M1 SM THORAX ED TG: Recherche in silico.

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Team IIA

L'unité de recherche de l'institut du thorax
Inserm UMR 1087 / CNRS UMR 6291
Nantes, France

Nantes, 27 Octobre 2021

1. Les analyses bibliographiques

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Que des sources scientifiques avérées …

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Le **Centre américain pour les informations biotechnologiques** (NCBI), en anglais National Center for Biotechnology Information, est un institut national américain pour l'information biologique moléculaire. Fondé en 1988 à Bethesda dans le Maryland, cet organisme fait partie de la Bibliothèque américaine de médecine, un des Instituts américains de la santé.

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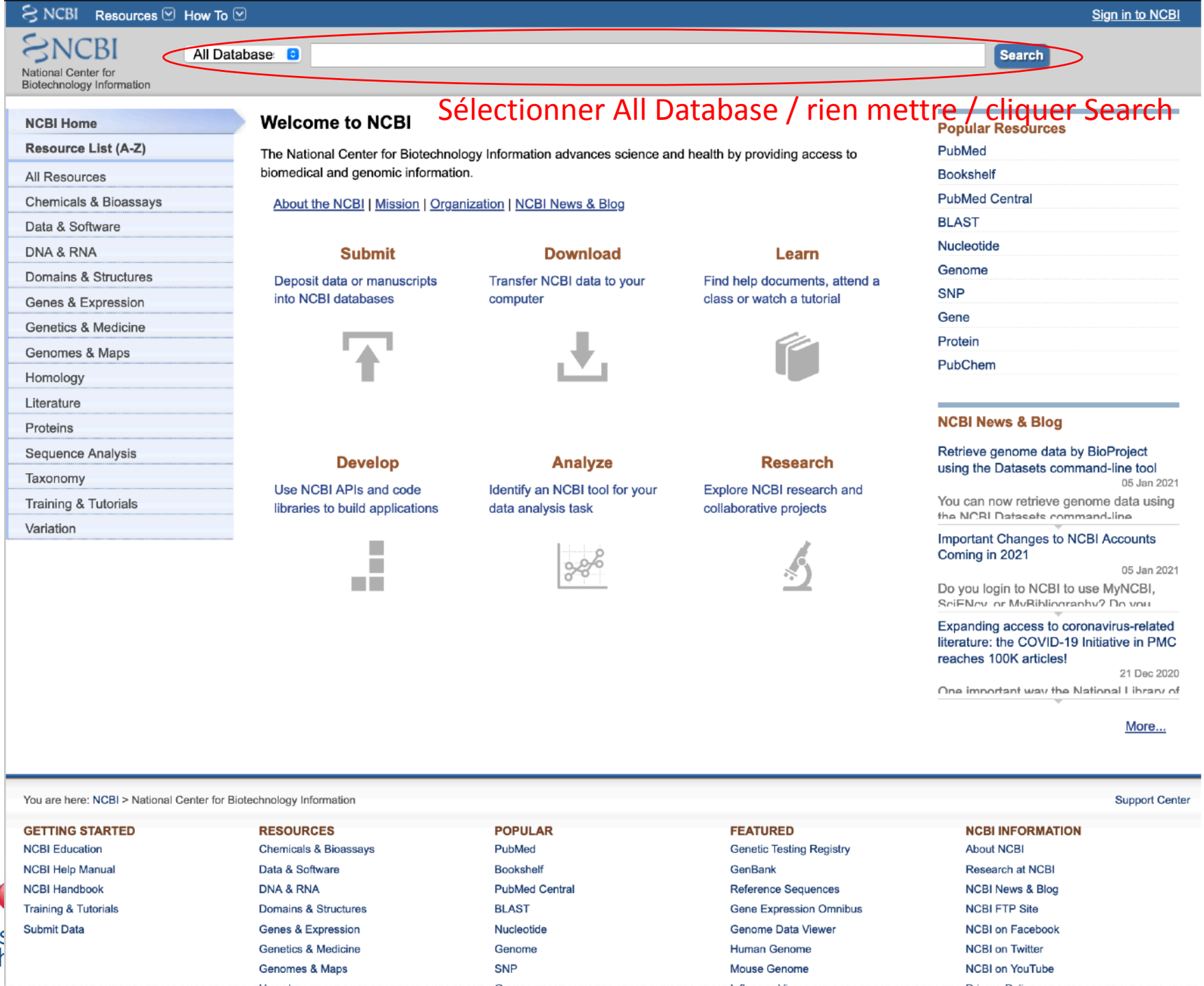
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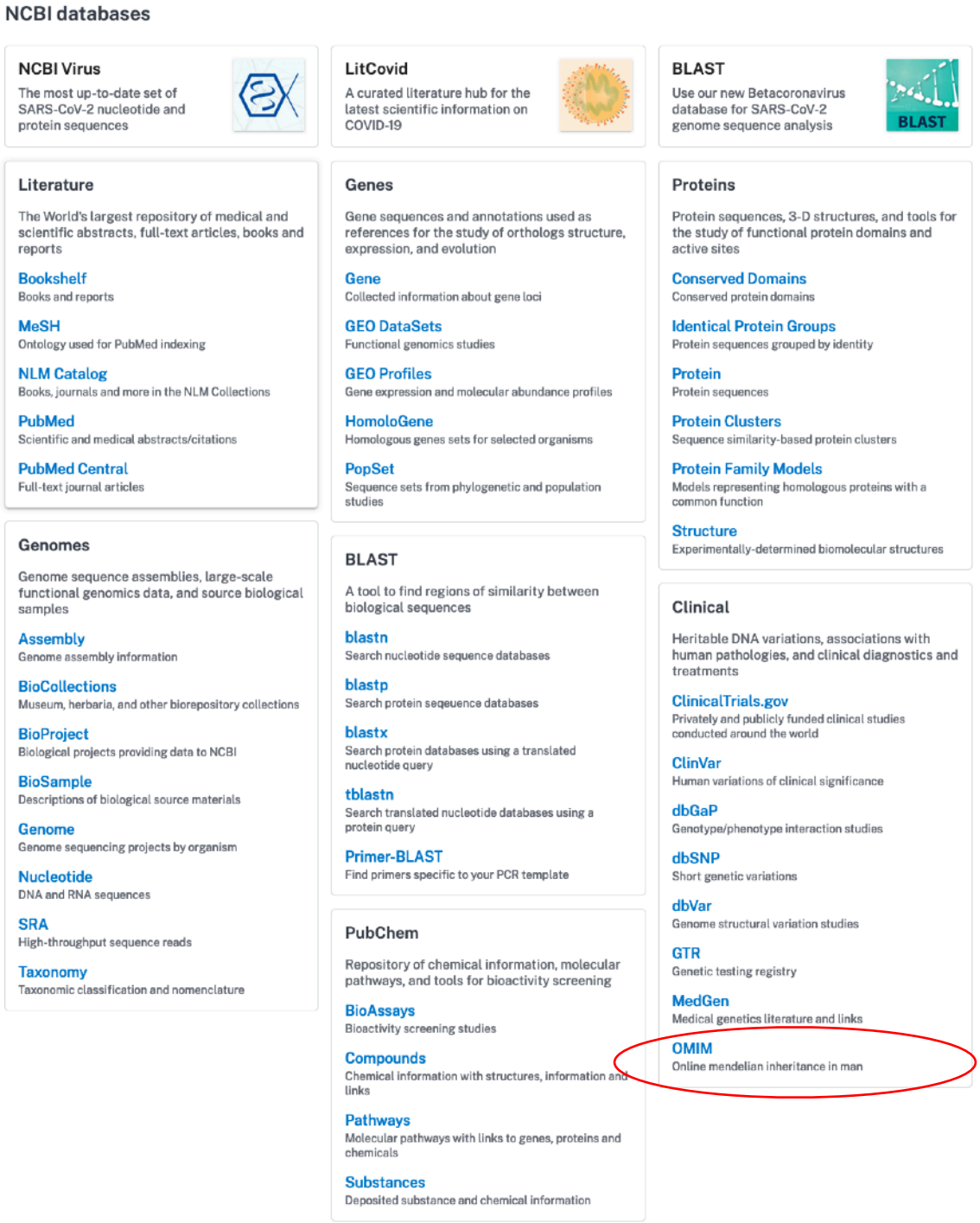

Le NCBI abrite une série de bases de données pertinentes pour la biotechnologie et la biomédecine et constitue une ressource importante pour les outils et services de bioinformatique.

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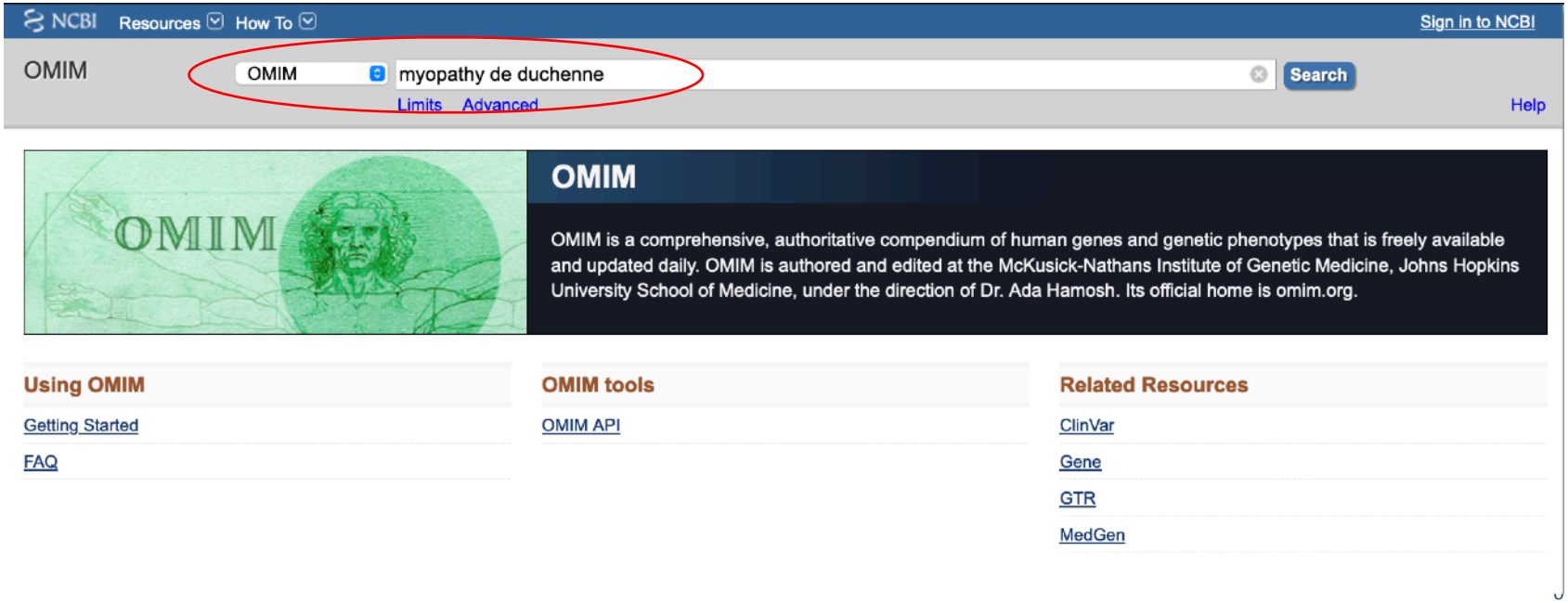
OMIM : Le projet Héritage mendélien chez l'humain (en anglais : Mendelian Inheritance in Man) est une base de données originellement compilée par Victor A. McKusick et qui dresse un catalogue de toutes les maladies connues qui relèvent de l'un ou l'autre composant génétique et — si possible — les relie aux gènes adéquats au sein du génome humain. Cette base de données est disponible sous forme d'un livre appelé Mendelian Inheritance in Man (MIM), qui en est à sa 13e édition.

La version en ligne est appelée Online Mendelian Inheritance in Man, OMIM, et peut être consultée à partir de la base de données Entrez1 de la National Library of Medicine2.



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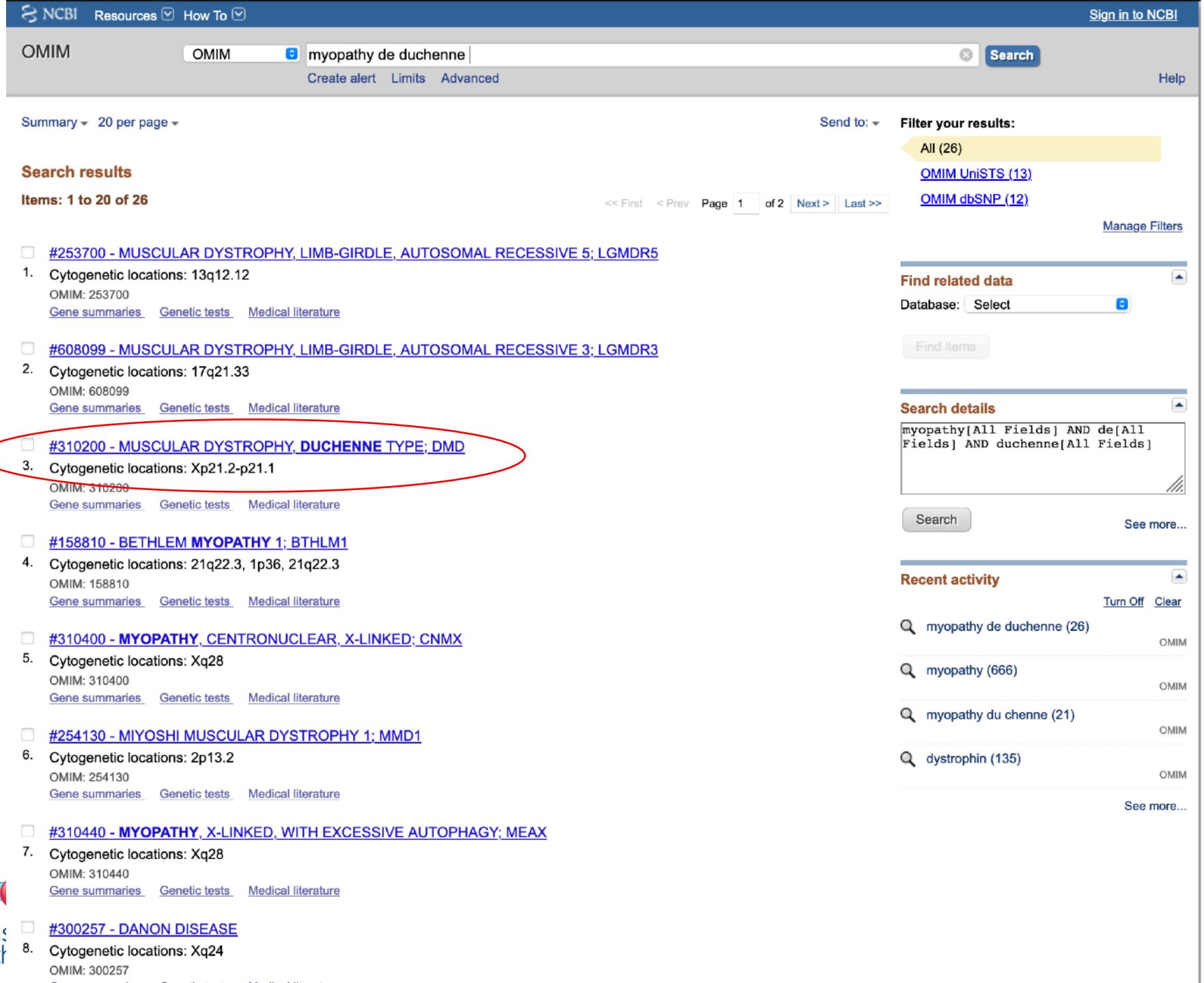
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1. Cytogenetic locations: 13q12.12
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#608099 - MUSCULAR DYSTROPHY, LIMB-GIRDLE, AUTOSOMAL RECESSIVE 3; LGMDR3
2. Cytogenetic locations: 17q21.33
OMIM: 608099
Gene summaries Genetic tests Medical literature
#310200 - MUSCULAR DYSTROPHY, DUCHENNE TYPE; DMD
3. Cytogenetic locations: Xp21.2-p21.1
OMIM: 310200
Gene summaries Genetic tests Medical literature
#158810 - BETHLEM MYOPATHY 1; BTHLM1
4. Cytogenetic locations: 21q22.3, 1p36, 21q22.3
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#310400 - MYOPATHY, CENTRONUCLEAR, X-LINKED; CNMX
5. Cytogenetic locations: Xq28
OMIM: 310400
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#254130 - MIYOSHI MUSCULAR DYSTROPHY 1; MMD1
6. Cytogenetic locations: 2p13.2
OMIM: 254130
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MUSCULAR DYSTROPHY, DUCHENNE TYPE; DMD

Alternative titles; symbols

DUCHENNE MUSCULAR DYSTROPHY
MUSCULAR DYSTROPHY, PSEUDOHYPERTROPHIC PROGRESSIVE, DUCHENNE TYPE

Phenotype-Gene Relationships

Location	Phenotype	Phenotype MIM number	Inheritance	Phenotype mapping key	Gene/Locus	Gene/Locus MIM number
Xp21.2-p21.1	Duchenne muscular dystrophy	310200	XLR	3	DMD	300377

Clinical Synopsis | PheneGene Graphics

TEXT

A number sign (#) is used with this entry because Duchenne muscular dystrophy is caused by mutation in the gene encoding dystrophin (DMD; 300377).

Description

Dystrophin-associated muscular dystrophies range from the severe Duchenne muscular dystrophy (DMD) to the milder Becker muscular dystrophy (BMD; 300376). Mapping and molecular genetic

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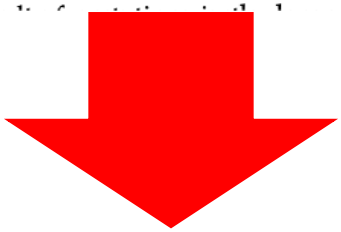

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Description

Dystrophin-associated muscular dystrophies range from the severe Duchenne muscular dystrophy (DMD) to the milder Becker muscular dystrophy (BMD; 300376). Mapping and molecular genetic studies indicate that both are the result of mutations in the huge gene that encodes dystrophin, also symbolized DMD. Approximately two-thirds of the mutations in both forms are deletions of one or many exons in the dystrophin gene. Although there is no clear correlation found between the extent of the deletion and the severity of the disorder, DMD deletions usually result in frameshift. Boland et al. (1996) studied a retrospective cohort of 33 male patients born between 1953 and 1983. The mean age at DMD diagnosis was 4.6 years; wheelchair dependency had a median age of 10 years; cardiac muscle failure developed in 15% of patients with a median age of 21.5 years; smooth muscle dysfunction in the digestive or urinary tract occurred in 21% and 6% of the patients, respectively, at a median age of 15 years. In this cohort, death occurred at a median age of 17 years. The authors commented that the diagnosis of DMD is being made at an earlier age but survival has not changed.

Clinical Features

Skeletal Muscle

The most distinctive feature of Duchenne muscular dystrophy is a progressive proximal muscular dystrophy with characteristic pseudohypertrophy of the calves. The bulbar (extraocular) muscles are spared but the myocardium is affected. There is massive elevation of creatine kinase levels in the blood, myopathic changes by electromyography, and myofiber degeneration with fibrosis and fatty infiltration on muscle biopsy.The onset of Duchenne muscular dystrophy usually occurs before age 3 years, and the victim is chairridden by age 12 and dead by age 20. The onset of Becker muscular dystrophy is often in the 20s and 30s and survival to a relatively advanced age is frequent.

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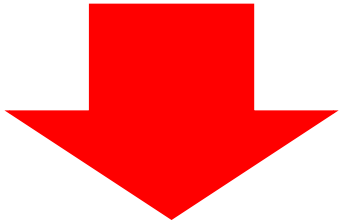

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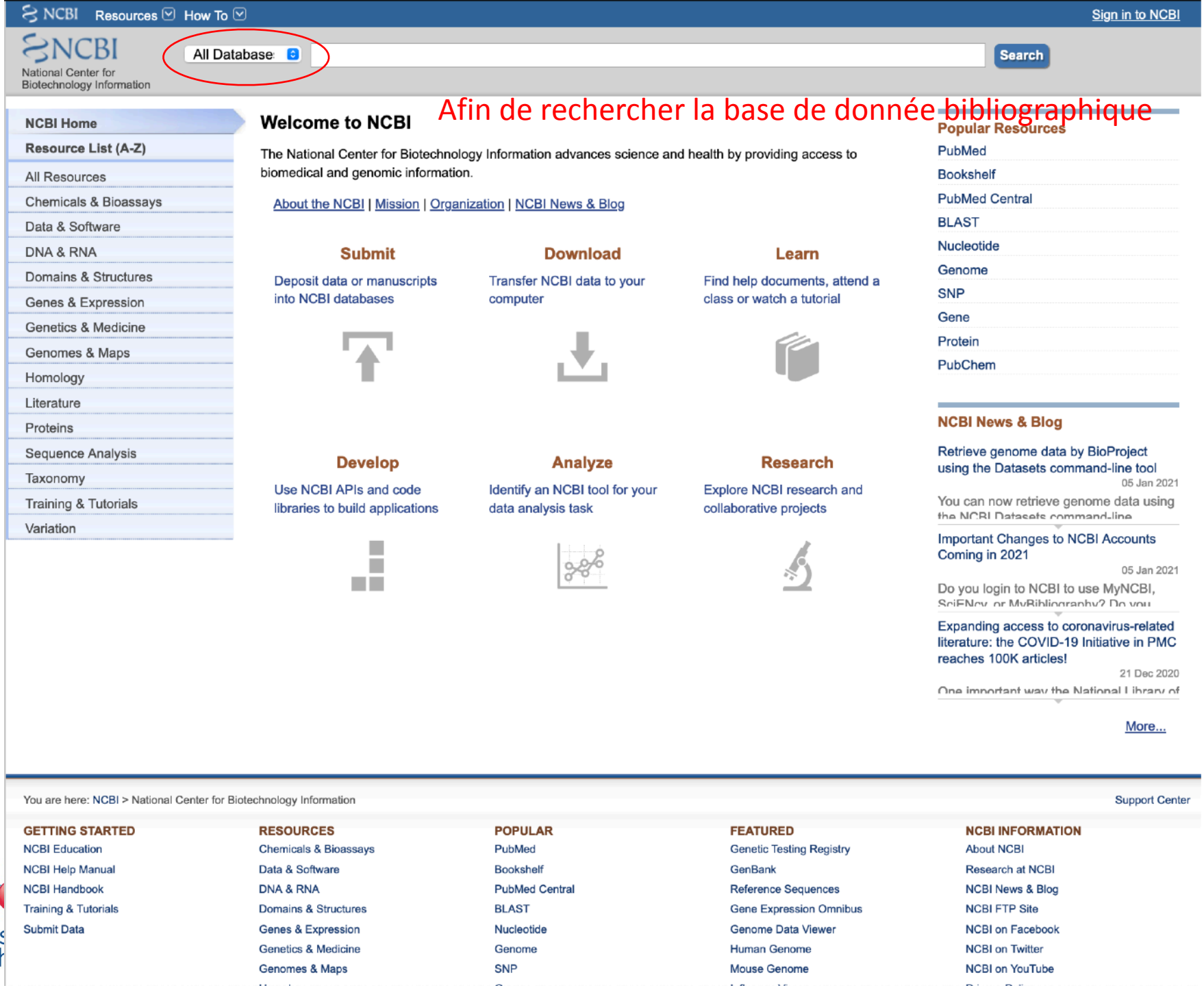
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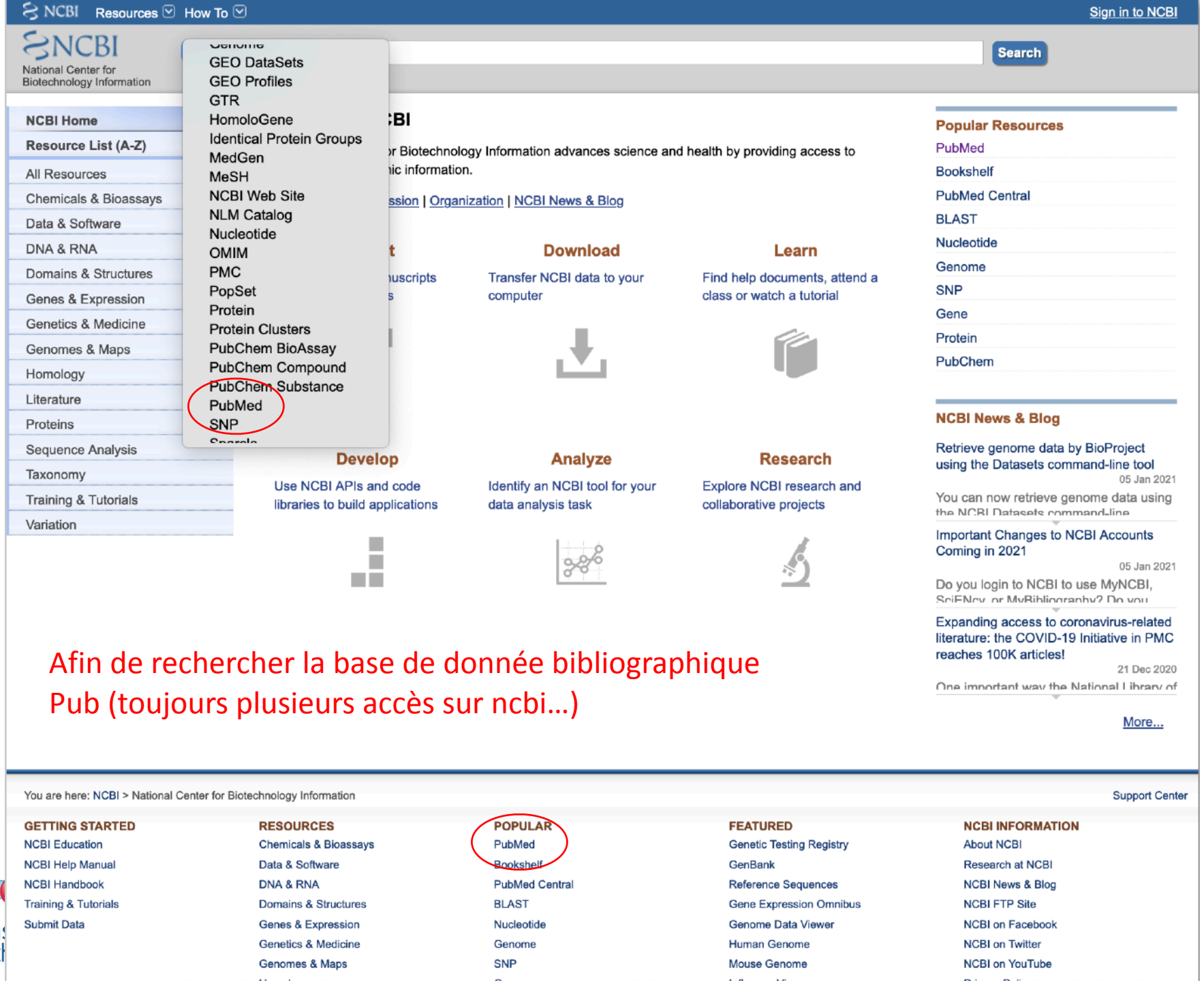

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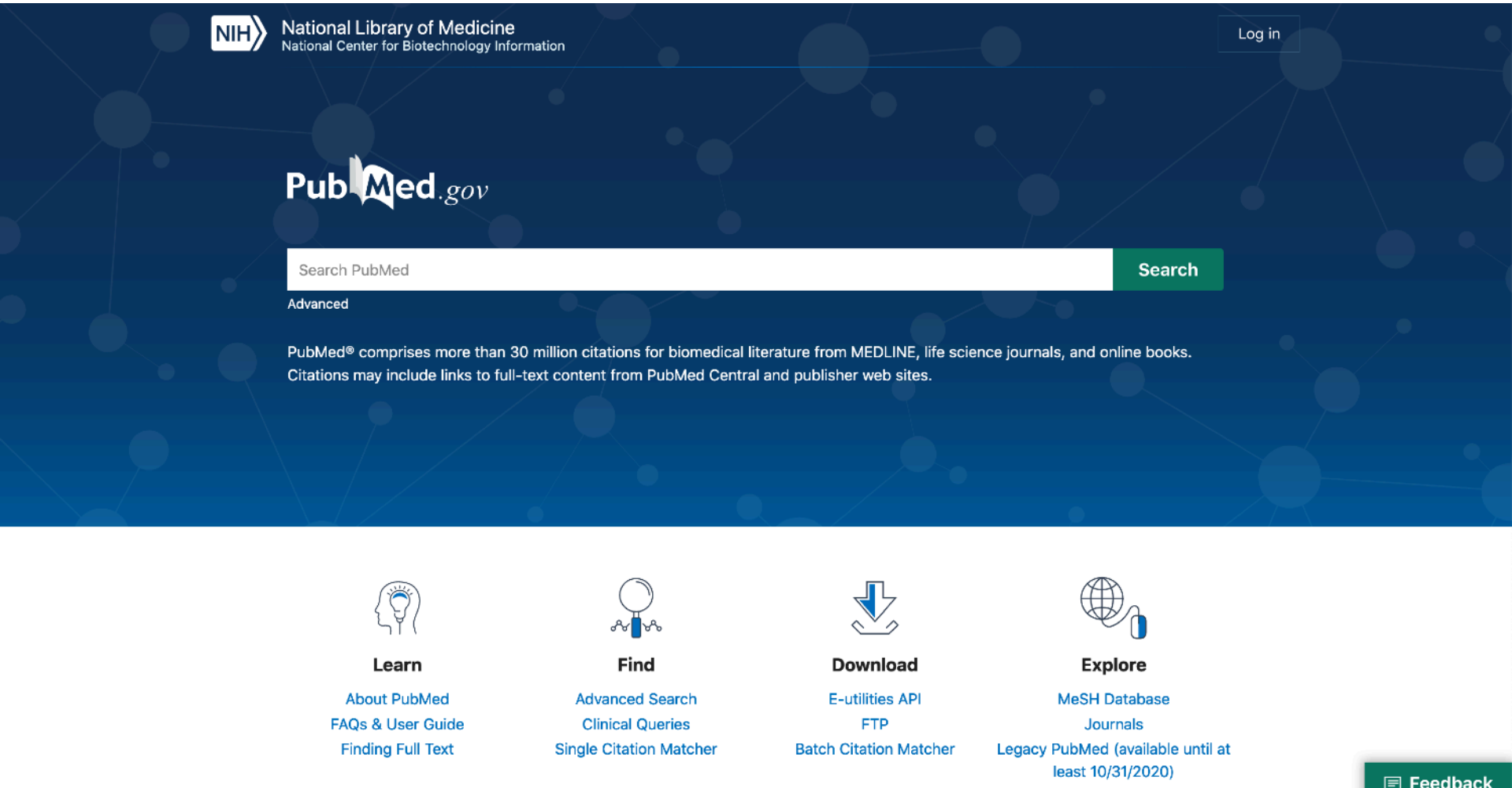

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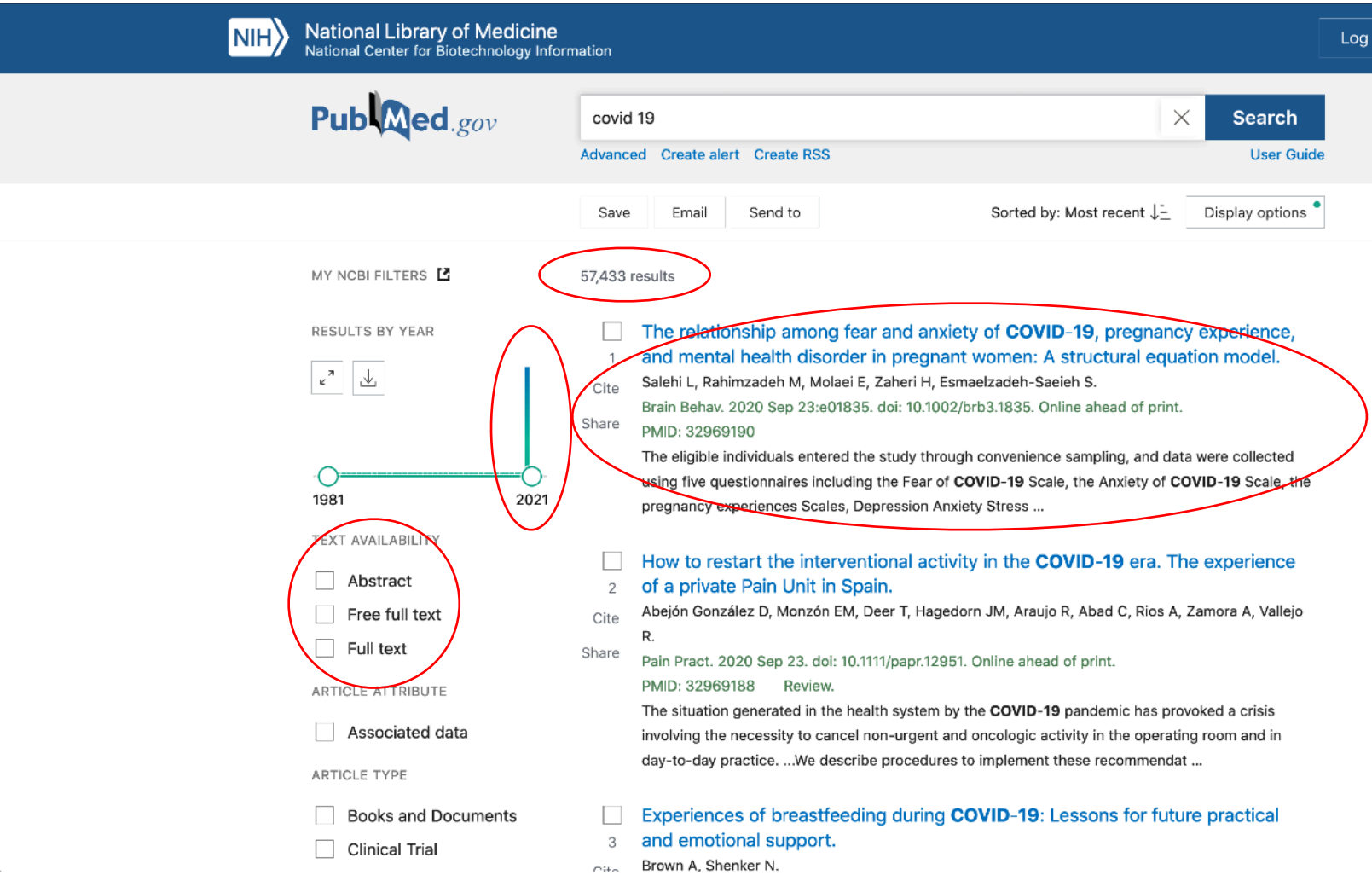

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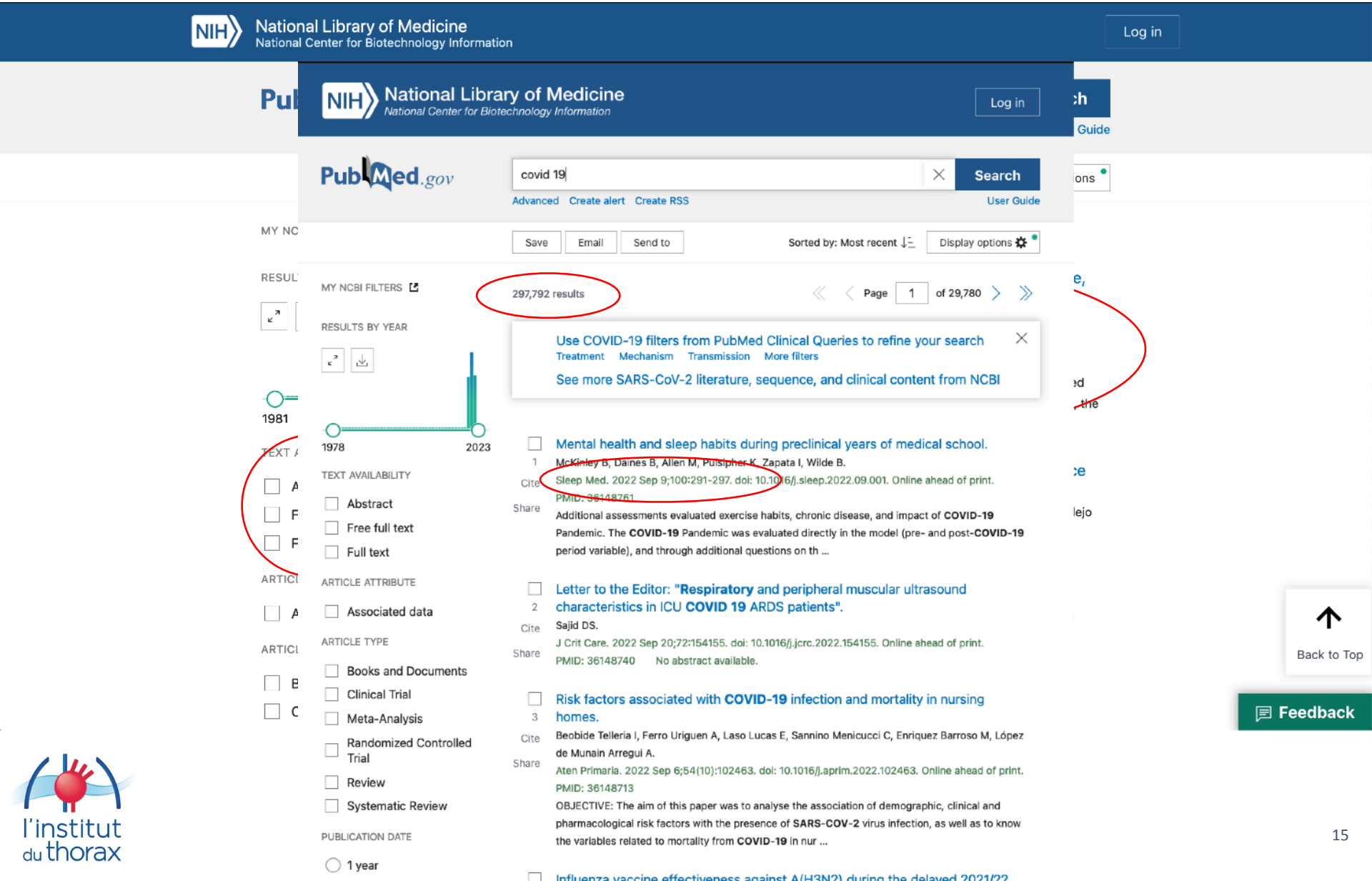

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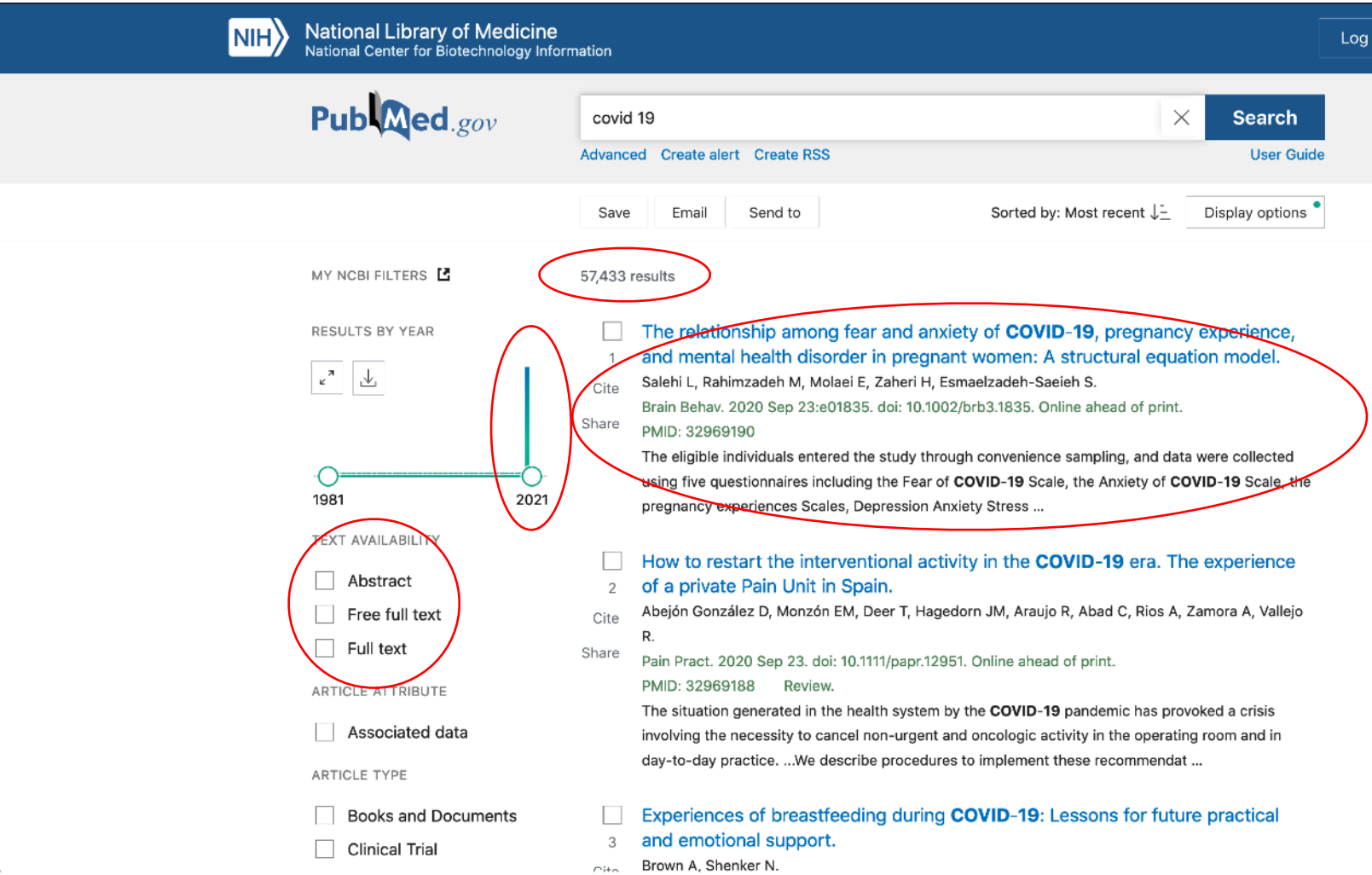

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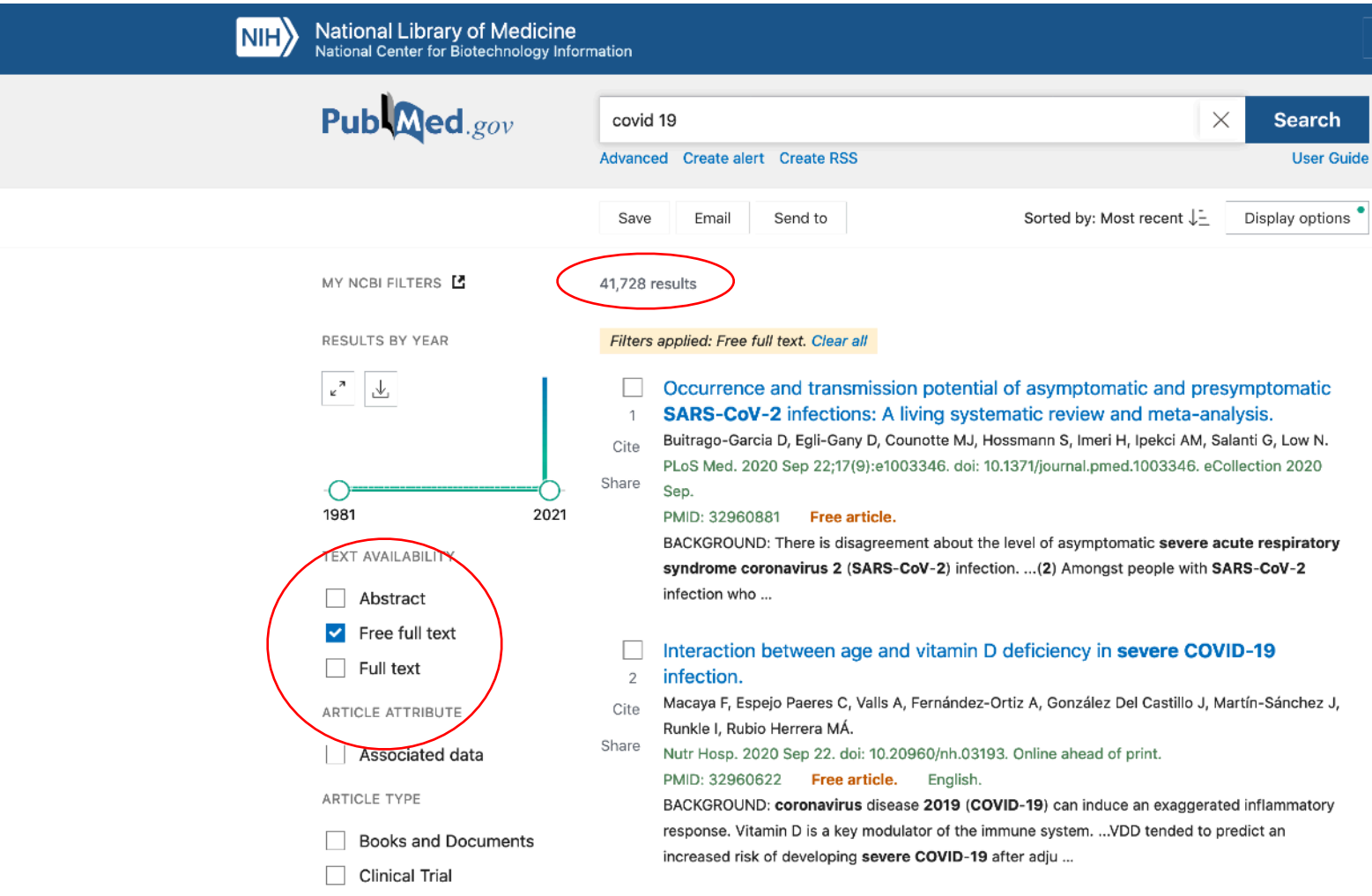

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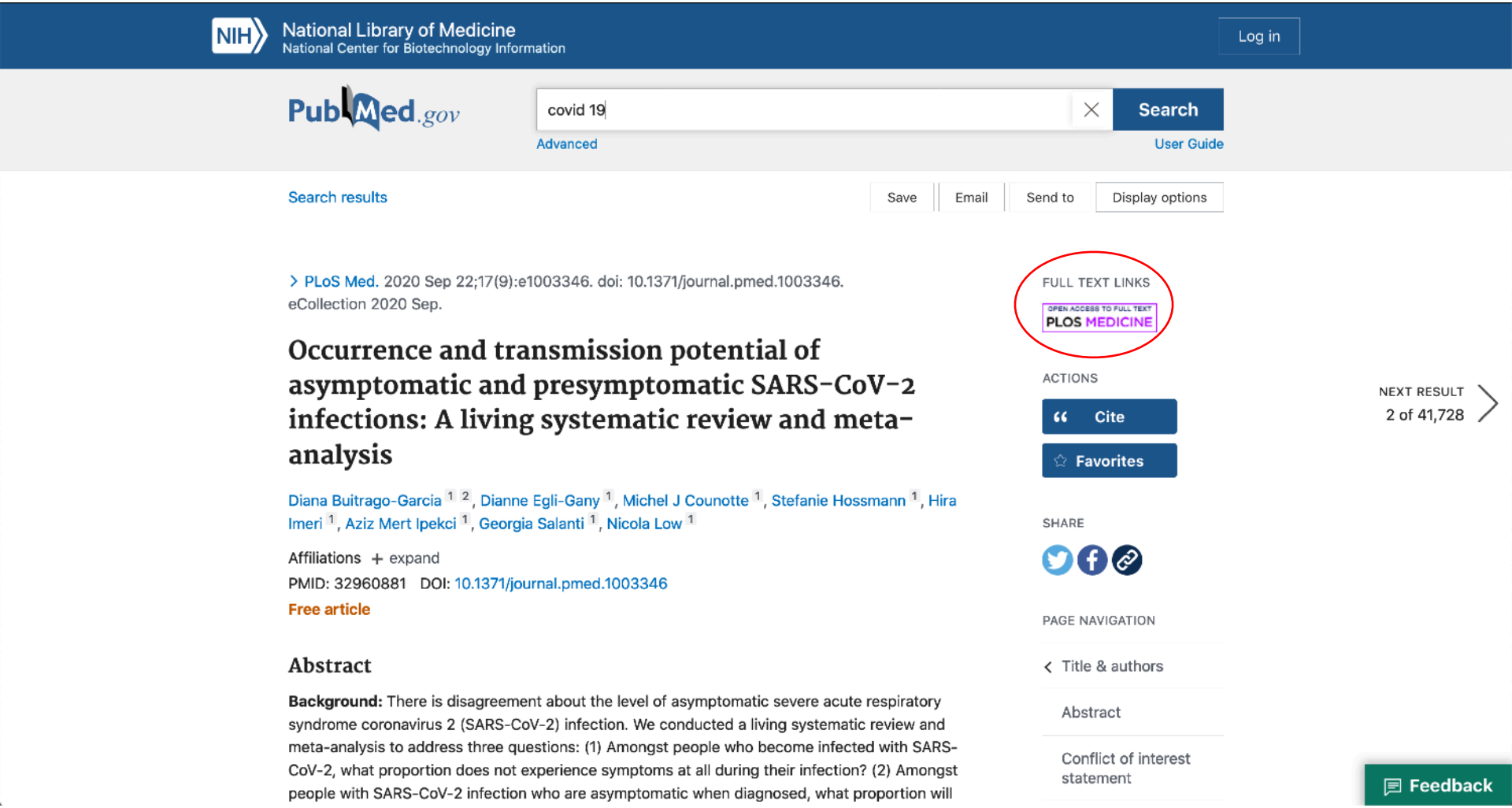

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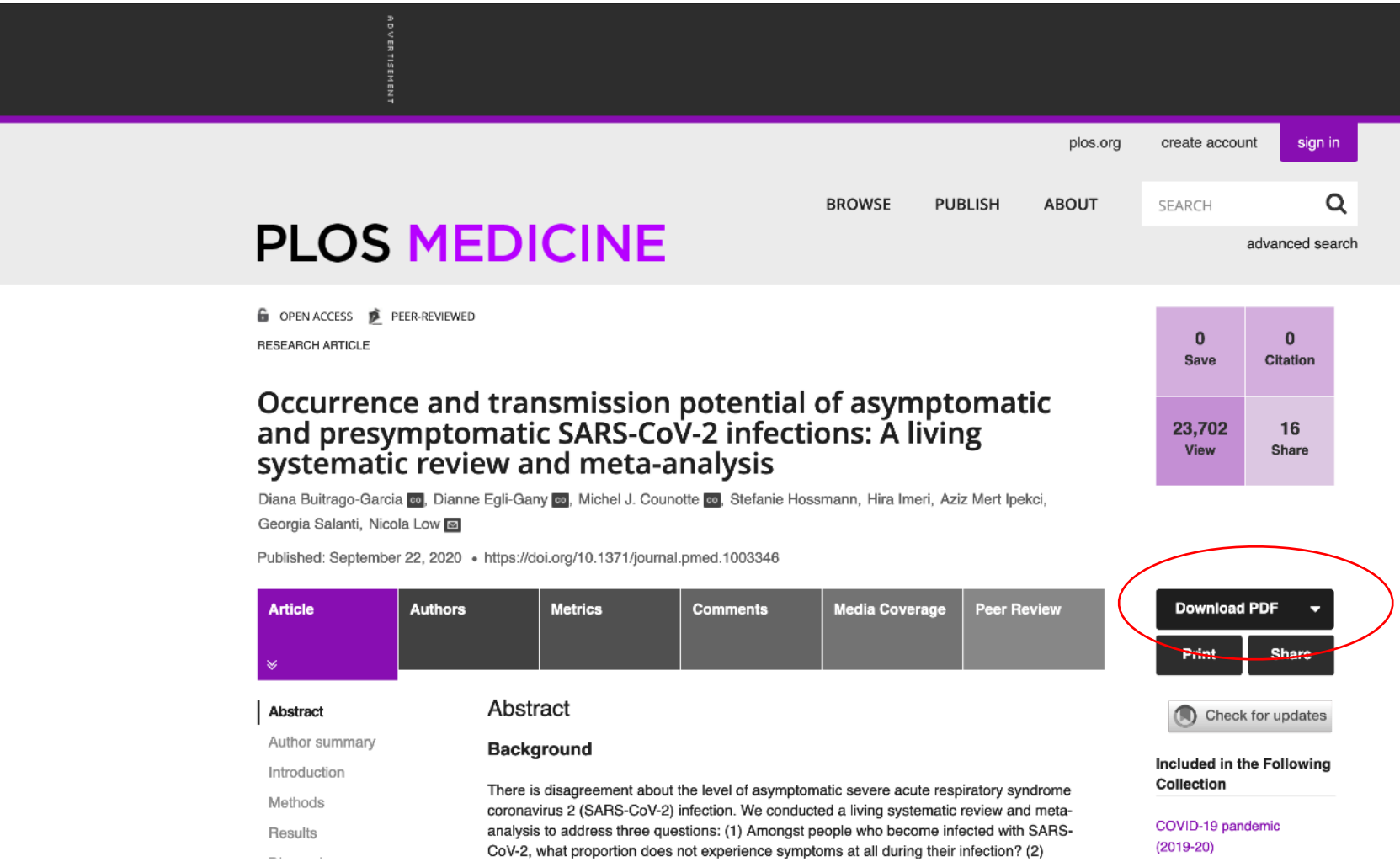

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PLOS MEDICINE

RESEARCH ARTICLE

Occurrence and transmission potential of asymptomatic and presymptomatic SARS-CoV-2 infections: A living systematic review and meta-analysis

Diana Buitrago-Garcia[1,2☯], Dianne Egli-Gany[1☯], Michel J. Counotte[1☯], Stefanie Hossmann[1], Hira Imeri[1], Aziz Mert Ipekci[1], Georgia Salanti[1], Nicola Low[1*]

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Academic Editor: Nathan Ford, World Health Organization, SWITZERLAND

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Peer Review History: PLOS recognizes the benefits of transparency in the peer review process; therefore, we enable the publication of all of the content of peer review and author responses alongside final, published articles. The editorial history of this article is available here: https://doi.org/10.1371/journal.pmed.1003346

Data Availability Statement: The file listing all included studies and files used for all analyses are available from the Harvard Dataverse database.

Abstract

Background

There is disagreement about the level of asymptomatic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. We conducted a living systematic review and meta-analysis to address three questions: (1) Amongst people who become infected with SARS-CoV-2, what proportion does not experience symptoms at all during their infection? (2) Amongst people with SARS-CoV-2 infection who are asymptomatic when diagnosed, what proportion will develop symptoms later? (3) What proportion of SARS-CoV-2 transmission is accounted for by people who are either asymptomatic throughout infection or presymptomatic?

Methods and findings

We searched PubMed, Embase, bioRxiv, and medRxiv using a database of SARS-CoV-2 literature that is updated daily, on 25 March 2020, 20 April 2020, and 10 June 2020. Studies of people with SARS-CoV-2 diagnosed by reverse transcriptase PCR (RT-PCR) that documented follow-up and symptom status at the beginning and end of follow-up or modelling studies were included. One reviewer extracted data and a second verified the extraction, with disagreement resolved by discussion or a third reviewer. Risk of bias in empirical studies was assessed with an adapted checklist for case series, and the relevance and credibility of modelling studies were assessed using a published checklist. We included a total of 94 studies. The overall estimate of the proportion of people who become infected with SARS-CoV-2 and remain asymptomatic throughout infection was 20% (95% confidence interval [CI] 17–25) with a prediction interval of 3%–67% in 79 studies that addressed this review question. There was some evidence that biases in the selection of participants influence the estimate. In seven studies of defined populations screened for SARS-CoV-2 and then

Table 1. Characteristics of studies reporting on proportion of asymptomatic SARS-CoV-2 infections.

Author	Country, location	Total SARS-CoV-2, *n*	Asymptomatic SARS-CoV-2, *n*	Sex of asymptomatic people	Age of asymptomatic people, years, median	Follow-up method[a]
Contact investigation, single						
Tong, ZD [44]	China, Zhejiang	5	3	2 F, 3 M	28 IQR 12–41	1, 3
Huang, R [74]	China, Suqian	2	1	1 F, 0 M	54	3
Jiang, XL [76]	China, Shandong	8	3	3 F, 0 M	35 IQR 0–53	3
Jiang, X [75]	China, Chongqing	3	1	1 F, 0 M	8	2
Liao, J [22]	China, Chongqing	12	3	NR	NR	1, 2
Hu, Z [21]	China, Nanjing	4	1	0 F, 1 M	64	2, 3
Luo, SH [23]	China, Anhui	4	1	1 F, 0 M	50	1, 2, 3
Chan, JF [18]	China, Guangdong	5	1	0 F, 1 M	10	1
Ye, F [49]	China, Sichuan	5	1	0 F, 1 M	28	1, 2
Bai, Y [17]	China, Anyang	6	1	1 F, 0 M	20	1
Luo, Y [85]	China, Wuhan	6	5	NR	37 IQR 7–62	1
Zhang, J [50]	China, Wuhan and Beijing	5	2	1 F, 1 M	NR	2
Zhang, B [110]	China, Guangdong	7	2	0 F, 2 M	13.5 IQR 13–14	3
Huang, L [73]	China, Gansu	7	2	2 F, 0 M	44 IQR 38.5–49.5	2
Qian, G [26]	China, Zhejiang	8	2	1 F, 1 M	30.5 IQR 1–60	1, 2
Gao, Y [70]	China, Wuxi	15	6	3 F, 3 M	50 IQR 48–51	1, 2
Contact investigation, aggregated						
Hijnen, D [72]	Germany	11	1	0 F, 1 M	49	1
Brandstetter, S [62]	Germany	36	2	NR	NR	2
Zhang, W2 [111]	China, Guiyang	12	4	NR	NR	1, 2, 3
Cheng, HY [66]	Taiwan	22	4	NR	NR	1
Wang, Z [47]	China, Wuhan	47	4	NR	NR	1
Wu, J [105]	China, Zhuhai	83	8	NR	NR	1, 2
Luo, L [36]	China, Guangzhou	129	8	NR	NR	1, 2, 3
Bi, Q [60]	China, Shenzhen	87	17	NR	NR	2, 3
Yang, R [108]	China, Wuhan	78	33	22 F, 11 M	37 IQR 26–45	3
Outbreak investigation						
Danis, K [32]	France	13	1	NR	NR	1, 2
Böhmer, MM [61]	Germany	16	1	NR	NR	1
Roxby, AC [94]	USA	6	3	NR	NR	1
Yang, N [48]	China, Xiaoshan	10	2	1 F, 1 M	NR	1, 2
Schwierzeck, V [95]	Germany	12	2	NR	NR	2
Arons, MM [58]	USA	47	3	NR	NR	2
Park, SY [90]	South Korea	97	4	NR	NR	2
Doru, AV [68]	USA	19	6	0 F, 6 M	75 IQR 72–75	3
Tian, S [43]	China, Shandong	24	7	NR	NR	3
Solbach, W [97]	Germany	97	10	NR	NR	2
Graham, N [71]	United Kingdom	126	46	NR	NR	2

(*Continued*)

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Accès à la totalité de la publication en mode pdf...

Notion de sources et citations…

S7 Fig. Assessment of credibility of mathematical modelling studies.
(PDF)

S1 Table. Types of study included in successive versions of the living systematic review, as of 10 June 2020.
(DOCX)

S2 Table. Location of studies contributing data to review questions 1 and 2.
(DOCX)

Author Contributions

Conceptualization: Diana Buitrago-Garcia, Dianne Egli-Gany, Nicola Low.

Data curation: Diana Buitrago-Garcia, Dianne Egli-Gany, Michel J. Counotte, Stefanie Hossmann, Hira Imeri, Nicola Low.

Formal analysis: Michel J. Counotte, Georgia Salanti.

Investigation: Aziz Mert Ipekci.

Methodology: Diana Buitrago-Garcia, Dianne Egli-Gany, Michel J. Counotte, Georgia Salanti, Nicola Low.

Project administration: Diana Buitrago-Garcia, Dianne Egli-Gany.

Supervision: Nicola Low.

Validation: Diana Buitrago-Garcia, Dianne Egli-Gany, Michel J. Counotte, Stefanie Hossmann, Hira Imeri, Aziz Mert Ipekci, Nicola Low.

Writing – original draft: Diana Buitrago-Garcia, Nicola Low.

Writing – review & editing: Diana Buitrago-Garcia, Dianne Egli-Gany, Michel J. Counotte, Stefanie Hossmann, Hira Imeri, Aziz Mert Ipekci, Georgia Salanti, Nicola Low.

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Figures pour vos rapports ou oraux.

Notion de citation

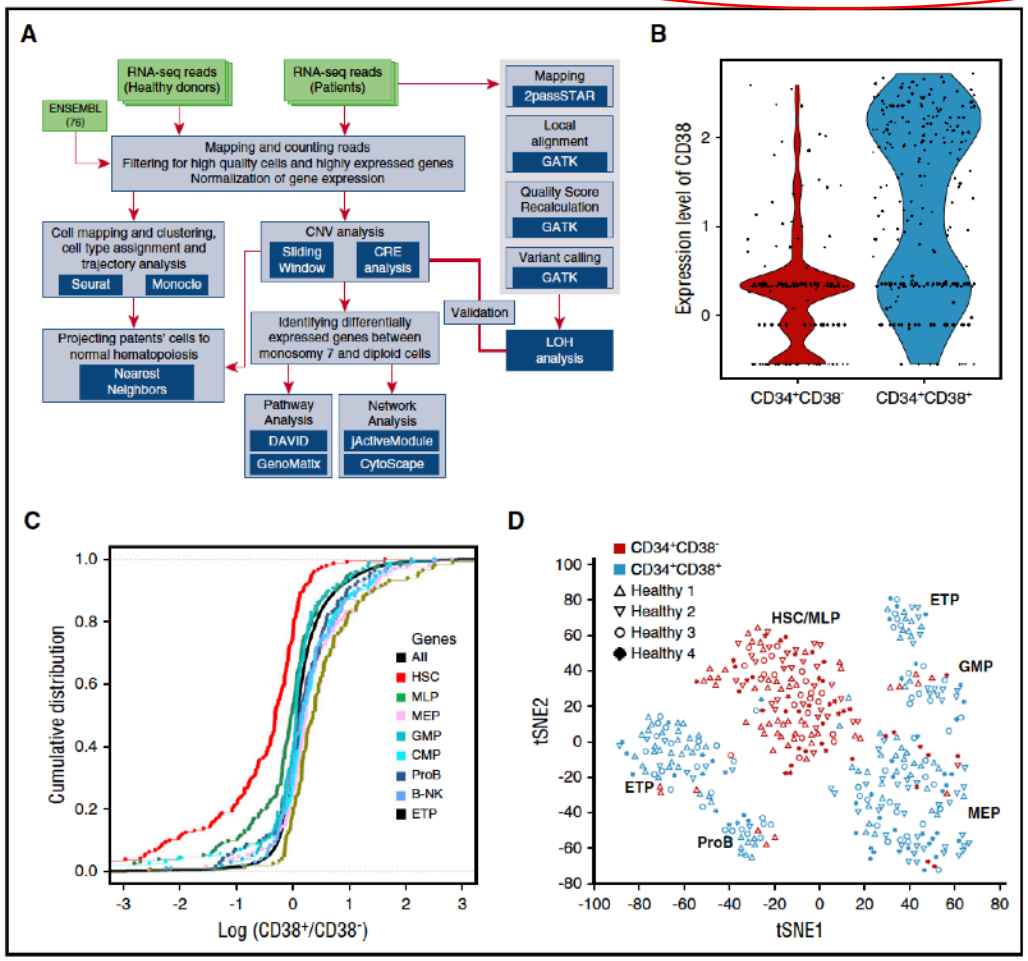

Figure 1. Hematopoietic heterogeneity in healthy donors quantified by scRNA-seq. $CD34^+CD38^-$ and $CD34^+CD38^+$ cells from 4 healthy donors (healthy 1-4) were sorted by surface-membrane markers and subjected to analyses. (A) The schematic pipeline consisting of 3 major analytic components: differentiation analysis with cells from healthy donors, identification and characterization of monosomy 7 cells with gene expression, and validation of monosomy identification with loss of reterozygosity (LOH). CNV, copy-number variation; CRE, chromosome relative expression; GATK, Genome Analysis Toolkit. (B) CD38 expression levels in $CD34^+CD38^-$ and $CD34^+CD38^+$ cells. Each dot represents a single cell. y-axis, batch-corrected gene expression levels. (C) Cumulative distribution of fold changes of expression of hematopoietic cell type signature genes between $CD34^+CD38^-$ and $CD34^+CD38^+$ cells. Each dot represents a gene. B-NK, B cell–natural killer cell precursor; CMP, common myeloid progenitor; ETP, earliest thymic progenitor; GMP, granulocyte-monocyte progenitor; MEP, megakaryocytic-erythroid progenitor; MLP, multilymphoid progenitor; ProB, pro–B cell. y-axis, cumulative distribution; x-axis, log (marker gene expression levels in $CD34^+CD38^+$ cells/marker gene expression levels in $CD34^+CD38^-$ cells). (D) t-distributed stochastic neighbor embedding (tSNE) plot of single-cell gene expression data. Single cells from 4 healthy donors (healthy 1-4) are represented by different symbols. Highly variable genes (1024) across all healthy donors were used in tSNE analysis.

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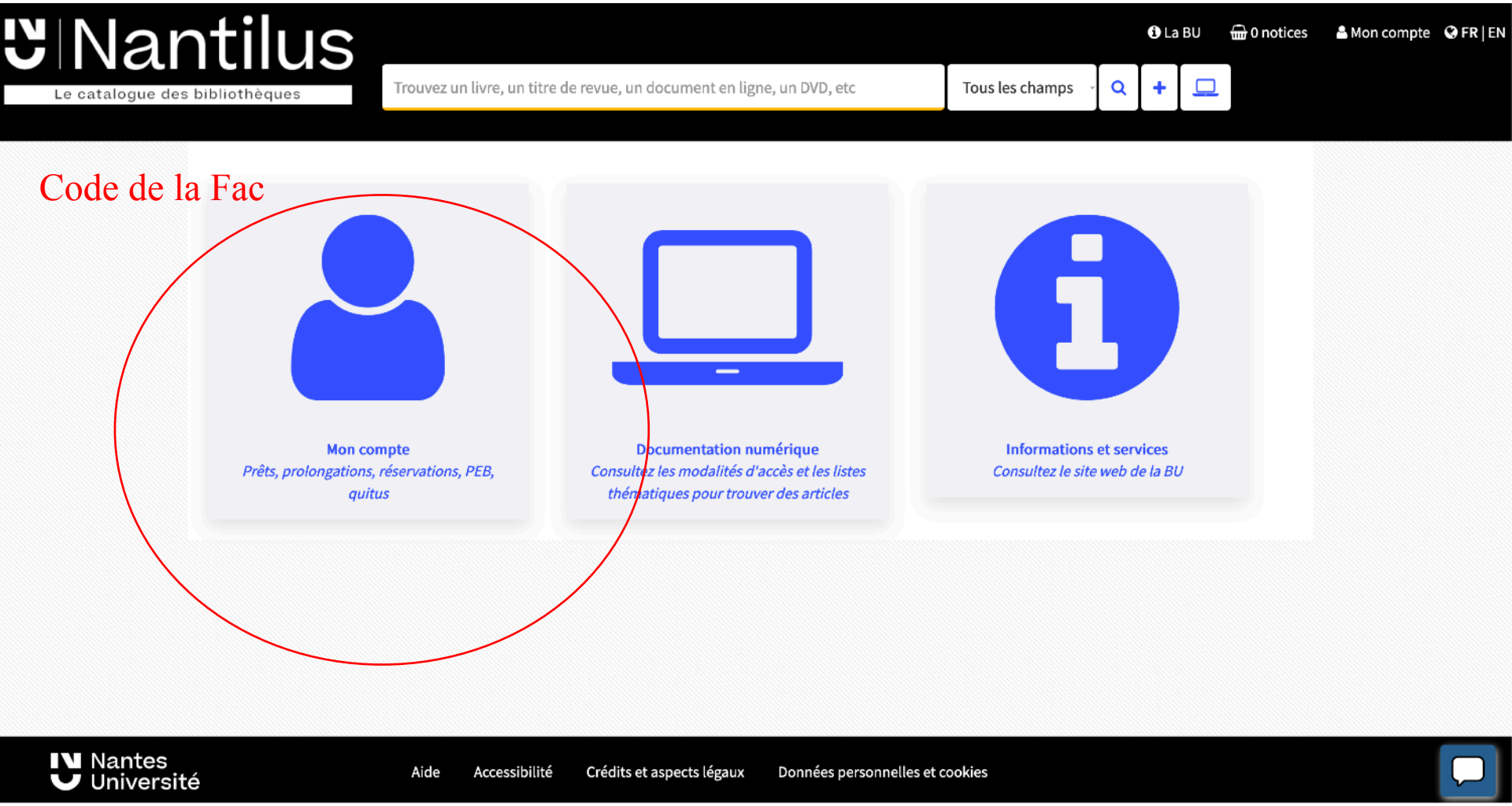
Un exemple de recherche: Covid19 ?



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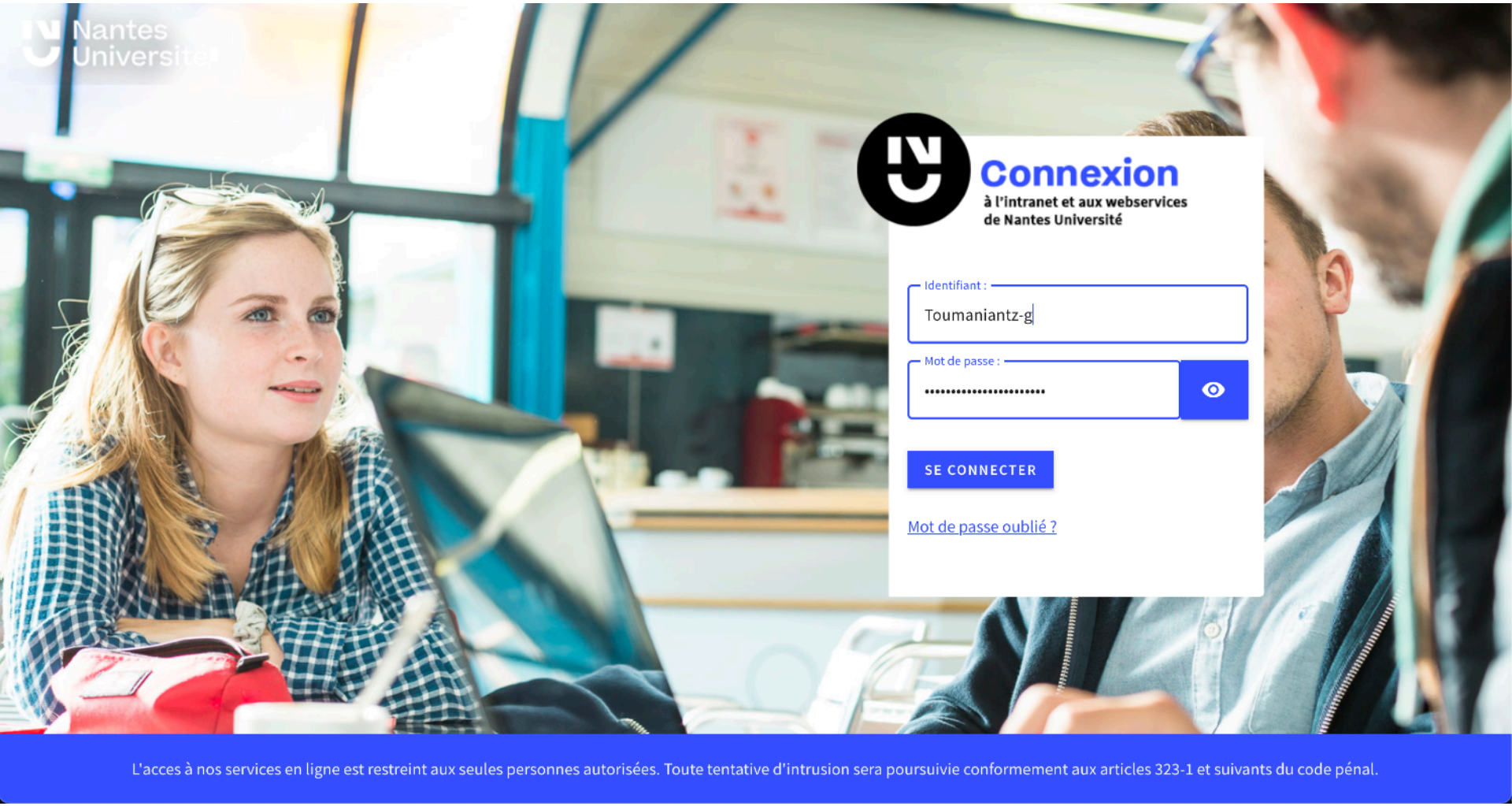

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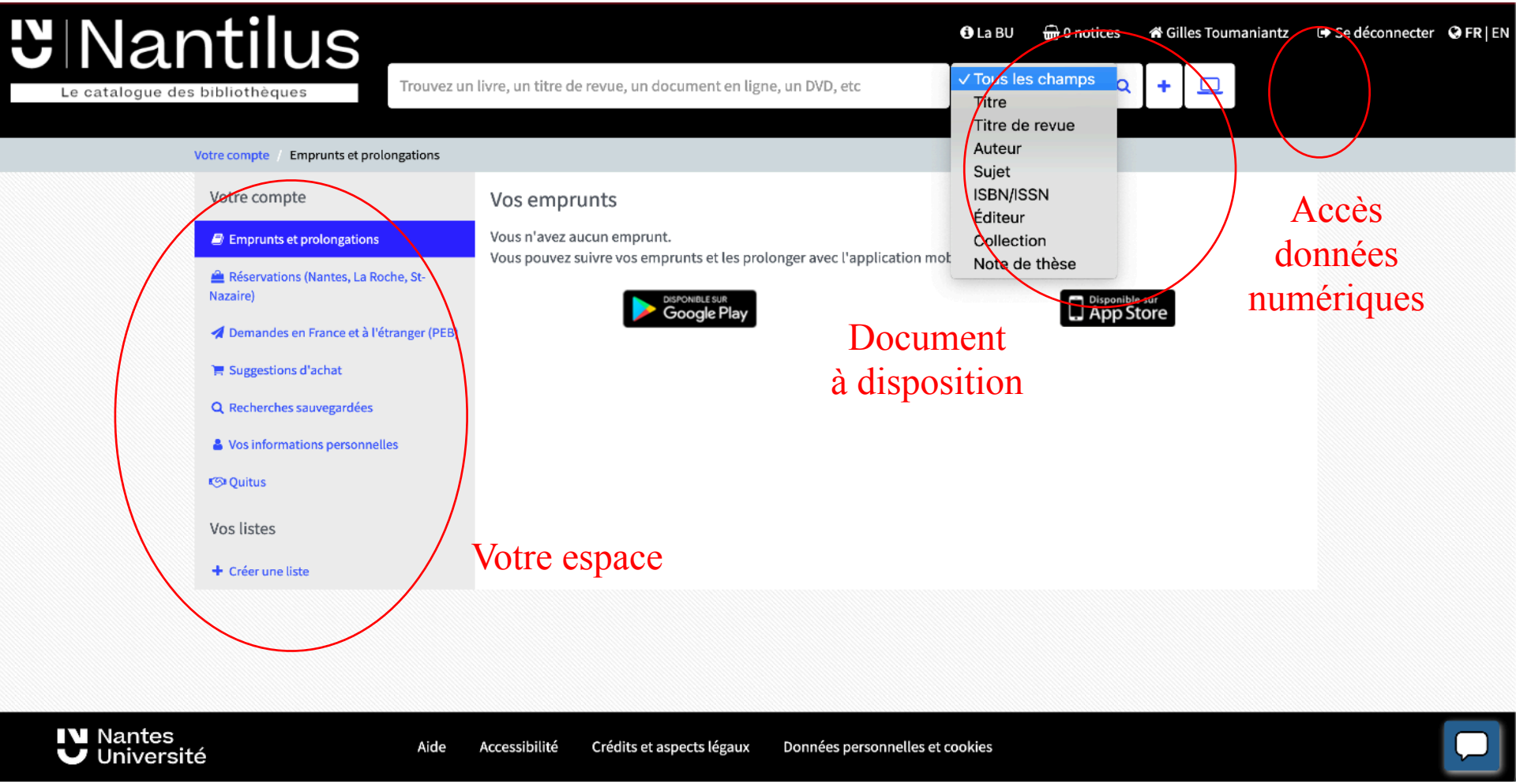
Un exemple de recherche: Covid19 ?



Accès données numériques

Document à disposition

Votre espace

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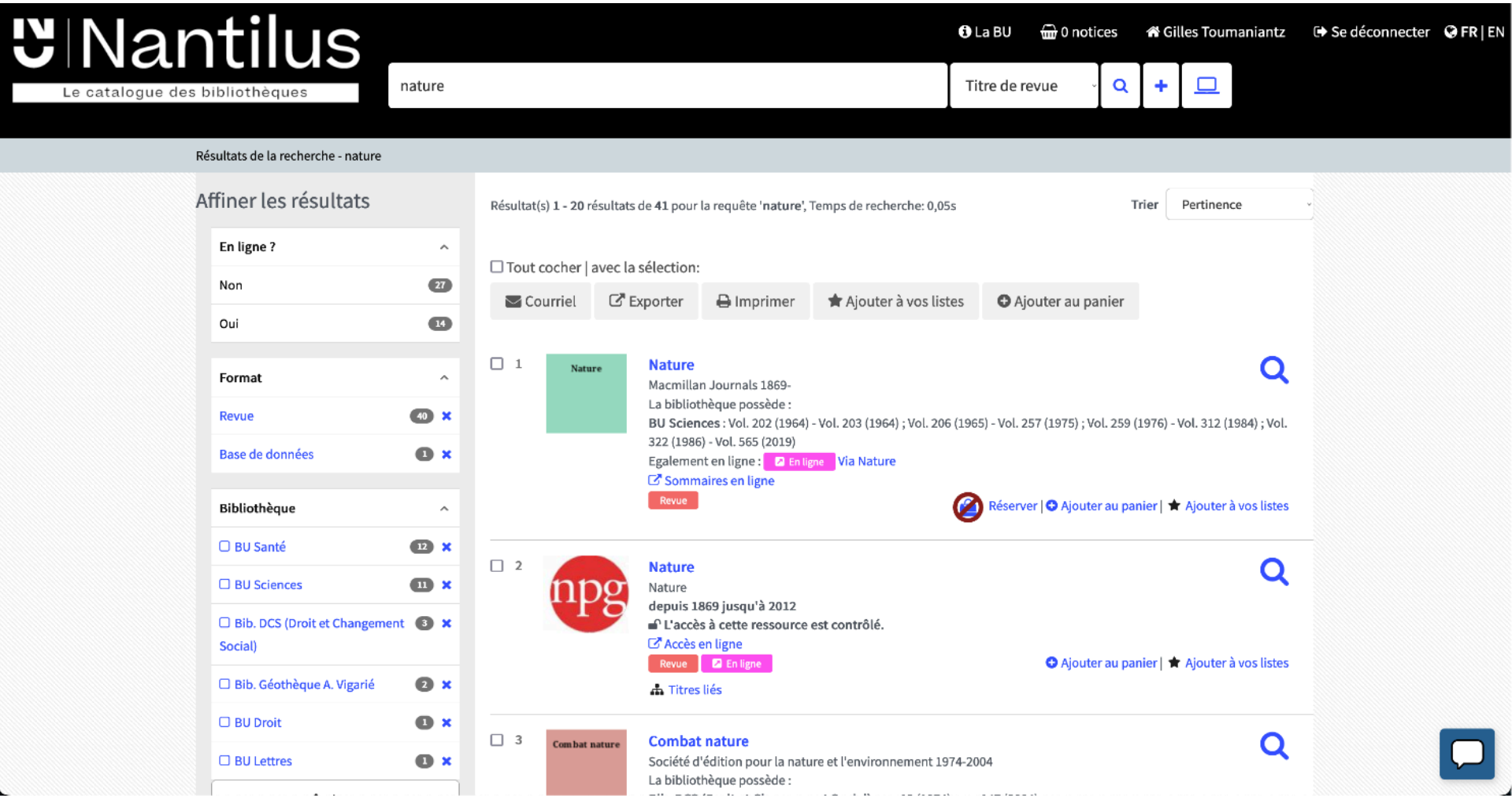
Un exemple de recherche: Covid19 ?



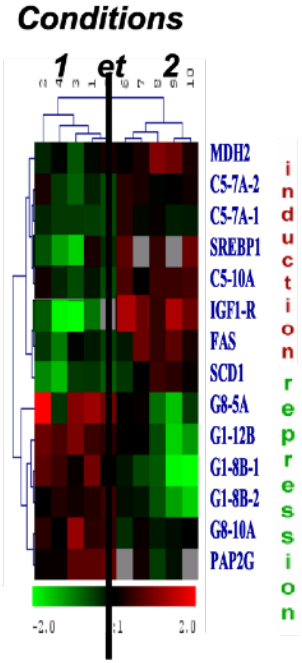
Mode d'emploi en ligne.
Attention pas Pub Med… Vous savez ce que vous cherchez.

Au labo : analyse à haut débit de cette bibliographie.

Les données expérimentales (en « omique ») => Biblio à au débit...

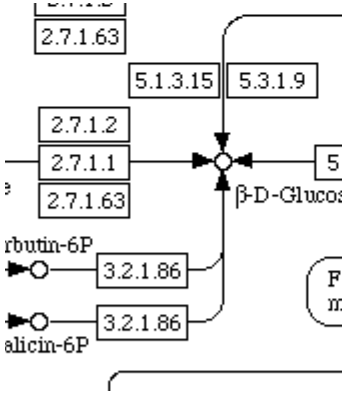
sont-elles cohérentes/incohérentes

avec les connaissances du moment ?



littérature

Les modèles intégrés



→ *Construire **un modèle intégré de connaissance***

→ *Développer des méthodes pour comparer données expé et modèle permettant de trouver les cohérences/incohérences*

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2. Les analyses nucléotidiques

Question à étudier:

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Question à étudier:

Suite à un protocole de puce à ADN cet ARNm a été décrit comme étant différentiel dans des préparations de coeur issues d'un modèle d'insuffisance cardiaque par ligature de coronaire chez le rat. Il est en effet sous-exprimé. Déterminez la nature de ce messager.

tcgctgctgcctccagccagcgagggctcagcgccgctgtcgcagcagtggaccgcgggtatgggccta
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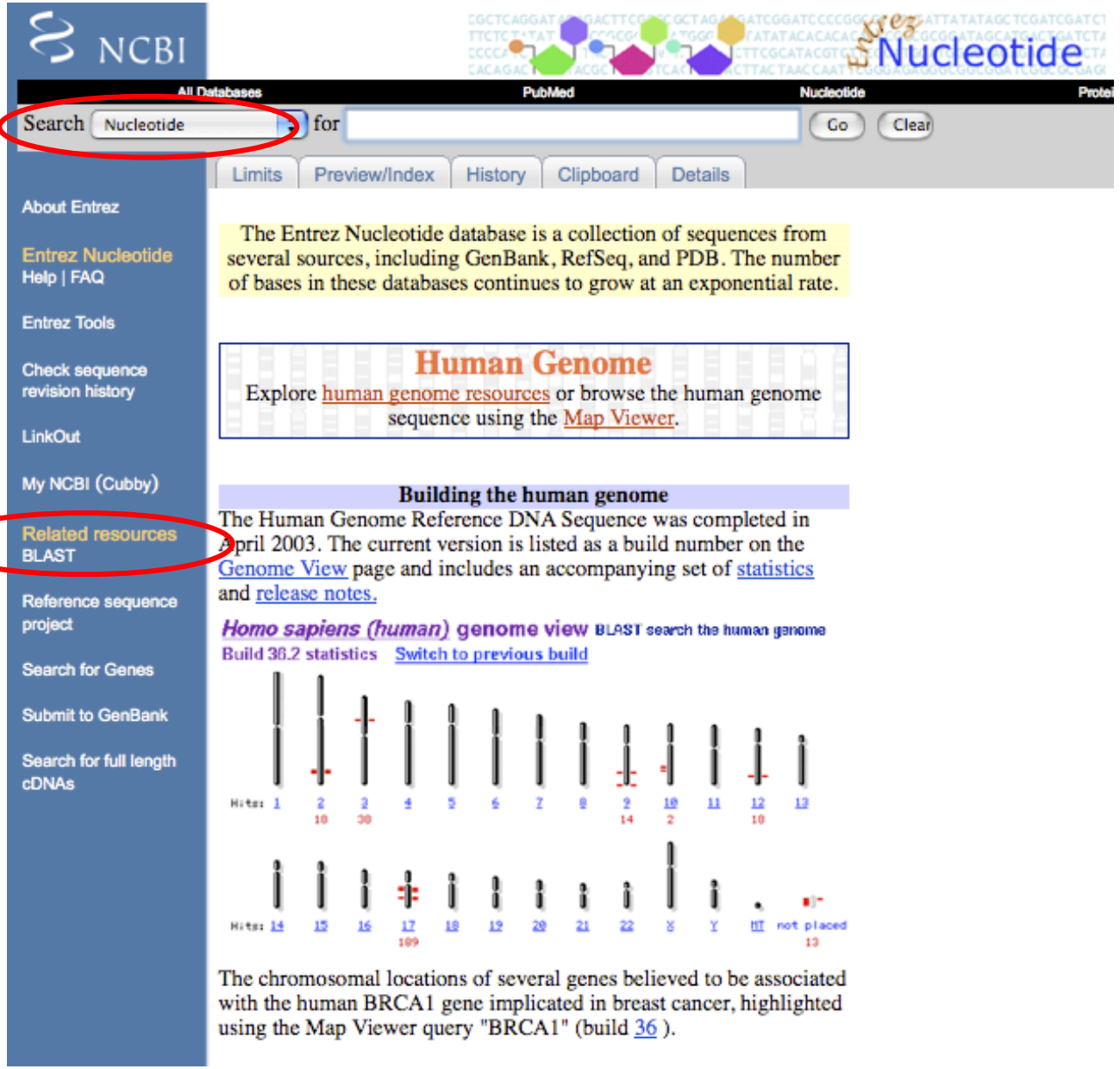
2. Les analyses nucléotidiques



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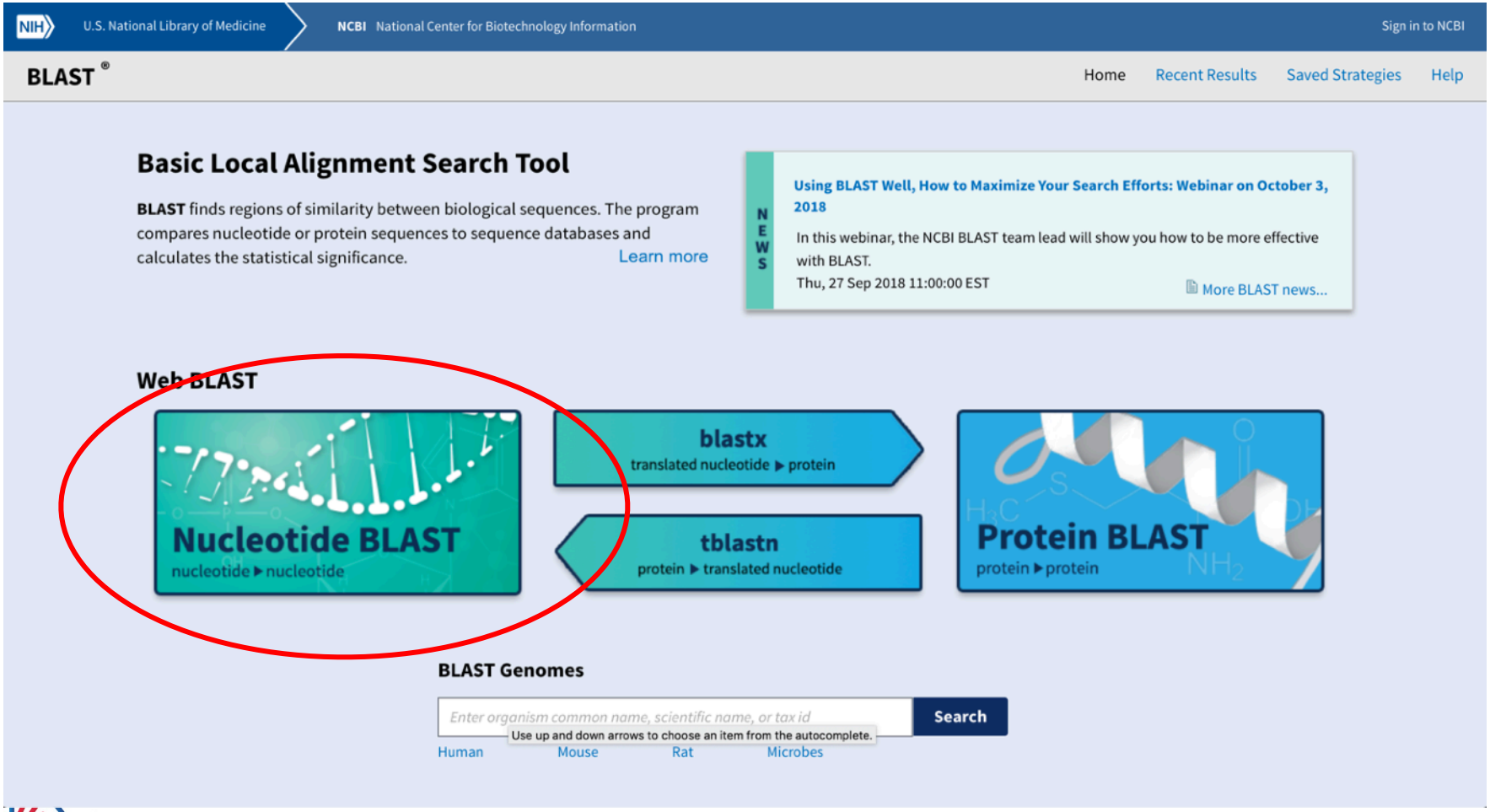

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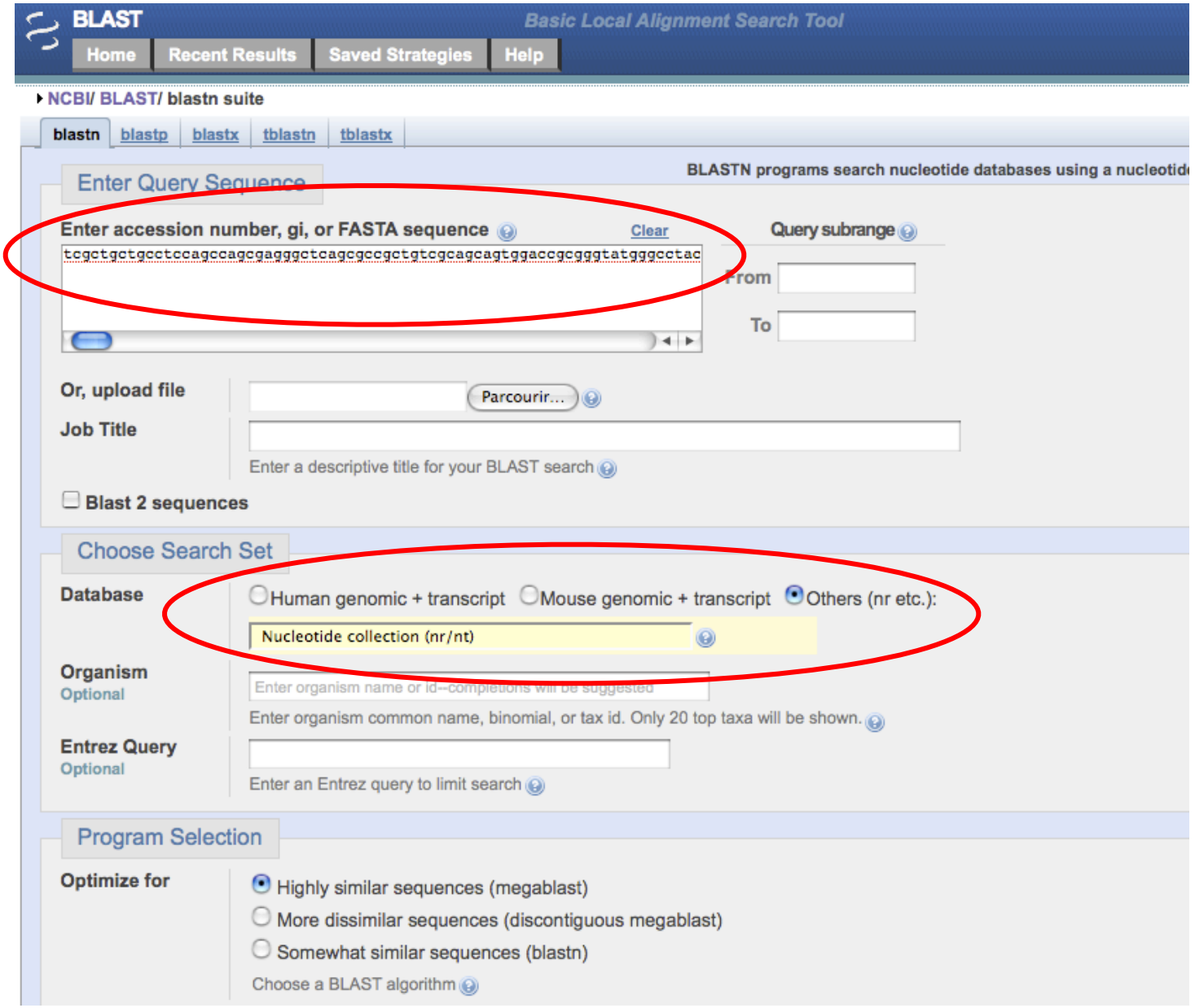

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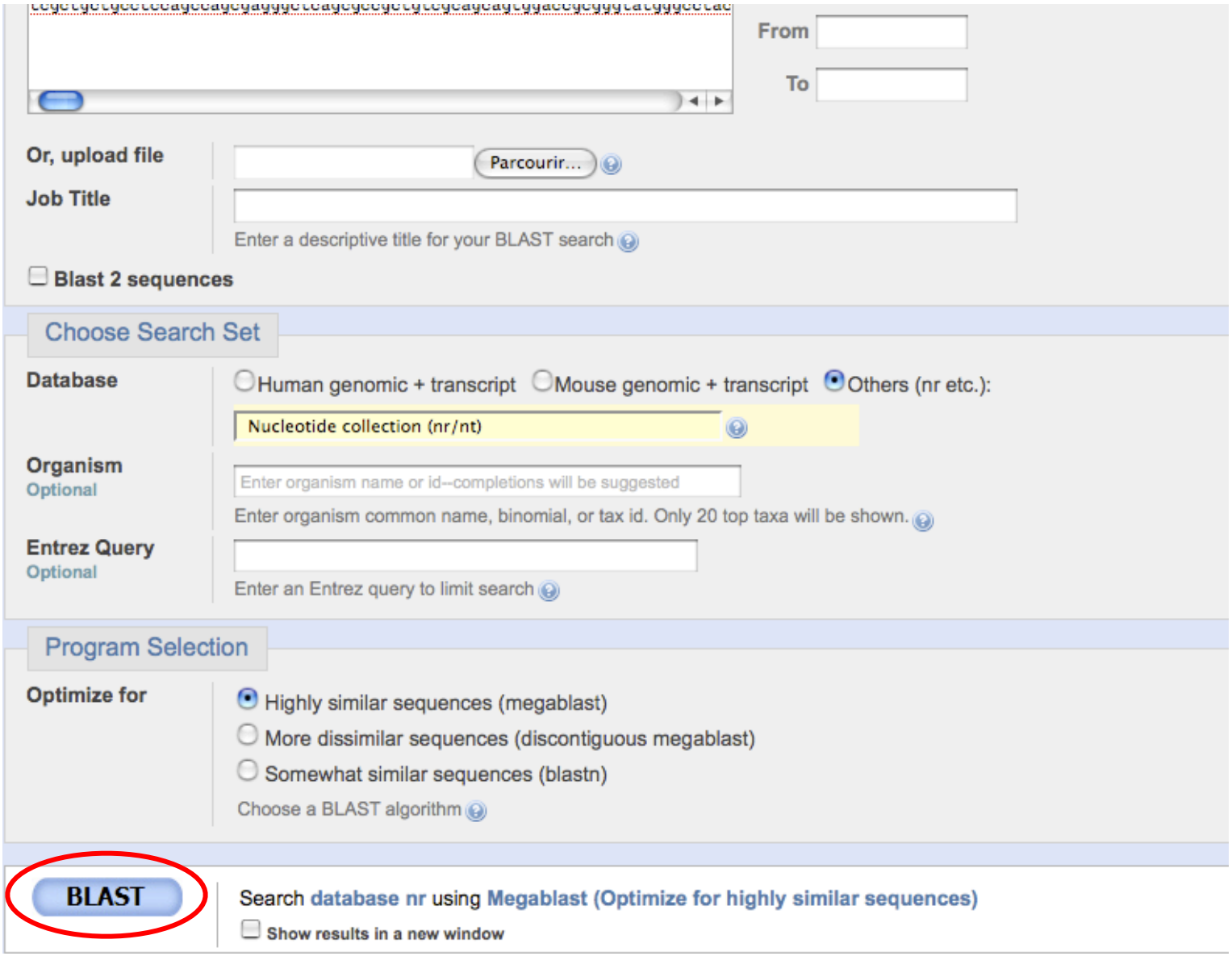
2. Les analyses nucléotidiques



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2. Les analyses nucléotidiques

BLAST Basic Local Alignment Search Tool

Home | Recent Results | Saved Strategies | Help

My NCBI [Sign In] [Register]

NCBI/ BLAST/ blastn suite/ **Formatting Results - HRDEGBVV01R**

Edit and Resubmit | Save Search Strategies | ▷Formatting options | ▷Download

Nucleotide Sequence (716 letters)

Query ID lcl|27220
Description None
Molecule type nucleic acid
Query Length 716

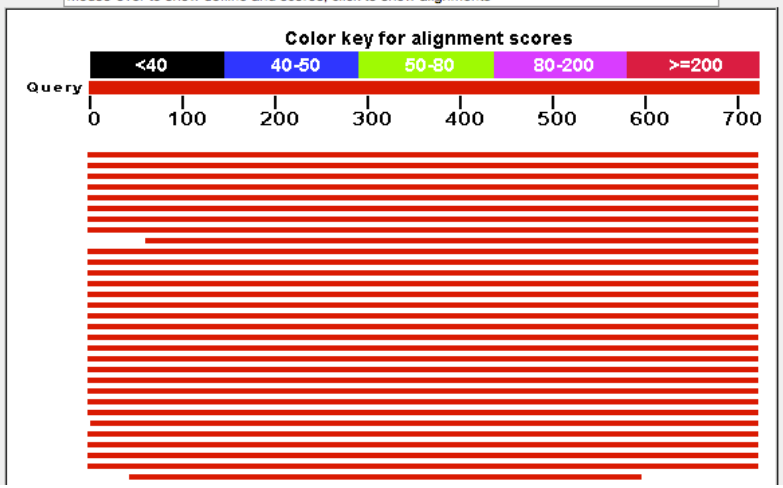
Database Name nr
Description All GenBank+EMBL+DDBJ+PDB sequences (but no EST, STS, GSS,environmental samples or phase 0, 1 or 2 HTGS sequences)
Program BLASTN 2.2.18+ ▷Citation

Other reports: ▷Search Summary [Taxonomy reports] [Distance tree of results]

▼ Graphic Summary

Distribution of 45 Blast Hits on the Query Sequence

Mouse-over to show defline and scores, click to show alignments



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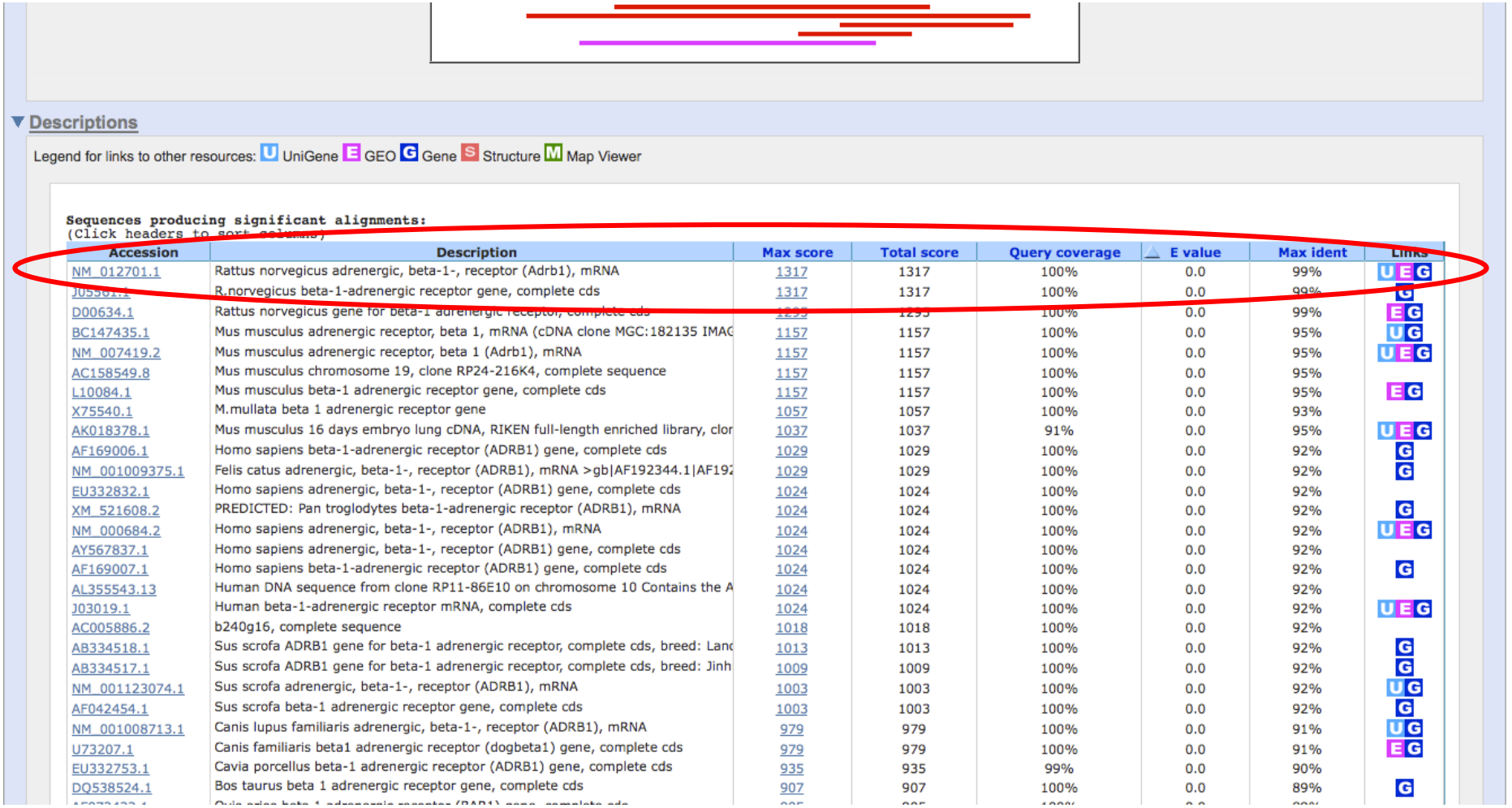
2. Les analyses nucléotidiques



▼ Descriptions

Legend for links to other resources: U UniGene E GEO G Gene S Structure M Map Viewer

Sequences producing significant alignments:
(Click headers to sort columns)

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
NM_012701.1	Rattus norvegicus adrenergic, beta-1-, receptor (Adrb1), mRNA	1317	1317	100%	0.0	99%	U E G
J05561.1	R.norvegicus beta-1-adrenergic receptor gene, complete cds	1317	1317	100%	0.0	99%	G
D00634.1	Rattus norvegicus gene for beta-1 adrenergic receptor, complete cds	1295	1295	100%	0.0	99%	E G
BC147435.1	Mus musculus adrenergic receptor, beta 1, mRNA (cDNA clone MGC:182135 IMAC	1157	1157	100%	0.0	95%	U G
NM_007419.2	Mus musculus adrenergic receptor, beta 1 (Adrb1), mRNA	1157	1157	100%	0.0	95%	U E G
AC158549.8	Mus musculus chromosome 19, clone RP24-216K4, complete sequence	1157	1157	100%	0.0	95%	
L10084.1	Mus musculus beta-1 adrenergic receptor gene, complete cds	1157	1157	100%	0.0	95%	E G
X75540.1	M.mullata beta 1 adrenergic receptor gene	1057	1057	100%	0.0	93%	
AK018378.1	Mus musculus 16 days embryo lung cDNA, RIKEN full-length enriched library, clor	1037	1037	91%	0.0	95%	U E G
AF169006.1	Homo sapiens beta-1-adrenergic receptor (ADRB1) gene, complete cds	1029	1029	100%	0.0	92%	G
NM_001009375.1	Felis catus adrenergic, beta-1-, receptor (ADRB1), mRNA >gb|AF192344.1|AF192	1029	1029	100%	0.0	92%	G
EU332832.1	Homo sapiens adrenergic, beta-1-, receptor (ADRB1) gene, complete cds	1024	1024	100%	0.0	92%	
XM_521608.2	PREDICTED: Pan troglodytes beta-1-adrenergic receptor (ADRB1), mRNA	1024	1024	100%	0.0	92%	G
NM_000684.2	Homo sapiens adrenergic, beta-1-, receptor (ADRB1), mRNA	1024	1024	100%	0.0	92%	U E G
AY567837.1	Homo sapiens adrenergic, beta-1-, receptor (ADRB1) gene, complete cds	1024	1024	100%	0.0	92%	
AF169007.1	Homo sapiens beta-1-adrenergic receptor (ADRB1) gene, complete cds	1024	1024	100%	0.0	92%	G
AL355543.13	Human DNA sequence from clone RP11-86E10 on chromosome 10 Contains the A	1024	1024	100%	0.0	92%	
J03019.1	Human beta-1-adrenergic receptor mRNA, complete cds	1024	1024	100%	0.0	92%	U E G
AC005886.2	b240g16, complete sequence	1018	1018	100%	0.0	92%	
AB334518.1	Sus scrofa ADRB1 gene for beta-1 adrenergic receptor, complete cds, breed: Lanc	1013	1013	100%	0.0	92%	G
AB334517.1	Sus scrofa ADRB1 gene for beta-1 adrenergic receptor, complete cds, breed: Jinh	1009	1009	100%	0.0	92%	G
NM_001123074.1	Sus scrofa adrenergic, beta-1-, receptor (ADRB1), mRNA	1003	1003	100%	0.0	92%	U G
AF042454.1	Sus scrofa beta-1 adrenergic receptor gene, complete cds	1003	1003	100%	0.0	92%	G
NM_001008713.1	Canis lupus familiaris adrenergic, beta-1-, receptor (ADRB1), mRNA	979	979	100%	0.0	91%	U G
U73207.1	Canis familiaris beta1 adrenergic receptor (dogbeta1) gene, complete cds	979	979	100%	0.0	91%	E G
EU332753.1	Cavia porcellus beta-1 adrenergic receptor (ADRB1) gene, complete cds	935	935	99%	0.0	90%	
DQ538524.1	Bos taurus beta 1 adrenergic receptor gene, complete cds	907	907	100%	0.0	89%	G

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2. Les analyses nucléotidiques

M14379.1	Turkey beta-adrenergic receptor mRNA, complete cds	501	501	89%	2e-138	81%	U
AF055349.1	Meriones unguiculatus beta-1-adrenergic receptor mRNA, partial cds	364	364	30%	2e-97	96%	
BC169226.1	Homo sapiens cDNA clone IMAGE:9093418, partial cds	261	261	24%	3e-66	93%	
AF041457.1	Cervus dama beta 1 adrenergic receptor mRNA, partial cds	228	228	20%	3e-56	95%	
U51098.1	Cavia porcellus beta3-adrenergic receptor mRNA, partial cds	132	132	52%	3e-27	74%	G

Alignments Select All Get selected sequences Distance tree of results

ref|NM_012701.1| Rattus norvegicus adrenergic, beta-1-, receptor (Adrb1), mRNA
Length=1401

GENE ID: 24925 Adrb1 | adrenergic, beta-1-, receptor [Rattus norvegicus]
(Over 10 PubMed links)

Score = 1317 bits (713), Expect = 0.0
Identities = 715/716 (99%), Gaps = 0/716 (0%)
Strand=Plus/Plus

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Query  1    TCGCTGCTGCCTCCAGCCAGCGAGGGCTCAGCGCCGCTGTCGCAGCAGTGGACCGCGGGT  60
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Position 470/début séquence référencée

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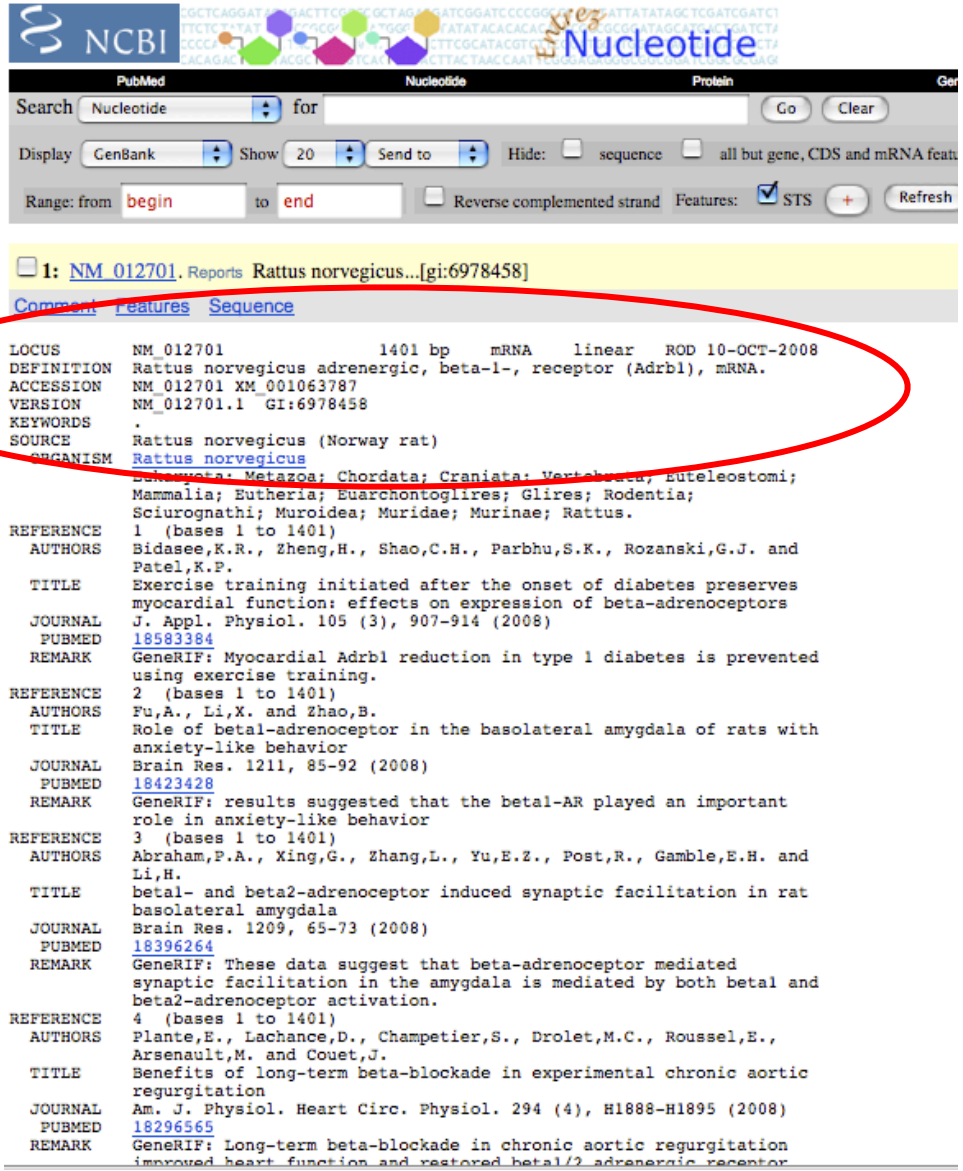
2. Les analyses nucléotidiques



Identité

Bibliographie

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2. Les analyses nucléotidiques

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Défaut en position 470/début séquence référencée

Phase ouverte de lecture

Protéine

Séquence nucléotidique

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2. Les analyses nucléotidiques

Question à étudier:

Mutation silencieuse, faux-sens ou non-sens chez mon patient ?

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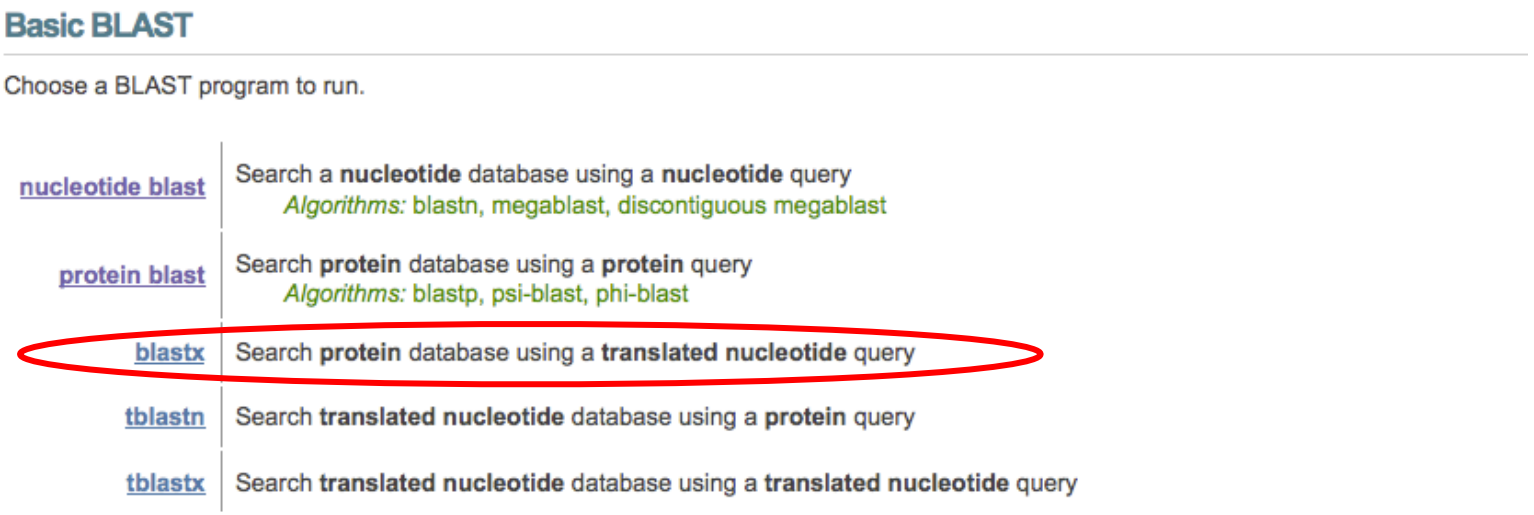
2. Les analyses nucléotidiques

Question à étudier:

Mutation silencieuse, faux-sens ou non-sens chez mon patient ?



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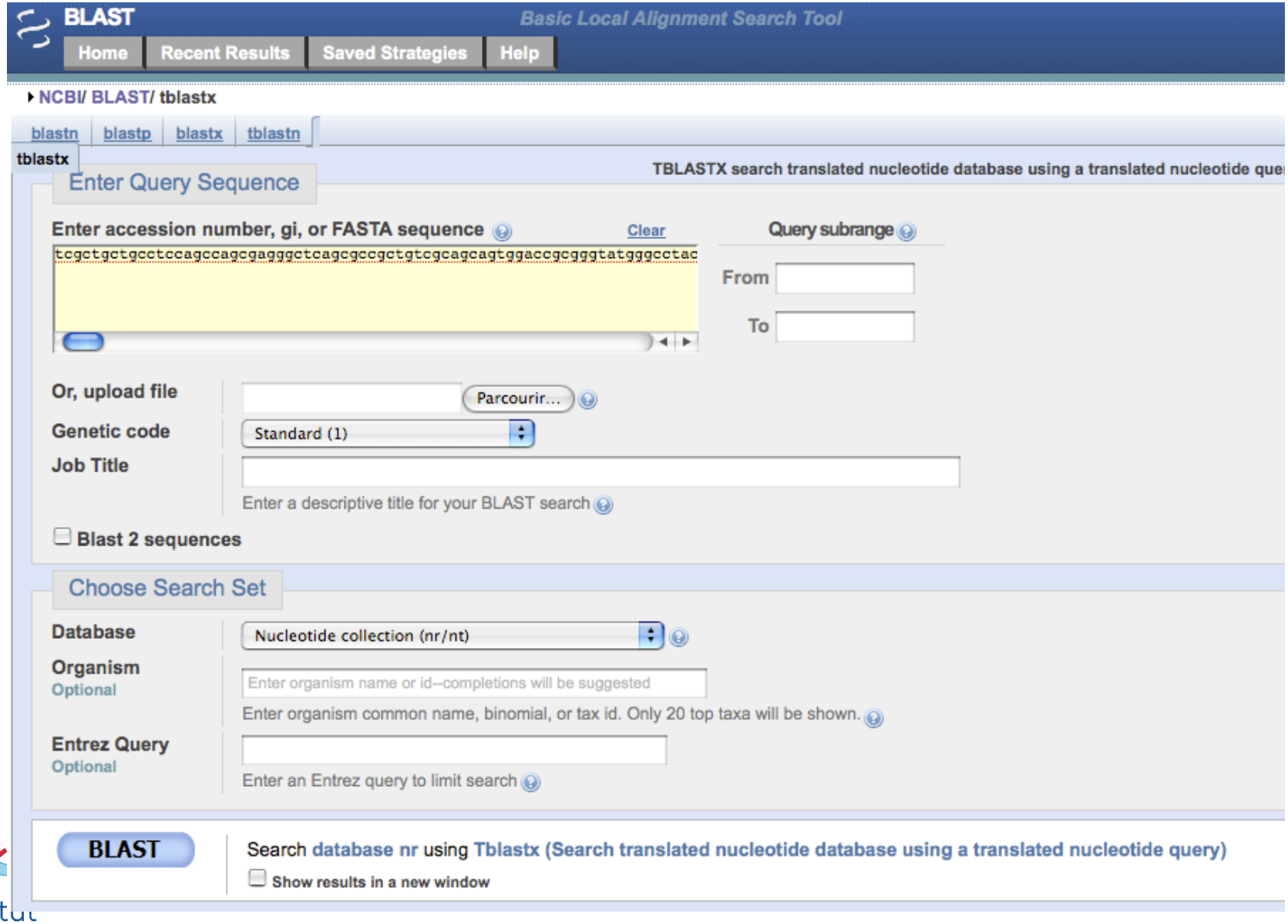
2. Les analyses nucléotidiques



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2. Les analyses nucléotidiques

BLAST Basic Local Alignment Search Tool

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My NCBI [Sign In] [Register]

NCBI/ BLAST/ tblastx/ Formatting Results - HRFE3ZC7015

Edit and Resubmit | Save Search Strategies | ▷Formatting options | ▷Download

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Description None
Molecule type nucleic acid
Query Length 716

Database Name nr
Description All GenBank+EMBL+DDBJ+PDB sequences (but no EST, STS, GSS,environmental samples or phase 0, 1 or 2 HTGS sequences)
Program TBLASTX 2.2.18+ ▷Citation

Other reports: ▷Search Summary [Taxonomy reports]

▼ Graphic Summary

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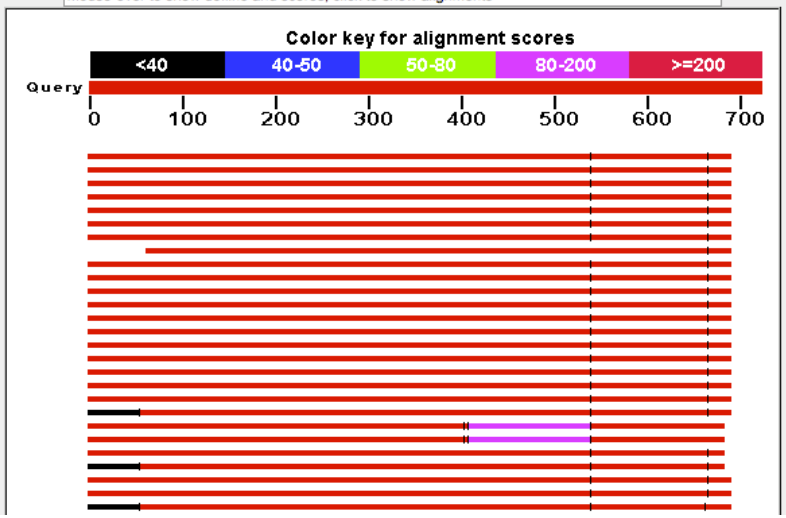

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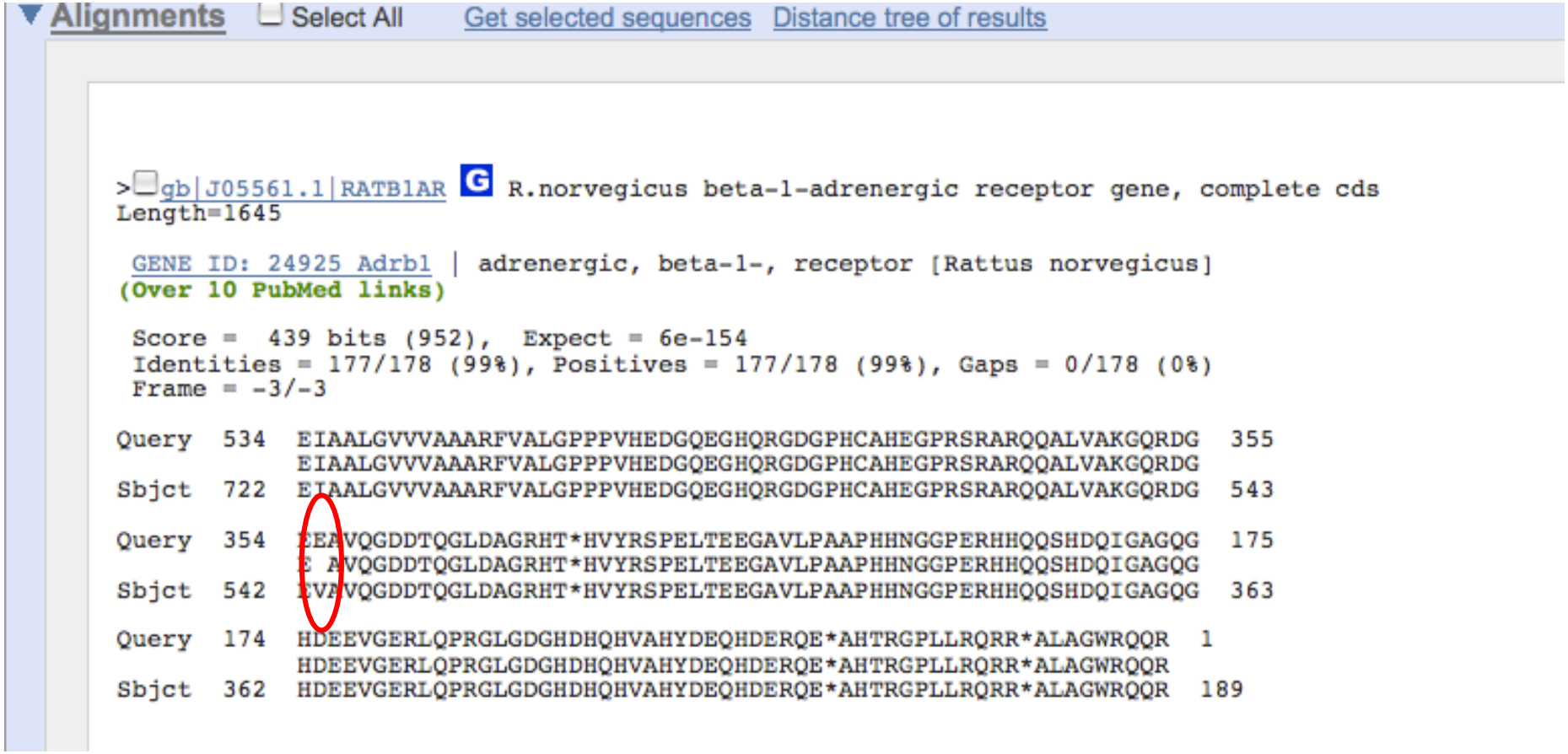
2. Les analyses nucléotidiques



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(Over 10 PubMed links)

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2. Les analyses nucléotidiques

Position sur le génome ???

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2. Les analyses nucléotidiques

Legend for links to other resources: U UniGene E GEO G Gene S Structure M Map Viewer

Position sur le génome ???

NCBI Entrez Gene

All Databases | PubMed | Nucleotide | Protein | Genome | Structure | OMIM | PMC | Journals | Books

Search Gene for 6978458[NUID] Go Clear Save Search

Limits | Preview/Index | History | Clipboard | Details

Display Full Report Show 20 Sort by Relevance Send to

All: 1 | Current Only: 1 | Genes Genomes: 1 | SNP GeneView: 0

1: Adrb1 adrenergic, beta-1-, receptor [*Rattus norvegicus*]

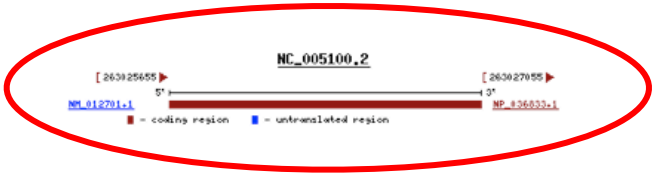
GeneID: 24925 updated 15-Oct-2008

Summary

Official Symbol	Adrb1 provided by RGD
Official Full Name	adrenergic, beta-1-, receptor provided by RGD
Primary source	RGD:2059
See related	Ensembl:ENSRNOG00000017002; RATMAP:34960
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	*Rattus norvegicus*
Lineage	*Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Sciurognathi; Muroidea; Muridae; Murinae; Rattus*
Also known as	B1AR; RATB1AR; Adrb1
Summary	binds beta-adrenergic receptor agonists isoproterenol, norepinephrine, and epinephrine; mediates adenylylcyclase induction; involved in regulation of calcium current [RGD]

Genomic regions, transcripts, and products

Go to reference sequence details Try our new Sequence Viewer



Entrez Gene Home

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- Summary
- Genomic regions, transcripts...
- Genomic context
- Bibliography
- Interactions
- General gene information
- General protein information
- Reference Sequences
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- Probe
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- PubMed (GeneRIF)
- Taxonomy
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- RATMAP
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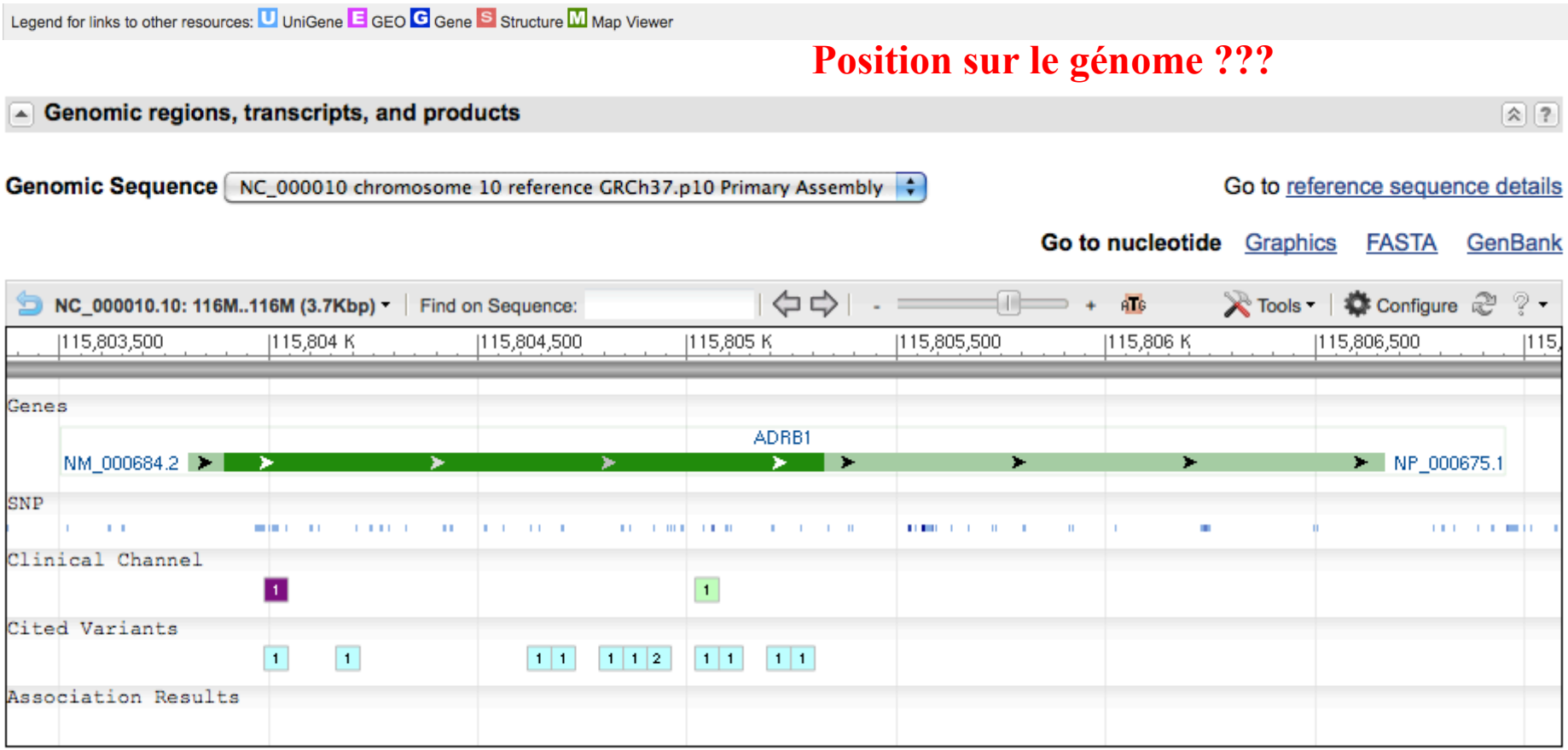
- LinkOut

Entrez Gene Info

2. Les analyses nucléotidiques



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2. Les analyses nucléotidiques

=> *Accès à toute séquence des génomes séquencés…*

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2. Les analyses nucléotidiques

Position sur le génome ???

La carte chromosomique…

3. Les pathologies héréditaires

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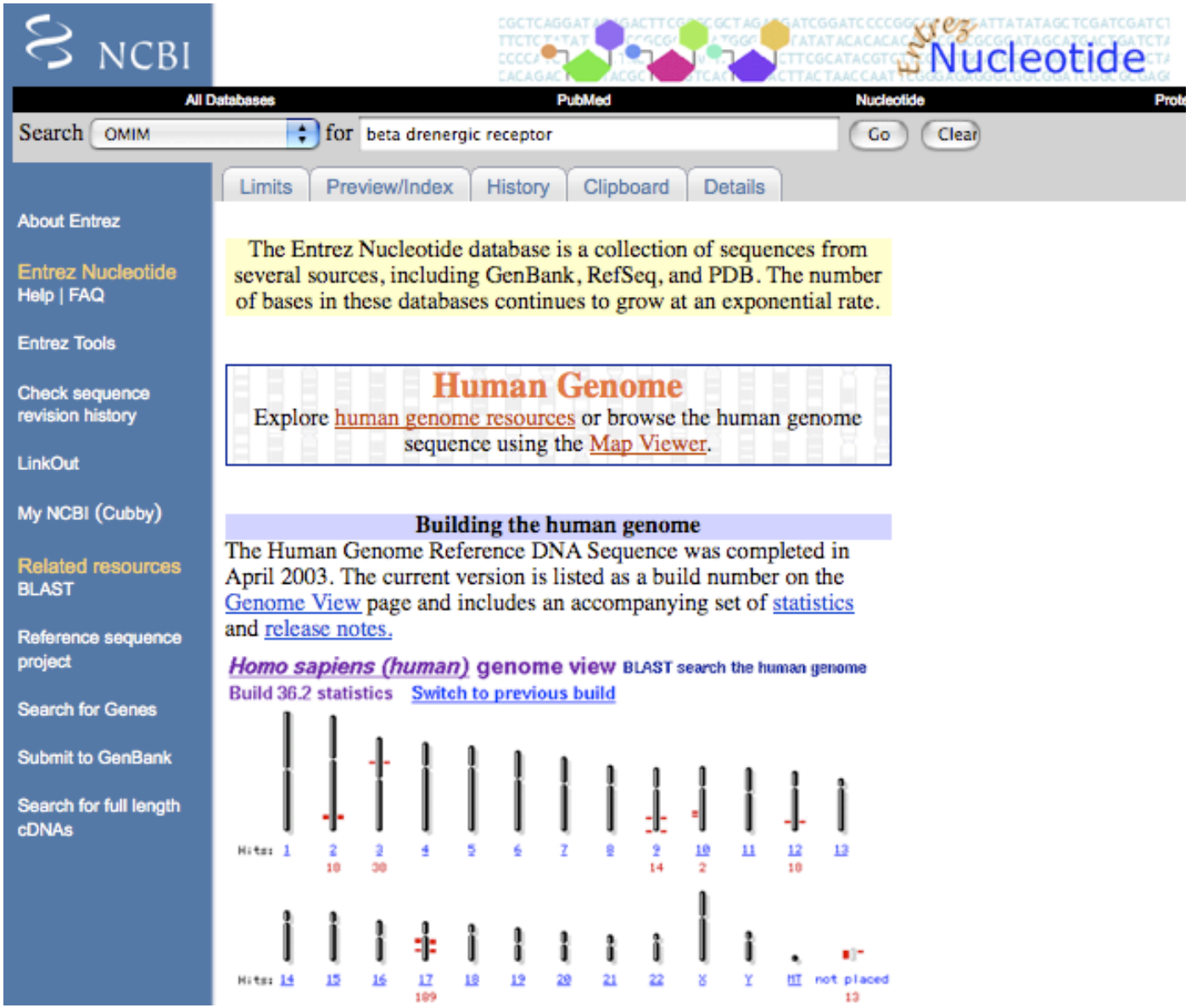
3. Les pathologies héréditaires



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3. Les pathologies héréditaires

L'onglet OMIM est un outils de synthèse

3. Les pathologies héréditaires

NCBI

OMIM
Online Mendelian Inheritance in Man
Johns Hopkins University

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Display Detailed Show 20 Send to

MIM *109691
Cloning
Gene Structure
Mapping
Molecular Genetics
Animal Model
Allelic Variants
• View List
References
Contributors
Creation Date
Edit History
• Gene map

Entrez Gene
N Nomenclature
R RefSeq
G GenBank
P Protein
U UniGene

LinkOut
HGVS
HGMD
GAD
MGI

Links

*109691
BETA-3-ADRENERGIC RECEPTOR; ADRB3

Gene map locus 8p12-p11.2

TEXT

CLONING

Emorine et al. (1989) isolated a third beta-adrenergic receptor, beta-3-adrenergic receptor (ADRB3). (See ADRB1 (109630) and ADRB2 (109690).) Exposure of eukaryotic cells transfected with this gene to adrenaline or noradrenaline promoted the accumulation of adenosine 3-prime,5-prime-monophosphate. The potency of beta-AR agonists and inhibitors was described.

Van Spronsen et al. (1993) demonstrated that the transcription start sites of the mouse and human ADRB3 mRNA are located in a region comprised between 150 and 200 nucleotides 5-prime from the ATG translation start codon. Motifs potentially implicated in heterologous regulation of ADRB3 expression by glucocorticoids and by beta-adrenergic agonists were identified upstream from these cap sites.

GENE STRUCTURE

Van Spronsen et al. (1993) described the exon/intron structure of the mouse and human ADRB3 genes. Their results suggested that utilization of alternate promoters and/or 3-prime untranslated regions may allow tissue-specific regulation of the expression of ADRB3.

MAPPING

Wilkie et al. (1993) presented a list of G protein-coupled receptor genes (their Table 3), indicating that the ADRB3 gene had been mapped to 8p12-p11.2 and the homologous gene to mouse chromosome 8.

MOLECULAR GENETICS

The beta-3-adrenergic receptor, located mainly in adipose tissue, is involved in the regulation of lipolysis and thermogenesis. The potential relevance of this receptor to obesity (see 601665) in humans led Clement et al. (1995) to screen obese patients for the mutation in the ADRB3 gene that results in replacement of tryptophan by arginine at position 64 (W64R; 109691.0001). They studied DNA extracted from leukocytes of 94 normal subjects and 185 unrelated patients with morbid obesity, as defined by a body-mass index (BMI; the weight in kilograms divided by the square of the height in meters) greater than 40. The mutation was detected by analysis of RFLPs with the restriction enzyme BstNI, which discriminates between the normal and mutant sequences. The frequency of the W64R variant was similar in the morbidly obese patients and the normal subjects: 0.08 and 0.10, respectively. However, patients with morbid obesity who were heterozygous for the allele had an increased capacity to gain weight: the mean weight in the 14 heterozygous patients was 140 kg, as compared with 126 kg in the 171 patients without the mutation ($P = 0.03$). There were no homozygotes in this sample. The cumulative 25-year change in weight (from the age of 20 years) was 67 kg in W64R heterozygotes, as compared with 51 kg in those without the mutation. The maximum weight differential (the maximal lifetime weight minus the weight at 20 years of age) in the heterozygotes was 74 kg, as compared with 59 kg in the patients without the mutation ($P = 0.02$). Clement et al. (1995) interpreted the findings as indicating that the ADRB3 gene mutation W64R increases the capacity to gain weight.

ANIMAL MODEL

To determine whether the sympathetic nervous system is the efferent arm of diet-induced thermogenesis, Bachman et al. (2002) created mice that lacked the beta-adrenergic receptors ADRB1, ADRB2, and ADRB3. Beta-less mice on a chow

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3. Les pathologies héréditaires

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ANIMAL MODEL

To determine whether the sympathetic nervous system is the efferent arm of diet-induced thermogenesis, Bachman et al. (2002) created mice that lacked the beta-adrenergic receptors ADRB1, ADRB2, and ADRB3. Beta-less mice on a chow diet had a reduced metabolic rate and were slightly obese. On a high-fat diet, beta-less mice, in contrast to wildtype mice, developed massive obesity that was due entirely to a failure of diet-induced thermogenesis. Bachman et al. (2002) concluded that the beta-adrenergic receptors are necessary for diet-induced thermogenesis and that this efferent pathway plays a critical role in the body's defense against diet-induced obesity.

ALLELIC VARIANTS
(selected examples)

.0001 OBESITY, SUSCEPTIBILITY TO [ADRB3, TRP64ARG]

Using a candidate gene approach to study the genetics of obesity (601665), Clement et al. (1995) found evidence suggesting that the trp64-to-arg (W64R) variant of the ADRB3 gene increases the capacity to gain weight. Gagnon et al. (1996) failed to find an association between W64R and obesity in studies in 2 cohorts: the Quebec Family Study (QFS) and the Swedish Obese Subjects (SOS).

Walston et al. (1995) found that Pima Indians homozygous for the W64R ADRB3 mutation had an earlier onset of noninsulin-dependent diabetes mellitus (NIDDM; 125853) and tended to have a lower resting metabolic rate. The authors suggested that the mutation may accelerate the onset of NIDDM by altering the balance of energy metabolism in visceral adipose tissue.

Elbein et al. (1996) tested the hypothesis that the beta-3-adrenergic receptor locus affects diabetes susceptibility, obesity as measured by body mass index (BMI), and components of the insulin (176730) resistance syndrome, by examining ADRB3 allele sharing in families ascertained for 2 or more sibs with NIDDM. They found no evidence for linkage to NIDDM as a dichotomous trait and no evidence for linkage to BMI, waist/hip ratio, insulin levels, or glucose levels as quantitative traits or to reported age of onset among NIDDM individuals. The W64R mutation present in 11% of the population also did not show linkage or association. They concluded that the beta-3-adrenergic receptor locus does not play an important role in NIDDM susceptibility or in the insulin resistance syndrome among members of families with a strong predisposition to NIDDM.

Kim-Motoyama et al. (1997) examined the frequency of the W64R variant in 278 Japanese men in relation to visceral obesity assessed by computerized tomography. They found that the mutation was more frequent in subjects with higher BMI. In subjects with a moderate degree of obesity, the mutation (homozygotes and heterozygotes) was associated with visceral obesity (higher ratio of visceral to subcutaneous fat area). Furthermore, the W64R variant was more frequent in subjects with lower serum triglyceride levels, and homozygotes, but not heterozygotes, exhibited lower triglyceride levels. Kim-Motoyama et al. (1997) suggested that the mutation may describe a subset of subjects characterized by decreased lipolysis in visceral adipose tissue.

To examine the effect of W64R on body weight during adult life, the ADRB3 genotypes of 186 unselected Japanese men, most of whom had records of body weight measured yearly from 25 to 53 years of age, were determined by Nagase et al. (1997). Of these subjects, 26 were diagnosed as having noninsulin-dependent diabetes mellitus (NIDDM) and 41 as having impaired glucose tolerance. The results suggested that ADRB3 is not a major contributing factor to obesity or NIDDM in Japanese men.

Buettner et al. (1998) examined the prevalence of the 2 ADRB3 alleles in Germany and looked for associations between the ADRB3 genotype and obesity and NIDDM. The frequencies of the different genotypes in the examined cohort were as follows: trp64/trp64, 88.3%; trp64/arg64, 10.8%; and arg64/arg64, 0.8%. The authors found no significant differences between the different genotypes when comparing age, BMI, weight, total and high density lipoprotein, cholesterol, fasting insulin, HbA1c, and blood pressure. They concluded that the NIDDM phenotype did not differ significantly between the different genotype groups in terms of age of diabetes onset or HbA1c.

Using hyperinsulinemic/euglycemic clamp methodology, Garcia-Rubi et al. (1998) measured insulin sensitivity in 13 obese women heterozygous for the W64R ADRB3 variant and in 14 women homozygous for the normal gene. Exogenous glucose infusion during the clamp was significantly lower (P = 0.03) in W64R heterozygotes (241 +/- 135 mg/min) compared with normal homozygotes (379 +/- 172 mg/min). They concluded that obese postmenopausal women who are heterozygous for the W64R variant have greater insulin resistance than women homozygous for the normal gene matched for age, body composition, and physical activity.

Mitchell et al. (1998) detected an effect of the W64R variant on obesity in a Mexican-American population. They had previously identified a major quantitative trait locus (QTL) influencing the serum concentrations of leptin on 2p in a Mexican-American population in south Texas (Comuzzie et al., 1997). They studied 45 sib pairs who were concordant (identical by descent) for this locus on chromosome 2, which had been shown previously to be tightly linked to obesity in this population. The W64R variant, detected by PCR-RFLP analysis, was present in 1 sib within each of the 45 sib pairs. Presence of the variant was associated with a significantly higher values in body mass index, fat mass, and waist circumference. The paired-sib design enhanced their ability to detect the effects of this variant by allowing them to account for variation attributable to another obesity susceptibility locus and to background genes.

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4. Les analyses génomiques

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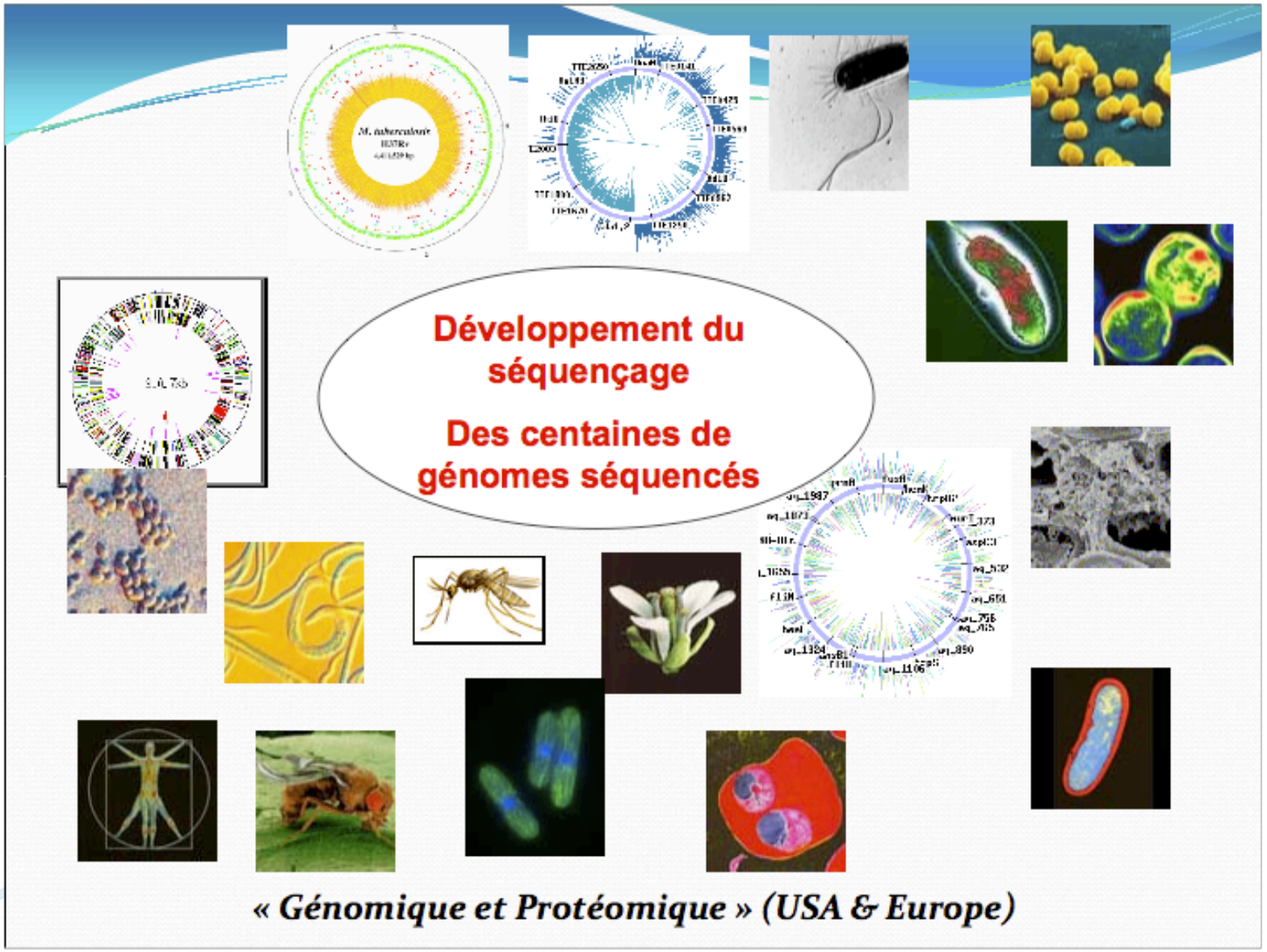
4. Les analyses génomiques



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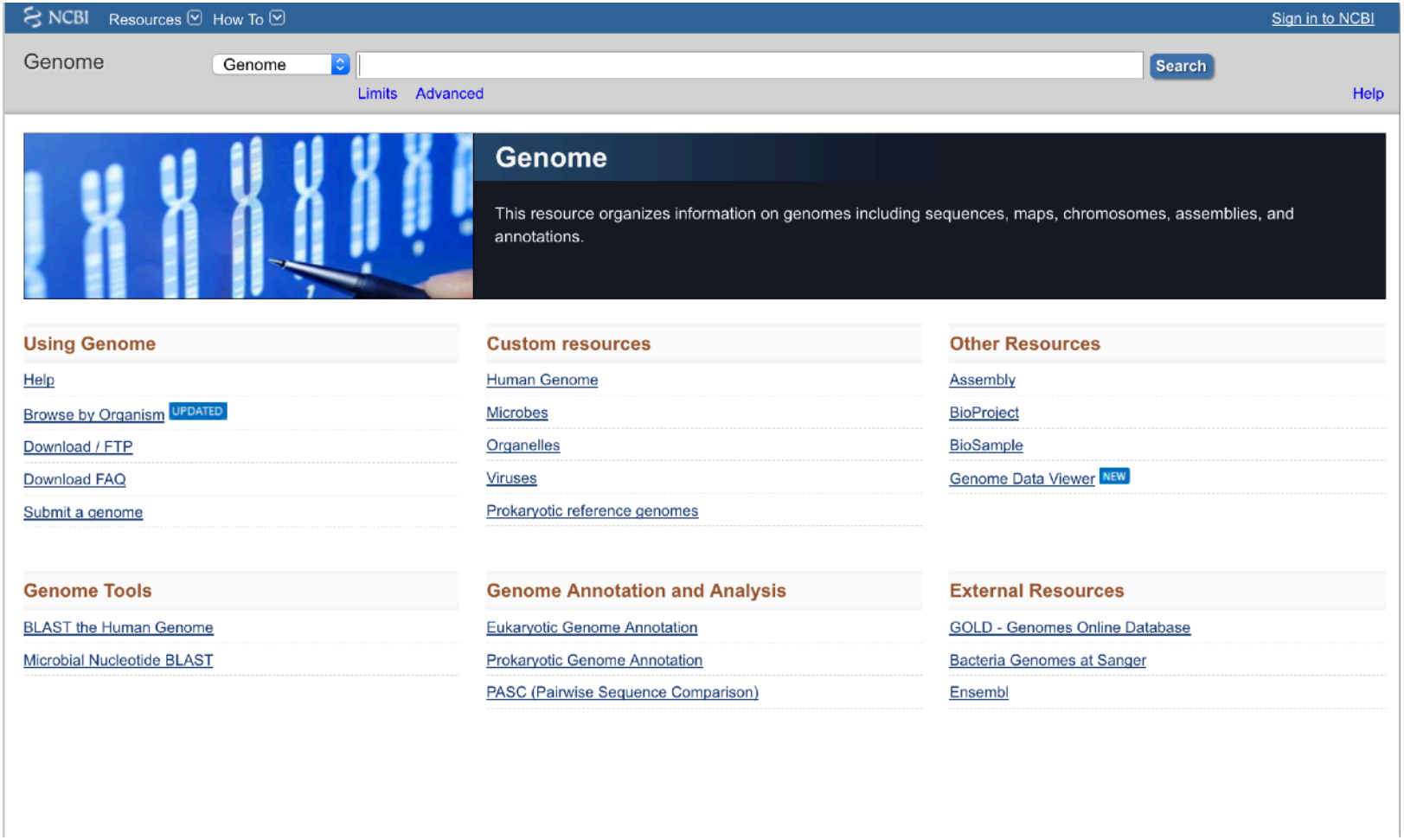

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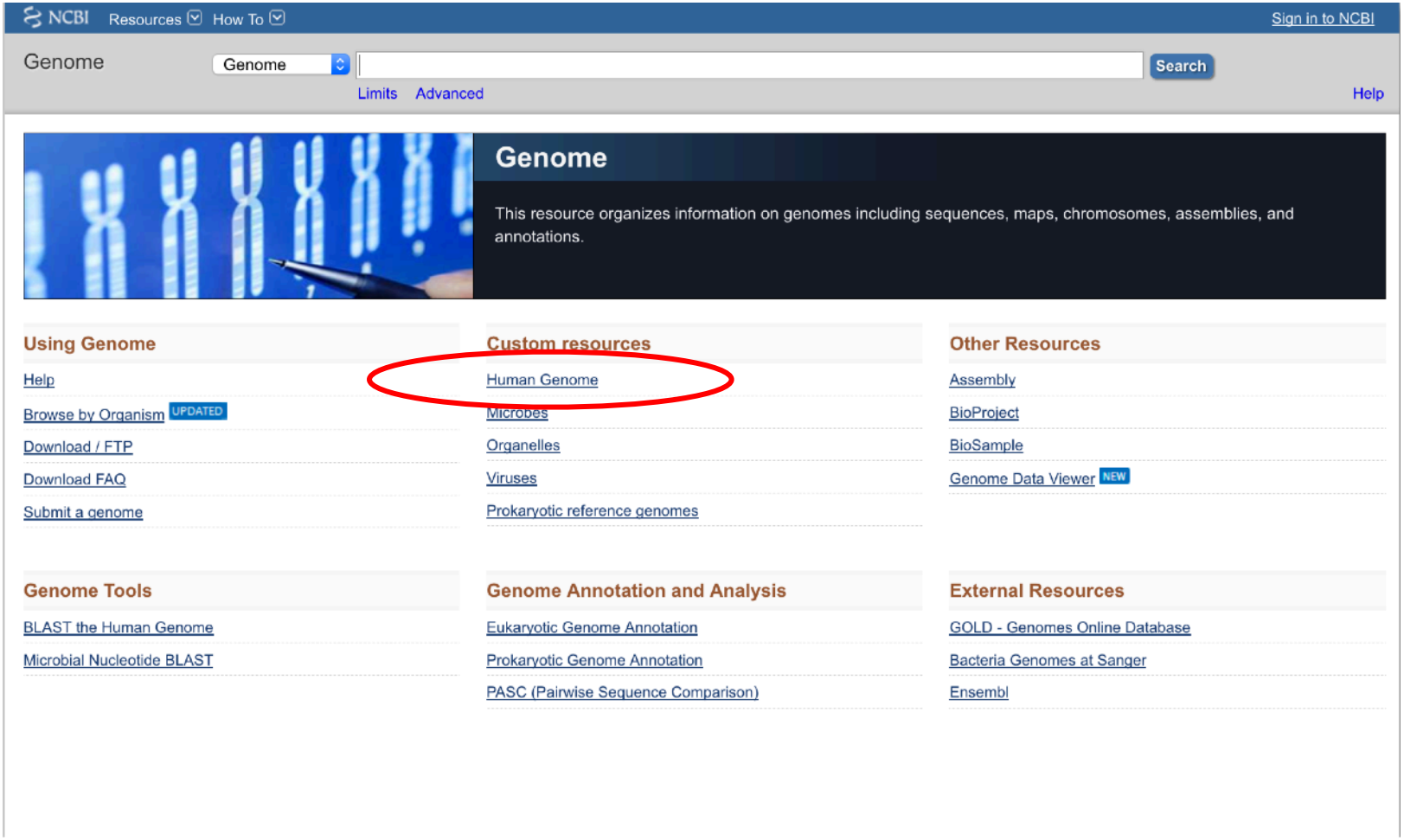

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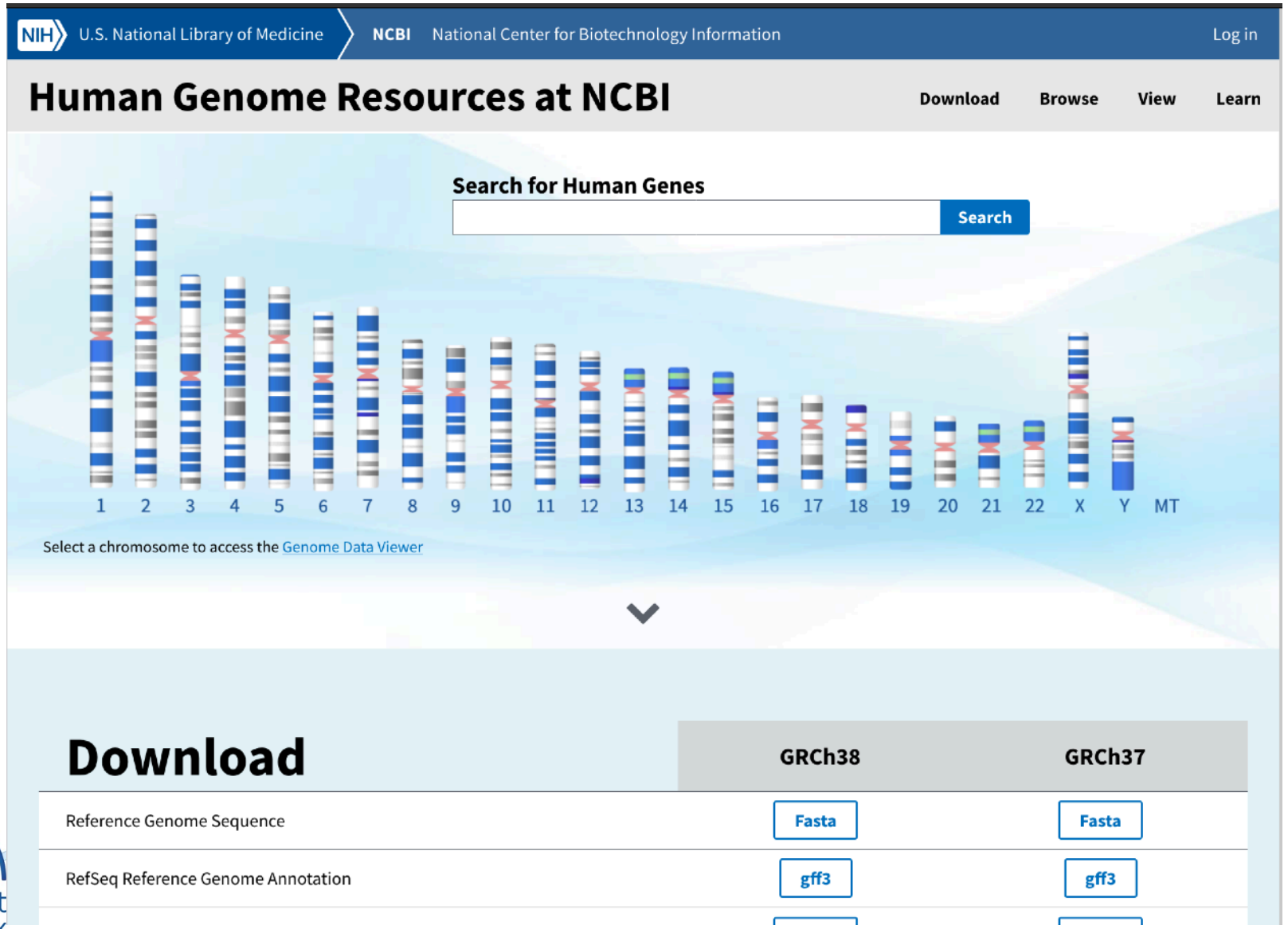

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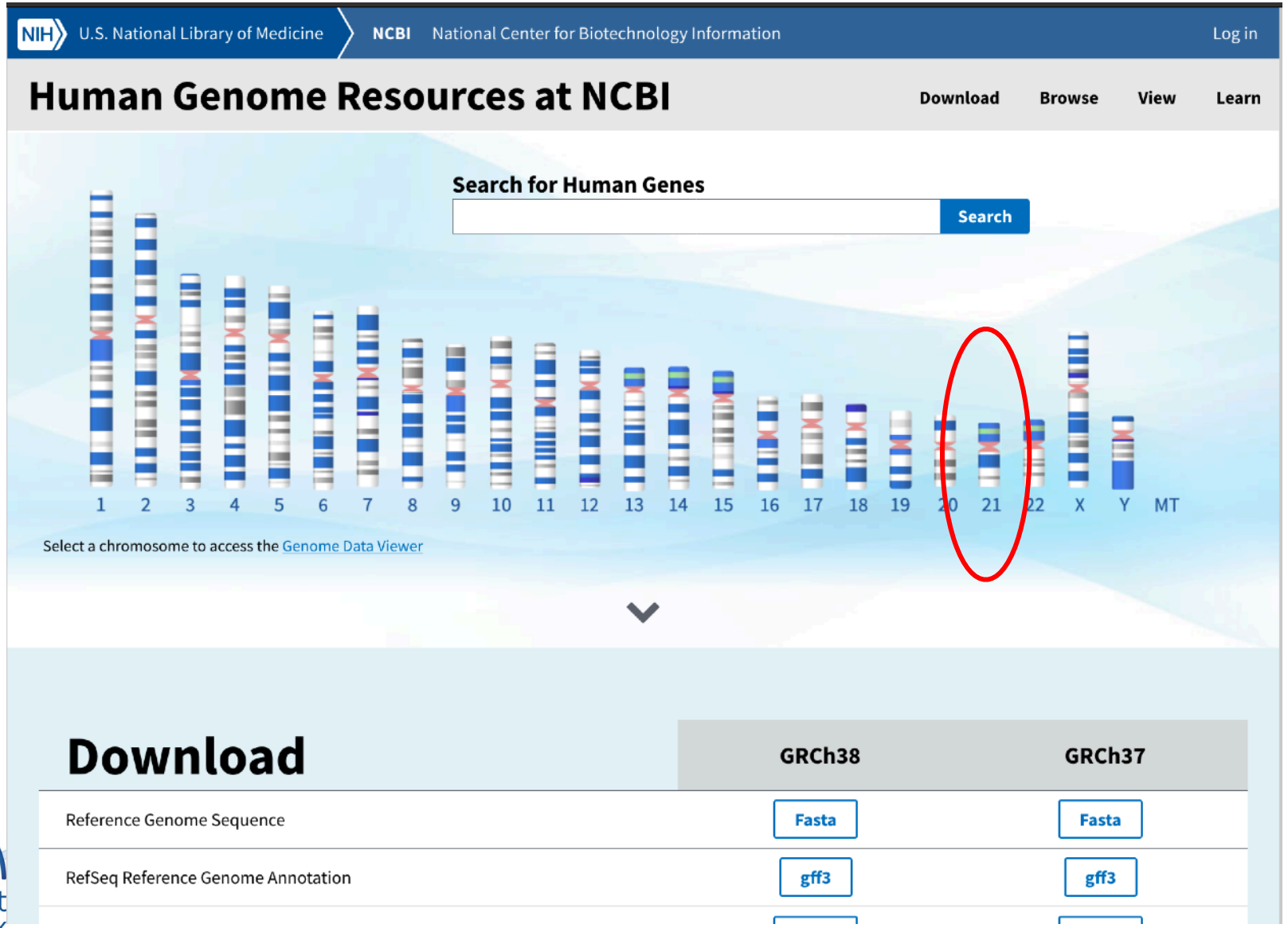

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4. Les analyses génomiques

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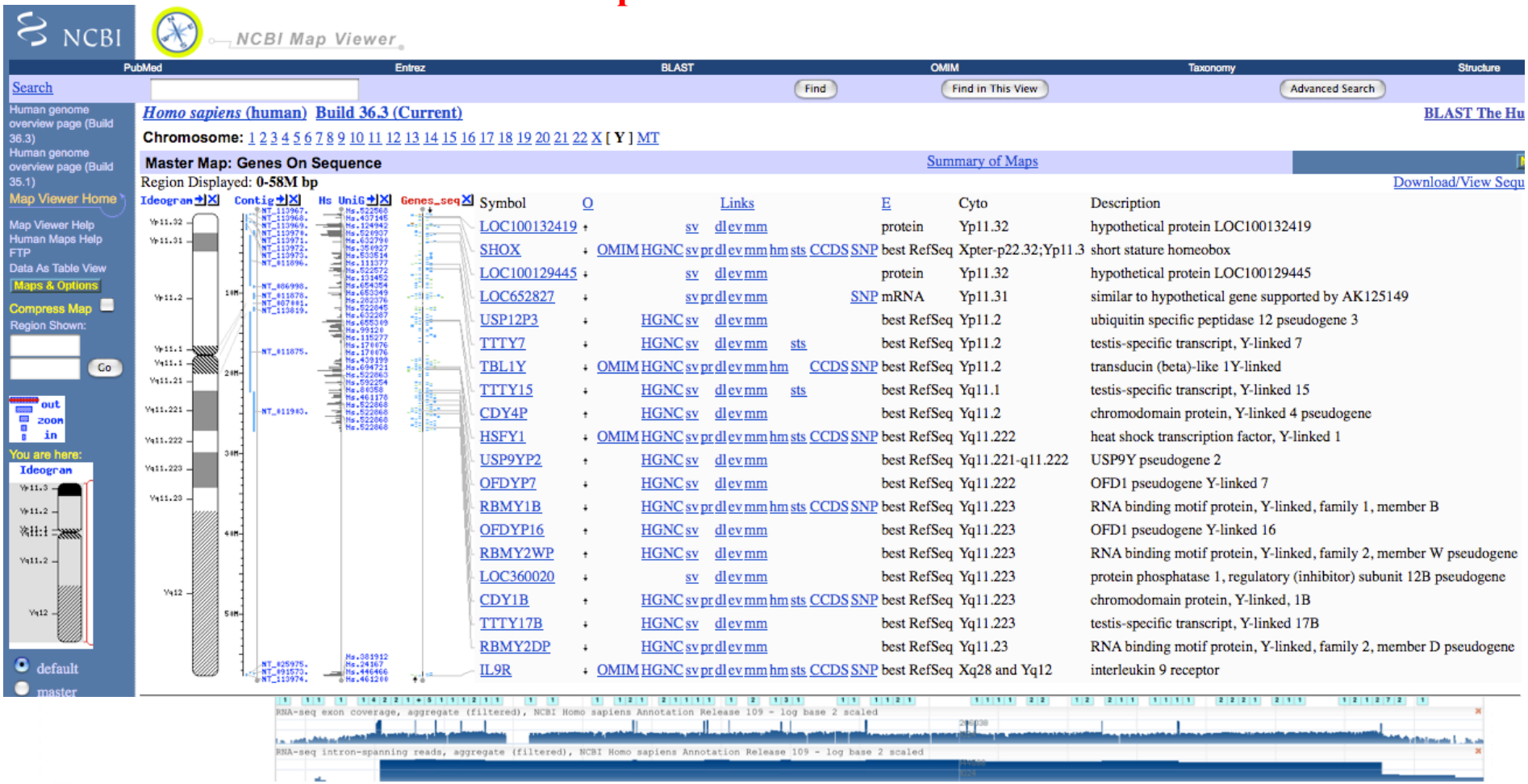
4. Les analyses génomiques

Ici un Map Viewer sur le chromosome Y.



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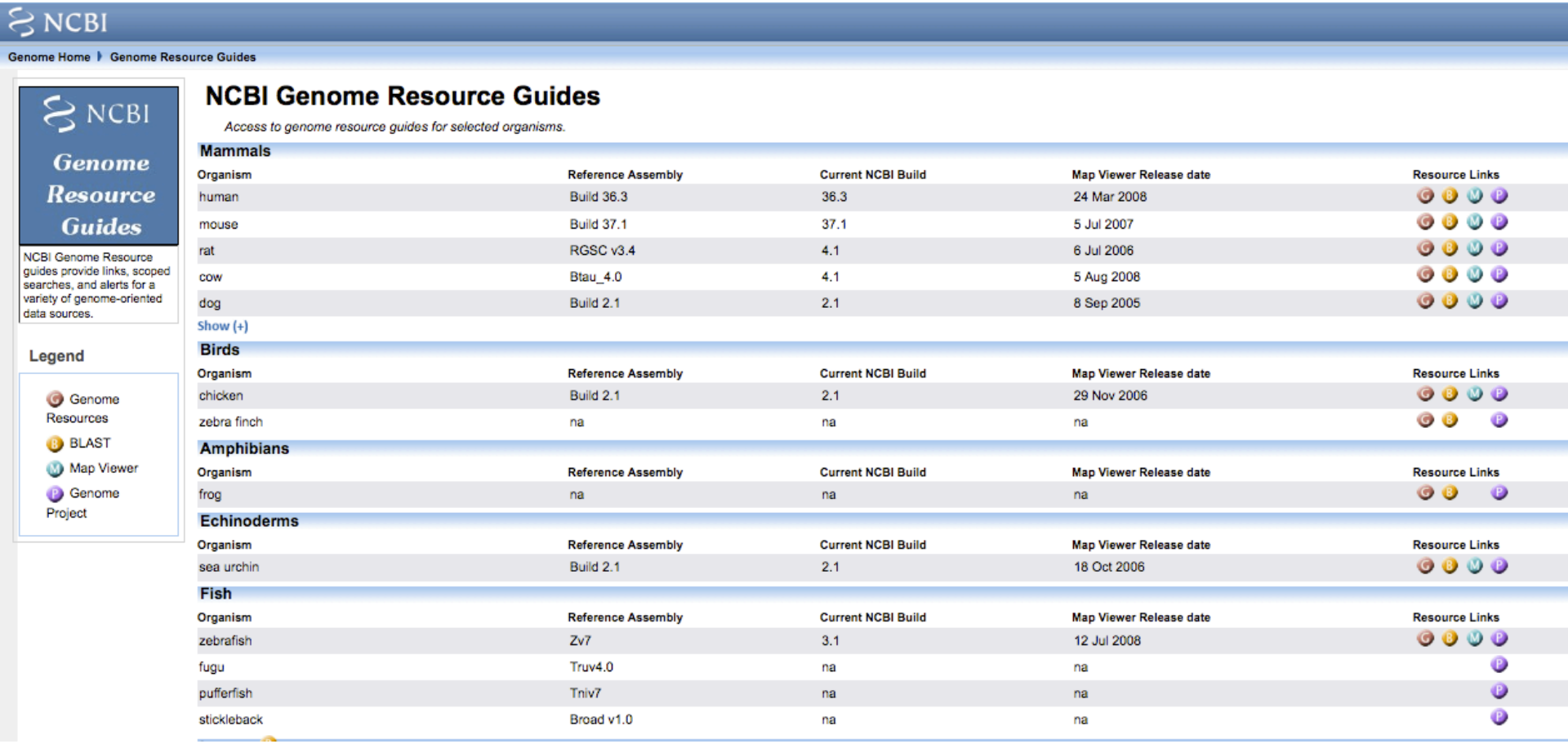
4. Les analyses génomiques



NCBI

Genome Home ▸ Genome Resource Guides

NCBI Genome Resource Guides

Access to genome resource guides for selected organisms.

NCBI Genome Resource Guides

NCBI Genome Resource guides provide links, scoped searches, and alerts for a variety of genome-oriented data sources.

Legend

- Genome Resources
- BLAST
- Map Viewer
- Genome Project

Mammals

Organism	Reference Assembly	Current NCBI Build	Map Viewer Release date	Resource Links
human	Build 36.3	36.3	24 Mar 2008	
mouse	Build 37.1	37.1	5 Jul 2007	
rat	RGSC v3.4	4.1	6 Jul 2006	
cow	Btau_4.0	4.1	5 Aug 2008	
dog	Build 2.1	2.1	8 Sep 2005	

Show (+)

Birds

Organism	Reference Assembly	Current NCBI Build	Map Viewer Release date	Resource Links
chicken	Build 2.1	2.1	29 Nov 2006	
zebra finch	na	na	na	

Amphibians

Organism	Reference Assembly	Current NCBI Build	Map Viewer Release date	Resource Links
frog	na	na	na	

Echinoderms

Organism	Reference Assembly	Current NCBI Build	Map Viewer Release date	Resource Links
sea urchin	Build 2.1	2.1	18 Oct 2006	

Fish

Organism	Reference Assembly	Current NCBI Build	Map Viewer Release date	Resource Links
zebrafish	Zv7	3.1	12 Jul 2008	
fugu	Truv4.0	na	na	
pufferfish	Tniv7	na	na	
stickleback	Broad v1.0	na	na	

Les autres espèces...

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5. Les analyses protéiques

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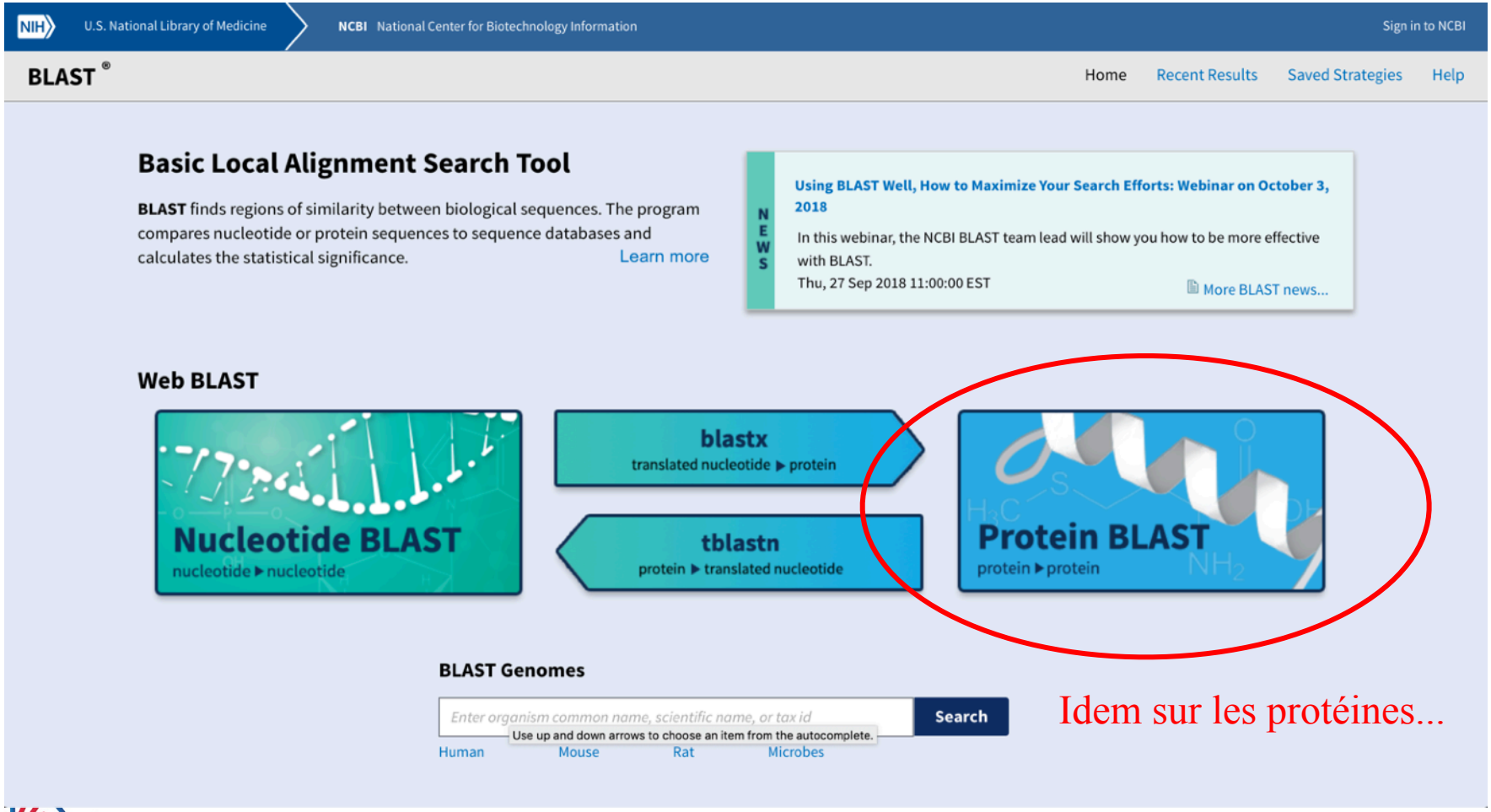
5. Les analyses protéiques



Idem sur les protéines...

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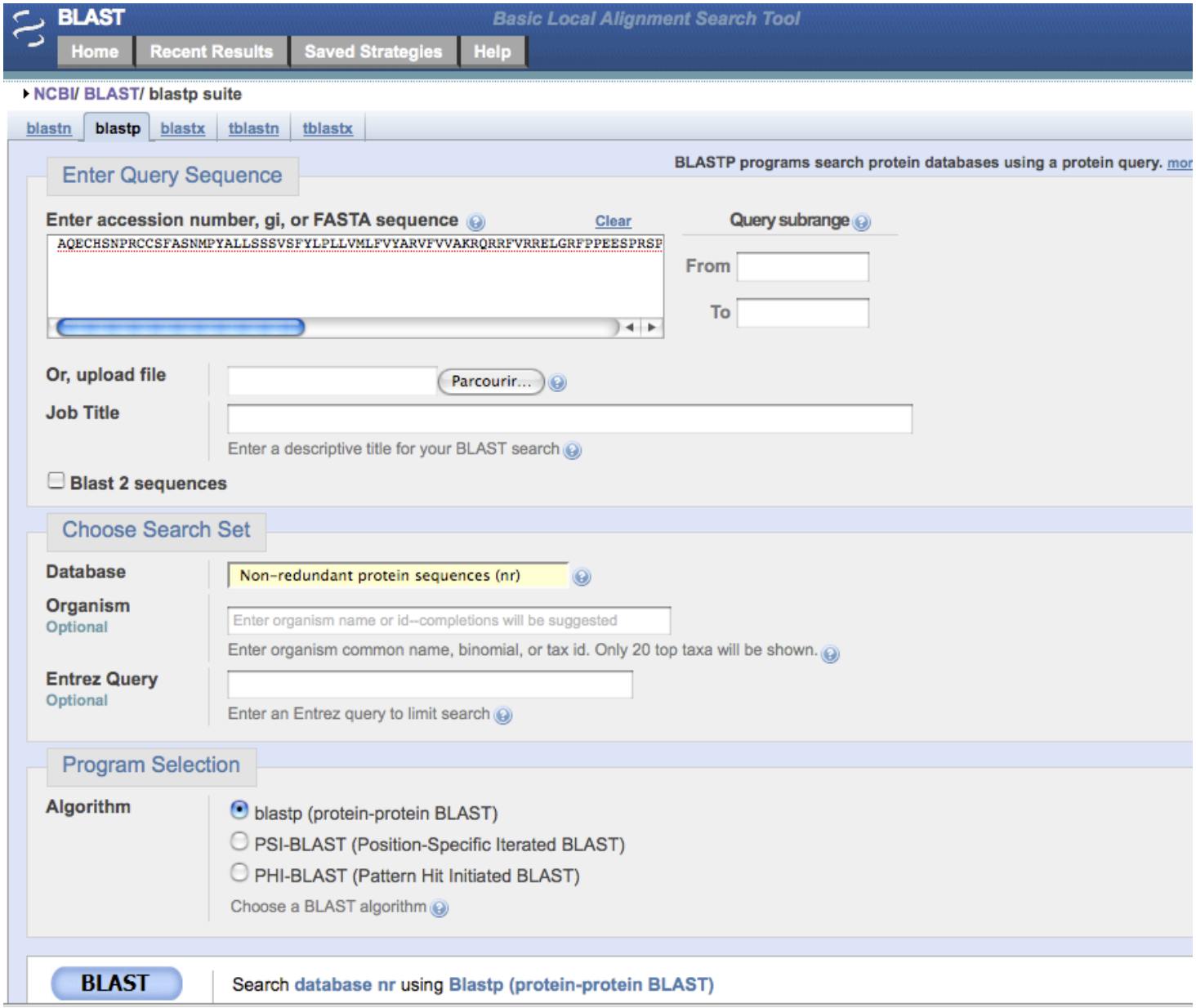
5. Les analyses protéiques



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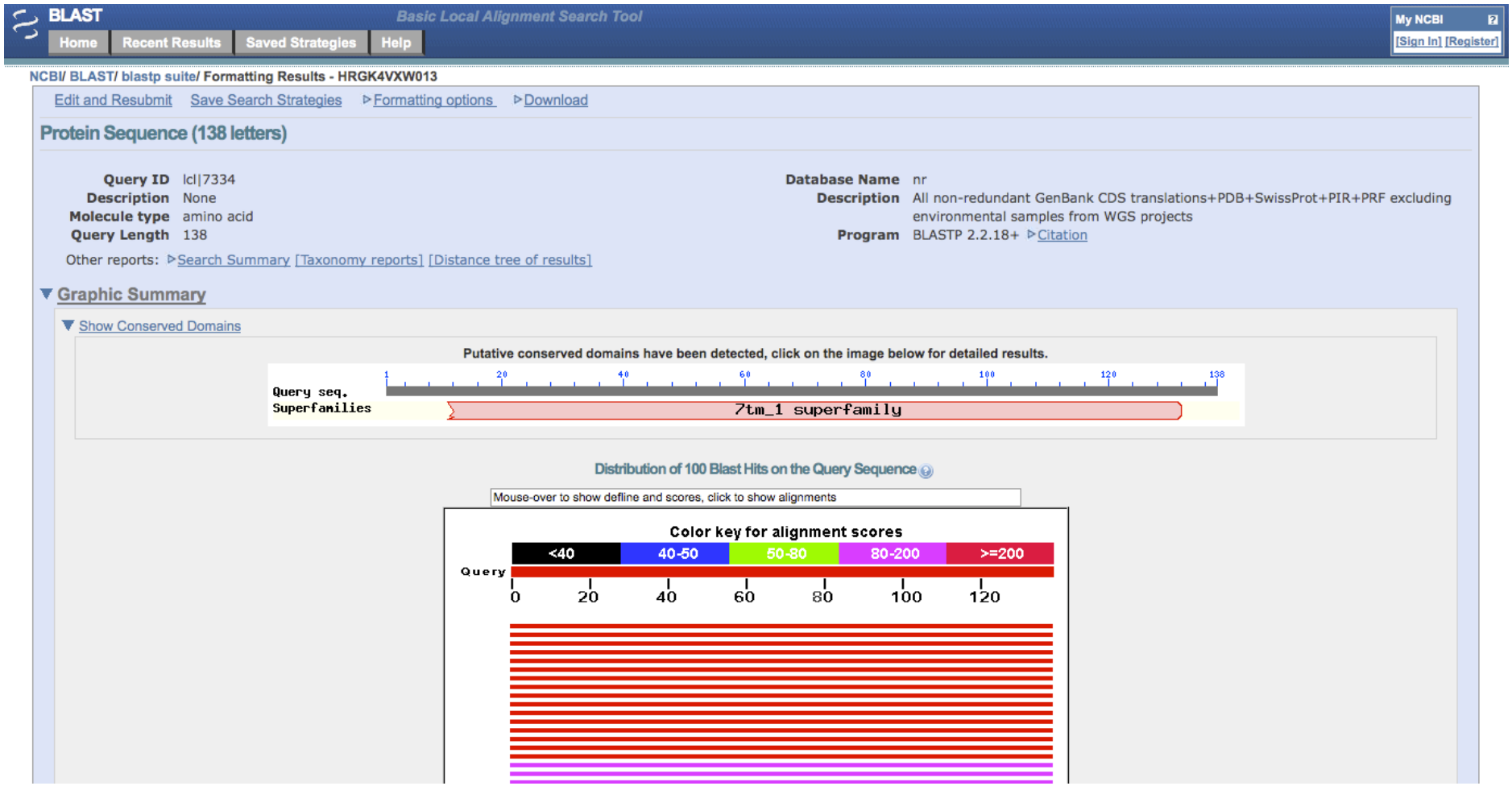
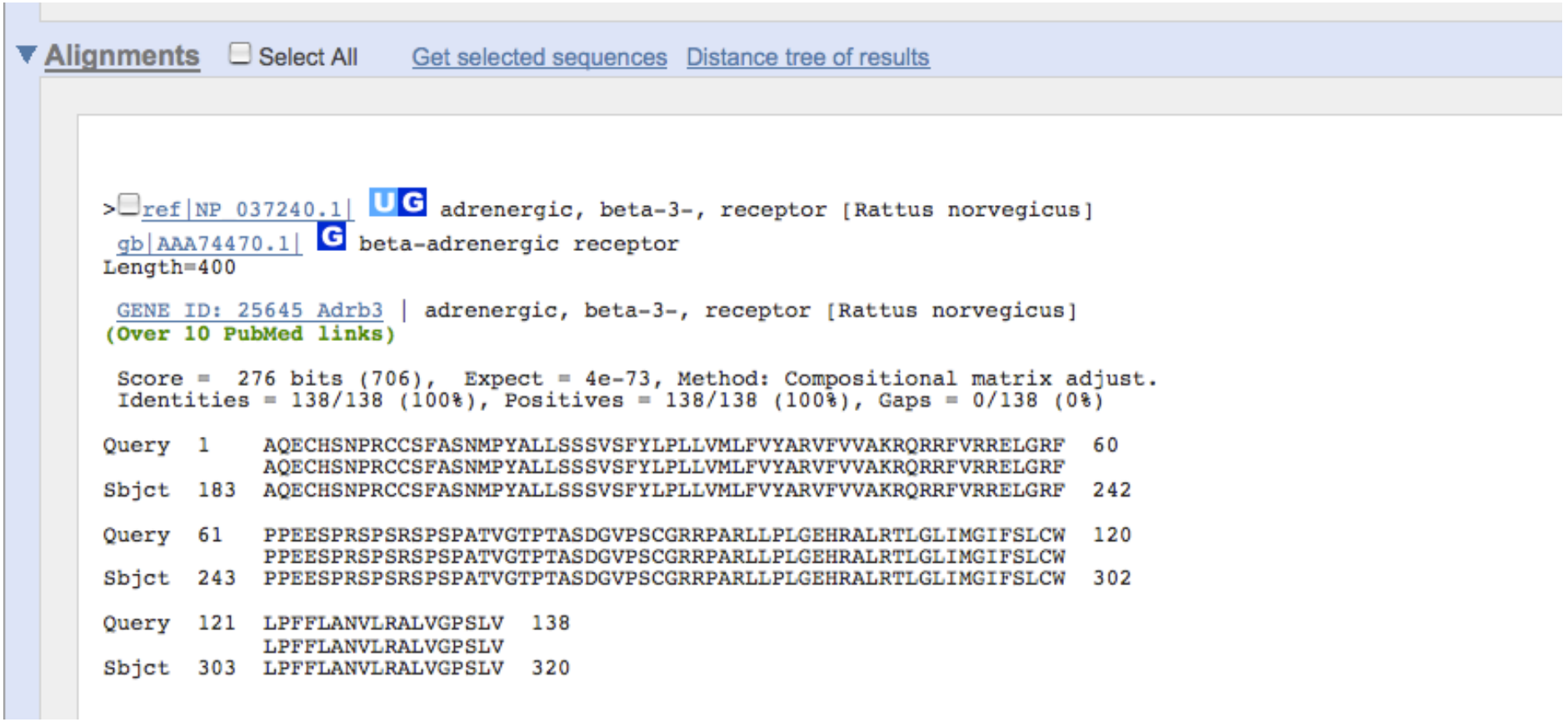
5. Les analyses protéiques



5. Les analyses protéiques



Alignments Select All Get selected sequences Distance tree of results

>ref|NP_037240.1| UG adrenergic, beta-3-, receptor [Rattus norvegicus]
gb|AAA74470.1| G beta-adrenergic receptor
Length=400

GENE ID: 25645 Adrb3 | adrenergic, beta-3-, receptor [Rattus norvegicus]
(Over 10 PubMed links)

Score = 276 bits (706), Expect = 4e-73, Method: Compositional matrix adjust.
Identities = 138/138 (100%), Positives = 138/138 (100%), Gaps = 0/138 (0%)

```
Query  1    AQECHSNPRCCSFASNMPYALLSSSVSFYLPLLVMLFVYARVFVVAKRQRRFVRRELGRF  60
            AQECHSNPRCCSFASNMPYALLSSSVSFYLPLLVMLFVYARVFVVAKRQRRFVRRELGRF
Sbjct  183  AQECHSNPRCCSFASNMPYALLSSSVSFYLPLLVMLFVYARVFVVAKRQRRFVRRELGRF  242

Query  61   PPEESPRSPSRSPSPATVGTPTASDGVPSCGRRPARLLPLGEHRALRTLGLIMGIFSLCW  120
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Sbjct  243  PPEESPRSPSRSPSPATVGTPTASDGVPSCGRRPARLLPLGEHRALRTLGLIMGIFSLCW  302

Query  121  LPFFLANVLRALVGPSLV  138
            LPFFLANVLRALVGPSLV
Sbjct  303  LPFFLANVLRALVGPSLV  320
```

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6. Exemple publication utilisant la bioinfo

OPEN ACCESS Freely available online

PLOS ONE

A Rapid Screening Assay to Search for Phosphorylated Proteins in Tissue Extracts

Ignazio Garaguso, Juergen Borlak*

Centre for Pharmacology and Toxicology, Hannover Medical School, Hannover, Germany

Abstract

Reversible protein phosphorylation is an essential mechanism in the regulation of diverse biological processes, nonetheless is frequently altered in disease. As most phosphoproteome studies are based on optimized *in-vitro* cell culture studies new methods are in need to improve *de novo* identification and characterization of phosphoproteins in extracts from tissues. Here, we describe a rapid and reliable method for the detection of phosphoproteins in tissue extract based on an experimental strategy that employs 1D and 2D SDS PAGE, Western immunoblotting of phosphoproteins, in-gel protease digestion and enrichment of phosphorpeptides using metal oxide affinity chromatography (MOAC). Subsequently, phosphoproteins are identified by MALDI-TOF-MS/MS with the CHCA-TL or DHB ML sample matrix preparation method and further characterized by various bioinformatic software tools to search for candidate kinases and phosphorylation-dependent binding motifs. The method was applied to mouse lung tissue extracts and resulted in an identification of 160 unique phosphoproteins. Notably, TiO_2 enrichment of pulmonary protein extracts resulted in an identification of additional 17 phosphoproteins and 20 phosphorylation sites. By use of MOAC, new phosphorylation sites were identified as evidenced for the advanced glycosylation end product-specific receptor. So far this protein was unknown to be phosphorylated in lung tissue of mice. Overall the developed methodology allowed efficient and rapid screening of phosphorylated proteins and can be employed as a general experimental strategy for an identification of phosphoproteins in tissue extracts.

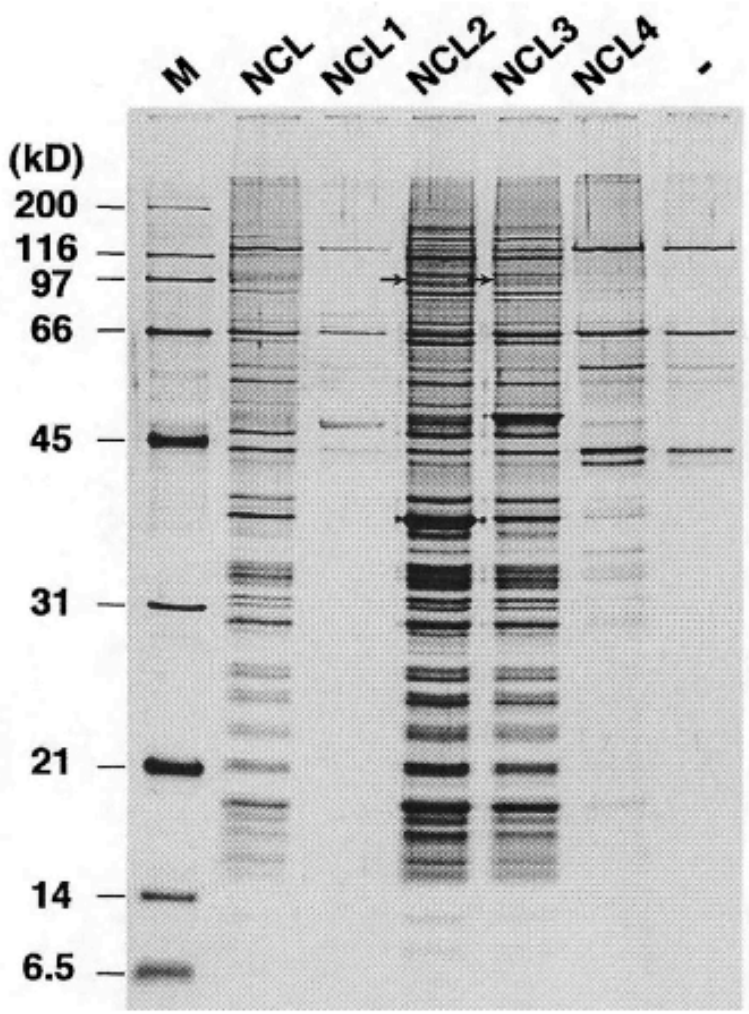
Citation: Garaguso I, Borlak J (2012) A Rapid Screening Assay to Search for Phosphorylated Proteins in Tissue Extracts. PLoS ONE 7(11): e50025. doi:10.1371/journal.pone.0050025

Editor: Christina Lynn Addison, Ottawa Hospital Research Institute, Canada

Received August 30, 2012; **Accepted** October 19, 2012; **Published** November 15, 2012

5. Les analyses protéiques

Migration de protéines: Gel en 1 dimension



Les protéines migrent selon leur poids moléculaire

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5. Les analyses protéiques

- L'électrophorèse bidimensionnelle consiste à séparer les protéines :
  - dans une première dimension par focalisation isoélectrique (IEF) selon leur pI
  - dans une deuxième direction, perpendiculaire à la première, selon leur poids moléculaire.

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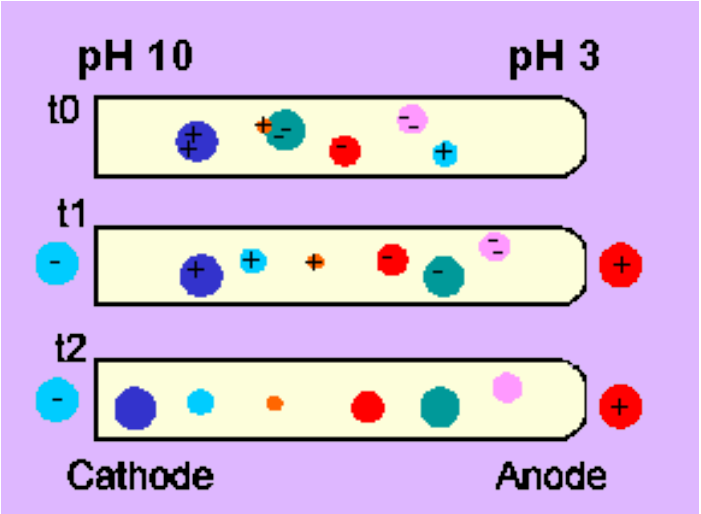
- Première dimension : IEF (électrofocalisation)
  - Gradient de pH sous l ’effet d ’un champ électrique
  - Migration des protéines à travers le gradient de pH jusqu ’à une charge nette nulle lorsqu ’elles atteignent leur pI



5. Les analyses protéiques

1ère dimension :
Électrofocalisation
(gradient de pH)

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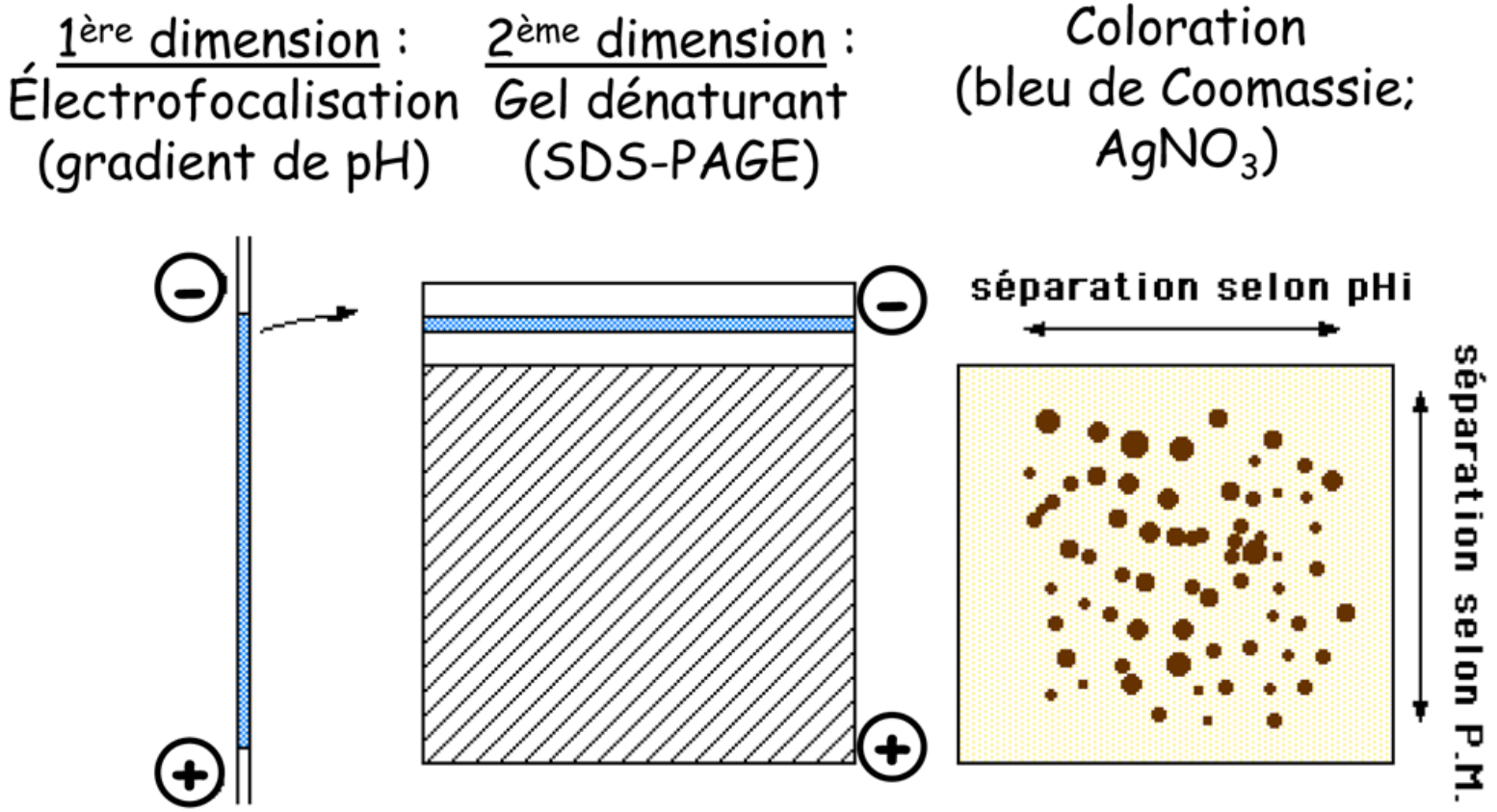
5. Les analyses protéiques



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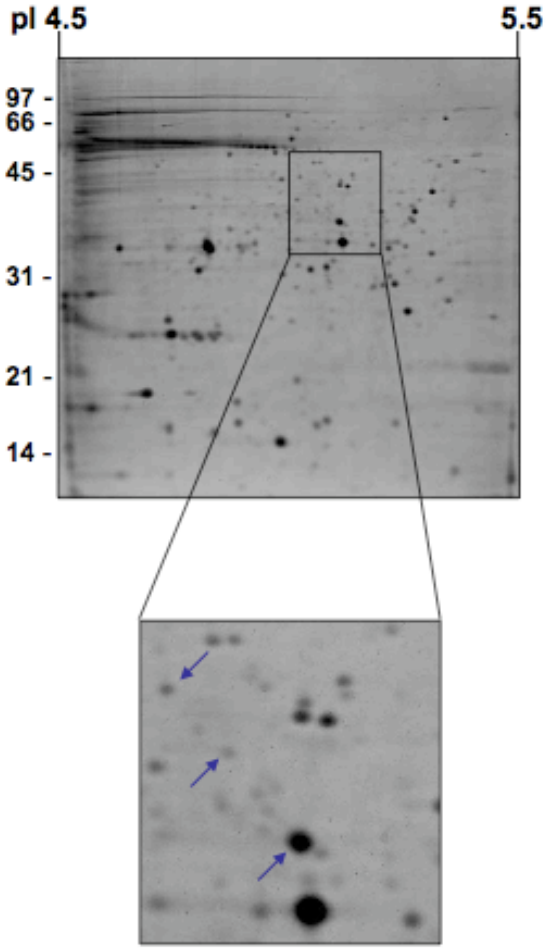
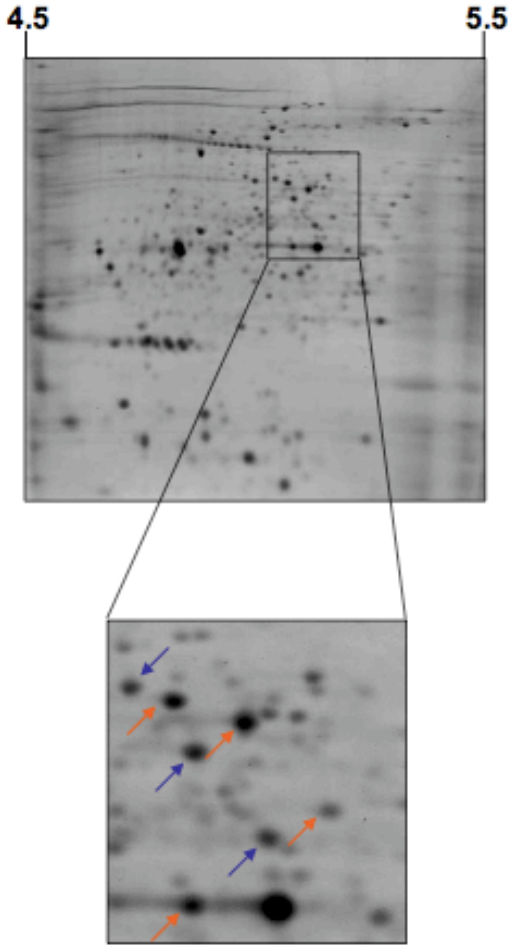
5. Les analyses protéiques

Etudes différentielles

Condition 1





Condition 2

stage-specific

altered expression level

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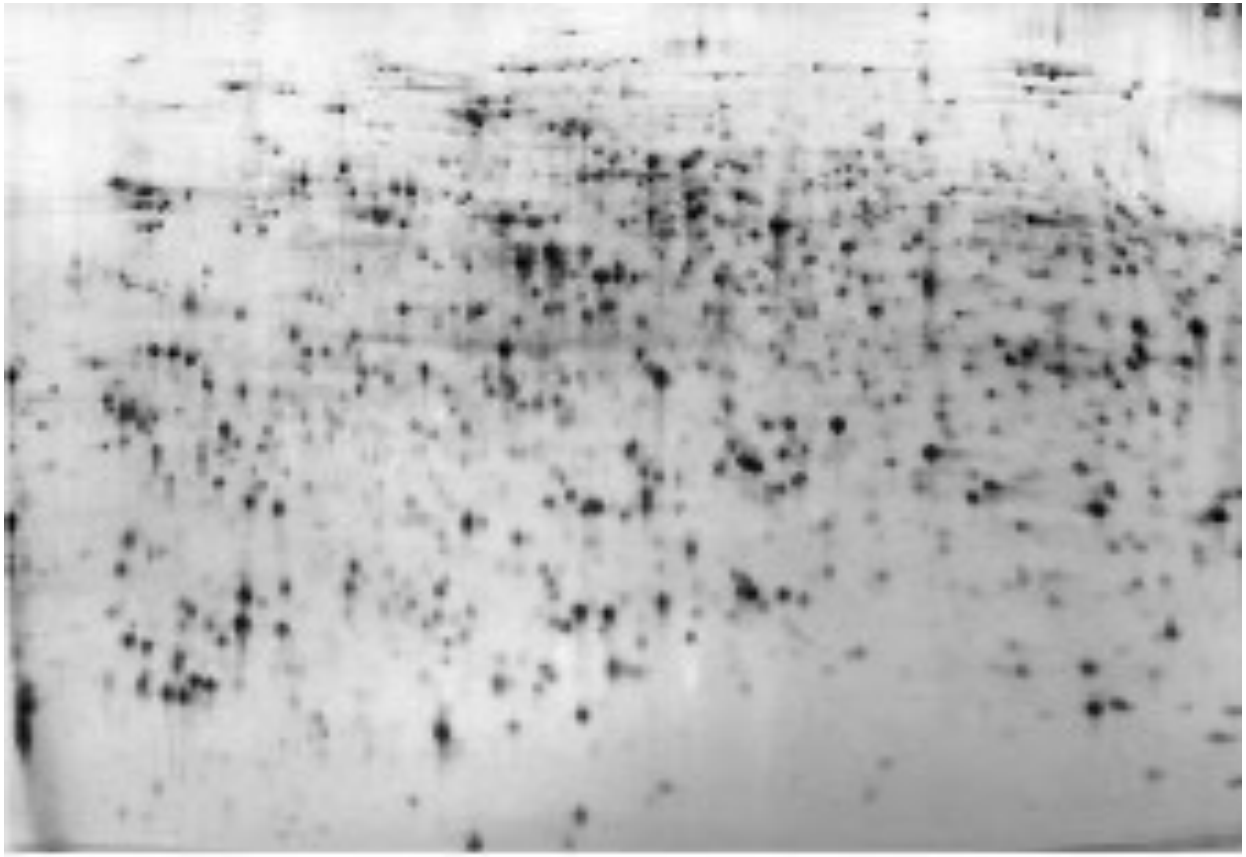
5. Les analyses protéiques

- Il existe des banques de données de résultats



E. coli

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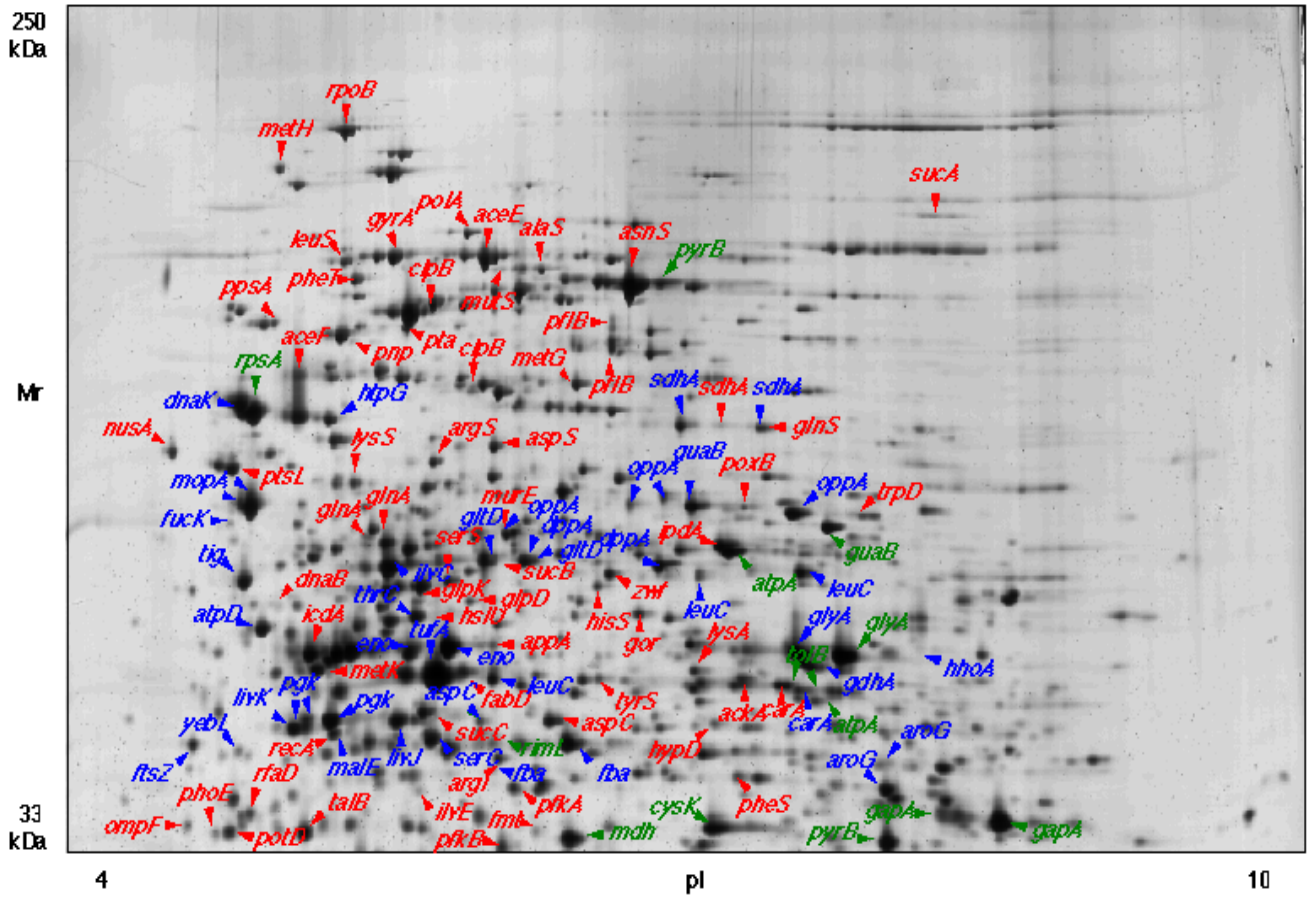
5. Les analyses protéiques

- Il existe des banques de données de résultats



E. coli

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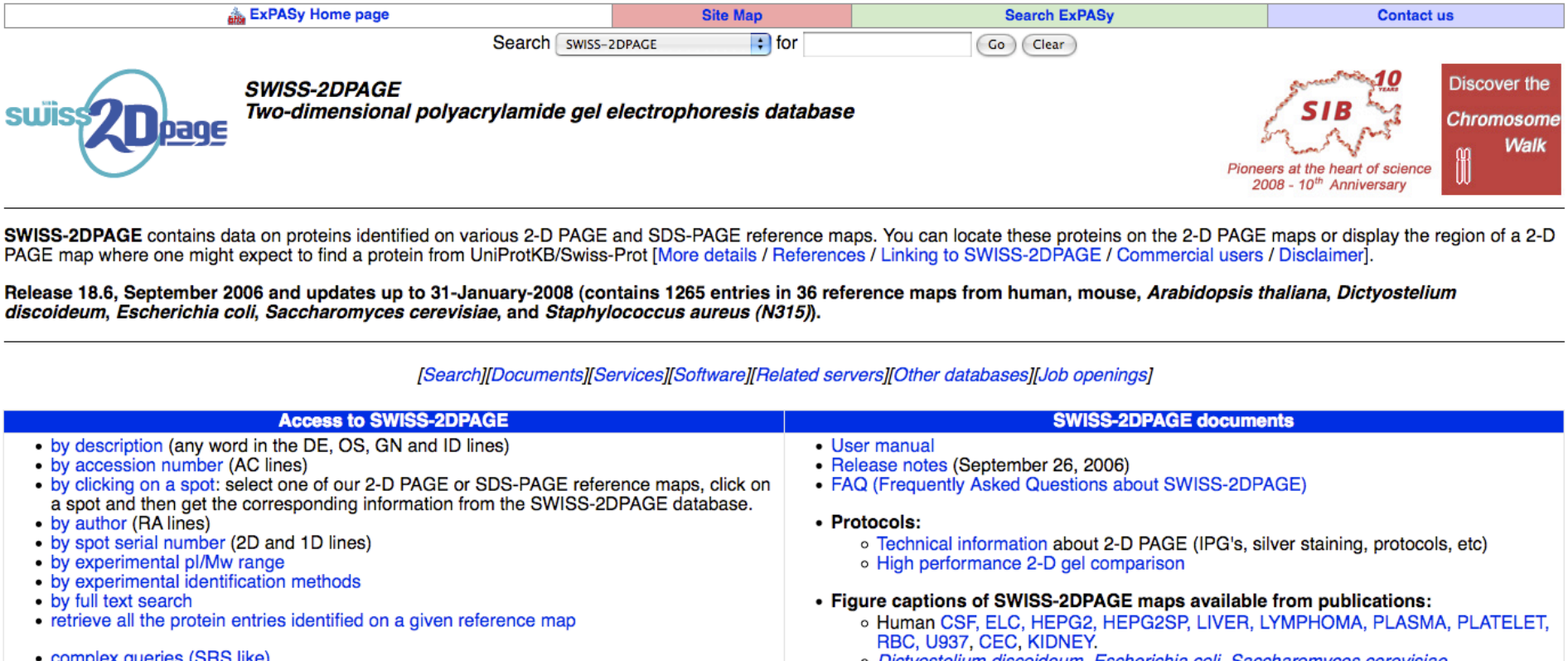
5. Les analyses protéiques

http://expasy.org/ch2d/



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Search SWISS-2DPAGE for Go Clear

SWISS-2DPAGE
Two-dimensional polyacrylamide gel electrophoresis database

SIB 10 YEARS — Pioneers at the heart of science 2008 - 10th Anniversary

Discover the Chromosome Walk

SWISS-2DPAGE contains data on proteins identified on various 2-D PAGE and SDS-PAGE reference maps. You can locate these proteins on the 2-D PAGE maps or display the region of a 2-D PAGE map where one might expect to find a protein from UniProtKB/Swiss-Prot [More details / References / Linking to SWISS-2DPAGE / Commercial users / Disclaimer].

Release 18.6, September 2006 and updates up to 31-January-2008 (contains 1265 entries in 36 reference maps from human, mouse, *Arabidopsis thaliana*, *Dictyostelium discoideum*, *Escherichia coli*, *Saccharomyces cerevisiae*, and *Staphylococcus aureus (N315)*).

[Search][Documents][Services][Software][Related servers][Other databases][Job openings]

Access to SWISS-2DPAGE

- by description (any word in the DE, OS, GN and ID lines)
- by accession number (AC lines)
- by clicking on a spot: select one of our 2-D PAGE or SDS-PAGE reference maps, click on a spot and then get the corresponding information from the SWISS-2DPAGE database.
- by author (RA lines)
- by spot serial number (2D and 1D lines)
- by experimental pI/Mw range
- by experimental identification methods
- by full text search
- retrieve all the protein entries identified on a given reference map
- complex queries (SRS like)
- compute estimated location on reference maps for a user-entered sequence

SWISS-2DPAGE documents

- User manual
- Release notes (September 26, 2006)
- FAQ (Frequently Asked Questions about SWISS-2DPAGE)
- **Protocols:**
  - Technical information about 2-D PAGE (IPG's, silver staining, protocols, etc)
  - High performance 2-D gel comparison
- **Figure captions of SWISS-2DPAGE maps available from publications:**
  - Human CSF, ELC, HEPG2, HEPG2SP, LIVER, LYMPHOMA, PLASMA, PLATELET, RBC, U937, CEC, KIDNEY.
  - *Dictyostelium discoideum*, *Escherichia coli*, *Saccharomyces cerevisiae*.

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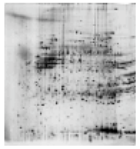
5. Les analyses protéiques

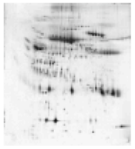
http://expasy.org/ch2d/

Homo sapiens (Human)

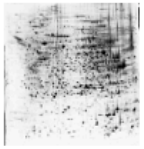
CEC_HUMAN
{ Colorectal epithelia cells }
Tissue: Colon epithelium



CSF_HUMAN
{ Cerebrospinal Fluid }
Tissue: Cerebrospinal fluid

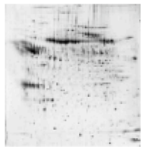


DLD1_HUMAN
{ Colorectal adenocarcinoma cell line (DL-1) }
Tissue: Colon adenocarcinoma

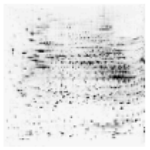


ELC_HUMAN
{ Erythroleukemia Cell }
Tissue: Erythroleukemia

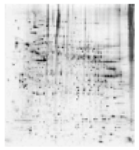
HEPG2SP_HUMAN
{ HepG2 Secreted Proteins }
Tissue: Hepatoblastoma



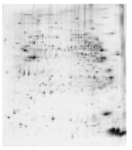
HEPG2_HUMAN
{ HepG2 }
Tissue: Hepatoblastoma



HL60_HUMAN
{ Promyelocytic leukemia cells }
Tissue: Promyelocytic leukemia

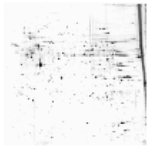


KIDNEY_HUMAN
{ Kidney }
Tissue: Kidney

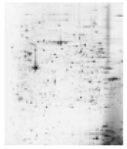


LIVER_HUMAN
{ Liver }
Tissue: Liver

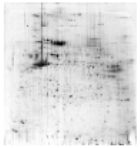
LYMPHOCYTE_HUMAN
{ Lymphocytes }
Tissue: Lymphocyte



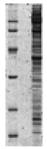
LYMPHOMA_HUMAN
{ Lymphoma }
Tissue: Lymphoma



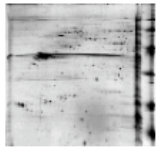
NUCLEI_LIVER_HUMAN
{ Soluble nuclear proteins and matrix from liver tissue }
Tissue: Liver



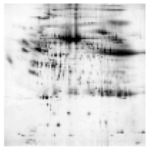
NUCLEOLI_HELA_1D_HUMAN
{ SDS-PAGE of nucleolar proteins from Human HeLa cells }
Tissue: Cervix carcinoma



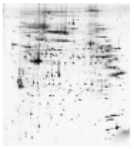
NUCLEOLI_HELA_2D_HUMAN
{ 2D-PAGE of nucleolar proteins from Human HeLa cells }
Tissue: Cervix carcinoma



PLASMA_HUMAN
{ Plasma }
Tissue: Plasma



PLATELET_HUMAN
{ Platelet }
Tissue: Platelet



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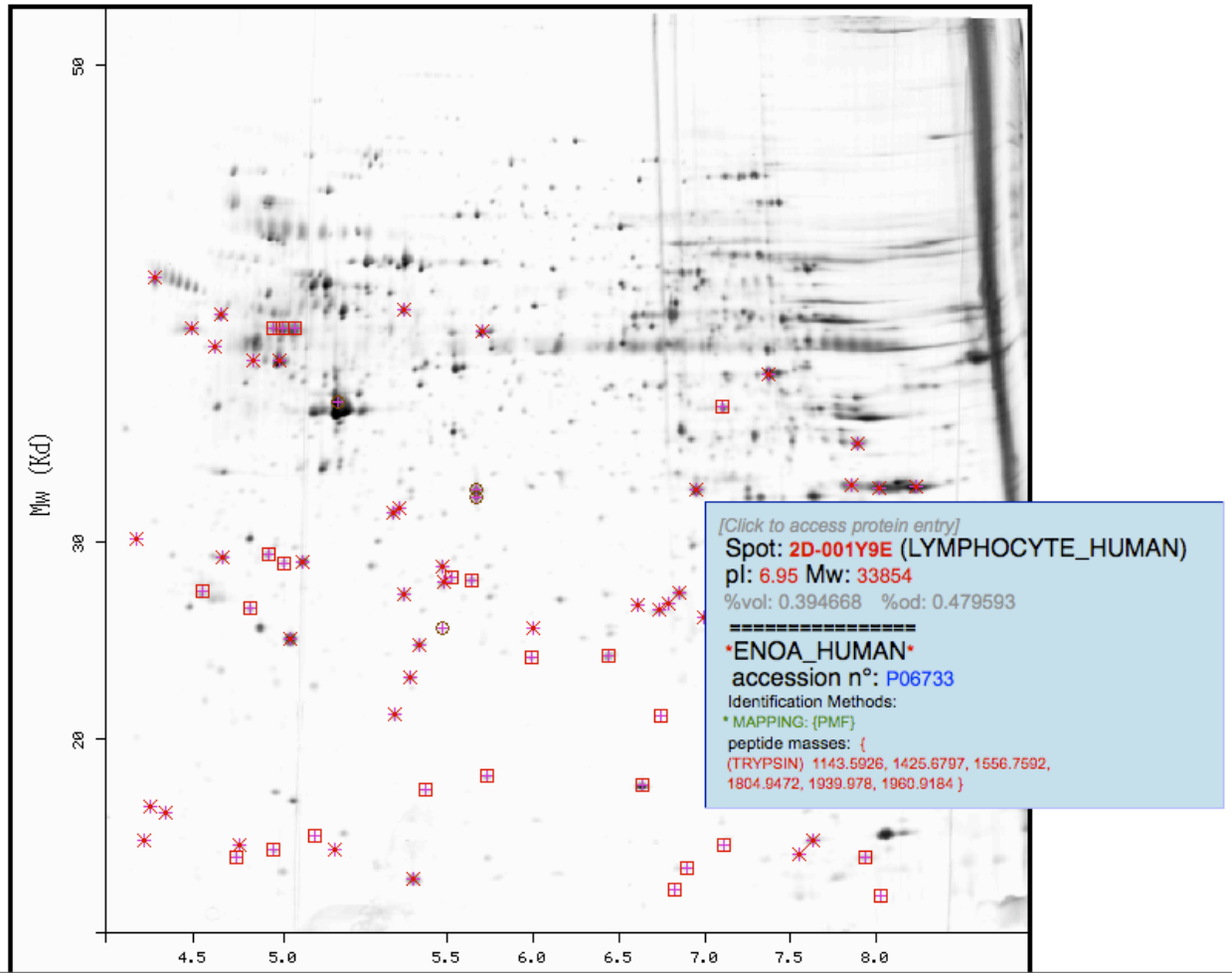
5. Les analyses protéiques

Get more information by dragging your mouse pointer over any spot, or click on a spot to access all its associated protein entries.



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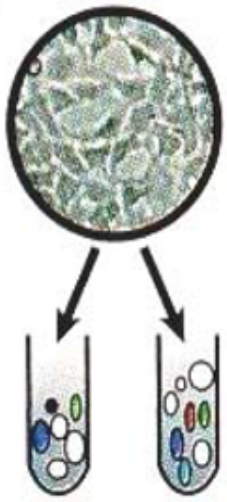
5. Les analyses protéiques



Fractionnement
et extraction
des protéines

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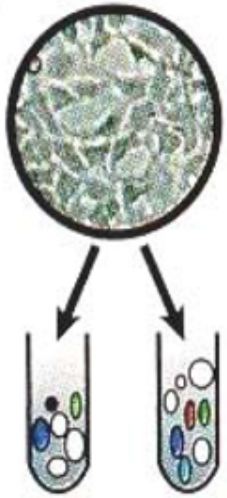
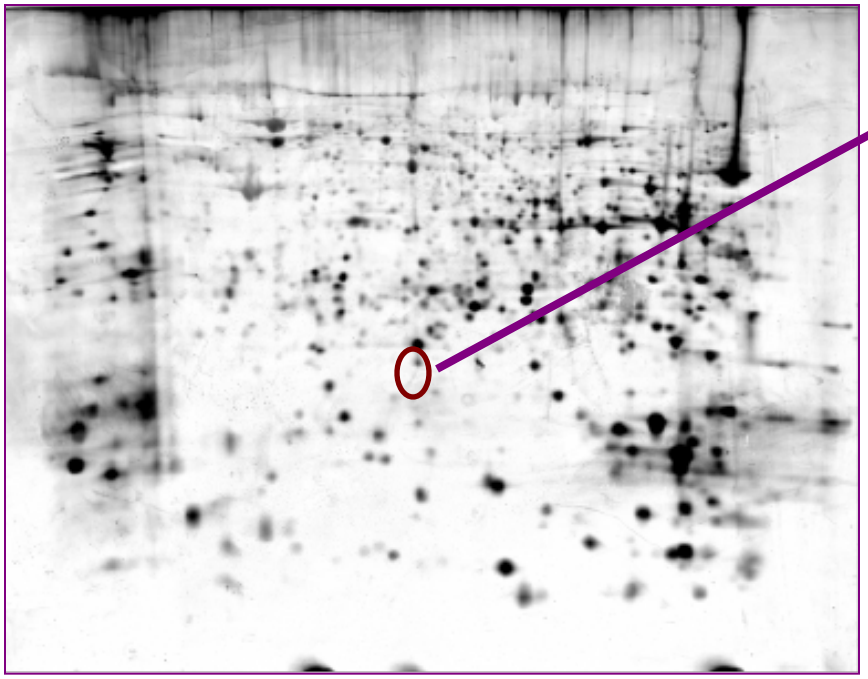
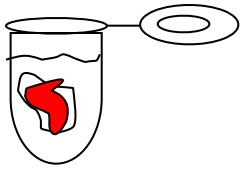
5. Les analyses protéiques

Après avoir repéré la protéine d'intérêt sur le gel 2D, on excise le spot



Fractionnement et extraction des protéines

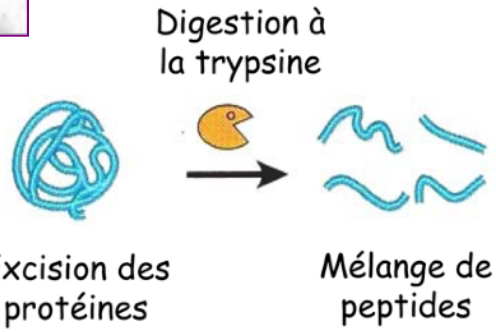




lavage;

déshydratation du morceau de gel prélevé ;

réhydratation par une solution contenant de la trypsine.



Une série de peptides résultant de la digestion de la protéine

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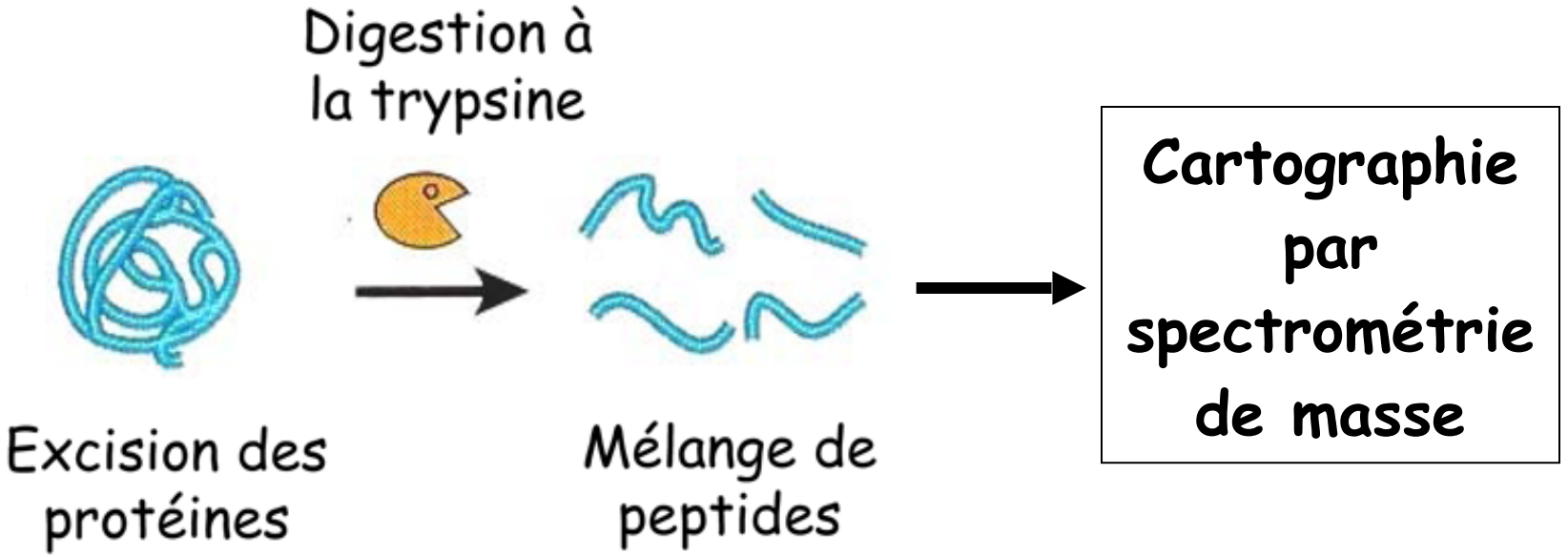
5. Les analyses protéiques



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- Définition

La spectrométrie de masse (mass spectrometry ou MS) est une technique physique d'analyse permettant de détecter et d'identifier des molécules d'intérêt par mesure de leur masse mono-isotopique.

De plus, la spectrométrie de masse permet de caractériser la structure chimique des molécules en les fragmentant.

5. Les analyses protéiques

- La spectrométrie de masse
  - Utilisée pour identifier une protéine, ou suivre ses modifications,
  - Mesure la masse des peptides issus d'une digestion trysique,
  - Les bases de données de séquences permettent de prédire la masse théorique des fragments de protéolyse : on peut identifier une protéine d 'après le spectre de masse de ses fragments de protéolyse (il faut disposer de la séquence).

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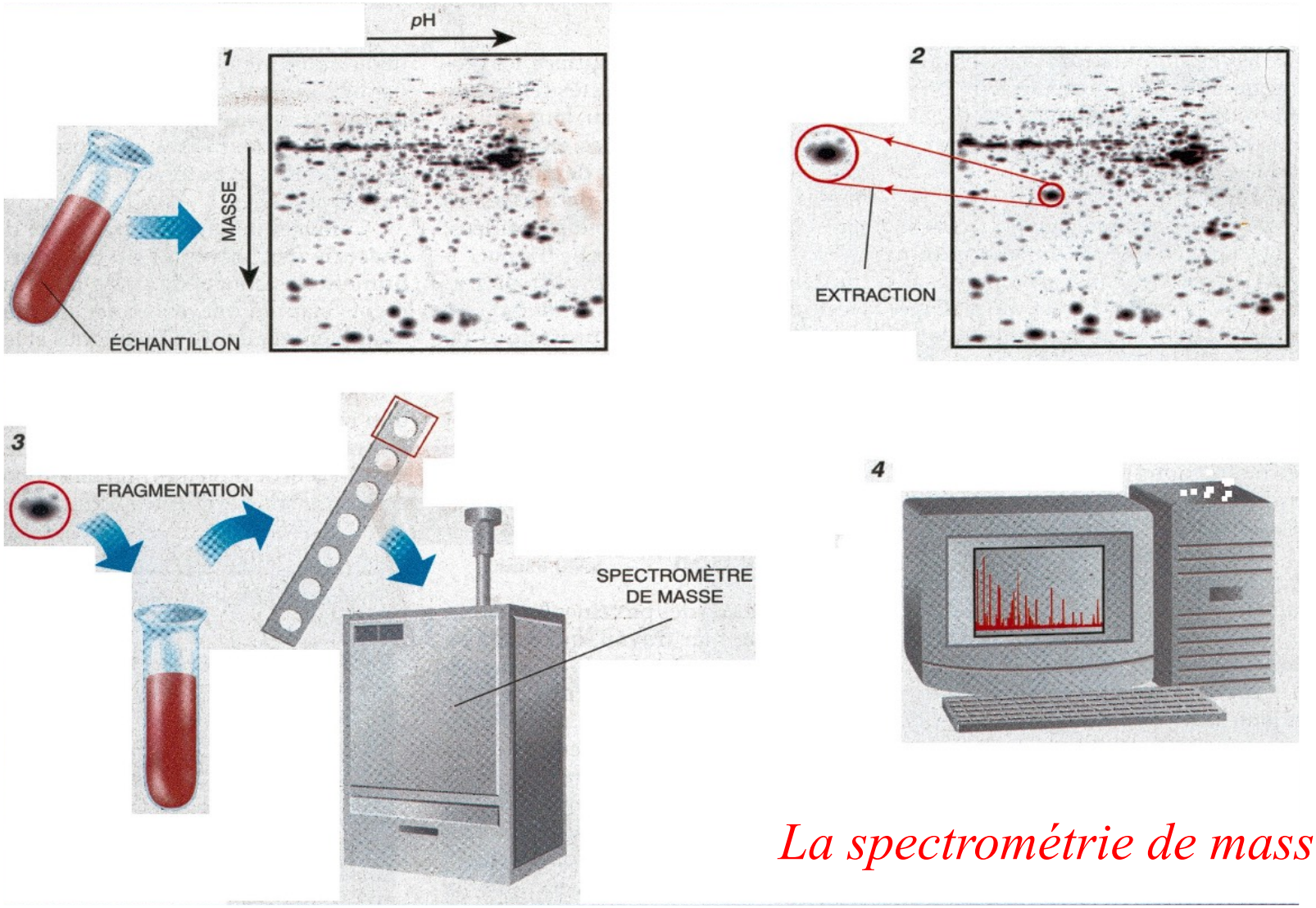
5. Les analyses protéiques

- *Principe* : **Son principe réside dans la séparation en phase gazeuse de molécules chargées (ions) en fonction de leur rapport masse/charge (*m/z*). La spectrométrie de masse est utilisée dans pratiquement tous les domaines scientifiques : physique, astrophysique, chimie en phase gazeuse, chimie organique, dosages, biologie, médecine...**

  - **Substance analysée en phase gazeuse (diff RMN subst condensée)**
    - **Importance des paramètres de la phase de vaporisation de l'échantillon**
  - **Mesure du déplacement de particules chargées dans un champs électromagnétique**
    - **Importance des paramètres définissants l'ionisation de l'échantillon**
  - **Obtention d'un spectre de masse qui montre la variation d'un courant ionique en fonction de m/z**
  - **Attention destruction de l'échantillon analysé !**

5. Les analyses protéiques



La spectrométrie de masse

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5. Les analyses protéiques

La spectrométrie de masse

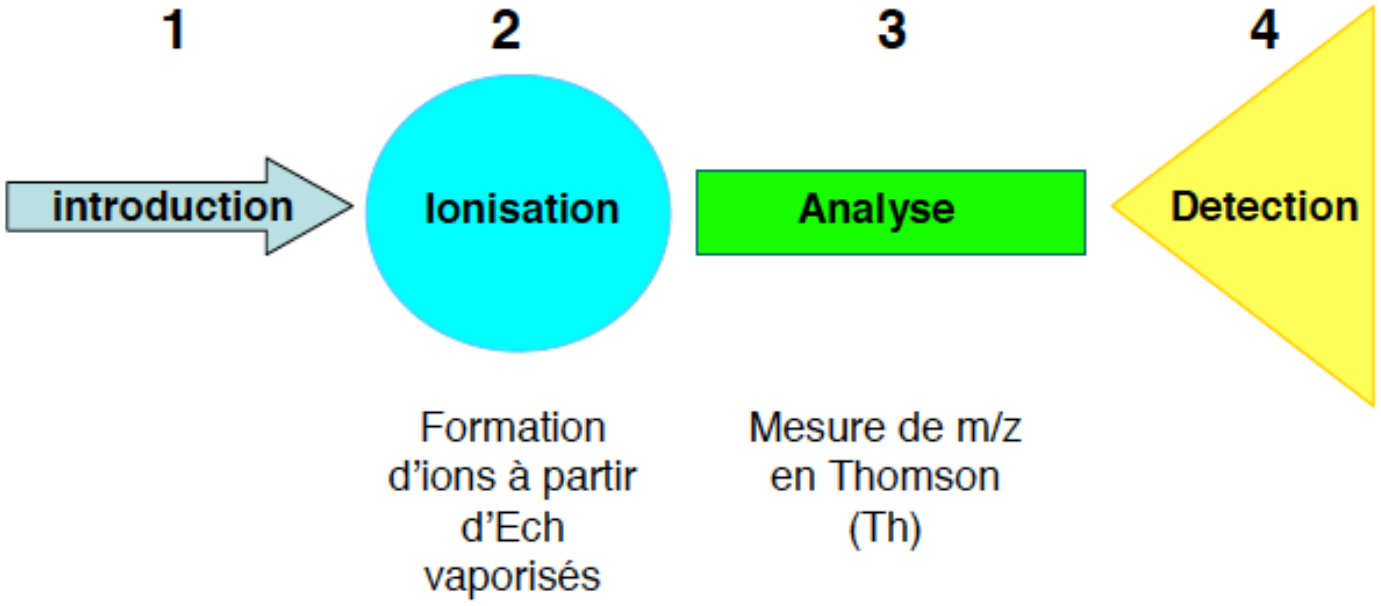
Le spectromètre de masse se compose donc de quatre parties :

1- Le système d'introduction de l'échantillon

2- La source d'ionisation: elle consiste à vaporiser les molécules et à les ioniser.

3- L'analyseur

4- Le détecteur et système de traitement



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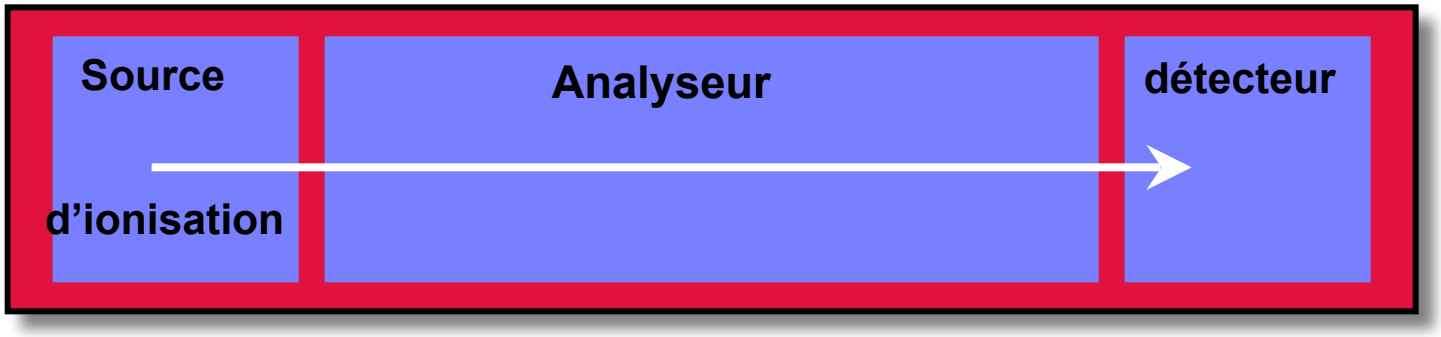
5. Les analyses protéiques

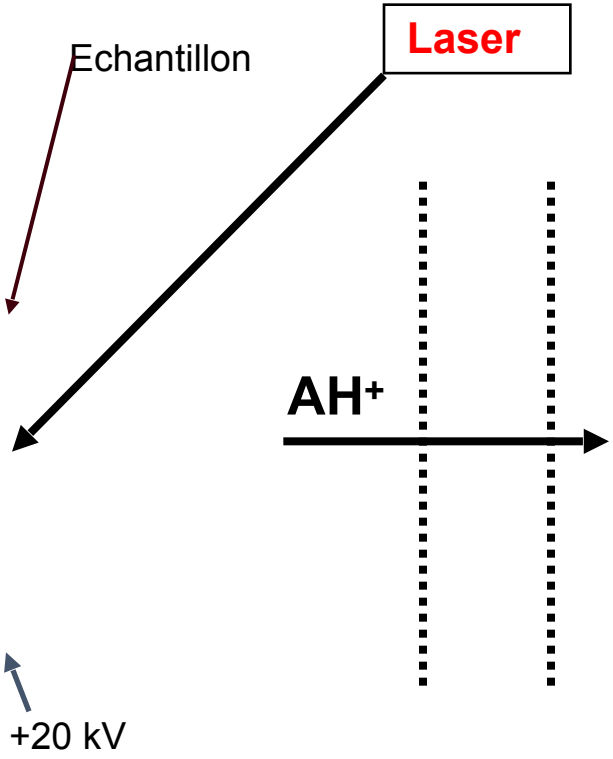
La spectrométrie de masse

1/2



Source d'ionisation :

Les molécules doivent passer en phase gazeuse et s'ioniser



$$MH^+ + A \rightarrow M + AH^+$$

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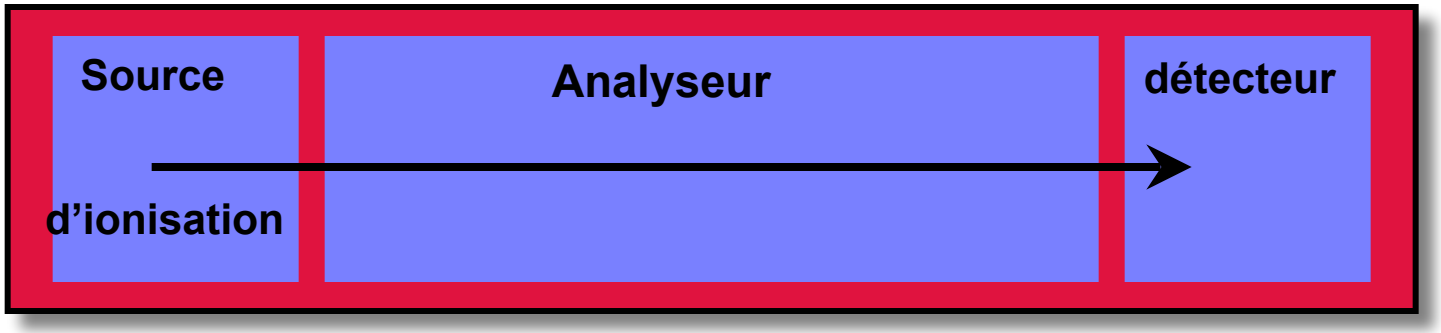
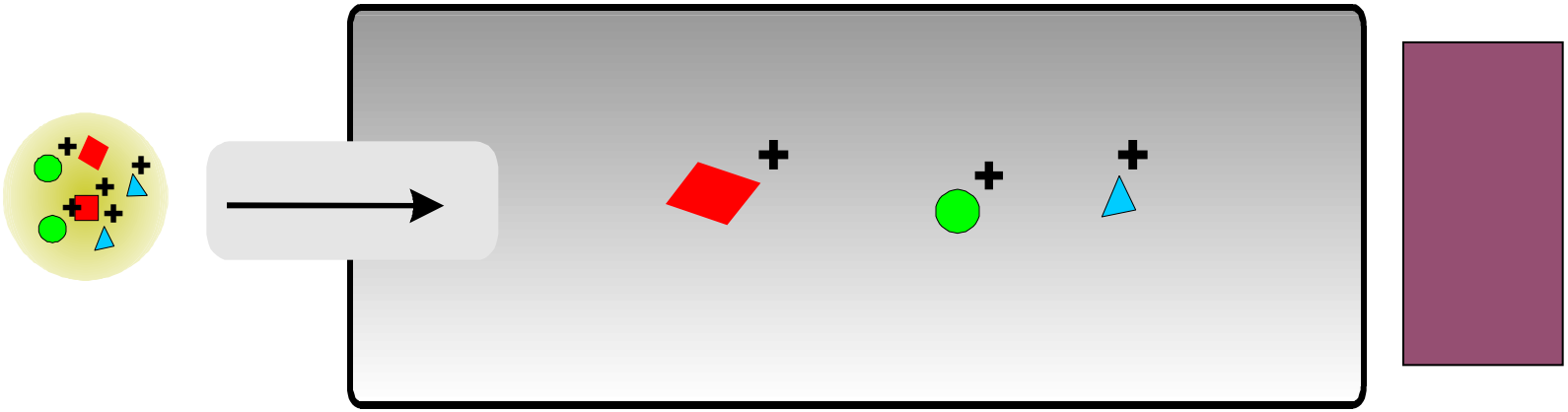
3 La spectrométrie de masse

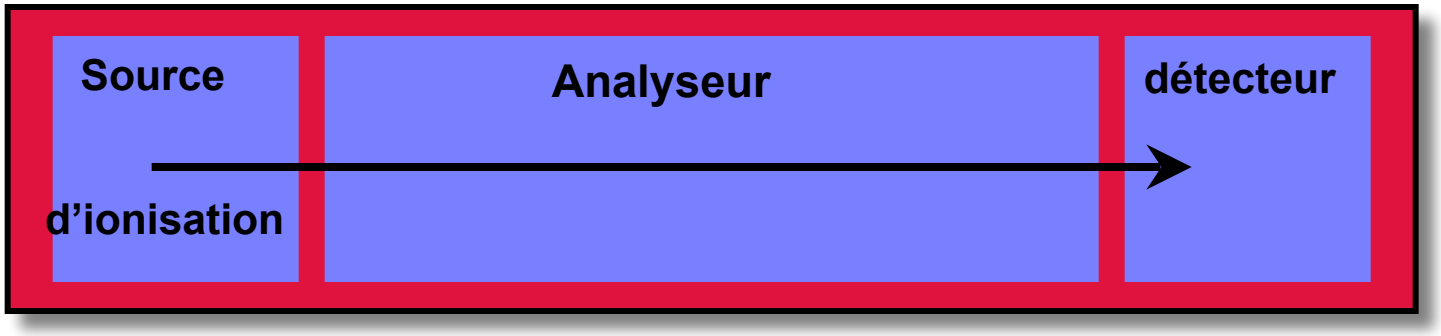


L'analyseur de masse

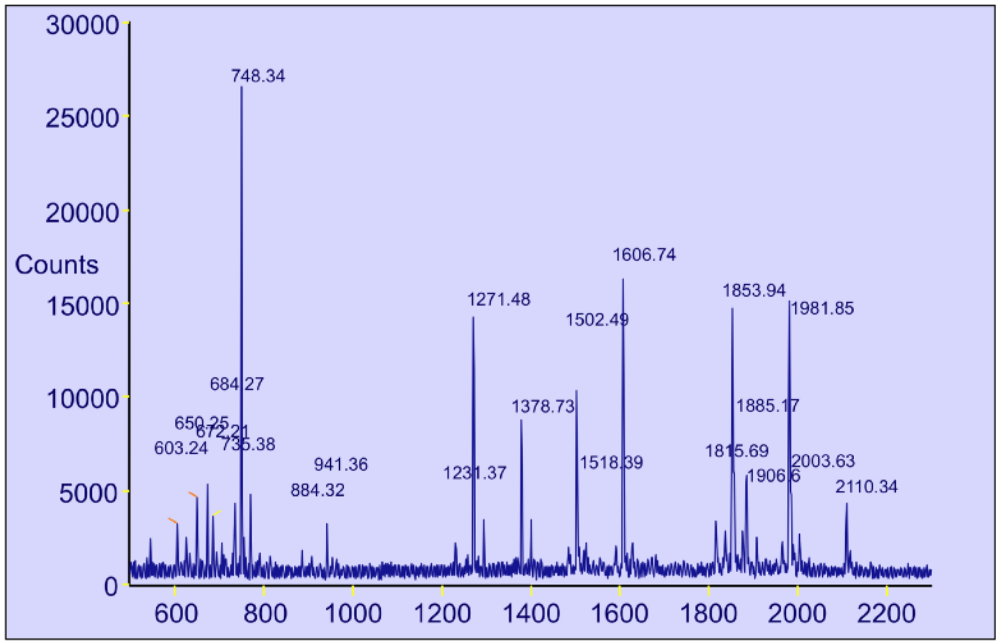
Détecteur



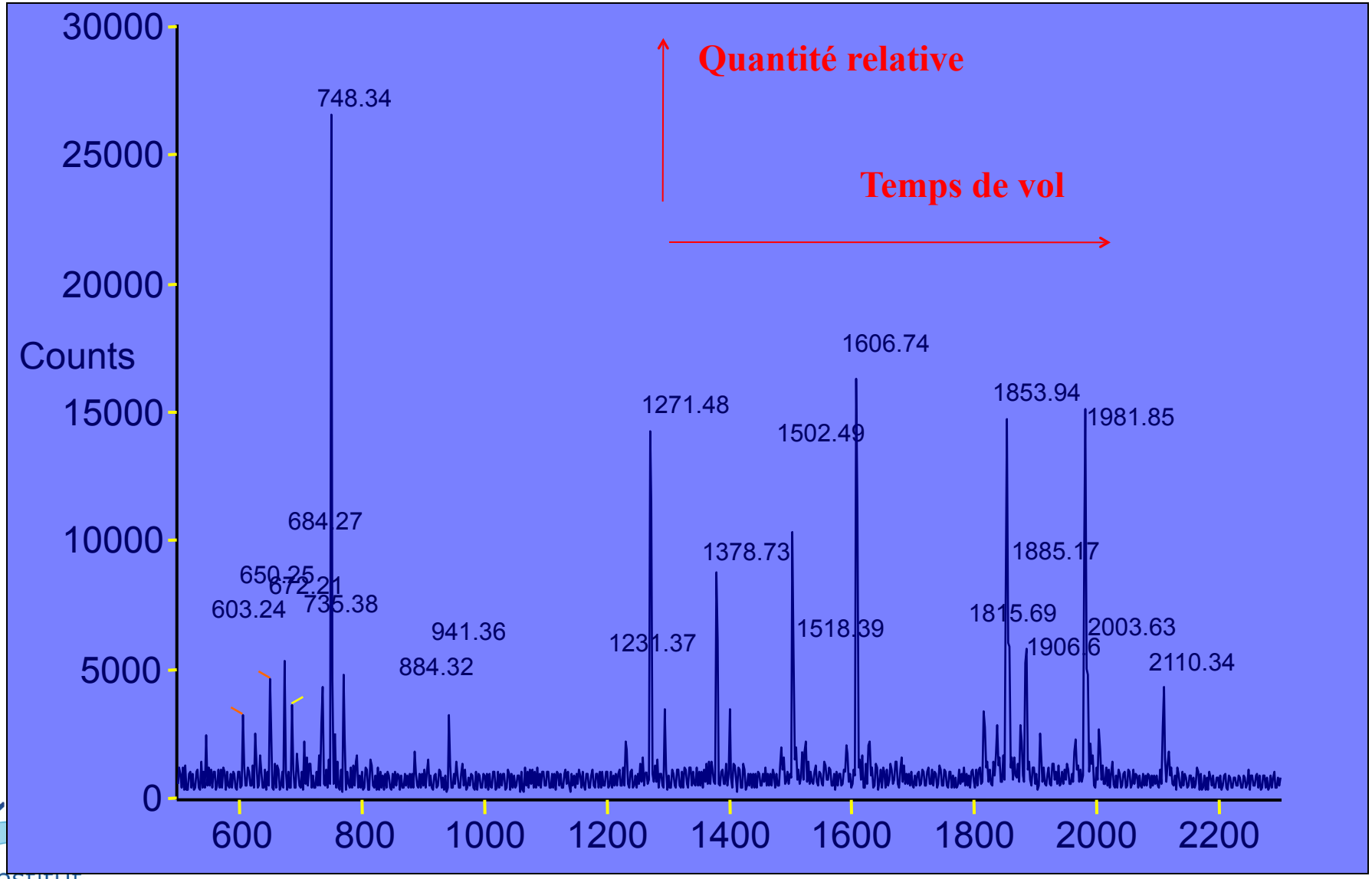
4 La spectrométrie de masse



Détecteur



5. Les analyses protéiques



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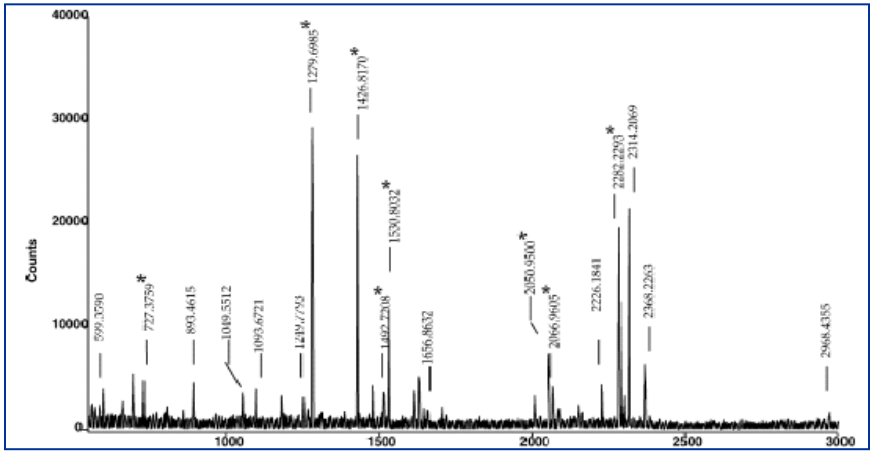
5. Les analyses protéiques

Cartographie massique par SM



4148.1061
3221.5113
3392.5756
2993.3522
2468.2415
2325.0207
2144.9770
1948.9585
2005.9800
1906.8817
1883.0497
1721.7765
1618.7577
1732.8006
1508.7889
1288.7259
1134.6742
1008.5360
1008.5295
1065.5509
950.4499
1007.4714
880.4159
832.3658
792.3709

La carte massique obtenue est **l'empreinte massique** de la protéine. Elle est très spécifique.

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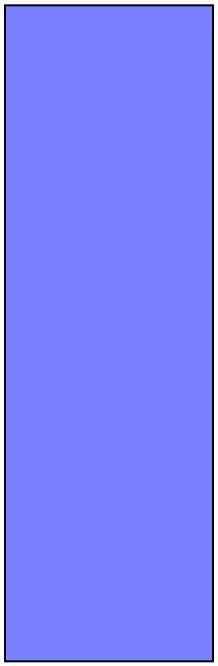
5. Les analyses protéiques

Liste de masses expérimentale



Interrogation des banques de données

Comparaison avec tous les profils théoriques des séquences de protéines connues présentes dans les banques

Sélection de la protéine la plus probable.

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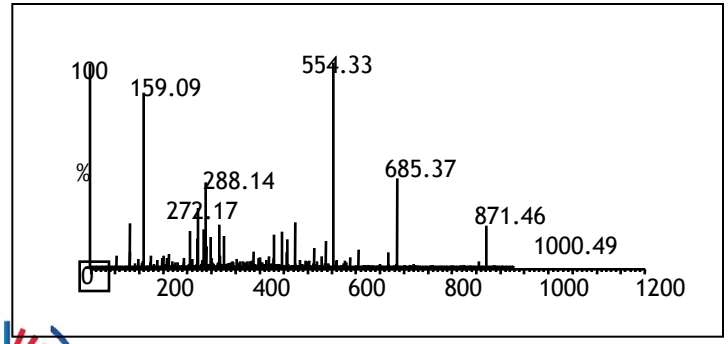
5. Les analyses protéiques

Données expérimentales

Masse du peptide trypsique (m)

+

Spectre MS/MS



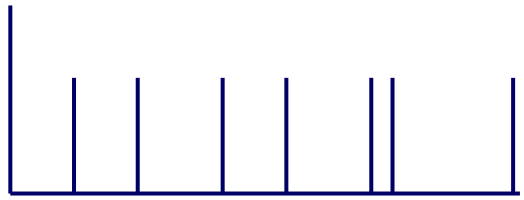
MASCOT

Données théoriques

Digestion trypsique *in silico* des banques protéiques

Sélection des n protéines pour lesquelles on attend un peptide trypsique de masse m

n spectres MS/MS théoriques



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6. Exemple publication utilisant la bioinfo

OPEN ACCESS Freely available online

PLOS ONE

A Rapid Screening Assay to Search for Phosphorylated Proteins in Tissue Extracts

Un papier de 2012 mais qui est représentatif du cours…

Ignazio Garaguso, Juergen Borlak*

Centre for Pharmacology and Toxicology, Hannover Medical School, Hannover, Germany

Abstract

Reversible protein phosphorylation is an essential mechanism in the regulation of diverse biological processes, nonetheless is frequently altered in disease. As most phosphoproteome studies are based on optimized *in-vitro* cell culture studies new methods are in need to improve *de novo* identification and characterization of phosphoproteins in extracts from tissues. Here, we describe a rapid and reliable method for the detection of phosphoproteins in tissue extract based on an experimental strategy that employs 1D and 2D SDS PAGE, Western immunoblotting of phosphoproteins, in-gel protease digestion and enrichment of phosphorpeptides using metal oxide affinity chromatography (MOAC). Subsequently, phosphoproteins are identified by MALDI-TOF-MS/MS with the CHCA-TL or DHB ML sample matrix preparation method and further characterized by various bioinformatic software tools to search for candidate kinases and phosphorylation-dependent binding motifs. The method was applied to mouse lung tissue extracts and resulted in an identification of 160 unique phosphoproteins. Notably, TiO_2 enrichment of pulmonary protein extracts resulted in an identification of additional 17 phosphoproteins and 20 phosphorylation sites. By use of MOAC, new phosphorylation sites were identified as evidenced for the advanced glycosylation end product-specific receptor. So far this protein was unknown to be phosphorylated in lung tissue of mice. Overall the developed methodology allowed efficient and rapid screening of phosphorylated proteins and can be employed as a general experimental strategy for an identification of phosphoproteins in tissue extracts.

Citation: Garaguso I, Borlak J (2012) A Rapid Screening Assay to Search for Phosphorylated Proteins in Tissue Extracts. PLoS ONE 7(11): e50025. doi:10.1371/journal.pone.0050025

Editor: Christina Lynn Addison, Ottawa Hospital Research Institute, Canada

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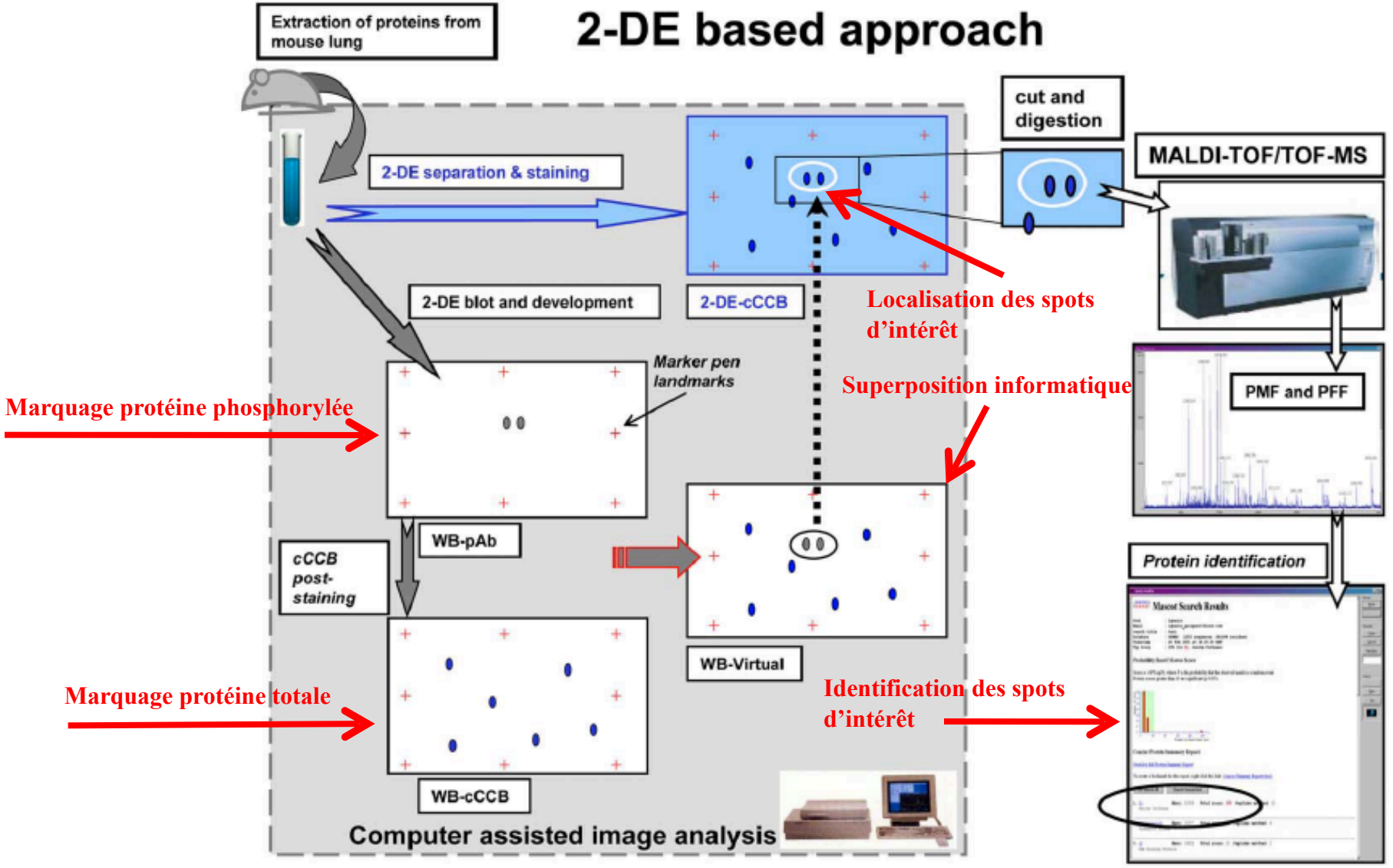

Figure 1. Description of the combined 2-DE-WB approach. 200 µg of tissue lysate protein extracts were separated on 2-DE, stained with Colloidal Coomassie G-250 and acquired as an image (2-DE-cCBB). In parallel 40 µg of total protein extract was separated by 2-DE and subsequently transferred onto PVDF membrane for incubation with antibodies directed against phosphorylated proteins. With a marker pen, landmarks points (crosses in Figure) were set around the membrane and the image of phosphorylated proteins was acquired (WB-pAb). Subsequently, the total proteins on the same membrane were revealed by cCCB-post staining and the image was recorded (WB-CBB). Using the marker added landmarks the two images were superimposed and combined by the image analysis software to create a virtual image showing the phosphoproteins and the total proteins together (WB-virtual). Several protein spots from this image, which are in common with the cCBB-2-DE image, were selected as additional landmarks and used to superimpose the WB-CCB image to the 2-DE-cCBB image. This allowed deciphering of phosphorylated proteins on the gel. Highlighted spots were then excised from the gel using a spot cutter, followed by in-gel digestion using trypsin. The proteins were identified using MALDI-TOF MS.

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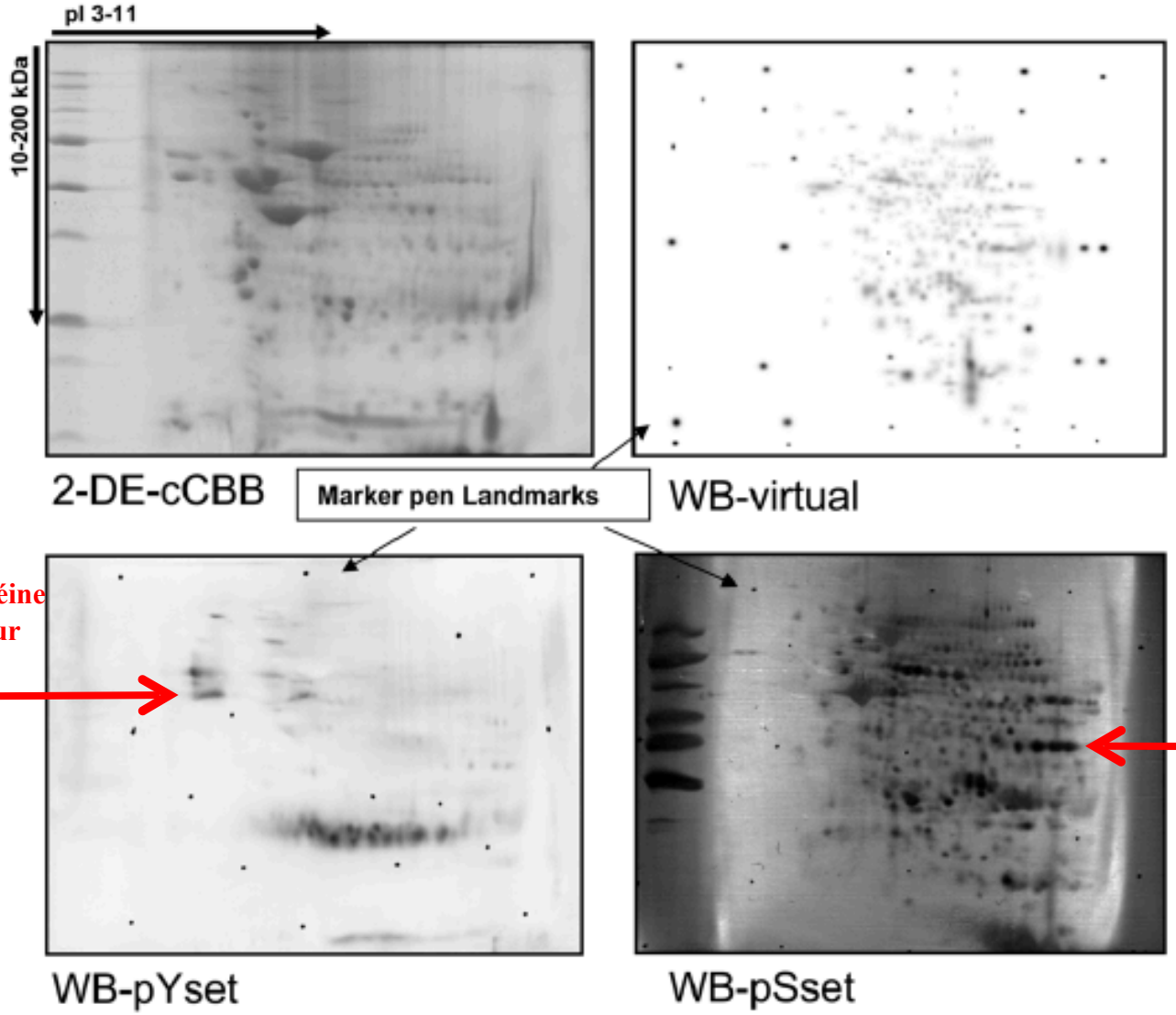

Marquage protéine phosphorylée sur tyrosine

Marquage protéine Phosphorylée sur sérine

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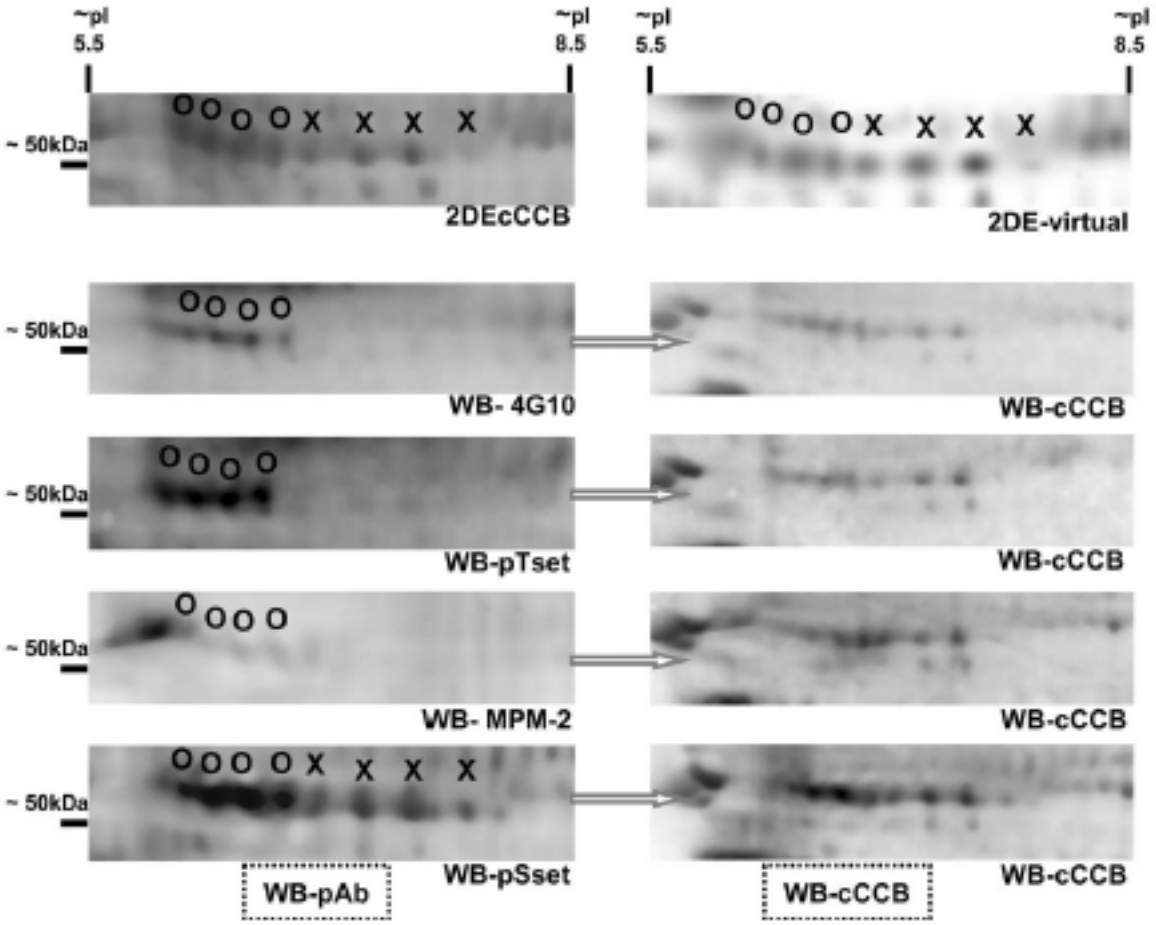

Une phosphorylation modifie une immunoréactivité: notion de comètes:

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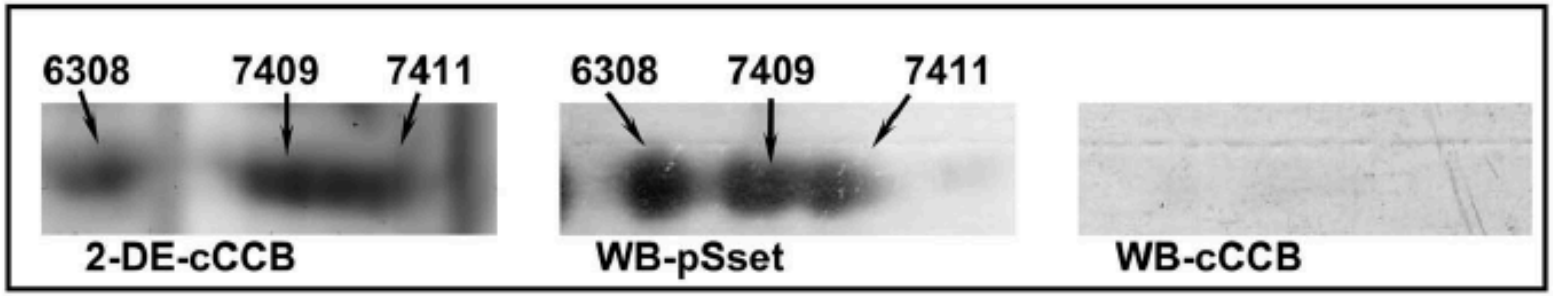

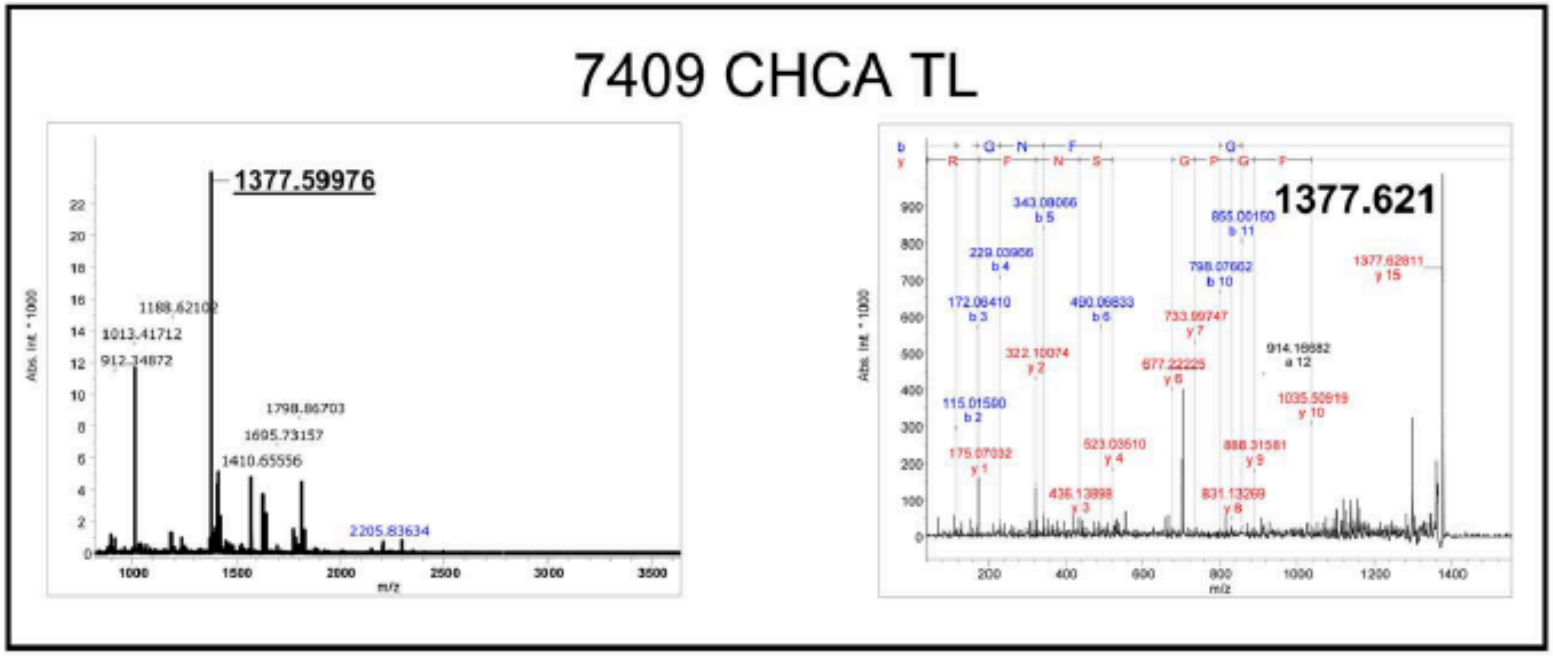

Analyse jusqu'à l'identification

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Table 1. Identified phosphopeptide by MALDI tandem MS using DHB ML sample preparation.

Protein Entry Name	Accession	Sequence	Start end	Meas. mass	Ion Score	Miss	MW
Angiotensin-converting enzyme	P09470	R.GPQFG**S**EVELR.H	1300–1310	1298.57765	63	0	151.888
Calnexin precursor	P35564	K.AEEDEILNR**S**PR.N	573–584	1508.67402	39	1	67.733
Advanced glycosylation end product-specific receptor	Q62151	R.KAPE**S**QEDEEER.A	373–384	1526.60058	60	1	43.068
Nascent polypeptide-associated complex alpha subunit	P70670	R.**S**V**T**DPA**M**APRTAK.N	588–600	1600.58786	20	1	221.603
Matrix-remodeling-associated protein 7	Q9CZH7	R.VAEPEE**S**EAEEPAAEGR.Q	73–89	1879.75921	82	0	19.516
Tensin	gi|226437589	R.**S**QSFPDVEPQLPQAPTR.G	790–806	1976.91134	56	0	203.317
Arginase-1	Q61176	-.**MSS**KPK**S**LEIIGAPFSK.G	1–17	2075.89248	35	0	34.999
60S acidic ribosomal protein	P99027	K.KEE**S**EE**S**DDD**M**GFGLFD.-	99–115	2125.68687	40	1	11.651
60S acidic ribosomal protein P1	P47955	K.KEE**S**EE**S**EDD**M**GFGLFD.-	98–114	2139.70251	40	1	11.610
Myosin phosphatase Rho-interacting protein	P97434	R.AEEQLPPLLSPP**S**PSTPHSR.R	280–299	2220.06959	27	0	117.357
Alpha-1 catenin	P26231	R.TPEELDD**S**DFETEDFDVR.S	634–651	2238.85983	127	0	100.896
Membrane-associated progesterone receptor component	O55022	K.EGEEPTVY**S**DDEEPKDETAR.K	173–192	2375.93983	112	1	21.692
60S acidic ribosomal protein P0	P14869	K.AEAKEE**S**EE**S**DED**M**GFGLFD.-	298–317	2410.81924	45	1	34.408
Septin-2	P42208	K.IYHLPDAE**S**DEDEDFKEQTR.L	210–229	2517.04527	115	0	41.783
EH domain-containing protein	Q8BH64	R.GPDEAIEDGEEG**S**EDDAEWVVTK.D	426–448	2557.01375	112	1	50.135
Elongation factor 1-delta	P57776	K.GATPAEDDEDKDIDLFG**S**DEEEEDKEAARL	145–173	3276.32225	144	2	31.916
Serum deprivation-response protein	Q63918	R.GNNSAVG**S**NADLTIEEDEEEEPVALQQAQ QVR.Y	356–387	3520.57109	135	0	46.806
		R.RGNNSAVG**S**NADLTIEEDEEEEPVALQQAQ QVR.Y	355–387	3676.67219	105	1	
		K.SSPFKV**S**PLSFGR.K	287–299	1488.72465	53	1	

Phosphopeptides identified by MALDI-TOF-MS/MS from 50 μg of protein extracted from mouse lung and separated by SDS-PAGE.
S=phosphorylated serine determined by MALDI TOF-MS/MS. M=oxidized methionine.
doi:10.1371/journal.pone.0050025.t001

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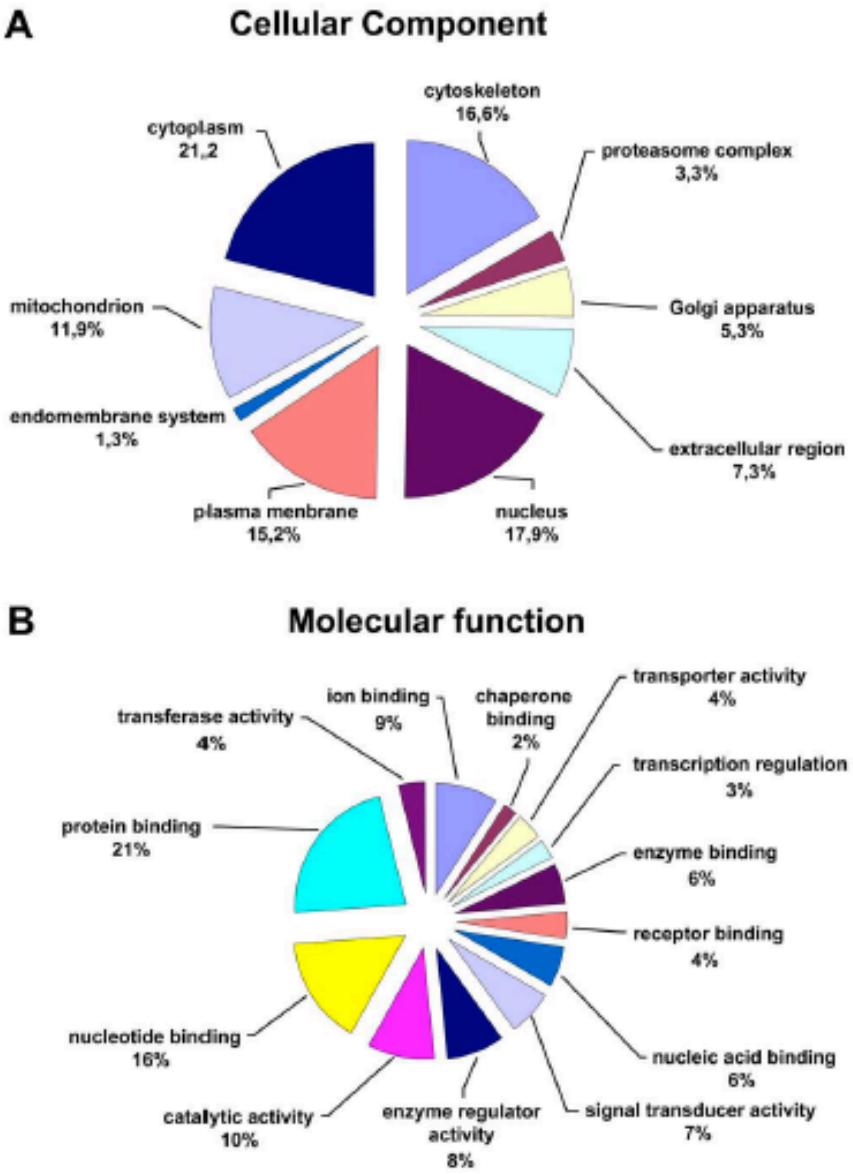

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MERCI à tous!

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L'unité de recherche de l'institut du thorax
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