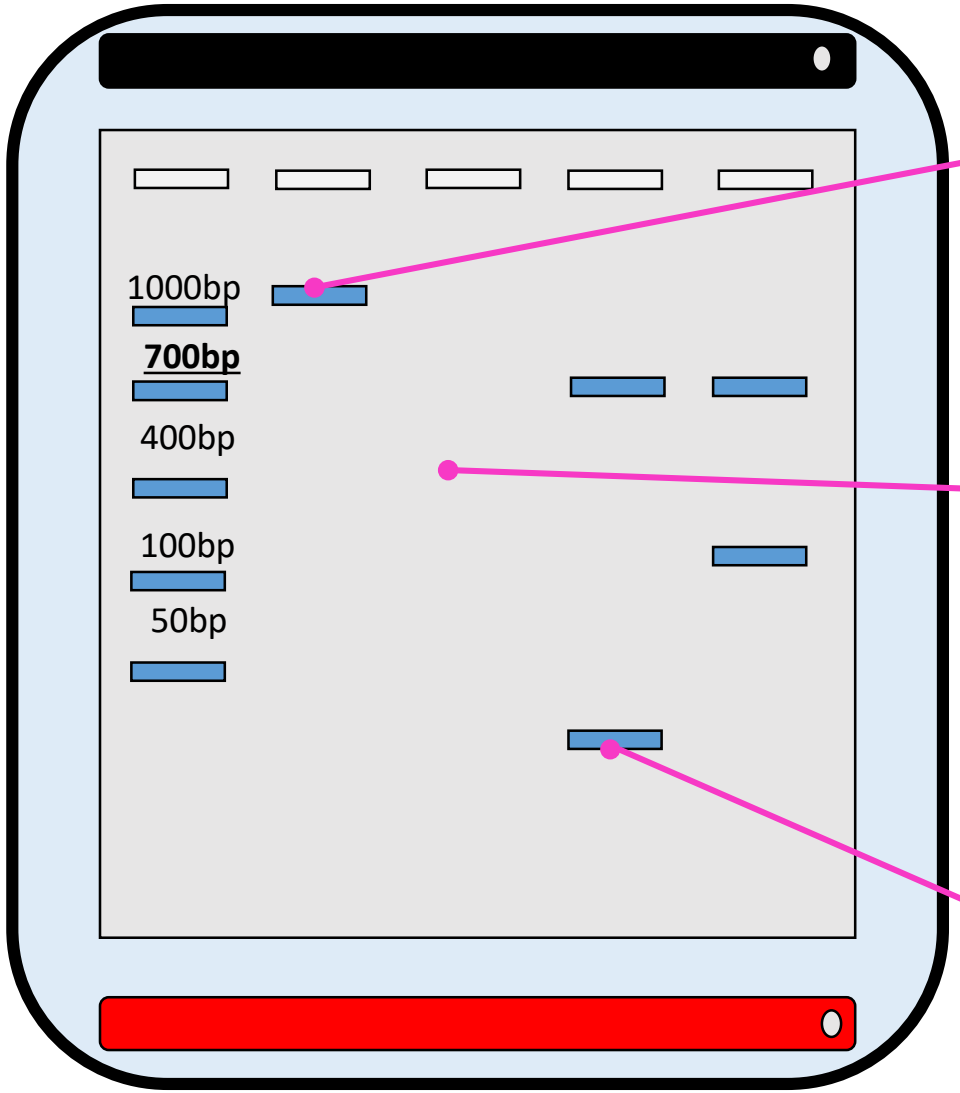


Comparer les tailles ?



Les résultats qui n'ont « pas marchés » comme on veut.

Diagnostic, Optimisation !



Bande trop grande taille
Une contamination ?
Vérifie l'amorce utilisé …
Tu as craché dans ton tube ?

Pas de bande
La PCR a tourné dans le vide :
Amorces pas adaptées
Trop de mutations / défaut de complémentarité
Essayer d'autres amorces
Baisser la température d'hybridation

ADN extrait trop sale / abîmé ?
Amplifier un fragment plus petit (autres amorces)
Changer le tampon PCR (moins de MgCl2)
Purification : Protéinase K ?
… Acheter un kit commercial …

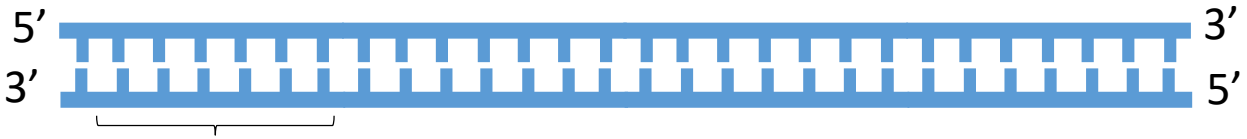
Plusieurs bandes dont la « bonne »
Amplification aspécifique : augmenter la température d'hybridation.

« Dimer » d'amorce
Les amorces se sont hybridées entre elles :
Pas grave : kit de nettoyage.

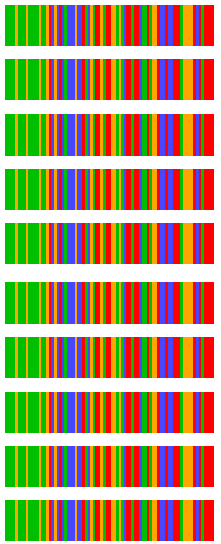
Et Apres ?

Résumé en une page

Extraction ADN

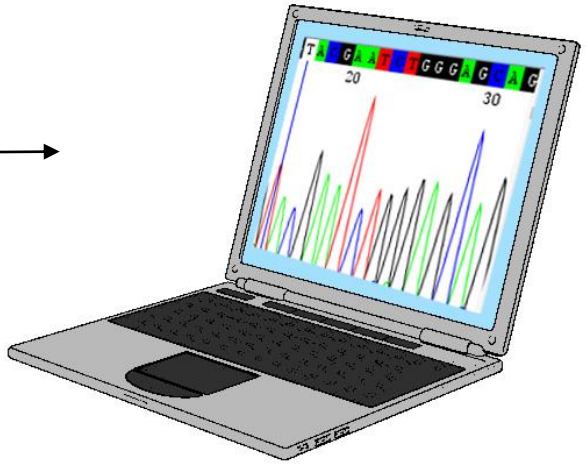


COI



Produit PCR

PCR :
Multi copie du Gène étudié.

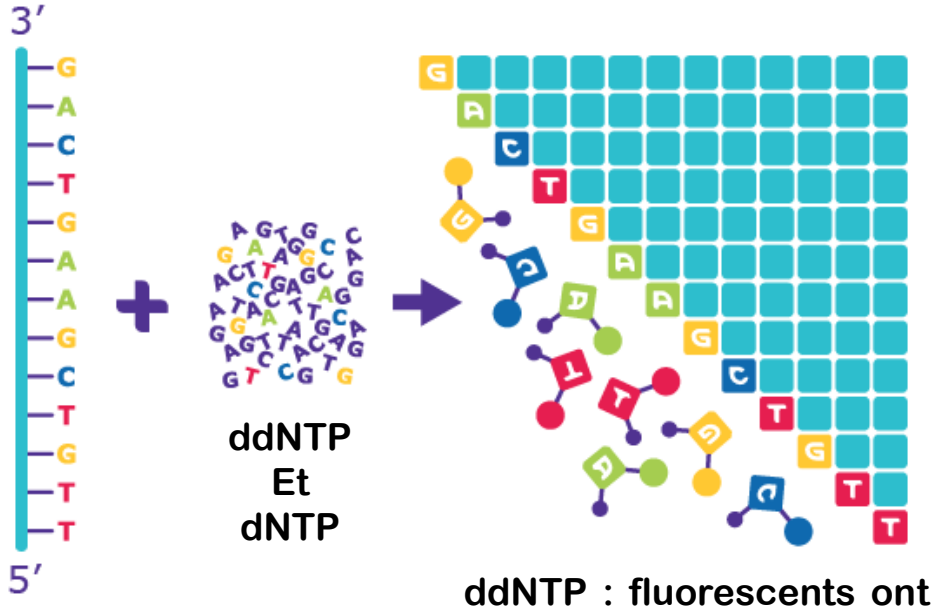


Séquençage :
Envoi à une plateforme de service

Séquençage ‘Sanger’

100-1200 bp

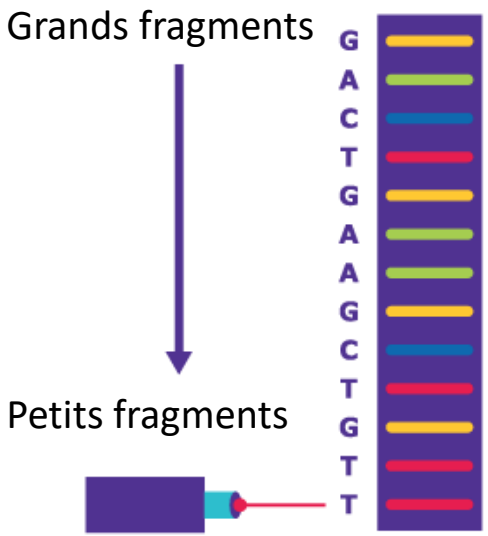
1 **PCR avec ddNTPs fluorescent et dNTPs classique.**



Notre produit PCR du gène COI + Nos amorces

ddNTP : fluorescents ont une terminaison arrêtant l’amplification pour le fragment

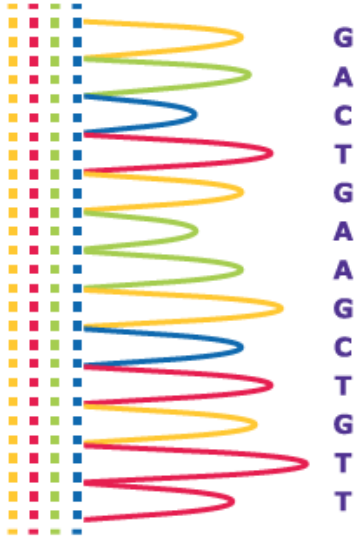
2 **Les fragments sont séparés par taille croissante par gel capillaires d’électrophorèse**



Laser d’excitation
Du fluorochrome

Capteur de la fluorescence

3 **Excitation au Laser et détection de fluorescence spécifique au A/T/C/G**

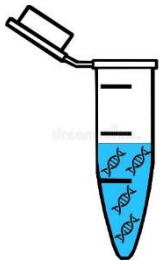


4 fluorochromes différents pour chaque base = Obtention d’un chromatographe correspondant à une séquence

Source de l’illustration : www.sigmaaldrich.com

Séquençage ‘Sanger’

100-1200 bp



Notre produit
PCR
Du gène COI

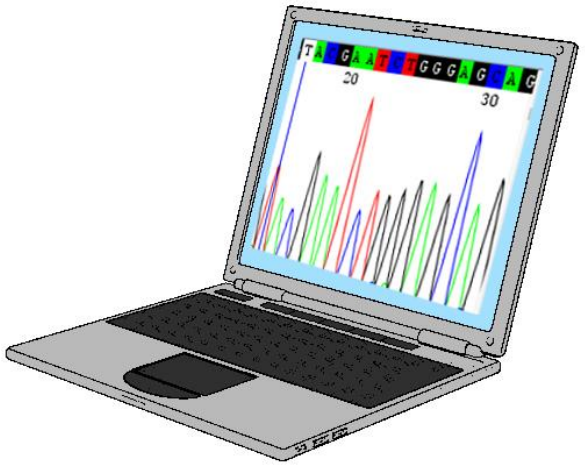
Plateforme de séquençage
~ 4 € la séquence
8 € l’échantillon x2 amorces

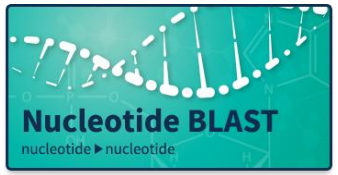
G
A
C
T
G
A
A
G
C
T
G
T
T

La séquence
en fichier texte

Identification de l’espèce

à partir de la séquence COI





> Echantillon inconnu n12
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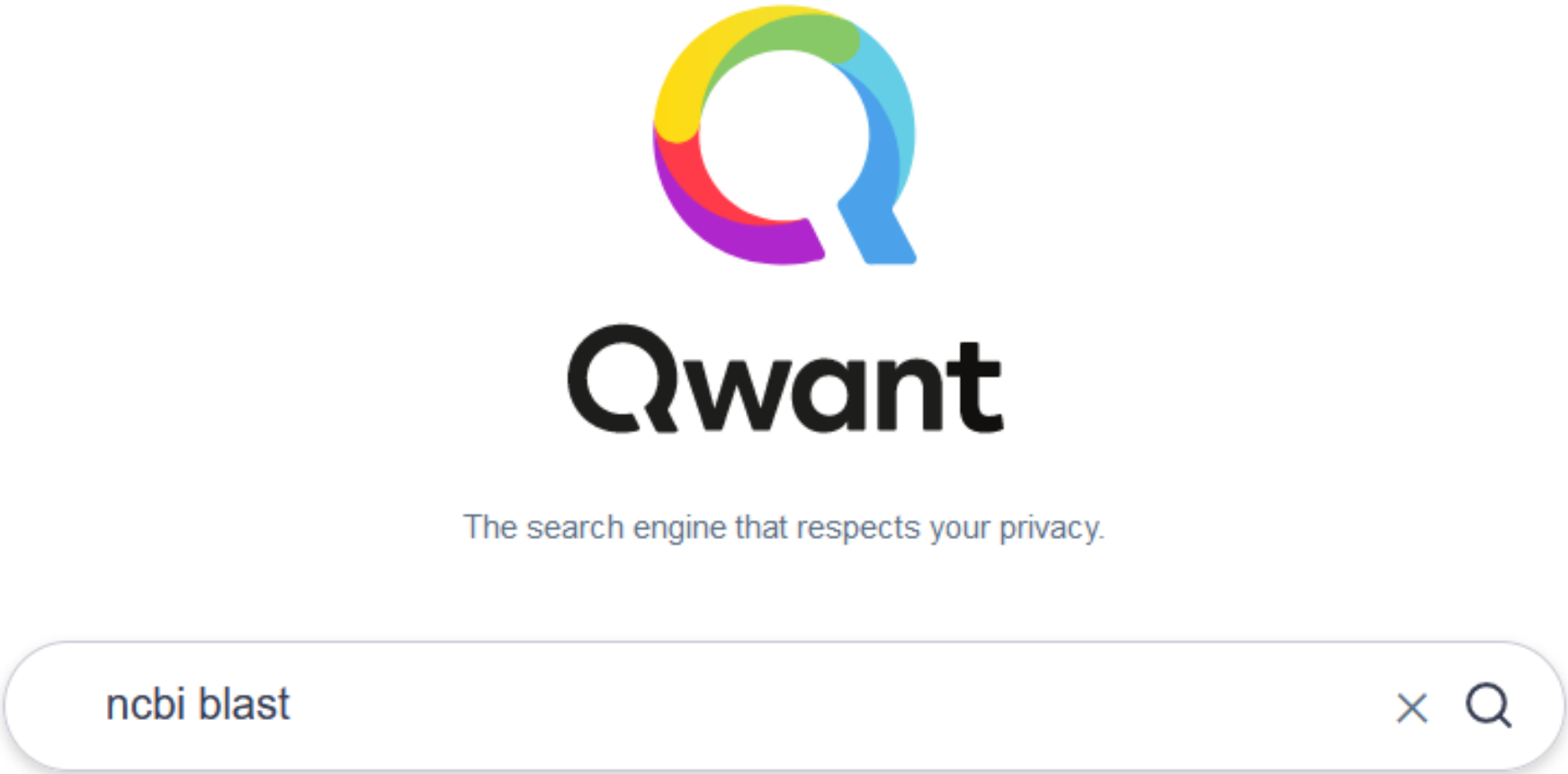
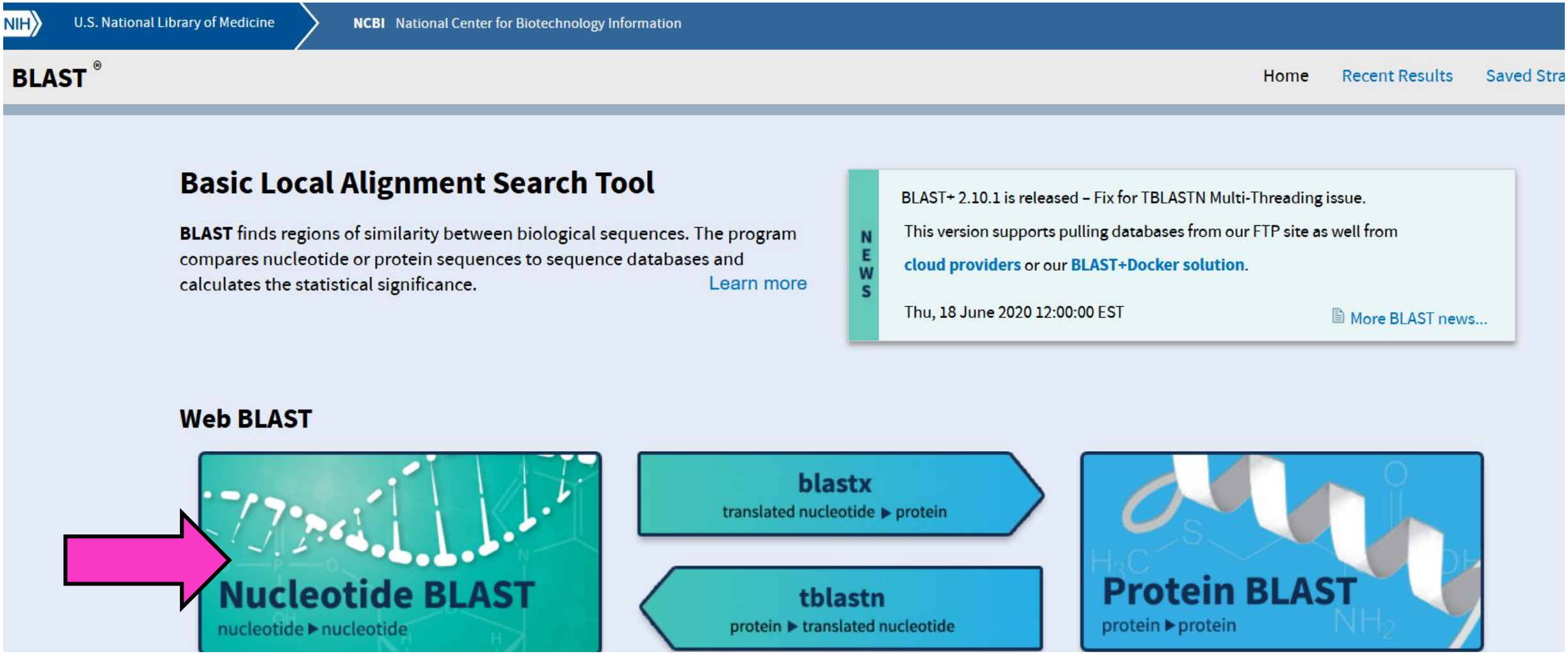
ATTAGCTGGTGCTATTACTATGTTGTTAACTGATCGTAATTTTAAT
ACCTCTTTTTTTGACCCTGCTGGCGGGGGGGATCCAGTGTTATT
TCAACATTTGTT

Nom de l’espèce ?
Identification ou confirmation

L’identification de l’espèce
Avec BLAST



L’identification de l’espèce



Standard Nucleotide BLAST

blastn | blastp | blastx | tblastn | tblastx

BLASTN programs search nucleotide databases using a nucleotide query. more...

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s)

Clear

> Echantillon inconnu n12
TCGTATGAATAATTTGAGTTTTTGGTTATTACCCCCTTCTTTATTTTTATTAGTGGTTTCTAGAATAG
TTGAAATAGGAGTAGGAACAGGTTGGACTGTGTATCCTCCATTGTCTTCTAATGTAGGGCATGCTTTT
GTGTCGGTTGATTTTGCTATTTTTTCTCTTCATTTAGCTGGGGCTTCTTCTATTATAGGGGCTGTTAA
TTTCATTACTACTATTATTAATATGCGAACTTTTGGTATGAGTATGGAAAAGGTTCCCTTGTTTGTTT
GATCAGTGTTAGTGACTGCTGTTTTGTTGTTGTTATCTTTACCTGTATTAGCTGGTGCTATTACTATG

Query subrange

From

To

Or, upload file — Parcourir... Aucun fichier sélectionné.

Job Title — Echantillon inconnu n12

Enter a descriptive title for your BLAST search

Align two or more sequences

Choose Search Set

Database — Standard databases (nr etc.): rRNA/ITS databases | Genomic + transcript databases | Betacoronavirus

Nucleotide collection (nr/nt)

Organism Optional — arthropods (taxid:6656) exclude +

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown

Exclude Optional — Models (XM/XP) Uncultured/environmental sample sequences

Limit to Optional — Sequences from type material

Entrez Query Optional — YouTube Create custom database

Enter an Entrez query to limit search

Program Selection

Optimize for

- Highly similar sequences (megablast)
- More dissimilar sequences (discontiguous megablast)
- Somewhat similar sequences (blastn)

Choose a BLAST algorithm

BLAST

Search database Nucleotide collection (nr/nt) using Megablast (Optimize for highly similar sequences)

Show results in a new window

CTRL-V

?? idée

Clickkk !

Résultats BLAST

Job Title	**Echantillon inconnu n12**
RID	M4DXJVG9014 *Search expires on 08-24 17:32 pm* Download All
Program	BLASTN Citation
Database	nt See details
Query ID	lcl|Query_32393
Description	Echantillon inconnu n12
Molecule type	dna
Query Length	420
Other reports	Distance tree of results MSA viewer

Filter Results

Organism *only top 20 will appear* exclude

Type common name, binomial, taxid or group name

+ Add organism

Percent Identity to **E value** to **Query Coverage** to

Filter **Reset**

Tri

Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments **Download** **Manage Columns** Show 100

select all *100 sequences selected* GenBank Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Leptoneta convexa voucher 9040712 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	776	776	100%	0.0	100.00%	JN378156.1
Leptoneta microphthalma voucher 9040711 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	593	593	100%	1e-166	92.14%	JN378155.1
Cybaeota nana cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	407	407	99%	1e-110	84.21%	FJ263787.1
Erigone autumnalis haplotype 1 cytochrome c oxidase subunit 1 (COI) gene, partial cds; mitochondrial	405	405	99%	5e-110	84.29%	MK521227.1
Neoramia sp. CG178 isolate ARACG000178 cytchrome oxidase subunit I (COI) gene, partial cds; mitochondrial	405	405	99%	5e-110	84.21%	KY017938.1

Clickk !

Download GenBank Graphics

Leptoneta convexa voucher 9040712 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial

Sequence ID: JN378156.1 **Length:** 817 **Number of Matches:** 1

Range 1: 1 to 420 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
776 bits(420)	0.0	420/420(100%)	0/420(0%)	Plus/Plus

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Query  1    TCGTATGAATAATTTGAGTTTTTGGTTATTACCCCCTTCTTTATTTTTATTAGTGGTTTC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    TCGTATGAATAATTTGAGTTTTTGGTTATTACCCCCTTCTTTATTTTTATTAGTGGTTTC  60

Query  61   TAGAATAGTTGAAATAGGAGTAGGAACAGGTTGGACTGTGTATCCTCCATTGTCTTCTAA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   TAGAATAGTTGAAATAGGAGTAGGAACAGGTTGGACTGTGTATCCTCCATTGTCTTCTAA  120

Query  121  TGTAGGGCATGCTTTTGTGTCGGTTGATTTTGCTATTTTTTCTCTTCATTTAGCTGGGGC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  TGTAGGGCATGCTTTTGTGTCGGTTGATTTTGCTATTTTTTCTCTTCATTTAGCTGGGGC  180

Query  181  TTCTTCTATTATAGGGGCTGTTAATTTCATTACTACTATTATTAATATGCGAACTTTTGG  240
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Sbjct  181  TTCTTCTATTATAGGGGCTGTTAATTTCATTACTACTATTATTAATATGCGAACTTTTGG  240

Query  241  TATGAGTATGGAAAAGGTTCCCTTGTTTGTTTGATCAGTGTTAGTGACTGCTGTTTTGTT  300
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Query  301  GTTGTTATCTTTACCTGTATTAGCTGGTGCTATTACTATGTTGTTAACTGATCGTAATTT  360
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Sbjct  301  GTTGTTATCTTTACCTGTATTAGCTGGTGCTATTACTATGTTGTTAACTGATCGTAATTT  360

Query  361  TAATACCTCttttttGACCCTGCTGGCggggggATCCAGTGTTATTTCAACATTTGTT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  TAATACCTCTTTTTTGACCCTGCTGGCGGGGGGGATCCAGTGTTATTTCAACATTTGTT  420
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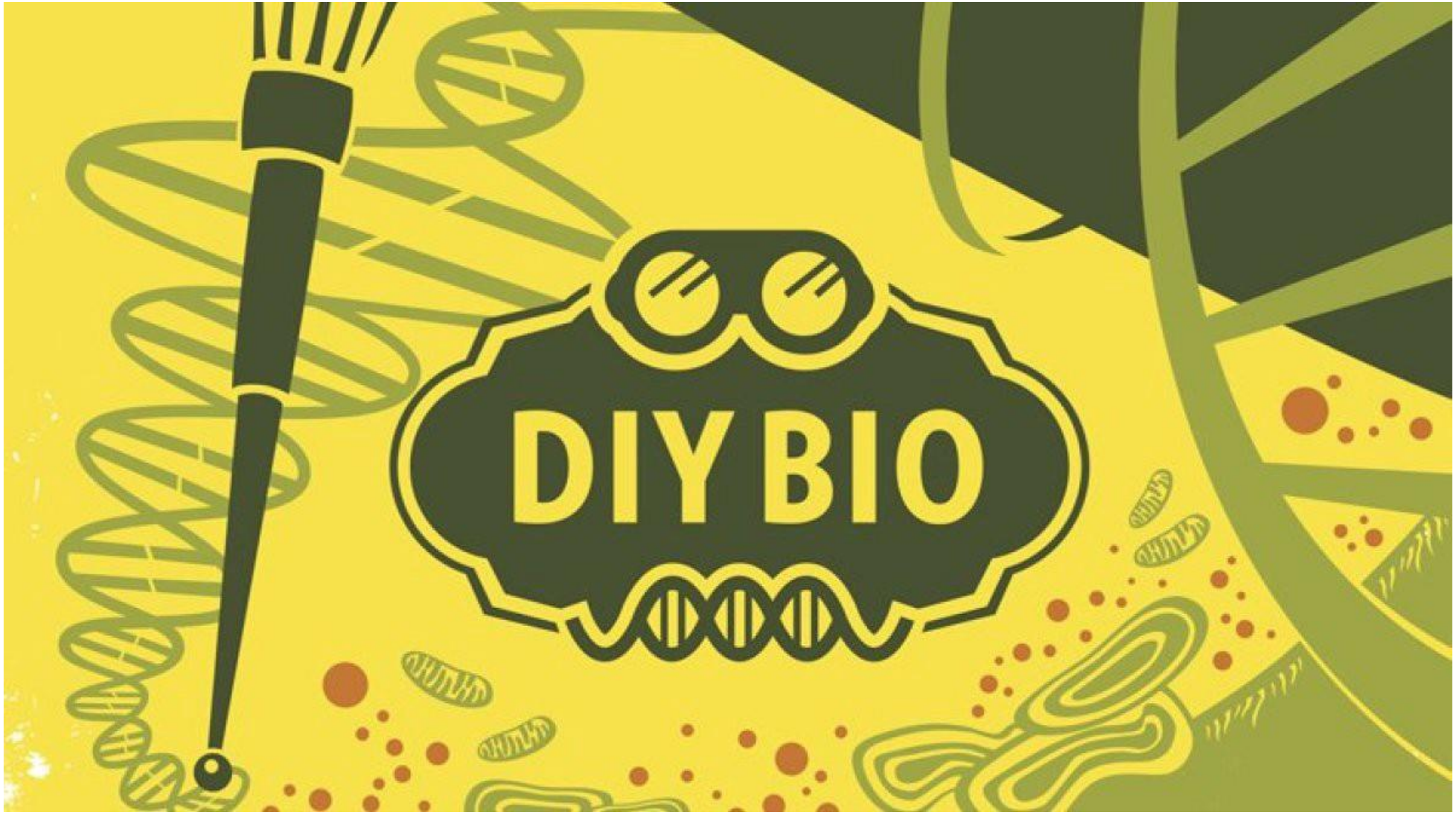
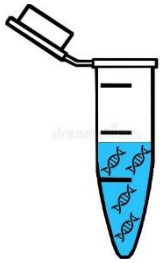
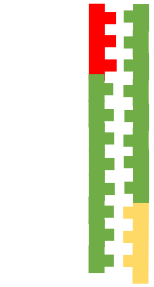

DIY BIO

Séquençage ‘Sanger’

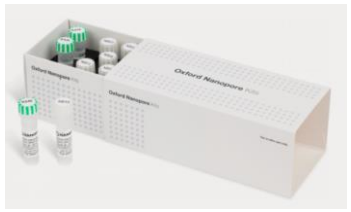
100-1200 bp



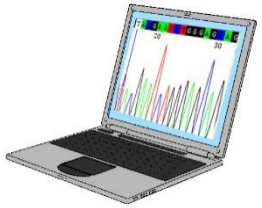


Notre produit
PCR
Du gène COI

MinION 900 euro



Consommables
539 euro



Ordi performant
3500 euro

G
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La séquence
en fichier texte