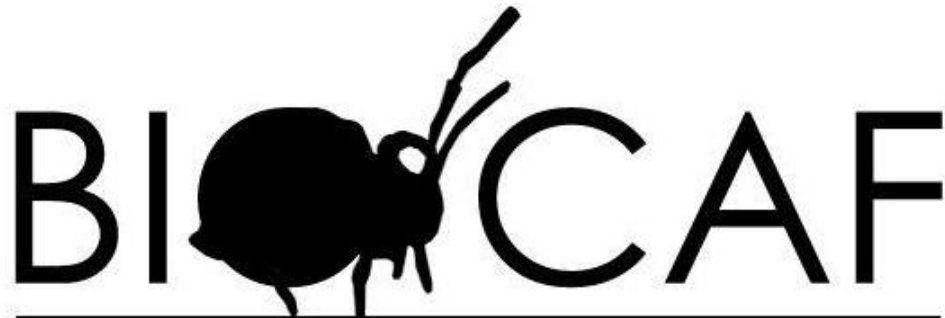


TP moléculaire :

Extraction ADN, PCR, électrophorèse

Stage biospéléo 12-13 septembre 2020

Marina FERRAND



INVENTAIRE BIOSPELEOLOGIQUE DES
CARRIERES SOUTERRAINES FRANCILIENNES



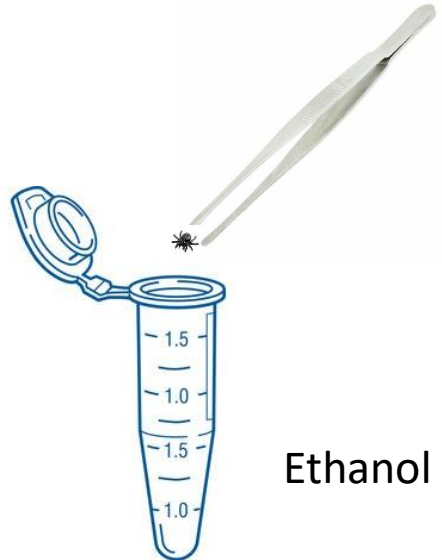
Travaux pratiques

TP1 : EXTRACTION DE L'ADN

Espèce collectée
à identifier



Extraction de
Son ADN



Ethanol 90%



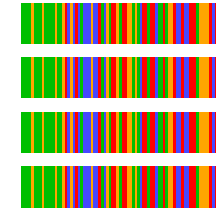
Tampon

TP2 : PCR

Amplifier le gène
Standard de barcoding

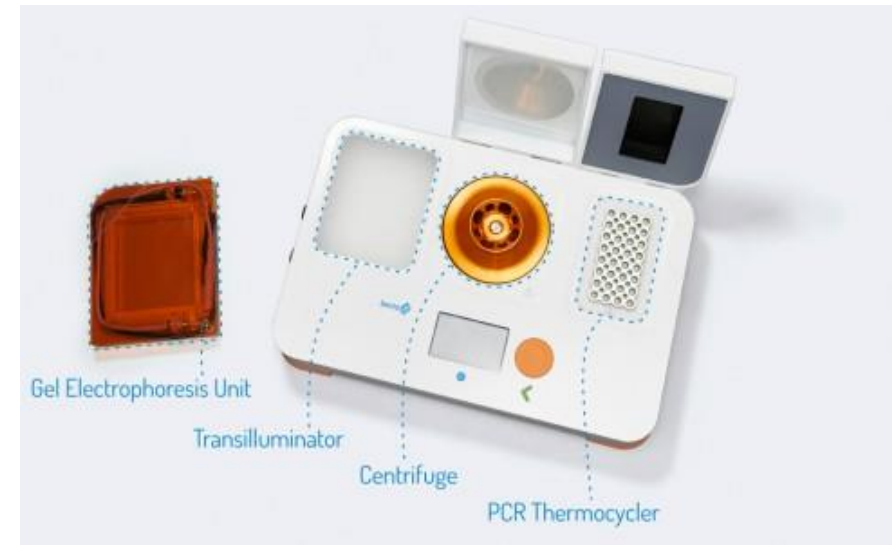


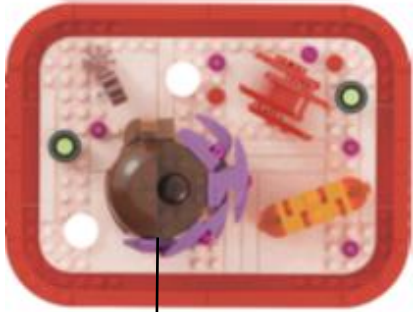
COI



Concentrer la
région cible
(pour la faire
séquencer
ultérieurement)

PCR

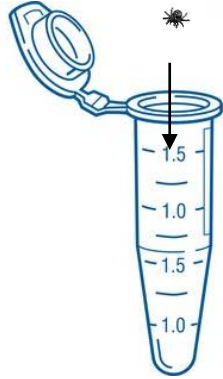




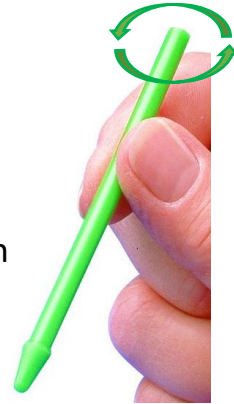
ECHANTILLON

1^{er} tube

échantillon



sable de
Fontainebleau
+200µL tampon
d'extraction



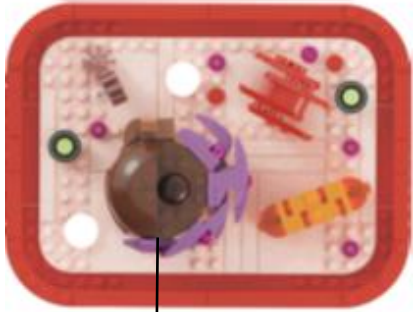
BROYAGE

Centrifugation
Séparation des débris

TP1 : Extraction ADN

Centrifugeuse

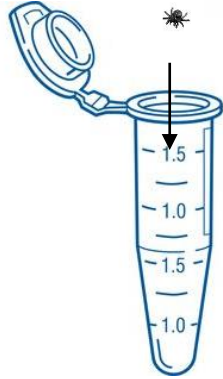




ECHANTILLON

1^{er} tube

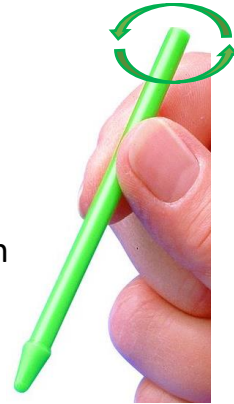
échantillon



sable de
Fontainebleau
+200µL tampon
d'extraction

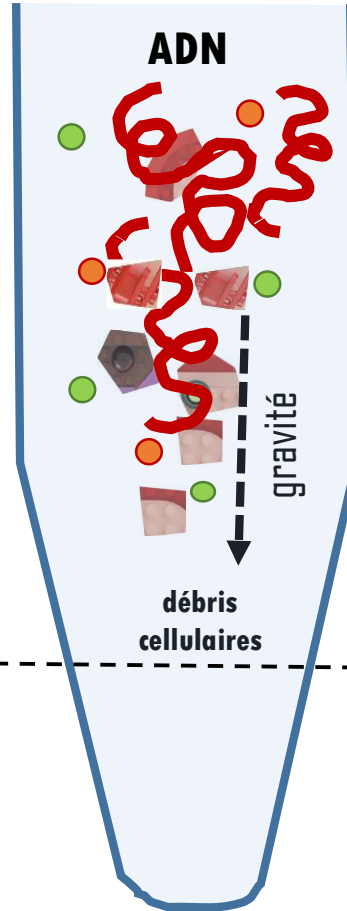


BROYAGE



10minutes de
centrifugation

surnageant



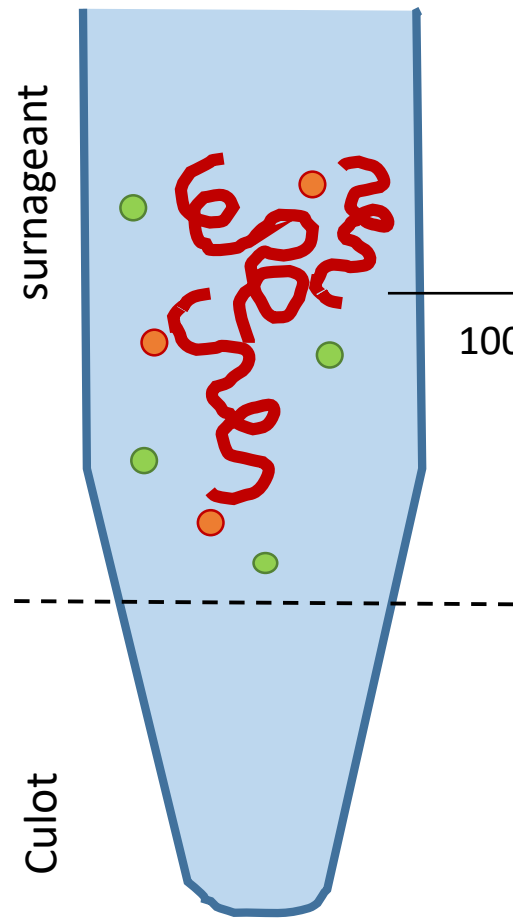
Culot

Centrifugation
Séparation des débris

TP1 : Extraction ADN

Centrifugeuse

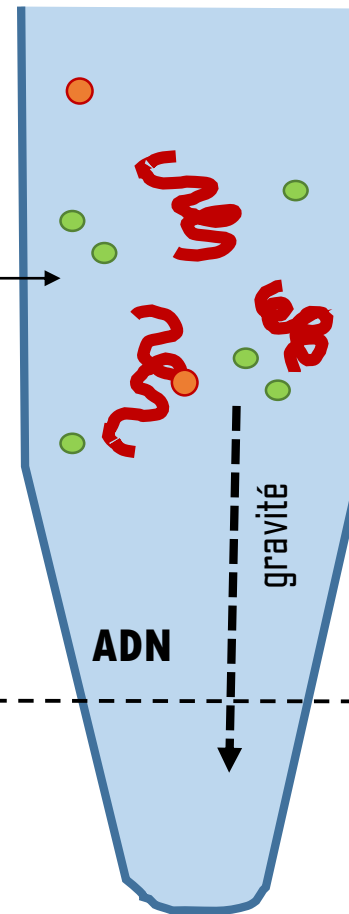




TUBE 1

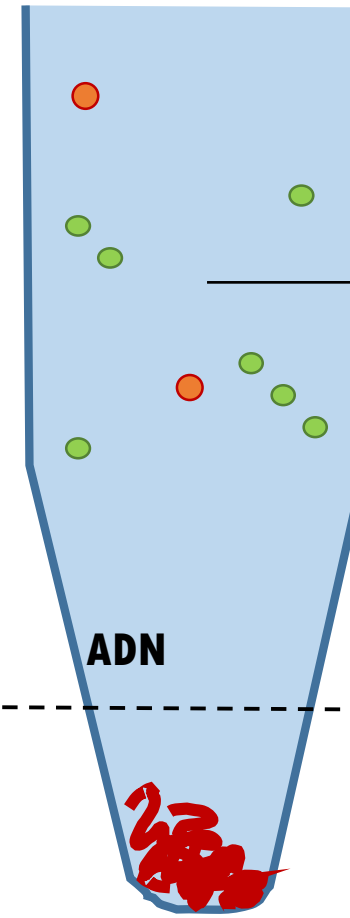
100µL de surnageant

2ème tube
100µL d'isopropanol
10min incubation
10min centrifugation

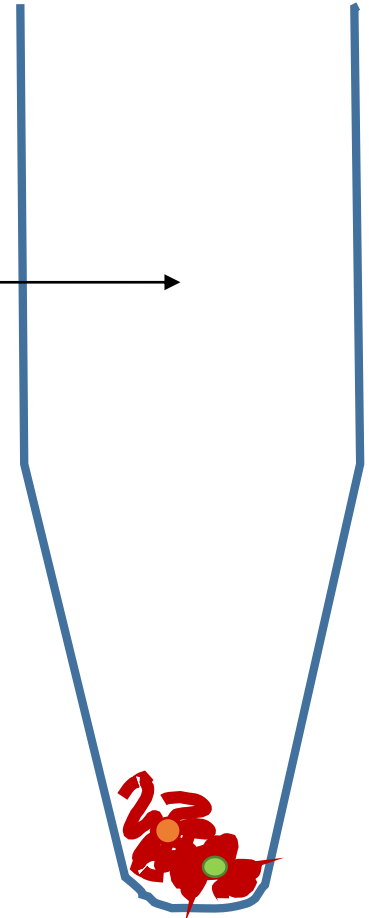


Précipitation
Isopropanol + centrifugation

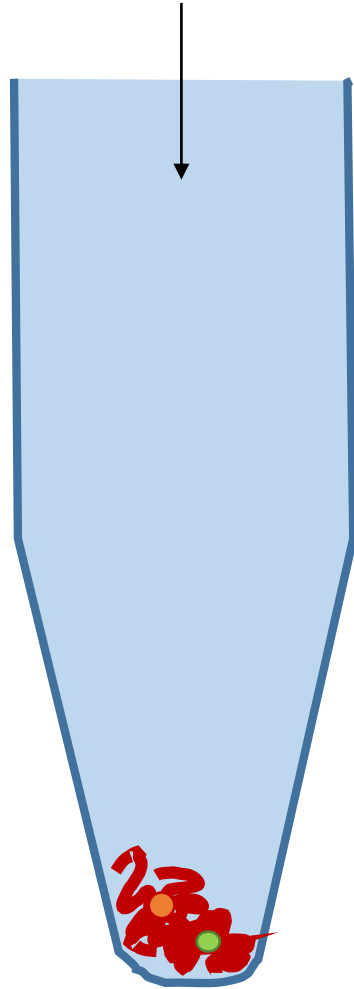
Vider le surnageant



Lavage
Ethanol + centrifugation

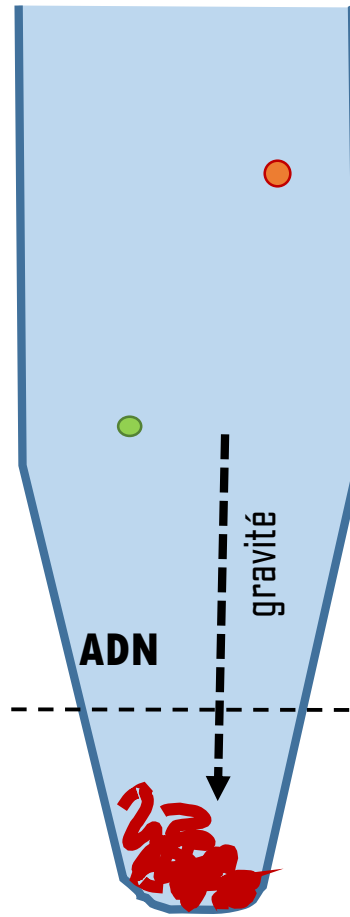


+50μL d'Ethanol 70%



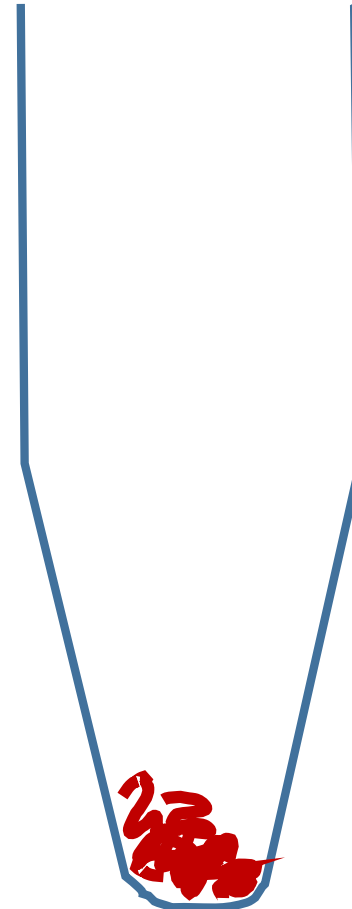
TUBE 2

5min centrifugation

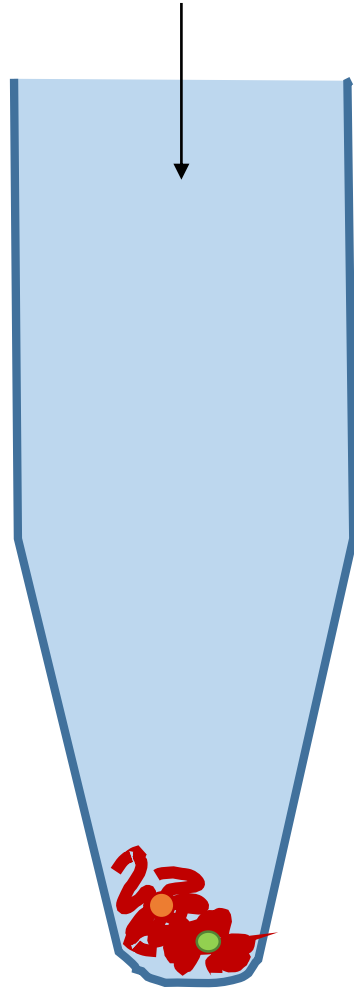


Lavage
Ethanol + centrifugation

Vider le surnageant

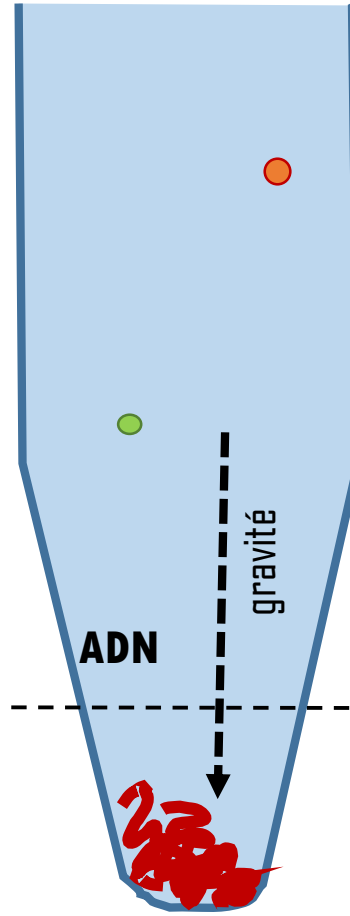


+50μL d'Ethanol 70%



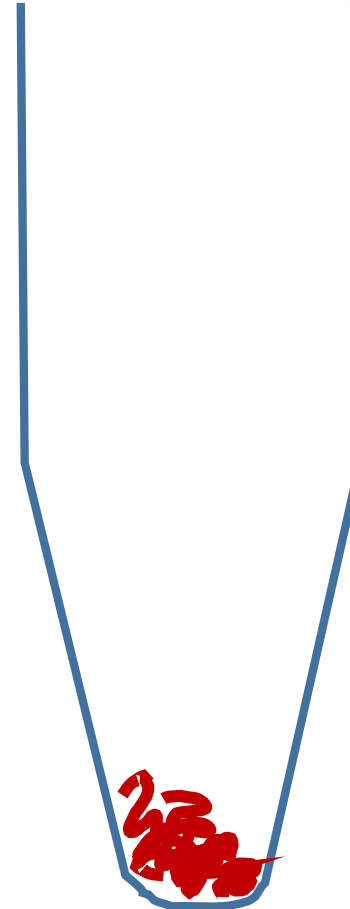
TUBE 2

5min centrifugation

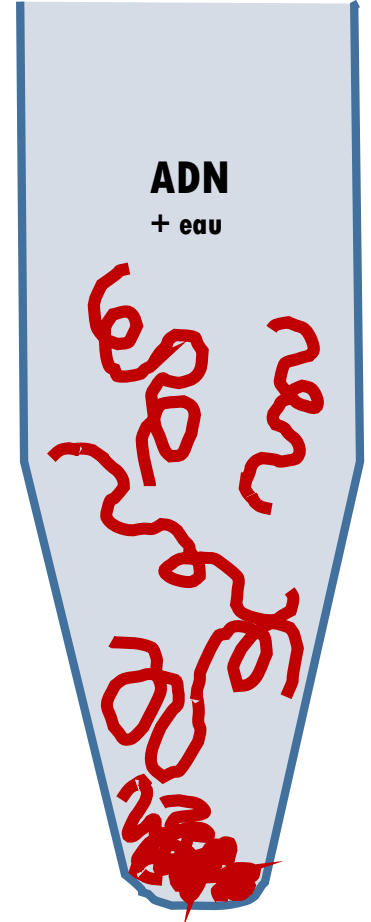


Lavage
Ethanol + centrifugation

Vider le surnageant



Vider le surnageant
Ajouter 50μL d'eau distillée



Suspension
Dans l'eau

PCR

Polymérase Chain
Reaction



ADN polymérase (Taq)



Thermus aquaticus

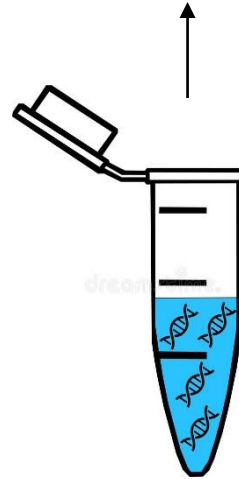
ADN total



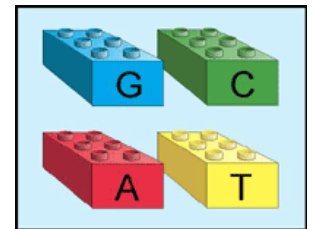
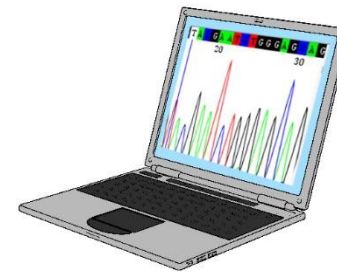
Amorces (primers)



Nucléotide (dntp)



ATTCGGGATTTCGGATTCA



PCR

Polymérase Chain
Reaction

X 30

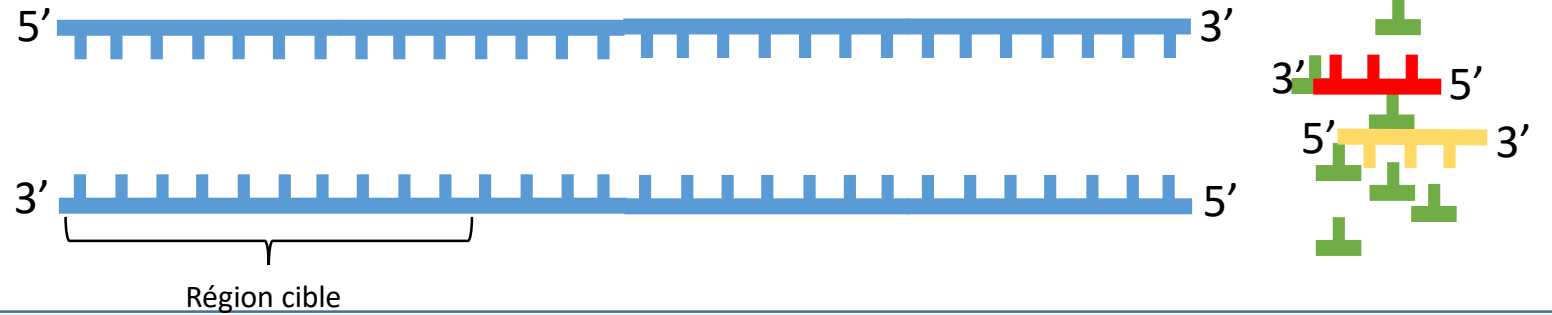
Dénaturation :

98°C



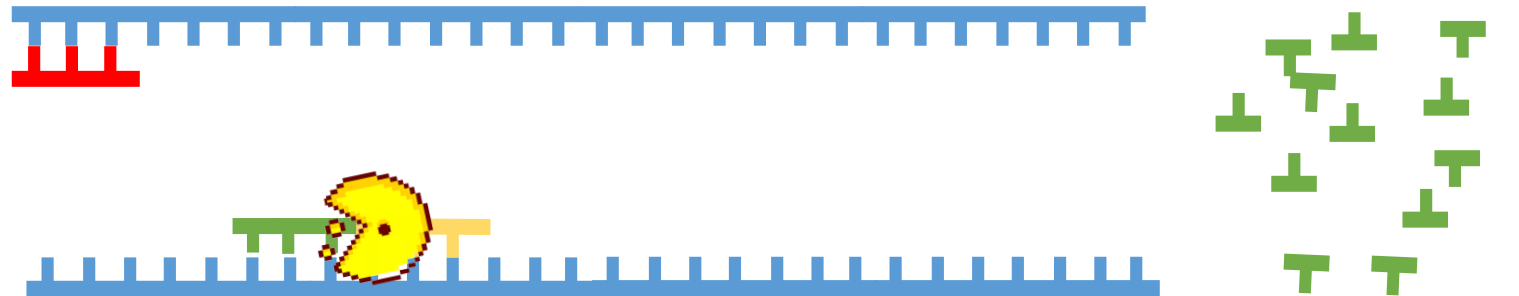
Hybridation :

45°C



Elongation :

72°C



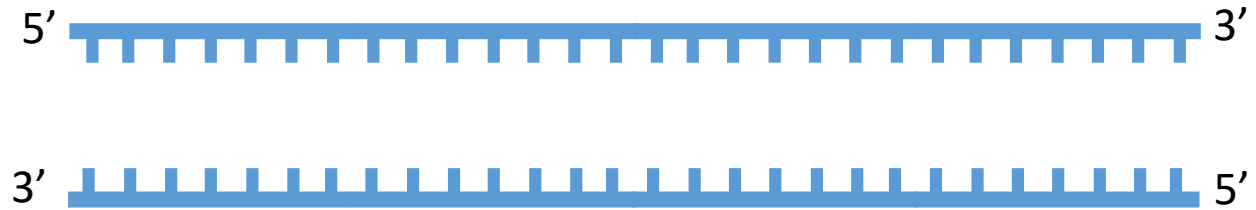
PCR

Polymérase Chain
Reaction

X 30

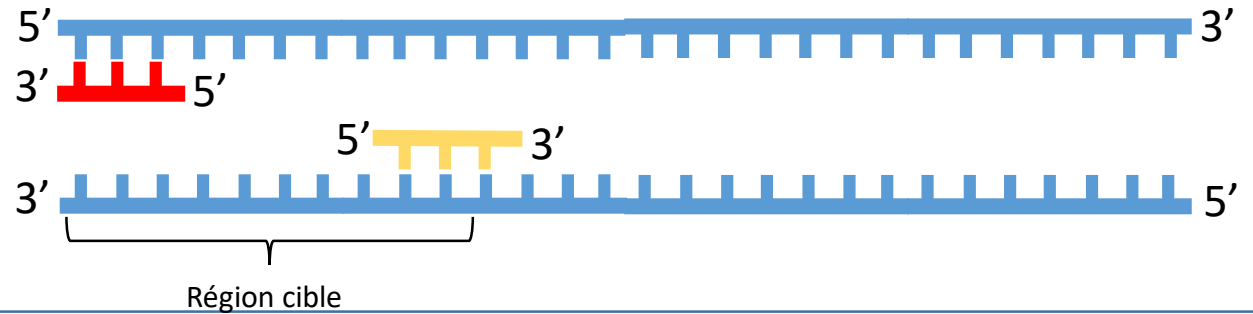
Dénaturation :

98°C



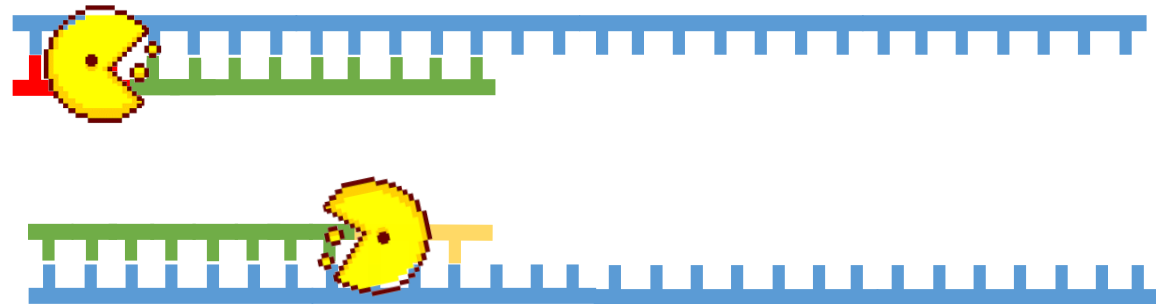
Hybridation :

40-60°C



Elongation :

72°C

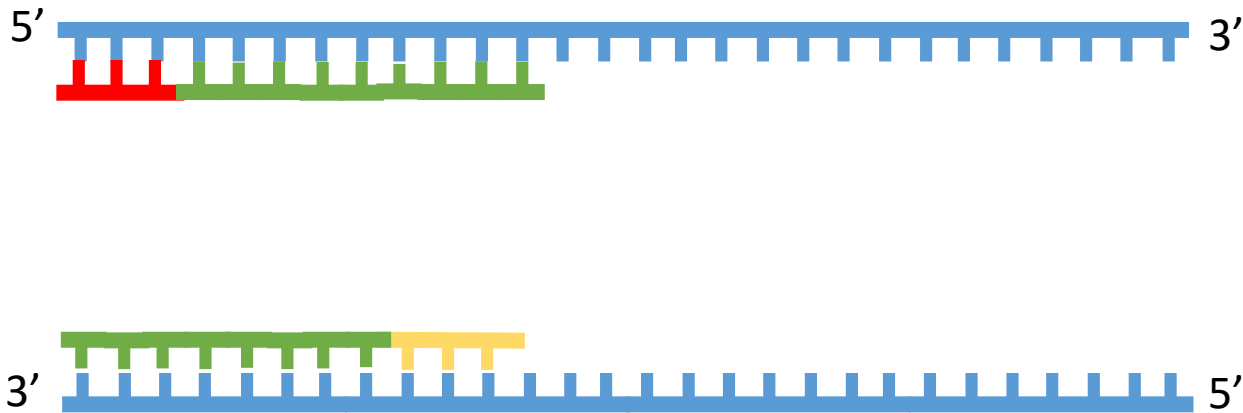


PCR

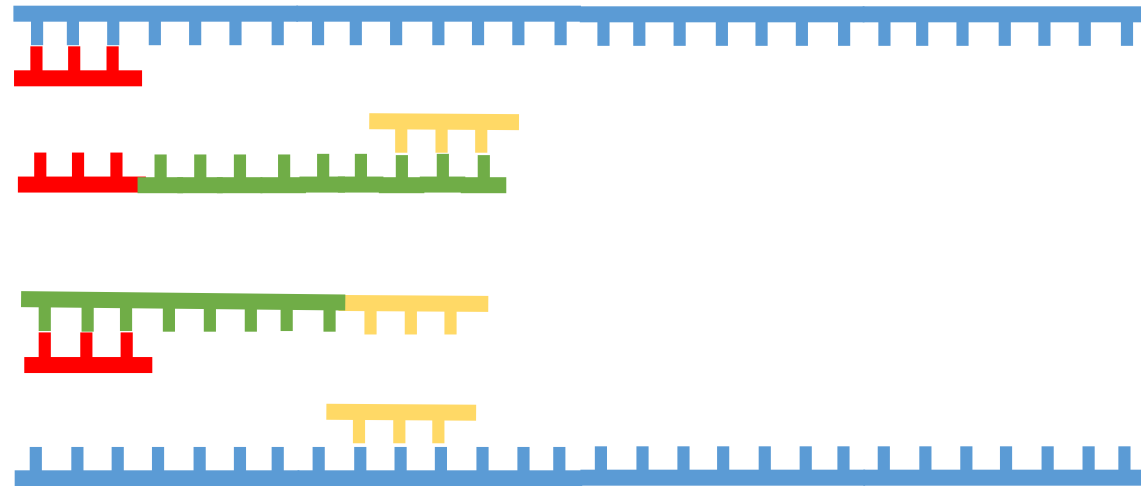
Polymérase Chain
Reaction

X 30

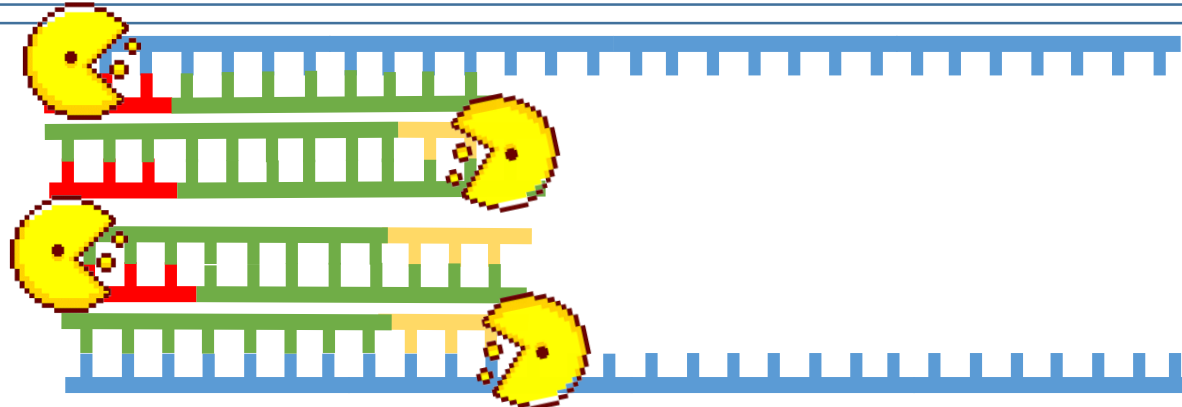
Dénaturation : 98°C



Hybridation : 40-60°C



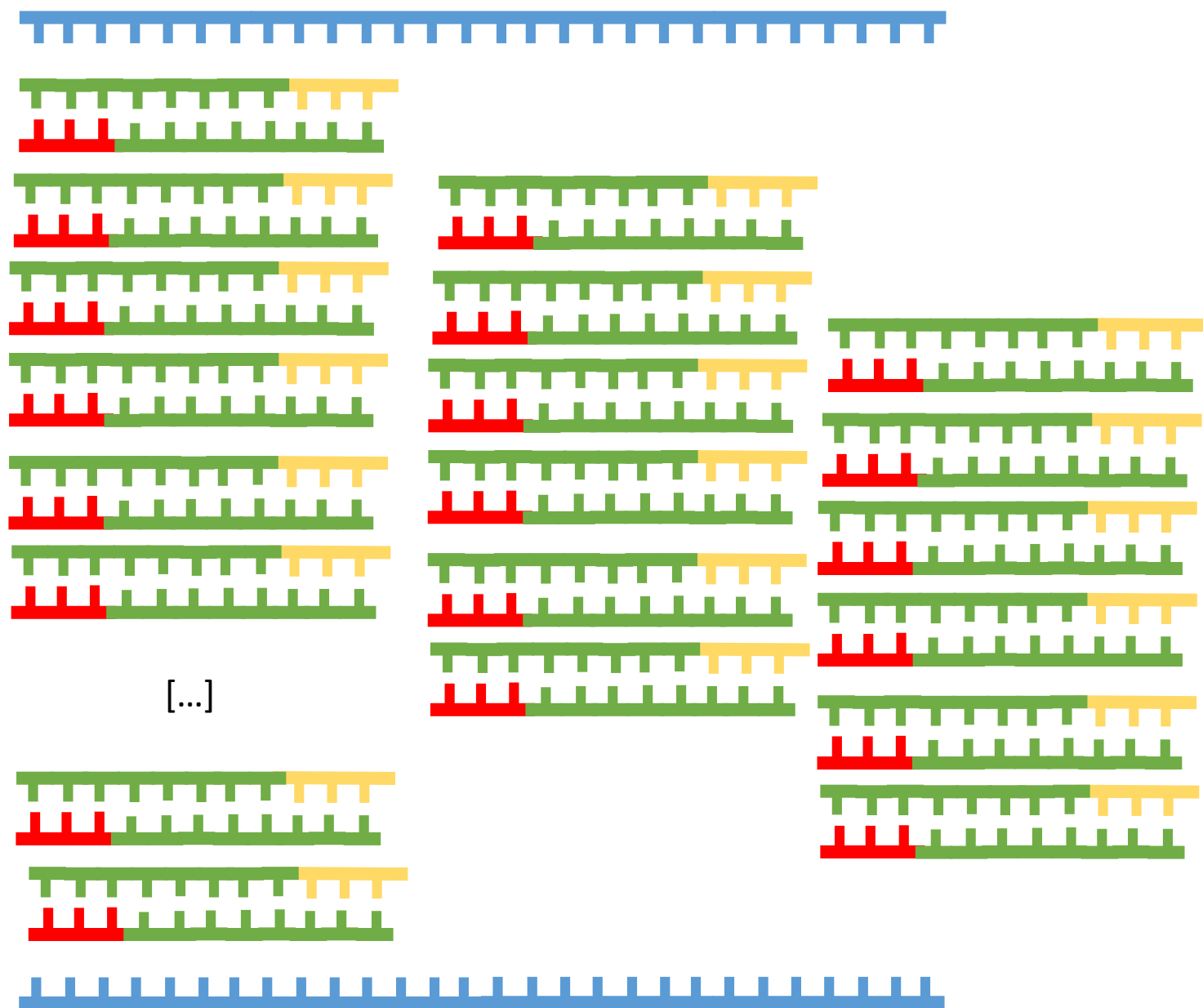
Elongation : 72°C



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.

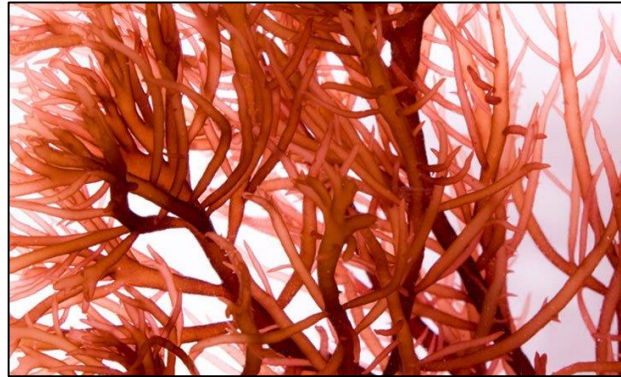
Vérifier que la PCR a fonctionné :

Est-ce que l'ADN est amplifié ?

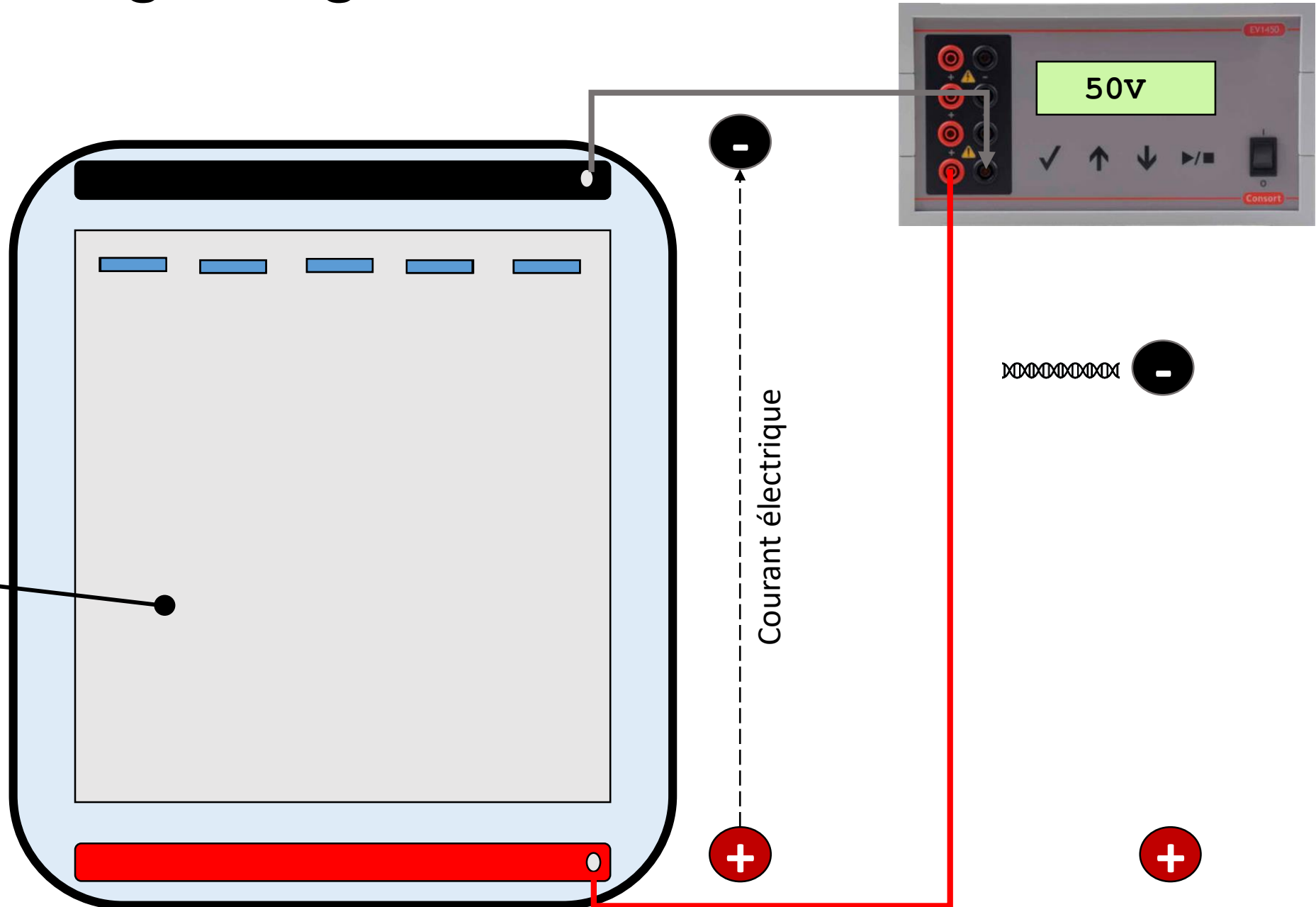
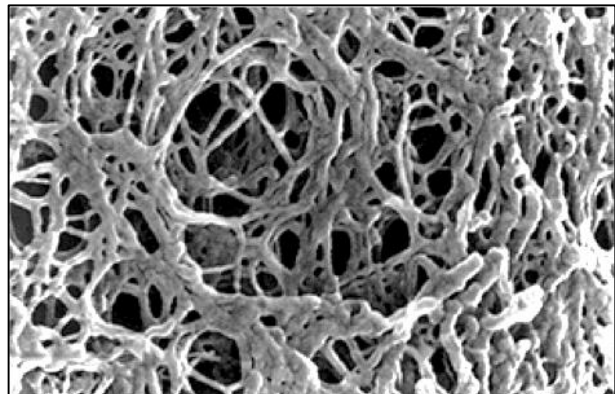
Est-ce que c'est la bonne région ?

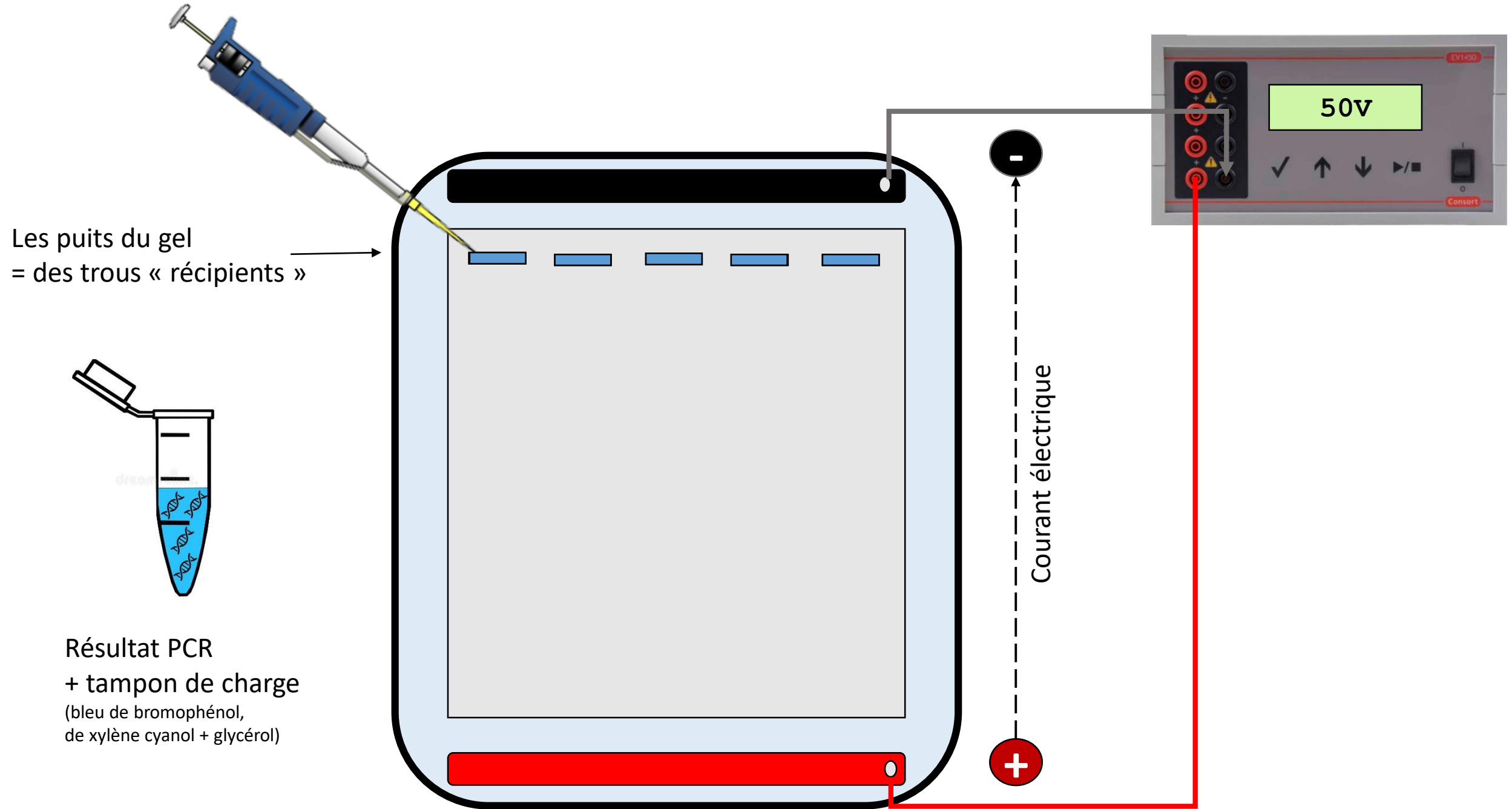


Electrophorèse sur gel d'agarose



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





Allumage LED : Fluorescence

Plus longue séquence

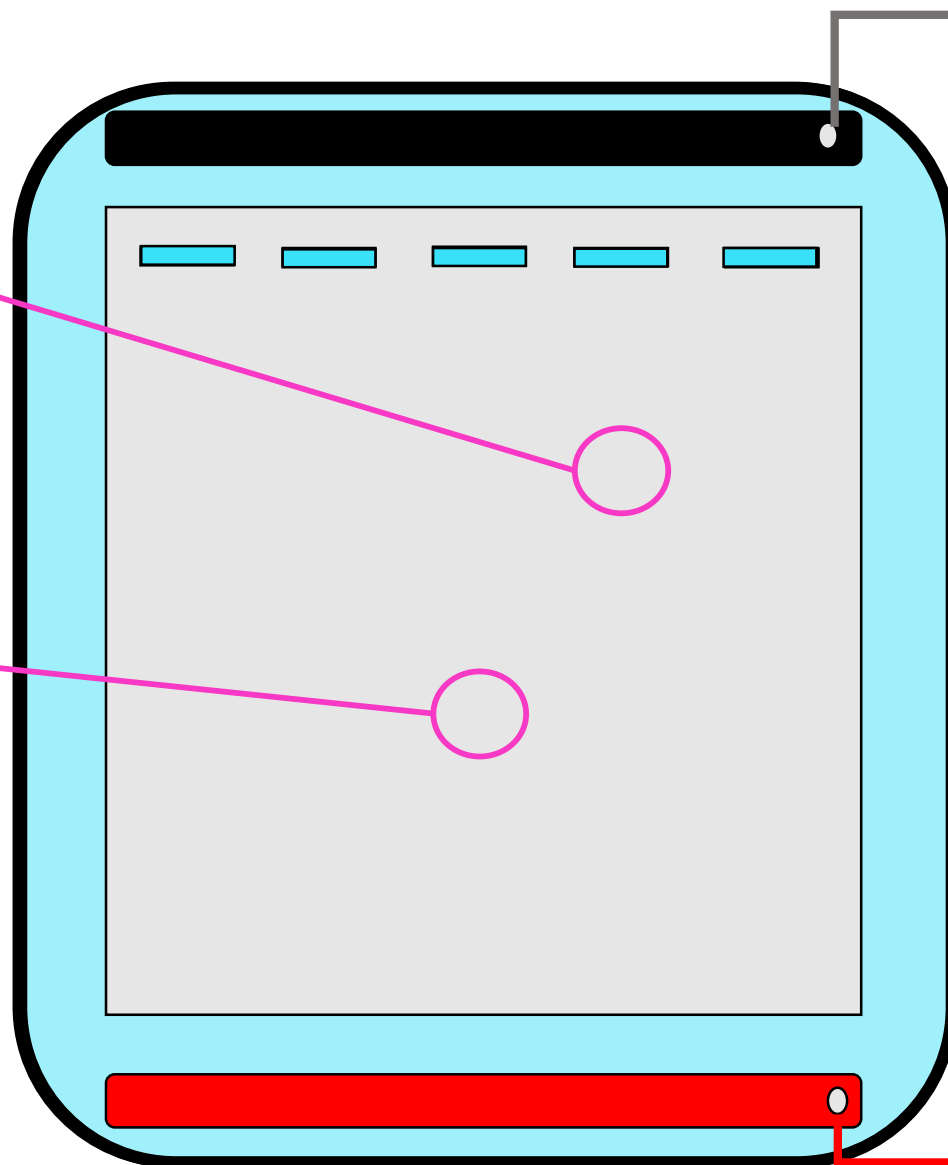


Molécule migre lentement

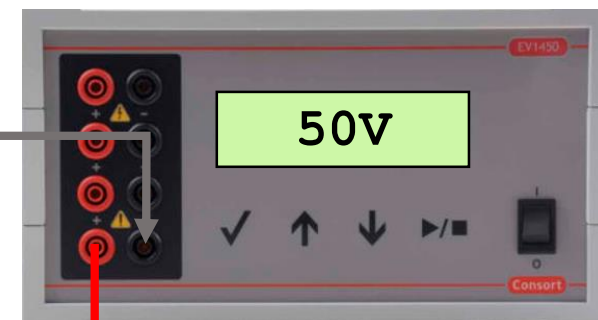
Plus courte séquence



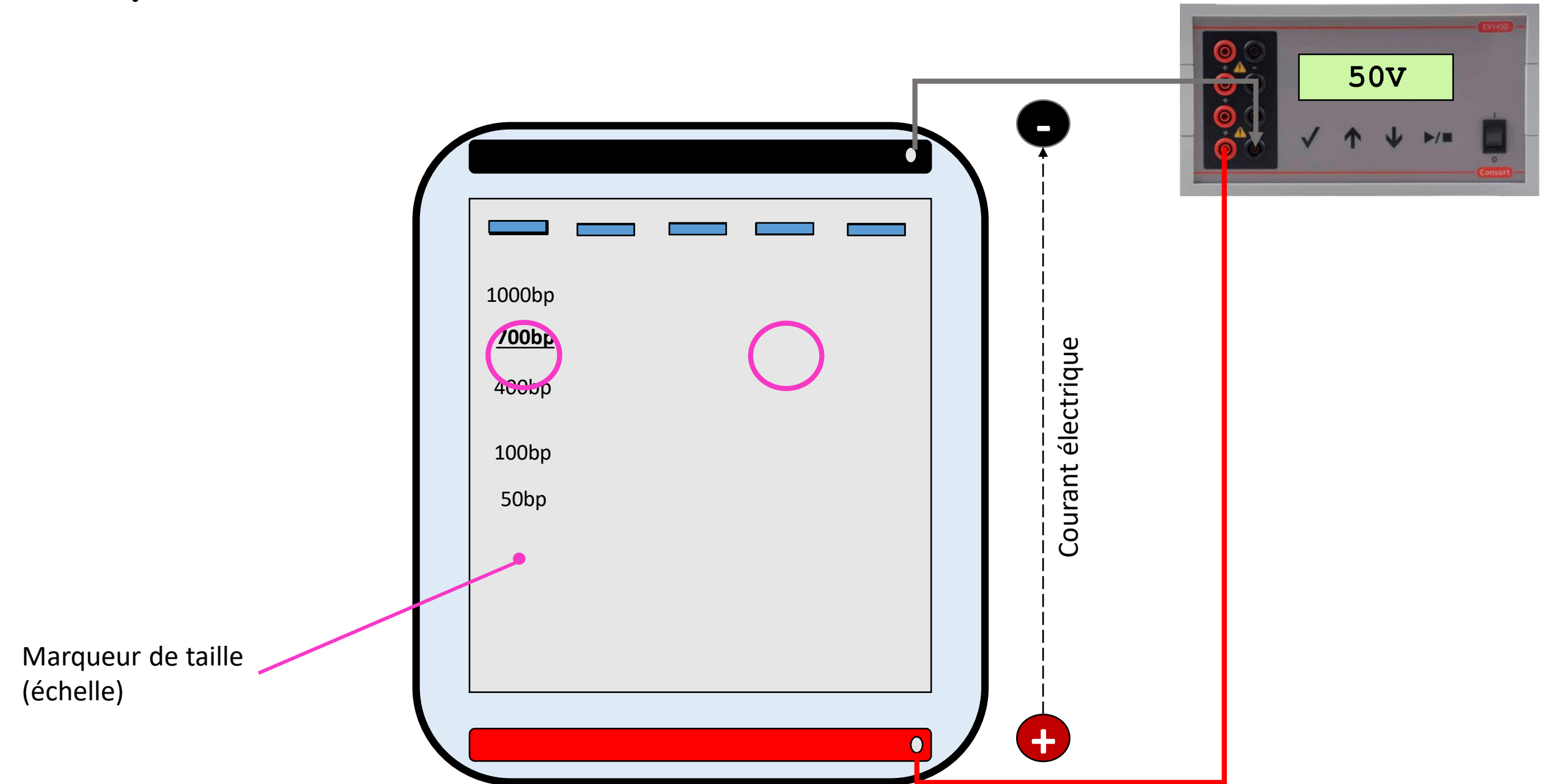
Molécule migre vite



Courant électrique

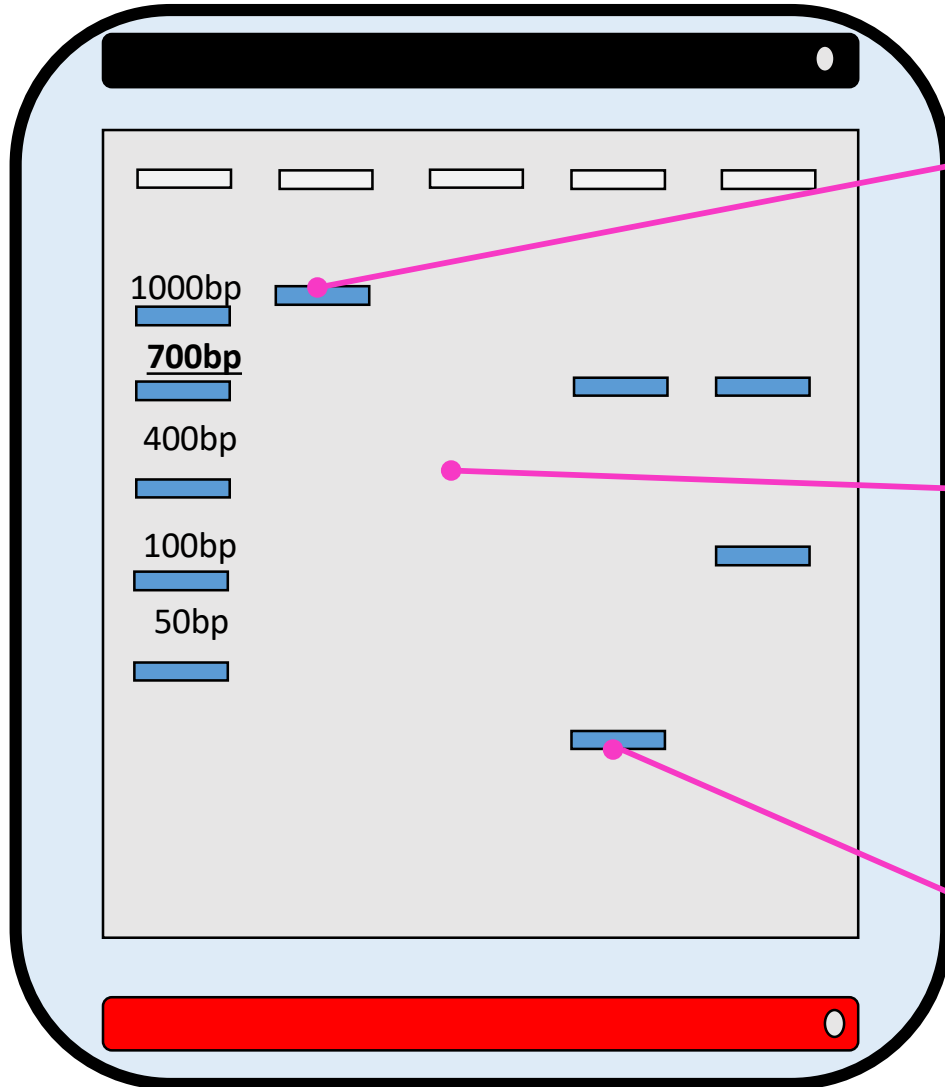


Comparer les tailles ?



Les résultats qui n'ont « pas marché » 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 $MgCl_2$)
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 ?

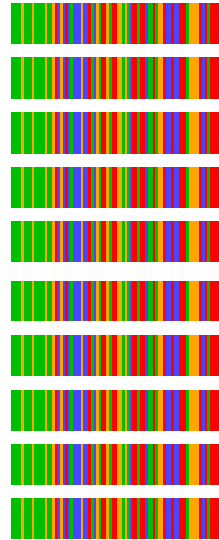
Résumé en une page



Extraction
ADN

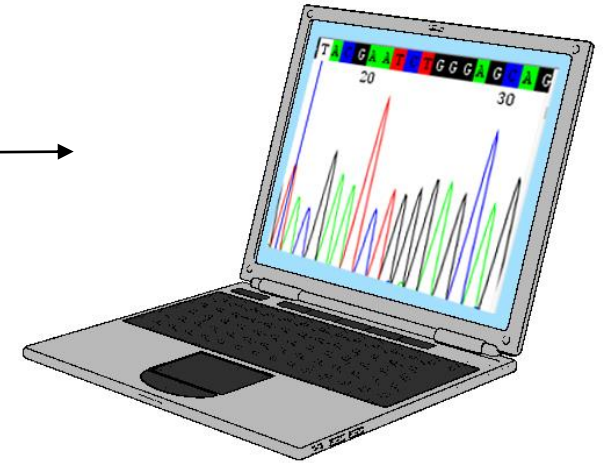


COI



Produit PCR

PCR :
Multi copie du
Gène étudié.



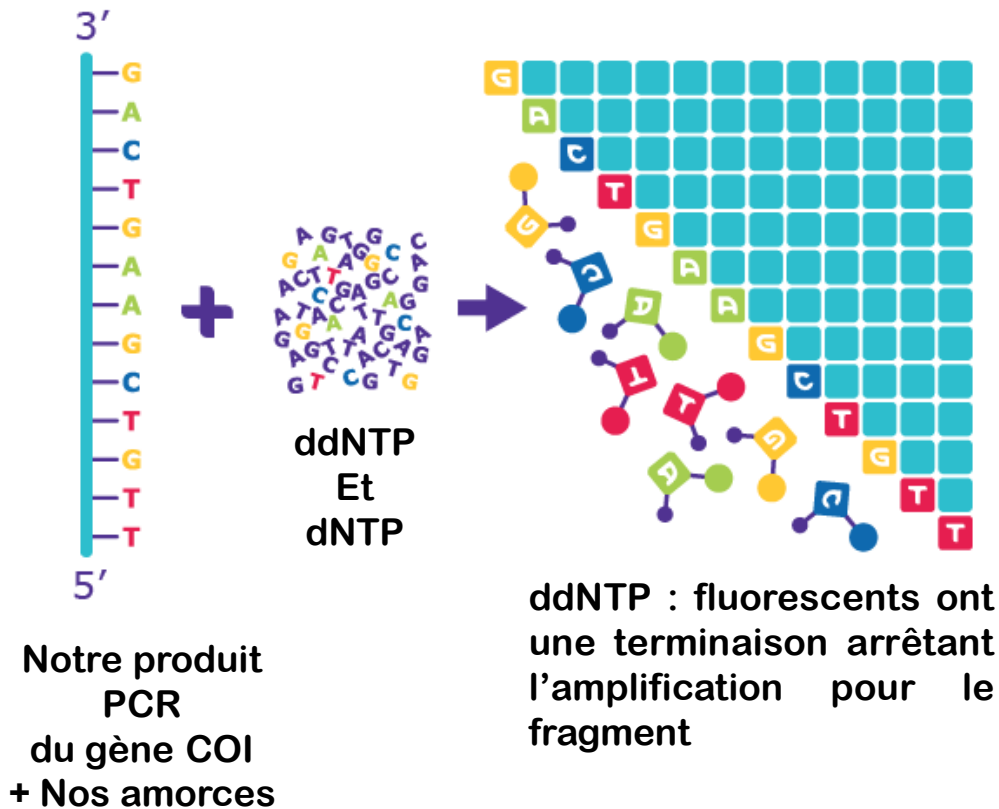
Séquençage :
Envoi à une plateforme de service

Séquençage 'Sanger'

100-1200 bp

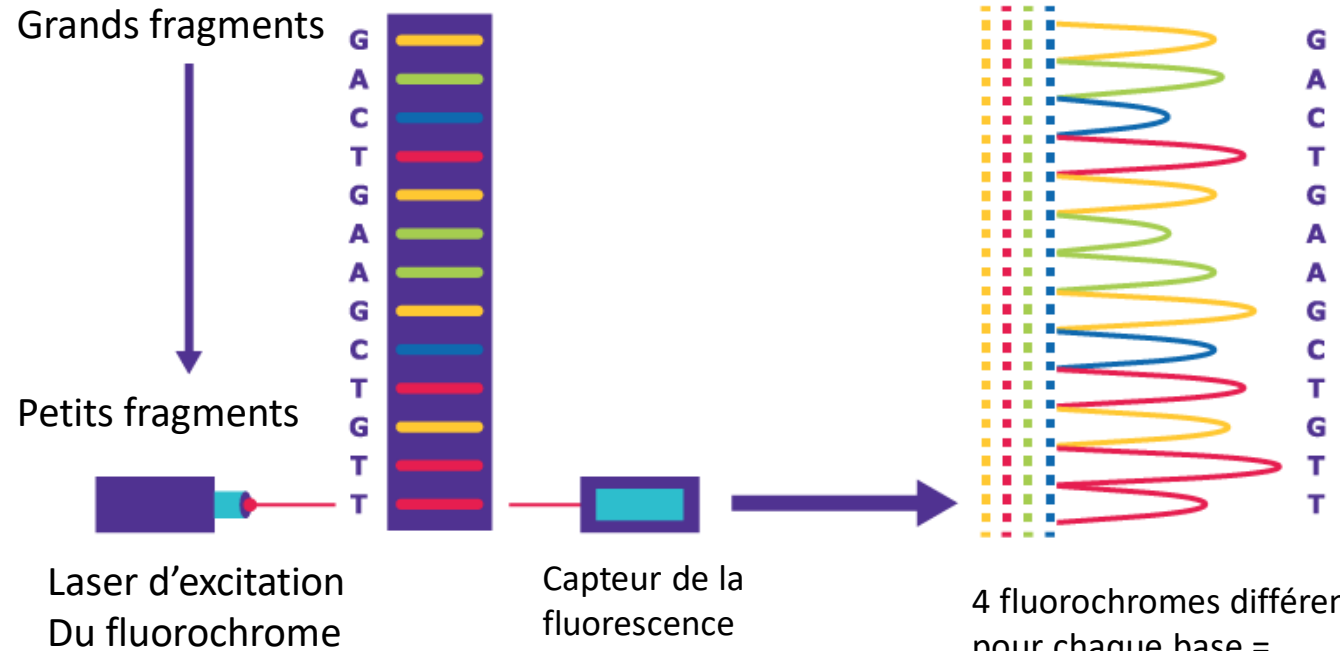
1

PCR avec ddNTPs fluorescent et dNTPs classique.



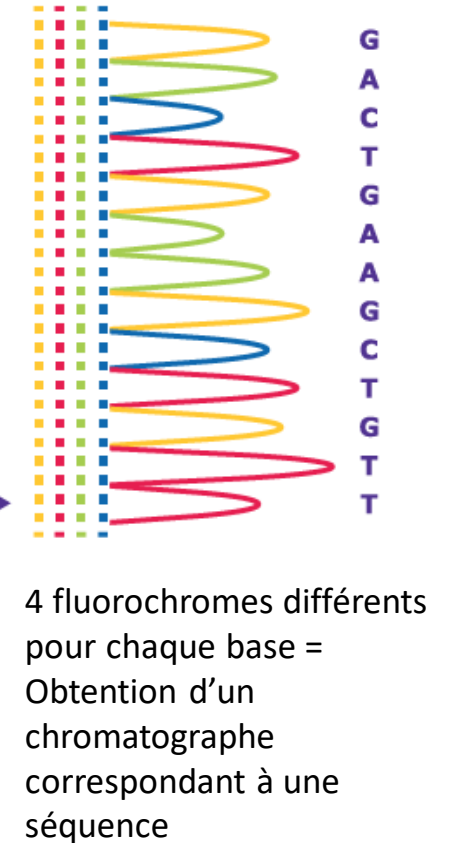
2

Les fragments sont séparés par taille croissante par gel capillaires d'électrophorèse



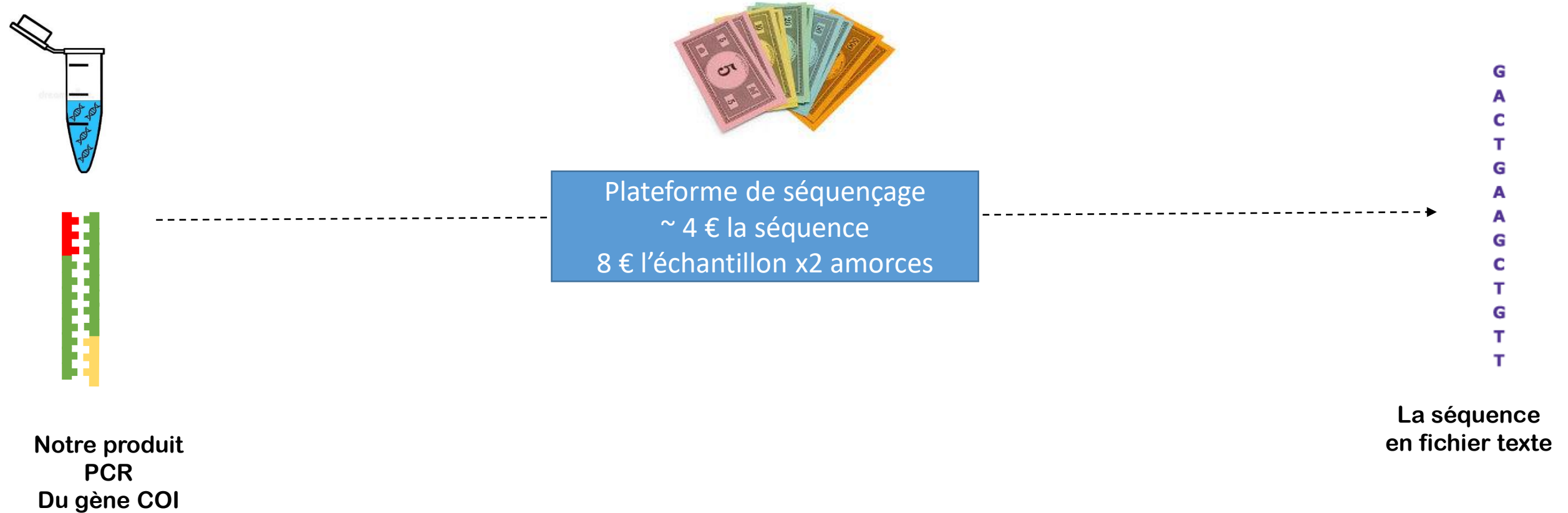
3

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



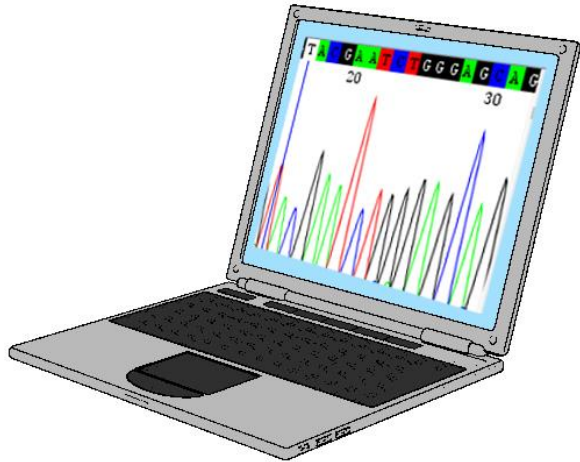
Séquençage 'Sanger'

100-1200 bp



Identification de l'espèce

à partir de la séquence COI



> Echantillon inconnu n12

```
TCGTATGAATAATTTGAGTTTTTGGTTATTACCCCCTTCTTTATTTT
TATTAGTGGTTTCTAGAATAGTTGAAATAGGAGTAGGAACAGGT
TGGACTGTGTATCCTCCATTGTCTTCTAATGTAGGGCATGCTTTT
GTGTCGGTTGATTTTGCTATTTTTTCTCTTCATTTAGCTGGGGCT
TCTTCTATTATAGGGGCTGTTAATTTCACTACTACTATTATTAATAT
GCGAACTTTTGGTATGAGTATGGAAAAGGTTCCCTTGTTTGTTT
GATCAGTGTTAGTGACTGCTGTTTTGTTGTTGTTATCTTTACCTGT
ATTAGCTGGTGCTATTACTATGTTGTTAACTGATCGTAATTTTAAT
ACCTCTTTTTTTGACCCTGCTGGCGGGGGGGATCCAGTGTTATT
TCAACATTTGTT
```

Nom de l'espèce ?
Identification ou confirmation

L'identification de l'espèce Avec BLAST



Qwant

The search engine that respects your privacy.

ncbi blast



L'identification de l'espèce

 U.S. National Library of Medicine

 National Center for Biotechnology Information

BLAST[®]

HomeRecent ResultsSaved Str

Basic Local Alignment Search Tool

BLAST finds regions of similarity between biological sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance.

[Learn more](#)

NEWS

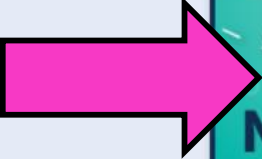
BLAST+ 2.10.1 is released – Fix for TBLASTN Multi-Threading issue.

This version supports pulling databases from our FTP site as well from [cloud providers](#) or our [BLAST+Docker solution](#).

Thu, 18 June 2020 12:00:00 EST

[More BLAST news...](#)

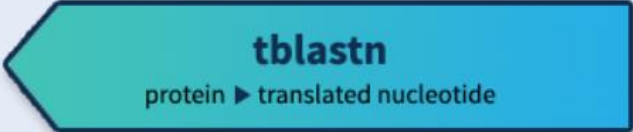
Web BLAST



Nucleotide BLAST
nucleotide ► nucleotide



blastx
translated nucleotide ► protein



tblastn
protein ► translated nucleotide



Protein BLAST
protein ► protein

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
Query subrange ⓘ

From
To

```
> Echantillon inconnu n12
TCGTATGAATAATTGAGTTTITGGTTATTACCCCTTCTTTATTTTTATTAGTGTTTCTAGAATAG
TTGAAATAGGAGTAGGAACAGGTGGACTGTGTATCCCTCATTTCTCTTAAGTAGGGCAATGCTTTI
GIGICGGTTGATTTTGCATTTTTCCTCCTCATTTAGCTGGGGCTTCTCTTATTATAGGGGCTGTTAA
TTTCATTACTACTATTATTATATGCGAAGCTTTGGTATGAGTATGAAAAGGTTCCCTTGTTTGTTT
GATCAGTGTAGTACTGCTGTGTTTGTGTTGTTATCTTTACCTGTATTAGCTGGTGTCTATTACTATG
TTCTTTTCTGCTTCTGCTTTCTGCTTTCTGCTTTCTGCTTTCTGCTTTCTGCTTTCTGCTTTCTGCTTT
```

Or, upload file

Aucun fichier sélectionné. ⓘ

Job Title

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

You [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 ⓘ



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

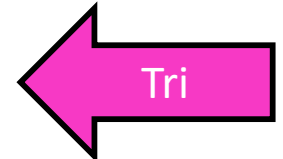
Organism *only top 20 will appear* ☐ exclude

Type common name, binomial, taxid or group name

[+ Add organism](#)

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

[Filter](#) [Reset](#)



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 microphthalmia 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 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	405	405	99%	5e-110	84.21%	KY017938.1



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](#)

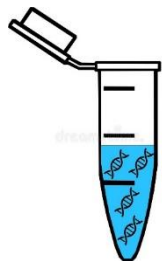
▼ [Next Match](#) ▲ [Previous Match](#)

Score	Expect	Identities	Gaps	Strand
776 bits(420)	0.0	420/420(100%)	0/420(0%)	Plus/Plus
Query 1	TCGTATGAATAATTTGAGTTTTTGGTTATTACCCCCTTCTTTATTTTTATTAGTGGTTTC	60		
Sbjct 1	TCGTATGAATAATTTGAGTTTTTGGTTATTACCCCCTTCTTTATTTTTATTAGTGGTTTC	60		
Query 61	TAGAATAGTTGAAATAGGAGTAGGAACAGGTTGGACTGTGTATCCTCCATTGTCTTCTAA	120		
Sbjct 61	TAGAATAGTTGAAATAGGAGTAGGAACAGGTTGGACTGTGTATCCTCCATTGTCTTCTAA	120		
Query 121	TGTAGGGCATGCTTTTGTGTCGGTTGATTTTGCTATTTTTCTCTTCATTTAGCTGGGGC	180		
Sbjct 121	TGTAGGGCATGCTTTTGTGTCGGTTGATTTTGCTATTTTTCTCTTCATTTAGCTGGGGC	180		
Query 181	TTCTTCTATTATAGGGGCTGTTAATTTTCACTACTATTATTAATATGCGAACTTTTGG	240		
Sbjct 181	TTCTTCTATTATAGGGGCTGTTAATTTTCACTACTATTATTAATATGCGAACTTTTGG	240		
Query 241	TATGAGTATGGAAAAGGTTCCCTTGTTTGTTGATCAGTGTTAGTGACTGCTGTTTTGTT	300		
Sbjct 241	TATGAGTATGGAAAAGGTTCCCTTGTTTGTTGATCAGTGTTAGTGACTGCTGTTTTGTT	300		
Query 301	GTTGTTATCTTTACCTGTATTAGCTGGTGCTATTACTATGTTGTTAACTGATCGTAATTT	360		
Sbjct 301	GTTGTTATCTTTACCTGTATTAGCTGGTGCTATTACTATGTTGTTAACTGATCGTAATTT	360		
Query 361	TAATACCTCttttttttGACCCTGCTGGCgggggggATCCAGTGTTATTTCAACATTTGTT	420		
Sbjct 361	TAATACCTCTTTTTTTGACCCTGCTGGCGGGGGGATCCAGTGTTATTTCAACATTTGTT	420		

Toutes les étapes par nous même ?



MinION 900 euro



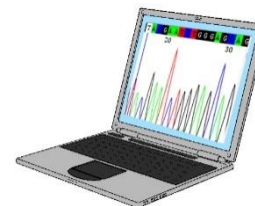
Notre produit
PCR
Du gène COI



Consommables
539 euro



5 échantillons



Ordi performant
3500 euro

= beaucoup plus cher, il faut avoir une vraie raison d'être si pressé, d'avoir le résultat sur le terrain (ex. diagnostic épidémie). En biospéléo y'a pas d'urgence.

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La séquence
en fichier texte



