Apport du séquençage long reads pour l'amélioration de la compatibilité HLA donneur/receveur en transplantation rénale

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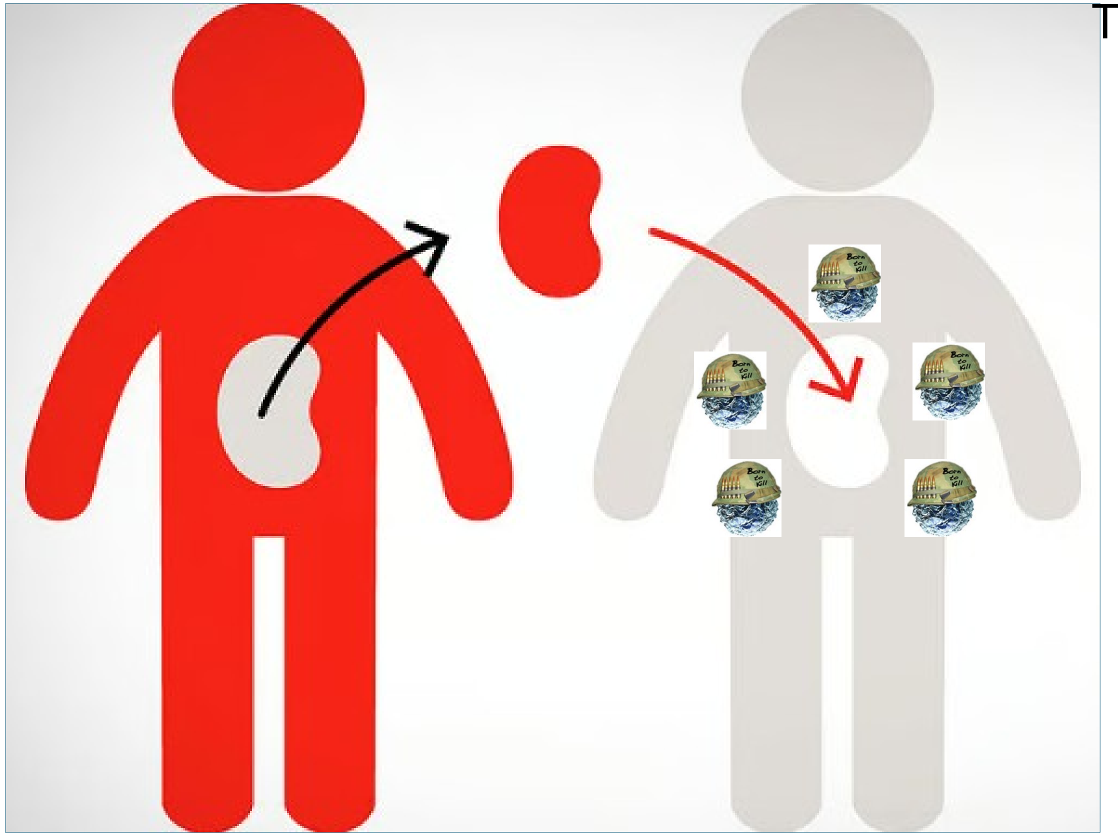
Séminaire BioInfoDiag – Montpellier – 01/04/2025

Barrières immunologiques en transplantation rénale

Système HLA (= CMH)



Transplantation d’un organe chez un individu non apparenté

→ Reconnaissance par le système immunitaire du receveur d’un « corps étranger »

→ Mise en route de mécanismes immunologiqu (alloréactivité)

→ Destruction du greffon (rejet)

Barrières immunologiques dans la transplantation: Complexe Majeur d’Histocompatibilité (CMH)
= système HLA (Human Leukocyte Antigen)

Expression des molecules du système HLA

ubiquitaire

Fonction:

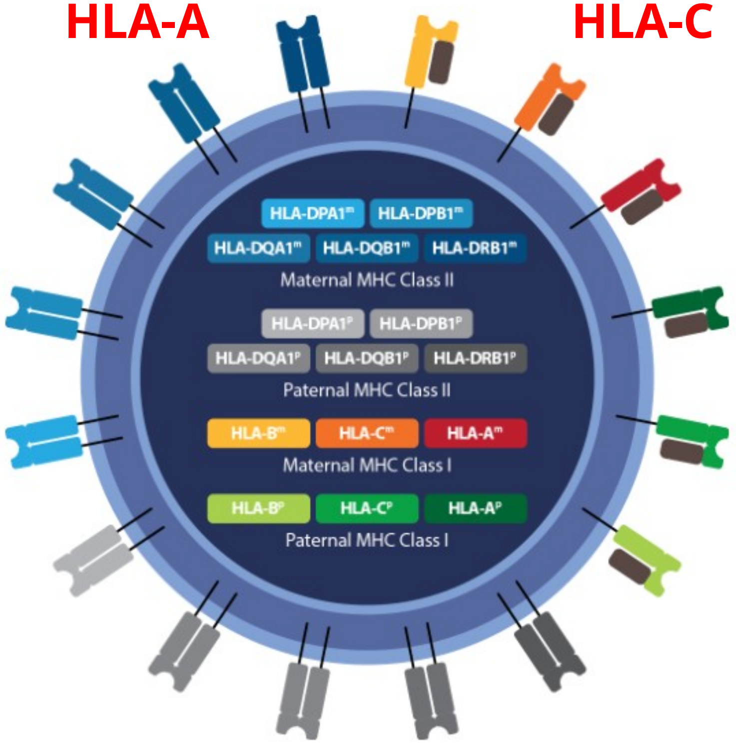
Présentation antigénique

Molécules HLA de classe I

HLA-A

HLA-B

HLA-C

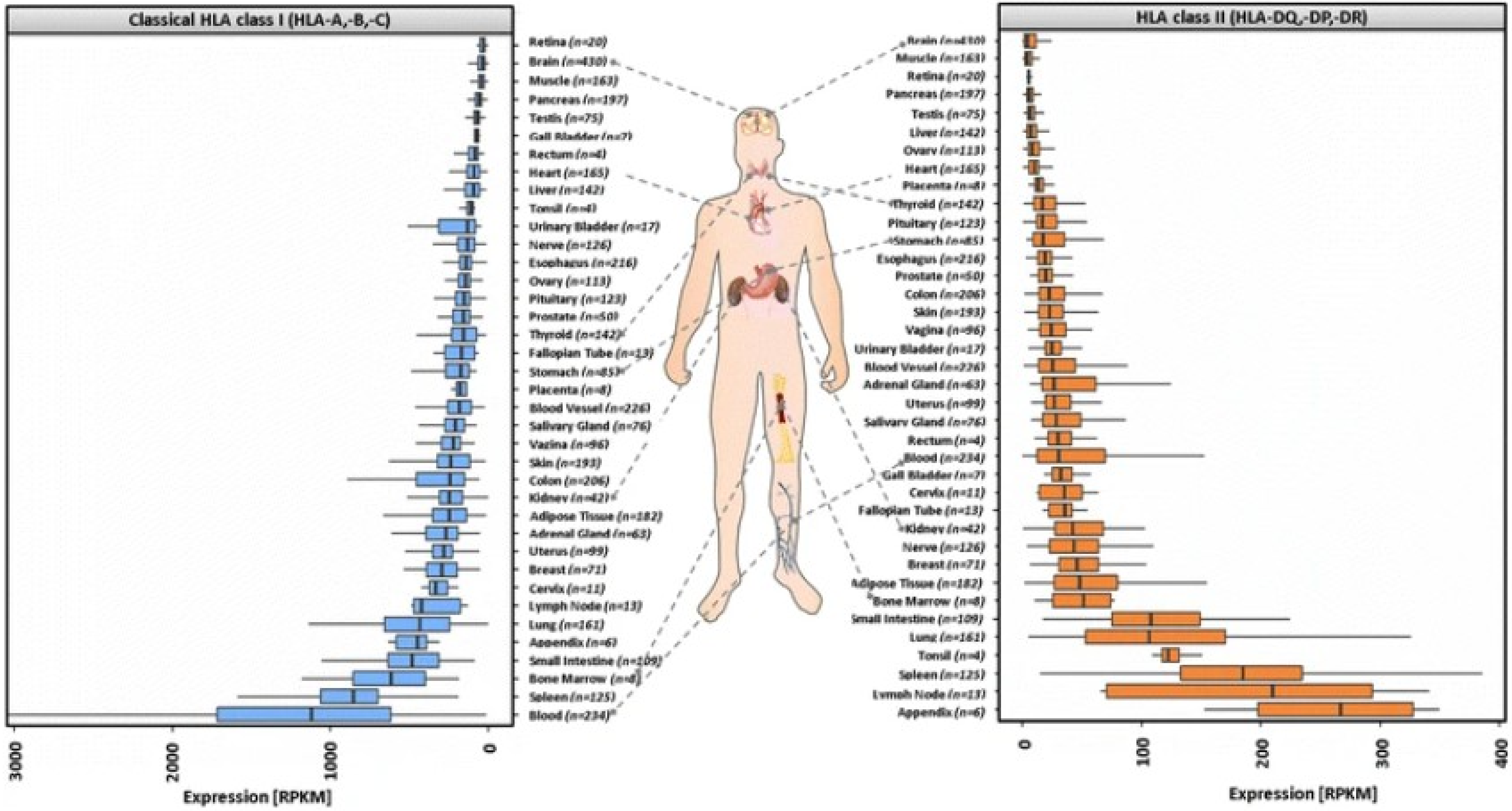


HLA-DR

HLA-DQ

HLA-DP

Molécules HLA de classe II

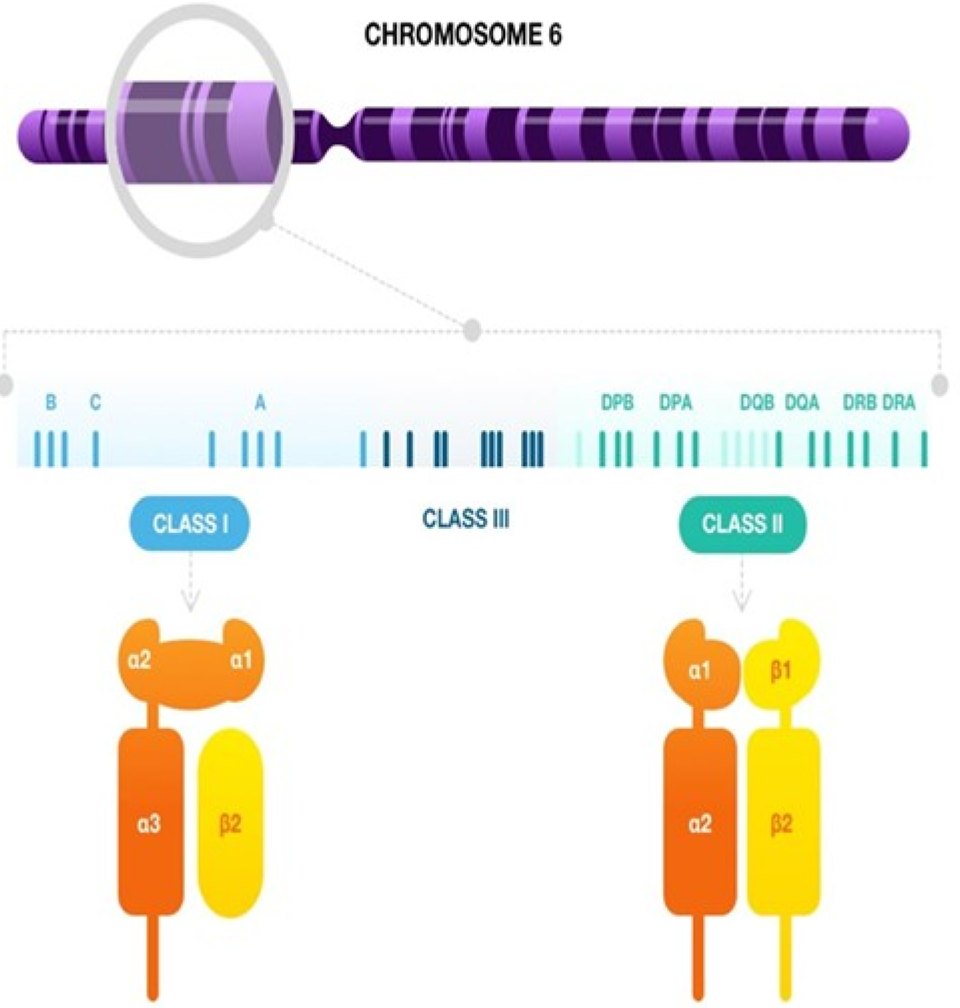


Boegel, S., Löwer, M., Bukur, T. *et al.* HLA and proteasome expression body map. *BMC Med Genomics* **11**, 36 (2018). https://doi.org/10.1186/s12920-018-0354-x

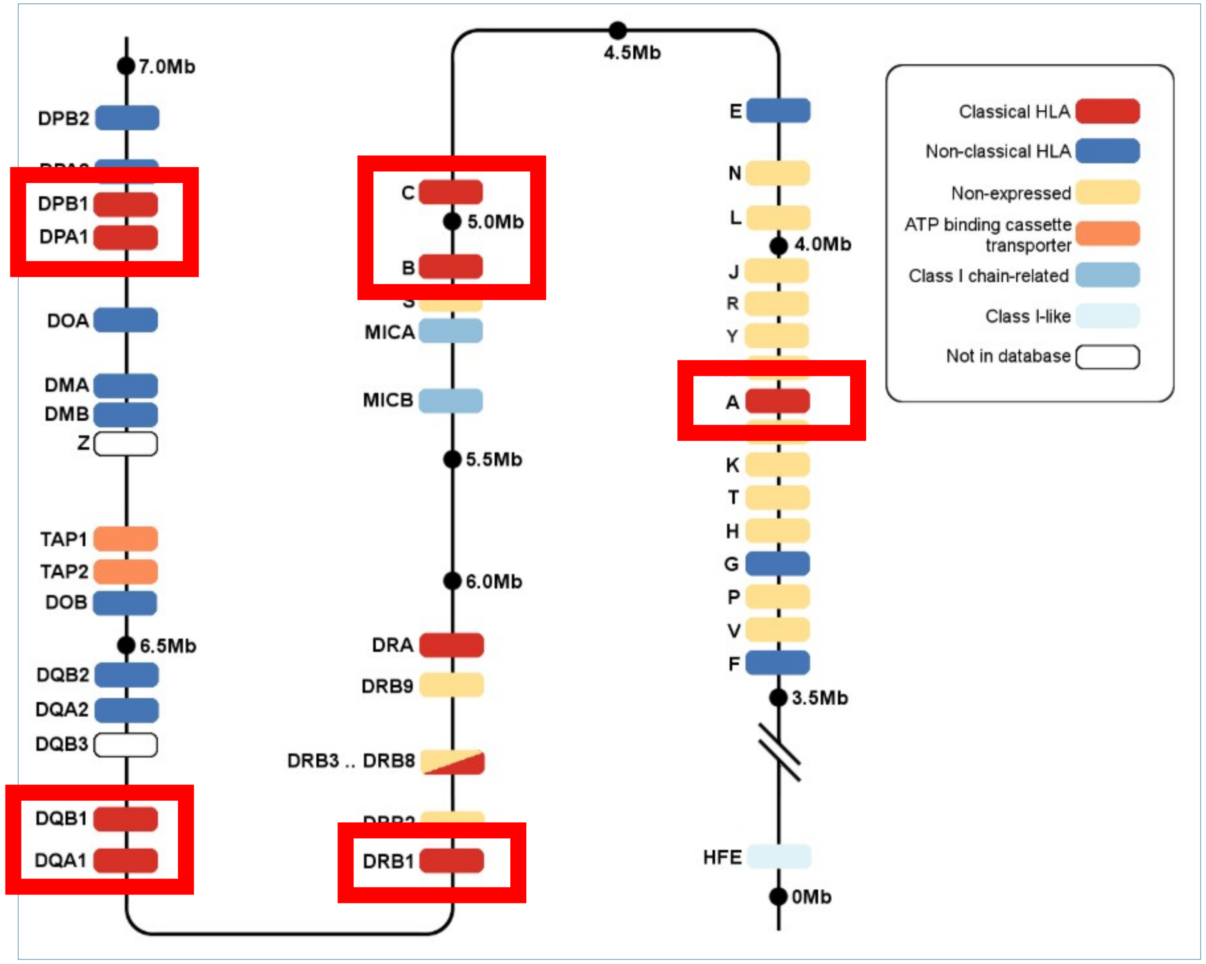
Génétique du système HLA

2 haplotypes parentaux
Expression codominante

Gènes d'intérêt en transplantation:
HLA-A, -B, -C, -DRB1, -DQA1, -DQB1, -DPA1, -DPB1

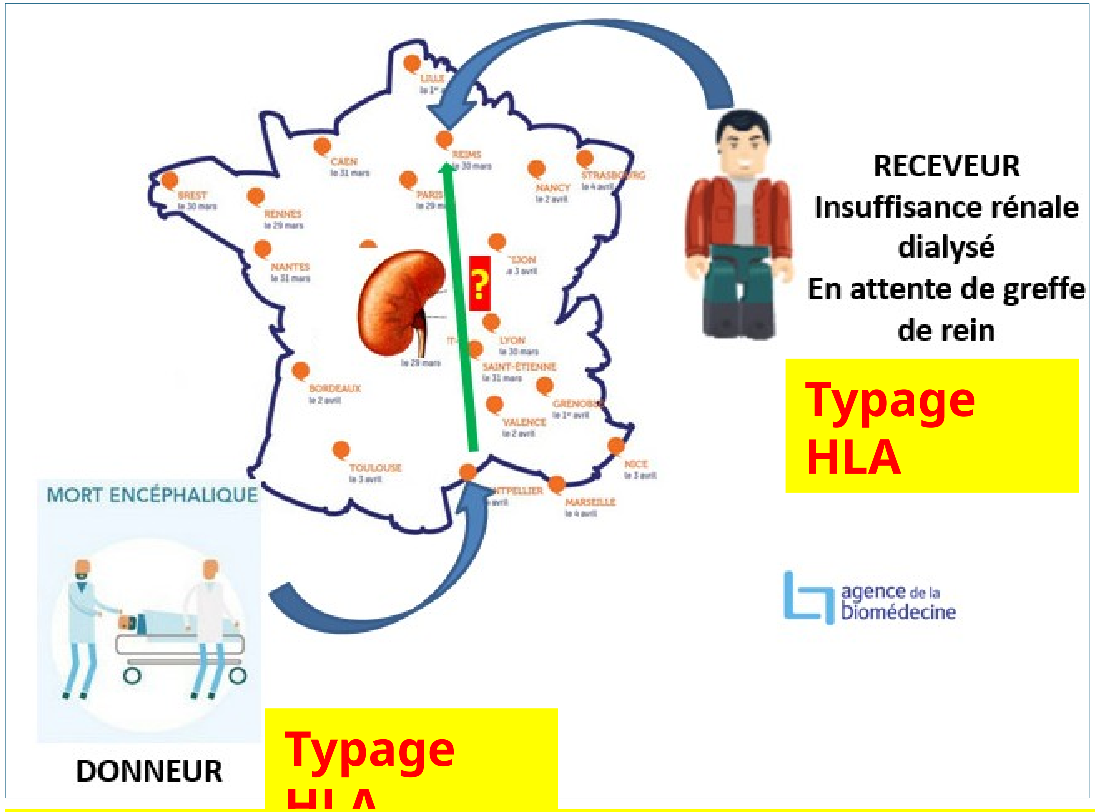


Muñiz-Castrillo *et al. Autoimmun Highlights* (2020) 11:2



Importance de la compatibilité donneur/receveur

Typage HLA

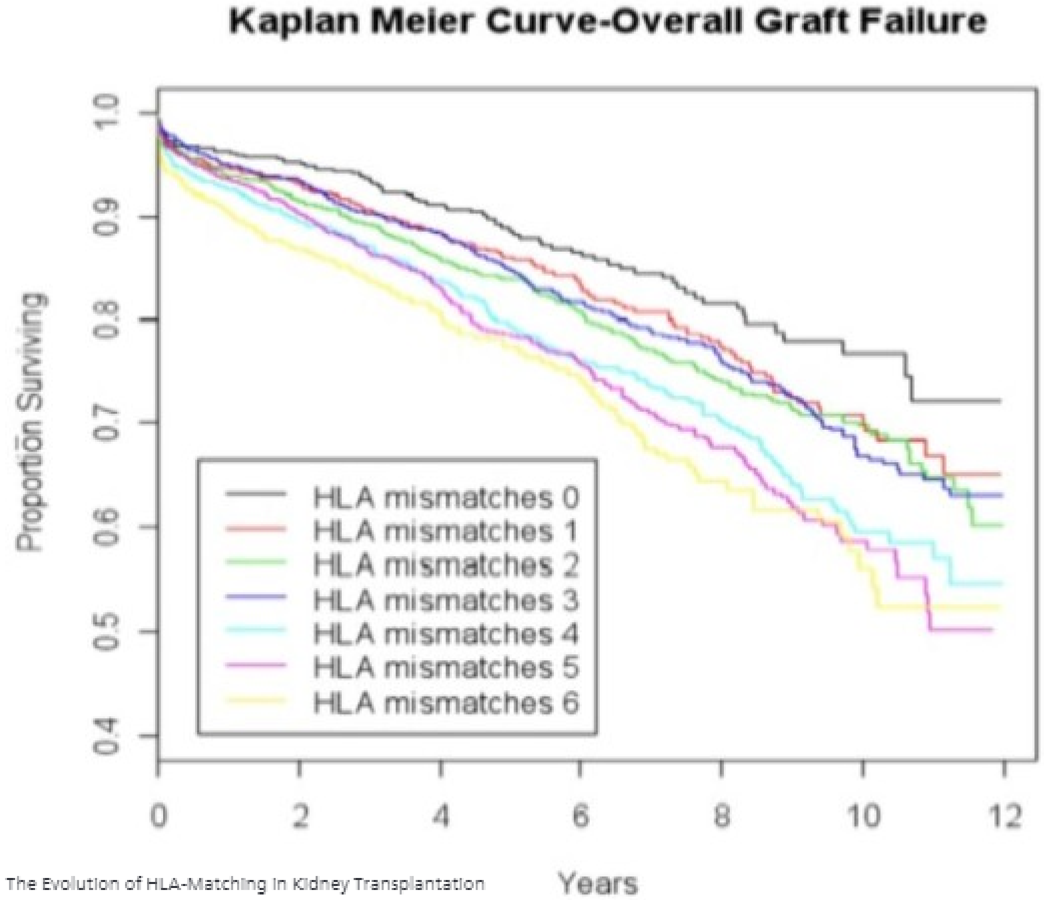


RECEVEUR
Insuffisance rénale dialysé
En attente de greffe de rein

Typage HLA

DONNEUR

Typage HLA

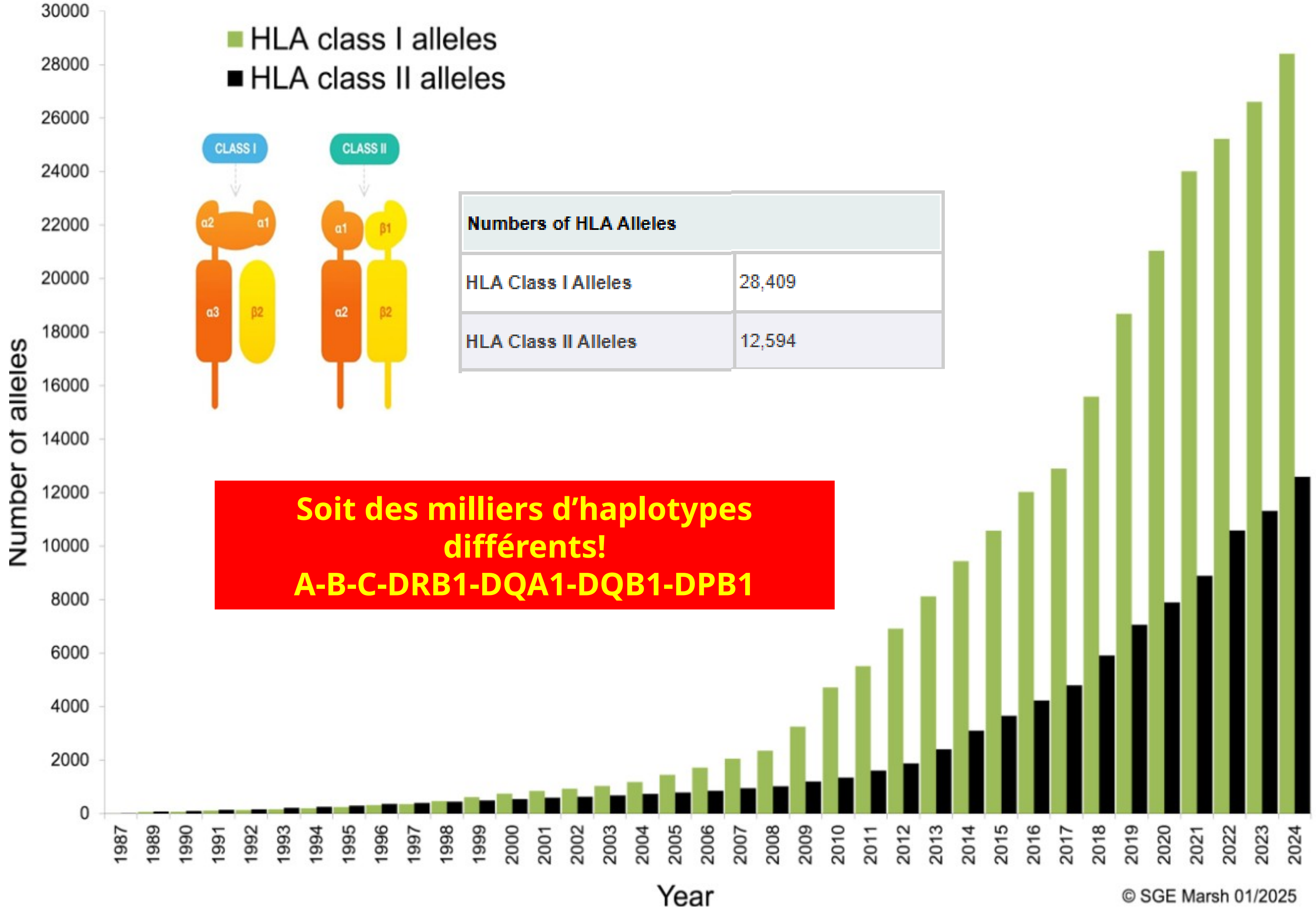


The Evolution of HLA-Matching in Kidney Transplantation
http://dx.doi.org/10.5772/54747

Plus la divergence HLA entre donneur et receveur est importante, plus le risque d'émergence d'anticorps dirigés contre les molécules HLA du greffon est élevé
→ REJET

Le Complexe Majeur d'Histocompatibilité est la region la plus polymorphe du génome

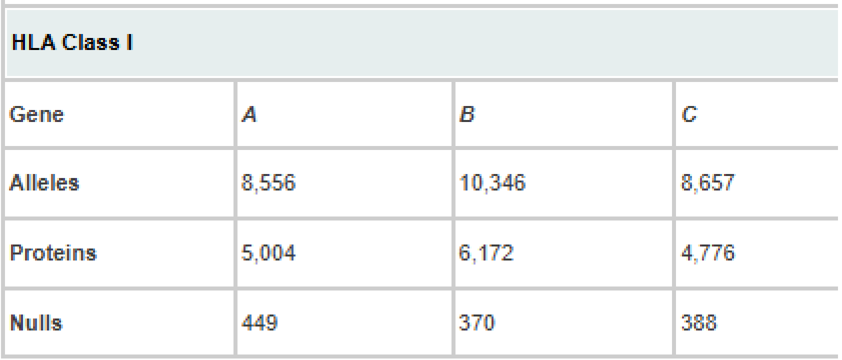
En Janvier 2025:
- 40 000 alleles décrits
- > 20 000 protéines différentes



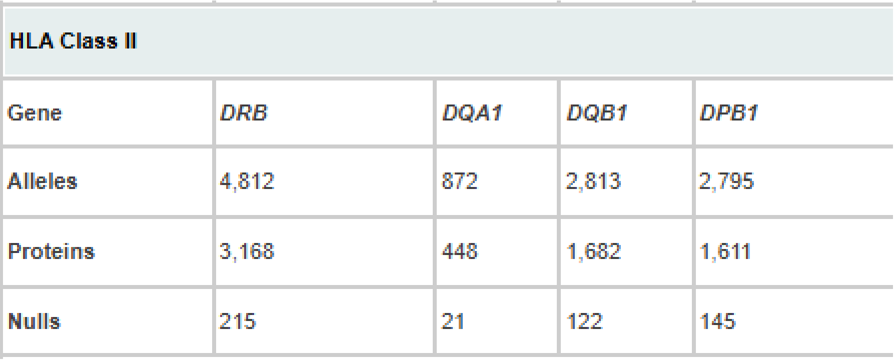
© SGE Marsh 01/2025

Numbers of HLA Alleles	
HLA Class I Alleles	28,409
HLA Class II Alleles	12,594

Soit des milliers d'haplotypes différents!
A-B-C-DRB1-DQA1-DQB1-DPB1



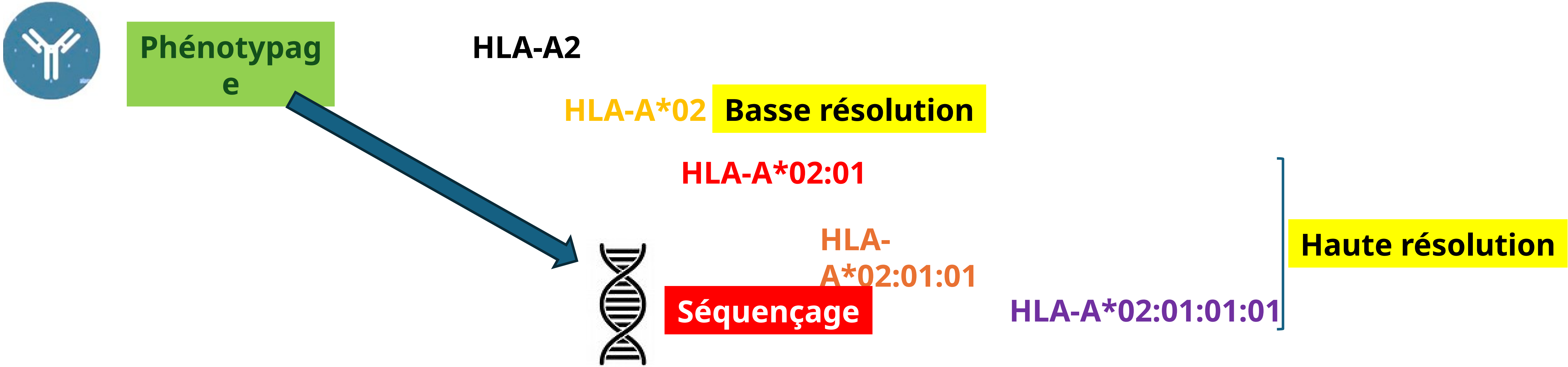
HLA Class I			
Gene	*A*	*B*	*C*
Alleles	8,556	10,346	8,657
Proteins	5,004	6,172	4,776
Nulls	449	370	388



HLA Class II				
Gene	*DRB*	*DQA1*	*DQB1*	*DPB1*
Alleles	4,812	872	2,813	2,795
Proteins	3,168	448	1,682	1,611
Nulls	215	21	122	145

Polymorphisme +++ des gènes HLA
= Obstacle important à la transplantation HLA-identique

Polymorphisme et nomenclature des gènes HLA



Phénotypage

HLA-A2

HLA-A*02 **Basse résolution**

HLA-A*02:01

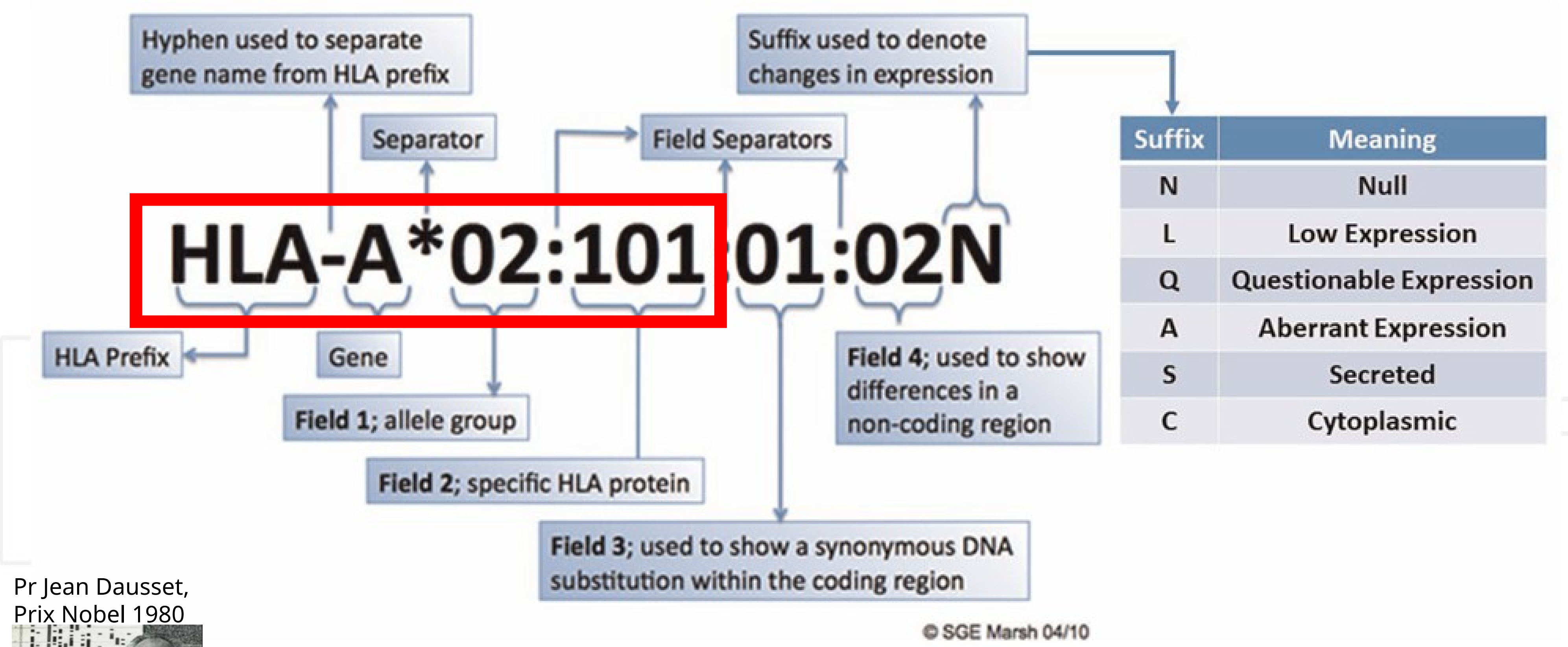
HLA-A*02:01:01

HLA-A*02:01:01:01

Haute résolution

Séquençage

Polymorphisme et nomenclature des gènes HLA

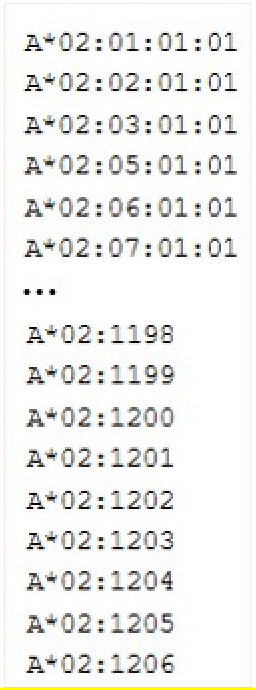


Field 3; used to show a synonymous DNA substitution within the coding region

Field 4; used to show differences in a non-coding region

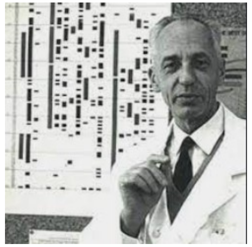
Suffix	Meaning
N	Null
L	Low Expression
Q	Questionable Expression
A	Aberrant Expression
S	Secreted
C	Cytoplasmic

© SGE Marsh 04/10



A*02:01:01:01
A*02:02:01:01
A*02:03:01:01
A*02:05:01:01
A*02:06:01:01
A*02:07:01:01
…
A*02:1198
A*02:1199
A*02:1200
A*02:1201
A*02:1202
A*02:1203
A*02:1204
A*02:1205
A*02:1206

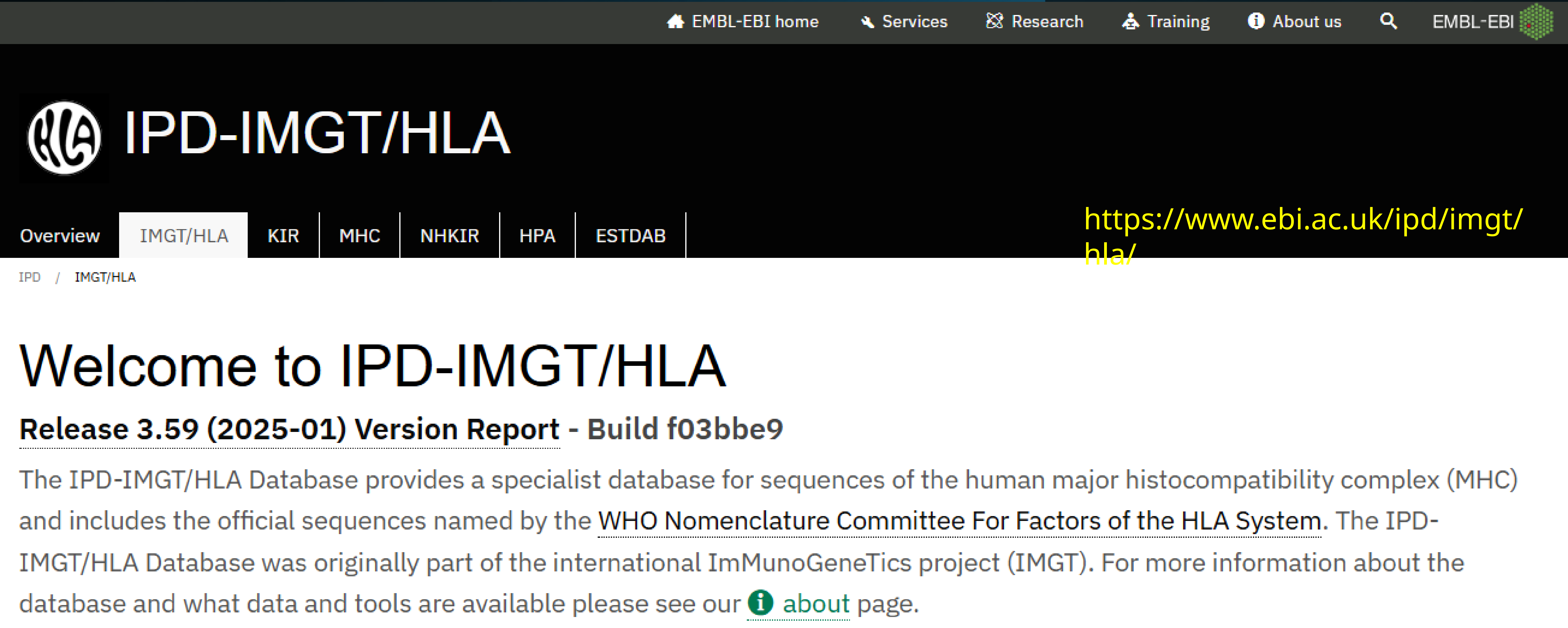
Pr Jean Dausset,
Prix Nobel 1980



Années 1950:
Découverte de la molécule HLA-A2

2025:
Il existe plus de 1200 molécules

Polymorphisme et nomenclature des gènes HLA



IPD-IMGT/HLA

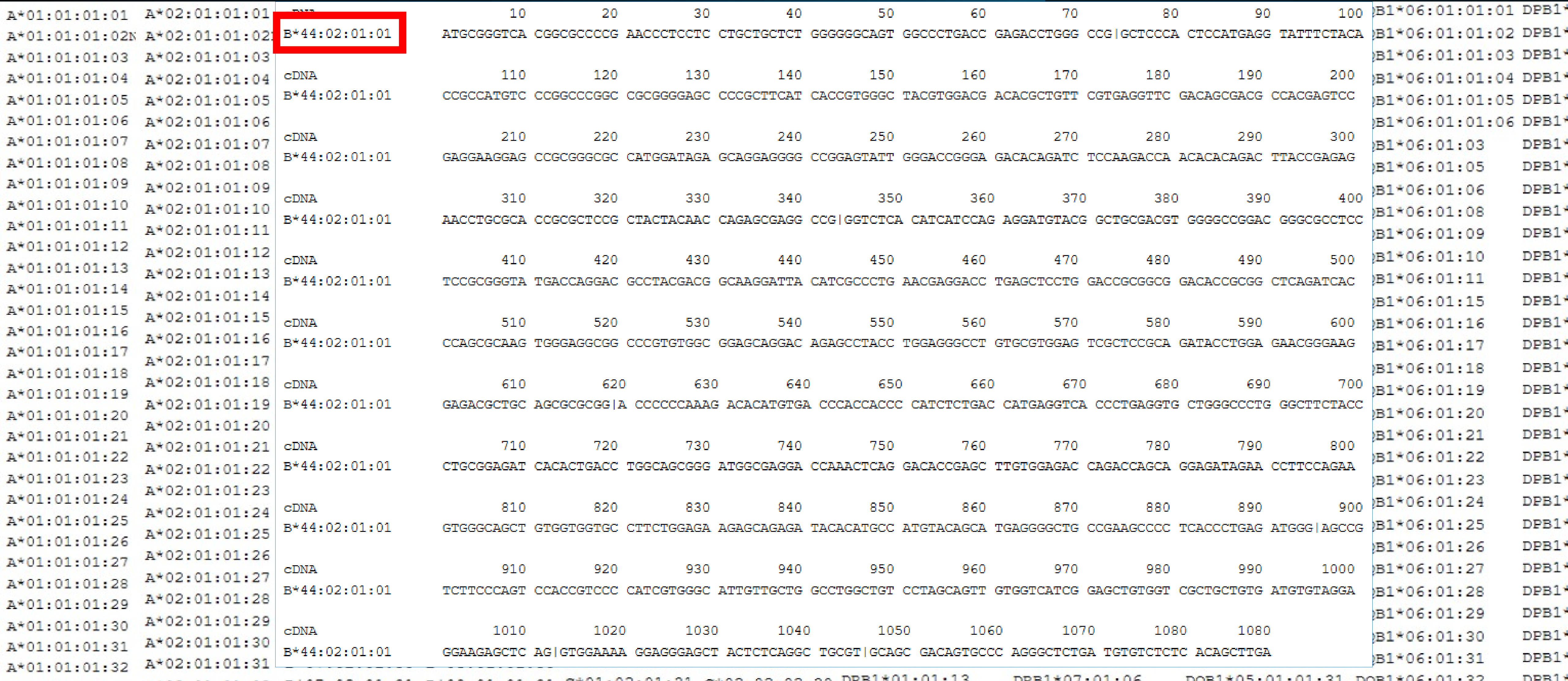
Welcome to IPD-IMGT/HLA

Release 3.59 (2025-01) Version Report - Build f03bbe9

The IPD-IMGT/HLA Database provides a specialist database for sequences of the human major histocompatibility complex (MHC) and includes the official sequences named by the WHO Nomenclature Committee For Factors of the HLA System. The IPD-IMGT/HLA Database was originally part of the international ImMunoGeneTics project (IMGT). For more information about the database and what data and tools are available please see our about page.

https://www.ebi.ac.uk/ipd/imgt/hla/

Polymorphisme et nomenclature des gènes HLA



```
A*01:01:01:01   A*02:01:01:01
A*01:01:01:02N  A*02:01:01:02
A*01:01:01:03   A*02:01:01:03
A*01:01:01:04   A*02:01:01:04
A*01:01:01:05   A*02:01:01:05
A*01:01:01:06   A*02:01:01:06
A*01:01:01:07   A*02:01:01:07
A*01:01:01:08   A*02:01:01:08
A*01:01:01:09   A*02:01:01:09
A*01:01:01:10   A*02:01:01:10
A*01:01:01:11   A*02:01:01:11
A*01:01:01:12   A*02:01:01:12
A*01:01:01:13   A*02:01:01:13
A*01:01:01:14   A*02:01:01:14
A*01:01:01:15   A*02:01:01:15
A*01:01:01:16   A*02:01:01:16
A*01:01:01:17   A*02:01:01:17
A*01:01:01:18   A*02:01:01:18
A*01:01:01:19   A*02:01:01:19
A*01:01:01:20   A*02:01:01:20
A*01:01:01:21   A*02:01:01:21
A*01:01:01:22   A*02:01:01:22
A*01:01:01:23   A*02:01:01:23
A*01:01:01:24   A*02:01:01:24
A*01:01:01:25   A*02:01:01:25
A*01:01:01:26   A*02:01:01:26
A*01:01:01:27   A*02:01:01:27
A*01:01:01:28   A*02:01:01:28
A*01:01:01:29   A*02:01:01:29
A*01:01:01:30   A*02:01:01:30
A*01:01:01:31   A*02:01:01:31
A*01:01:01:32
```

```
                        10         20         30         40         50         60         70         80         90        100
B*44:02:01:01   ATGCGGGTCA CGGCGCCCCG AACCCTCCTC CTGCTGCTCT GGGGGGCAGT GGCCCTGACC GAGACCTGGG CCG|GCTCCCA CTCCATGAGG TATTTCTACA

cDNA                   110        120        130        140        150        160        170        180        190        200
B*44:02:01:01   CCGCCATGTC CCGGCCCGGC CGCGGGGAGC CCCGCTTCAT CACCGTGGGC TACGTGGACG ACACGCTGTT CGTGAGGTTC GACAGCGACG CCACGAGTCC

cDNA                   210        220        230        240        250        260        270        280        290        300
B*44:02:01:01   GAGGAAGGAG CCGCGGGCGC CATGGATAGA GCAGGAGGGG CCGGAGTATT GGGACCGGGA GACACAGATC TCCAAGACCA ACACACAGAC TTACCGAGAG

cDNA                   310        320        330        340         350        360        370        380        390        400
B*44:02:01:01   AACCTGCGCA CCGCGCTCCG CTACTACAAC CAGAGCGAGG CCG|GGTCTCA CATCATCCAG AGGATGTACG GCTGCGACGT GGGGCCGGAC GGGCGCCTCC

cDNA                   410        420        430        440        450        460        470        480        490        500
B*44:02:01:01   TCCGCGGGTA TGACCAGGAC GCCTACGACG GCAAGGATTA CATCGCCCTG AACGAGGACC TGAGCTCCTG GACCGCGGCG GACACCGCGG CTCAGATCAC

cDNA                   510        520        530        540        550        560        570        580        590        600
B*44:02:01:01   CCAGCGCAAG TGGGAGGCGG CCCGTGTGGC GGAGCAGGAC AGAGCCTACC TGGAGGGCCT GTGCGTGGAG TCGCTCCGCA GATACCTGGA GAACGGGAAG

cDNA                   610         620        630        640        650        660        670        680        690        700
B*44:02:01:01   GAGACGCTGC AGCGCGCGG|A CCCCCCAAAG ACACATGTGA CCCACCACCC CATCTCTGAC CATGAGGTCA CCCTGAGGTG CTGGGCCCTG GGCTTCTACC

cDNA                   710        720        730        740        750        760        770        780        790        800
B*44:02:01:01   CTGCGGAGAT CACACTGACC TGGCAGCGGG ATGGCGAGGA CCAAACTCAG GACACCGAGC TTGTGGAGAC CAGACCAGCA GGAGATAGAA CCTTCCAGAA

cDNA                   810        820        830        840        850        860        870        880        890         900
B*44:02:01:01   GTGGGCAGCT GTGGTGGTGC CTTCTGGAGA AGAGCAGAGA TACACATGCC ATGTACAGCA TGAGGGGCTG CCGAAGCCCC TCACCCTGAG ATGGG|AGCCG

cDNA                   910        920        930        940        950        960        970        980        990       1000
B*44:02:01:01   TCTTCCCAGT CCACCGTCCC CATCGTGGGC ATTGTTGCTG GCCTGGCTGT CCTAGCAGTT GTGGTCATCG GAGCTGTGGT CGCTGCTGTG ATGTGTAGGA

cDNA                  1010        1020        1030        1040        1050       1060       1070       1080       1080
B*44:02:01:01   GGAAGAGCTC AG|GTGGAAAA GGAGGGAGCT ACTCTCAGGC TGCGT|GCAGC GACAGTGCCC AGGGCTCTGA TGTGTCTCTC ACAGCTTGA
```

```
B1*06:01:01:01 DPB1*
B1*06:01:01:02 DPB1*
B1*06:01:01:03 DPB1*
B1*06:01:01:04 DPB1*
B1*06:01:01:05 DPB1*
B1*06:01:01:06 DPB1*
B1*06:01:03    DPB1*
B1*06:01:05    DPB1*
B1*06:01:06    DPB1*
B1*06:01:08    DPB1*
B1*06:01:09    DPB1*
B1*06:01:10    DPB1*
B1*06:01:11    DPB1*
B1*06:01:15    DPB1*
B1*06:01:16    DPB1*
B1*06:01:17    DPB1*
B1*06:01:18    DPB1*
B1*06:01:19    DPB1*
B1*06:01:20    DPB1*
B1*06:01:21    DPB1*
B1*06:01:22    DPB1*
B1*06:01:23    DPB1*
B1*06:01:24    DPB1*
B1*06:01:25    DPB1*
B1*06:01:26    DPB1*
B1*06:01:27    DPB1*
B1*06:01:28    DPB1*
B1*06:01:29    DPB1*
B1*06:01:30    DPB1*
B1*06:01:31    DPB1*
```

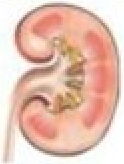
En cas de proposition d’un greffon…

PRE-INSCRIPTION

INSCRIPTION

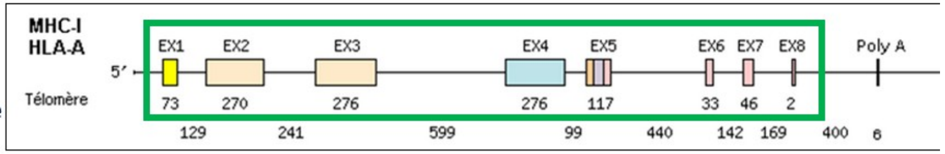
Anticorps anti-HLA
Immunisation contre B*44:02
Mais pas contre B*44:03

PROPOSITION D’UN GREFFON



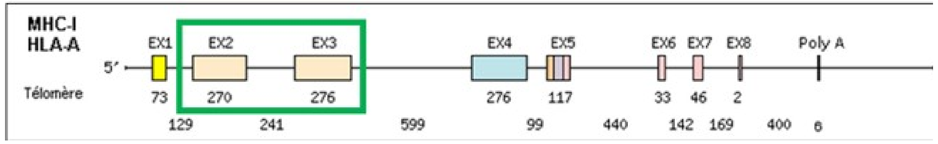
Typage HLA du receveur
NGS → Haute résolution

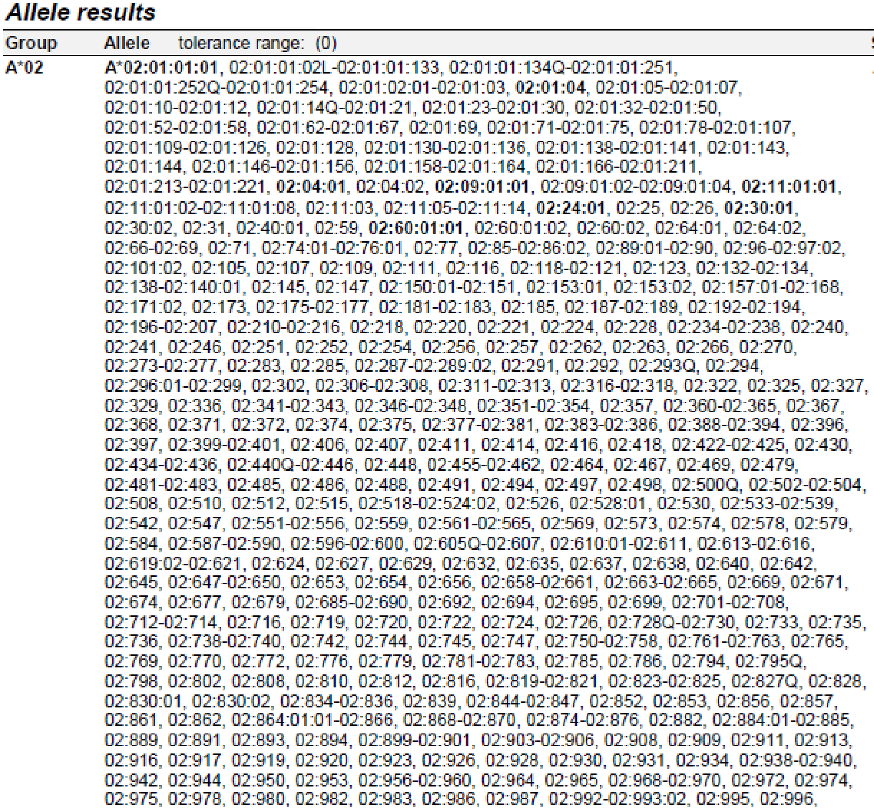
A*02:01, A*24:03
B*07:05, B*57:01
C*07:01, C*08:01
DRB1*01:01,
DRB1*15:01
DQB1*05:02,



Typage en urgence 24h/24
qPCR → basse résolution

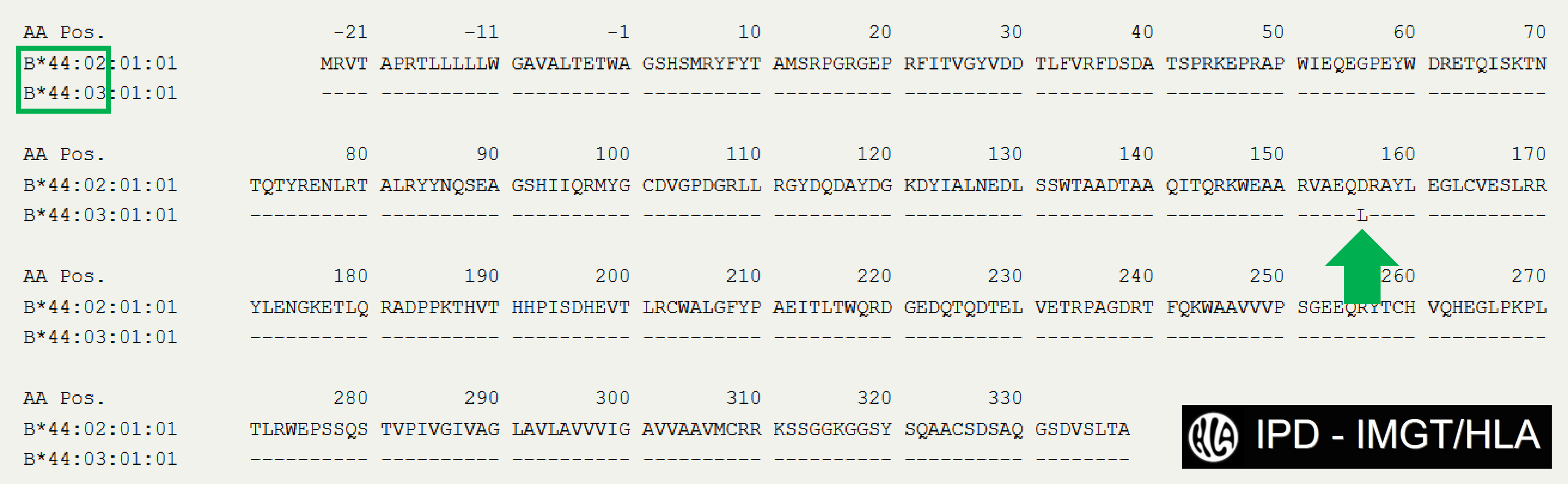
A*02, A*24
B*08 **B*44:02 ou B*44:03 ?**
C*07, C*04
DRB1*10, DRB1*11
DQB1*03, DQB1*05
DPB1*02, DPB1*04





Allele results

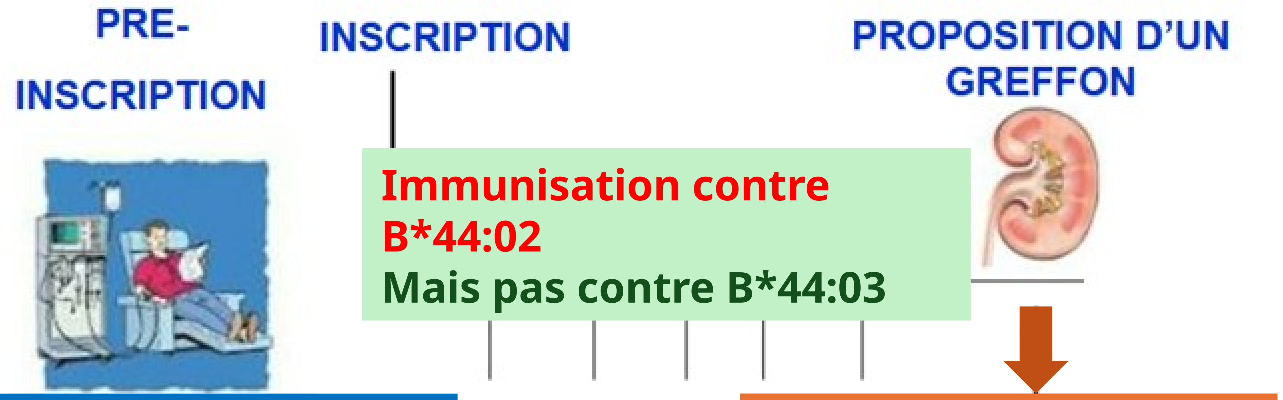
Group	Allele tolerance range: (0)
A*02	**A*02:01:01:01**, 02:01:01:02L-02:01:01:133, 02:01:01:134Q-02:01:01:251, 02:01:01:252Q-02:01:01:254, 02:01:02:01-02:01:03, **02:01:04**, 02:01:05-02:01:07, 02:01:10-02:01:12, 02:01:14Q-02:01:21, 02:01:23-02:01:30, 02:01:32-02:01:50, 02:01:52-02:01:58, 02:01:62-02:01:67, 02:01:69, 02:01:71-02:01:75, 02:01:78-02:01:107, 02:01:109-02:01:126, 02:01:128, 02:01:130-02:01:136, 02:01:138-02:01:141, 02:01:143, 02:01:144, 02:01:146-02:01:156, 02:01:158-02:01:164, 02:01:166-02:01:211, 02:01:213-02:01:221, **02:04:01**, 02:04:02, **02:09:01:01**, 02:09:01:02-02:09:01:04, **02:11:01:01**, 02:11:01:02-02:11:01:08, 02:11:03, 02:11:05-02:11:14, **02:24:01**, 02:25, 02:26, **02:30:01**, 02:30:02, 02:31, 02:40:01, 02:59, **02:60:01:01**, 02:60:01:02, 02:60:02, 02:64:01, 02:64:02, 02:66-02:69, 02:71, 02:74:01-02:76:01, 02:77, 02:85-02:86:02, 02:89:01-02:90, 02:96-02:97:02, 02:101:02, 02:105, 02:107, 02:109, 02:111, 02:116, 02:118-02:121, 02:123, 02:132-02:134, 02:138-02:140:01, 02:145, 02:147, 02:150:01-02:151, 02:153:01, 02:153:02, 02:157:01-02:168, 02:171:02, 02:173, 02:175-02:177, 02:181-02:183, 02:185, 02:187-02:189, 02:192-02:194, 02:196-02:207, 02:210-02:216, 02:218, 02:220, 02:221, 02:224, 02:228, 02:234-02:238, 02:240, 02:241, 02:246, 02:251, 02:252, 02:254, 02:256, 02:257, 02:262, 02:263, 02:266, 02:270, 02:273-02:277, 02:283, 02:285, 02:287-02:289:02, 02:291, 02:292, 02:293Q, 02:294, 02:296:01-02:299, 02:302, 02:306-02:308, 02:311-02:313, 02:316-02:318, 02:322, 02:325, 02:327, 02:329, 02:336, 02:341-02:343, 02:346-02:348, 02:351-02:354, 02:357, 02:360-02:365, 02:367, 02:368, 02:371, 02:372, 02:374, 02:375, 02:377-02:381, 02:383-02:386, 02:388-02:394, 02:396, 02:397, 02:399-02:401, 02:406, 02:407, 02:411, 02:414, 02:416, 02:418, 02:422-02:425, 02:430, 02:434-02:436, 02:440Q-02:446, 02:448, 02:455-02:462, 02:464, 02:467, 02:469, 02:479, 02:481-02:483, 02:485, 02:486, 02:488, 02:491, 02:494, 02:497, 02:498, 02:500Q, 02:502-02:504, 02:508, 02:510, 02:512, 02:515, 02:518-02:524:02, 02:526, 02:528:01, 02:530, 02:533-02:539, 02:542, 02:547, 02:551-02:556, 02:559, 02:561-02:565, 02:569, 02:573, 02:574, 02:578, 02:579, 02:584, 02:587-02:590, 02:596-02:600, 02:605Q-02:607, 02:610:01-02:611, 02:613-02:616, 02:619:02-02:621, 02:624, 02:627, 02:629, 02:632, 02:635, 02:637, 02:638, 02:640, 02:642, 02:645, 02:647-02:650, 02:653, 02:654, 02:656, 02:658-02:661, 02:663-02:665, 02:669, 02:671, 02:674, 02:677, 02:679, 02:685-02:690, 02:692, 02:694, 02:695, 02:699, 02:701-02:708, 02:712-02:714, 02:716, 02:719, 02:720, 02:722, 02:724, 02:726, 02:728Q-02:730, 02:733, 02:735, 02:736, 02:738-02:740, 02:742, 02:744, 02:745, 02:747, 02:750-02:758, 02:761-02:763, 02:765, 02:769, 02:770, 02:772, 02:776, 02:779, 02:781-02:783, 02:785, 02:786, 02:794, 02:795Q, 02:798, 02:802, 02:808, 02:810, 02:812, 02:816, 02:819-02:821, 02:823-02:825, 02:827Q, 02:828, 02:830:01, 02:830:02, 02:834-02:836, 02:839, 02:844-02:847, 02:852, 02:853, 02:856, 02:857, 02:861, 02:862, 02:864:01:01-02:866, 02:868-02:870, 02:874-02:876, 02:882, 02:884:01-02:885, 02:889, 02:891, 02:893, 02:894, 02:899-02:901, 02:903-02:906, 02:908, 02:909, 02:911, 02:913, 02:916, 02:917, 02:919, 02:920, 02:923, 02:926, 02:928, 02:930, 02:931, 02:934, 02:938-02:940, 02:942, 02:944, 02:950, 02:953, 02:956-02:960, 02:964, 02:965, 02:968-02:970, 02:972, 02:974, 02:975, 02:978, 02:980, 02:982, 02:983, 02:986, 02:987, 02:992-02:993:02, 02:995, 02:996,



```
AA Pos.                   -21        -11         -1         10         20         30         40         50         60         70
B*44:02:01:01            MRVT APRTLLLLLW GAVALTETWA GSHSMRYFYT AMSRPGRGEP RFITVGYVDD TLFVRFDSDA TSPRKEPRAP WIEQEGPEYW DRETQISKTN
B*44:03:01:01            ---- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

AA Pos.                    80         90        100        110        120        130        140        150        160        170
B*44:02:01:01      TQTYRENLRT ALRYYNQSEA GSHIIQRMYG CDVGPDGRLL RGYDQDAYDG KDYIALNEDL SSWTAADTAA QITQRKWEAA RVAEQDRAYL EGLCVESLRR
B*44:03:01:01      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----L---- ----------

AA Pos.                   180        190        200        210        220        230        240        250        260        270
B*44:02:01:01      YLENGKETLQ RADPPKTHVT HHPISDHEVT LRCWALGFYP AEITLTWQRD GEDQTQDTEL VETRPAGDRT FQKWAAVVVP SGEEQRYTCH VQHEGLPKPL
B*44:03:01:01      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

AA Pos.                   280        290        300        310        320        330
B*44:02:01:01      TLRWEPSSQS TVPIVGIVAG LAVLAVVVIG AVVAAVMCRR KSSGGKGGSY SQAACSDSAQ GSDVSLTA
B*44:03:01:01      ---------- ---------- ---------- ---------- ---------- ---------- --------
```

Changement d’un ou plusieurs AA → situation potentiellement immunogène en fonction des propriétés physico-chimiques

En cas de proposition d'un greffon…



Immunisation contre B*44:02
Mais pas contre B*44:03

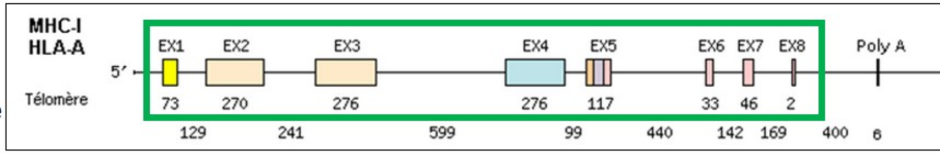
Typage HLA du receveur
NGS → Haute résolution

A*02:01, A*24:03
B*07:05, B*57:01
C*07:01, C*08:01
DRB1*01:01,
DRB1*15:01
DQB1*05:02,

Typage en urgence 24h/24
qPCR → basse résolution

A*02, A*24
B*08 **B*44:02 ou B*44:03 ?**
C*07, C*04
DRB1*10, DRB1*11
DQB1*03, DQB1*05
DPB1*02, DPB1*04

Proposition acceptable ?



En cas de proposition d'un greffon…

Typage HLA du receveur

A*02:01, A*24:03
B*07:05, B*57:01
C*07:01, C*08:01
DRB1*01:01,
DRB1*15:01
DQB1*05:02,

Immunisation contre B*44:02
Mais pas contre B*44:03

Proposition acceptable ?

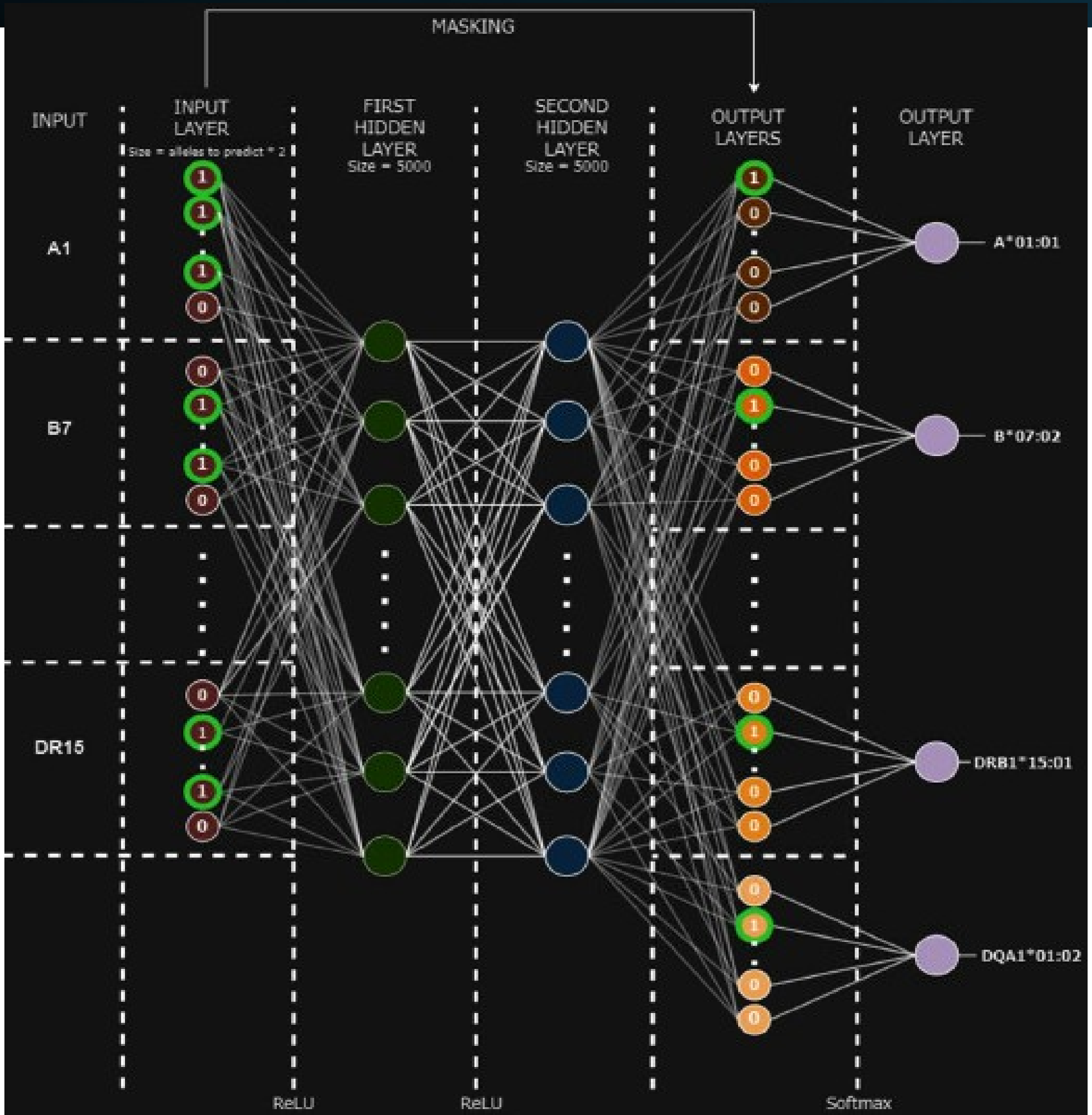
Typage du donneur

A*02, A*24
B*08, B*44
C*07, C*04
DRB1*10, DRB1*11
DQB1*03, DQB1*05
DPB1*02, DPB1*04

Prédire
le typage haute résolution ??

En cas de proposition d’un greffon…

Prédire le typage haute résolution



HaploDeep

Romain LHOTTE
(Laboratoire HLA Hôpital Saint-Louis/Centrale Supele

https://github.com/JasonMendoza2008/HLATypingImputationBenchmarks/releases/tag/HaploDee

Deep Learning
Réseau de 5 neurones
5 x 86 798 858 paramètres

Entrainement sur > 150 000 typages HLA
A-B-C-DRB1-DRB345-DQA1-DQB1-DPA1-DPB1

→ Extrapolation A-B-C-DRB1-DQB1 en haute résolution

A*02, A*24
B*08, B*44
C*07, C*04
DRB1*04, DRB1*13
DQB1*03,
DQB1*05

→

A*02:01, A*24:02
B*08:01, B*44:02
C*07:01, C*04:01
DRB1*04:03, DRB1*13:02
DQB1*03:02,
DQB1*06:04

En cas de proposition d’un greffon…

Typage HLA du receveur

A*02:01, A*24:03
B*07:05, B*57:01
C*07:01, C*08:01
DRB1*01:01,
DRB1*15:01
DQB1*05:02,

Immunisation contre B*44:02
Mais pas contre B*44:03

Proposition acceptable ?

Typage du donneur

A*02, A*24
B*08, B*44
C*07, C*04
DRB1*10, DRB1*11
DQB1*03, DQB1*05
DPB1*02, DPB1*04

Prédire le typage haute résolution

Avoir le typage haute résolution du donneur

En cas de proposition d’un greffon…

Typage HLA du receveur

A*02:01, A*24:03
B*07:05, B*57:01
C*07:01, C*08:01
DRB1*01:01,
DRB1*15:01
DQB1*05:02,

Immunisation contre B*44:02
Mais pas contre B*44:03

Proposition acceptable ?

haute résolution du donneur

Typage du donneur

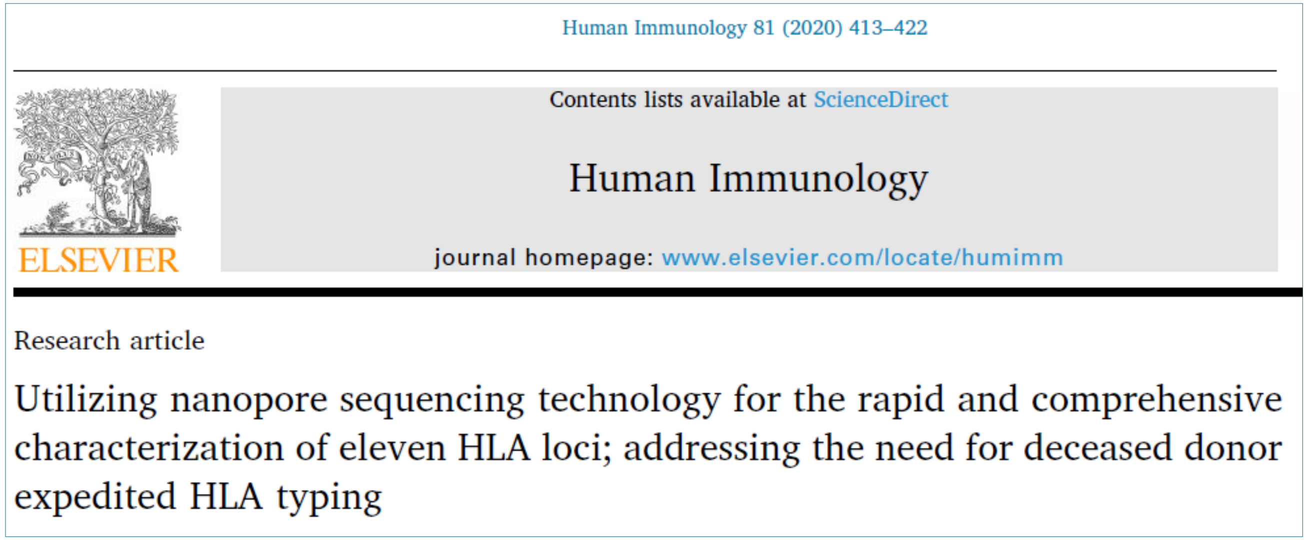
A*02, A*24
B*08, B*44
C*07, C*04
DRB1*10, DRB1*11
DQB1*03, DQB1*05
DPB1*02, DPB1*04

✓ Technique rapide

✓ Réalisable 24h/24

✓ Haute résolution

Améliorer la compatibilité donneur/receveur
Typage HLA par séquençage nanopore


Human Immunology 81 (2020) 413–422

Contents lists available at ScienceDirect

Human Immunology

journal homepage: www.elsevier.com/locate/humimm

ELSEVIER

Research article

Utilizing nanopore sequencing technology for the rapid and comprehensive characterization of eleven HLA loci; addressing the need for deceased donor expedited HLA typing

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Received: 7 February 2020 | Revised: 7 April 2020 | Accepted: 8 April 2020

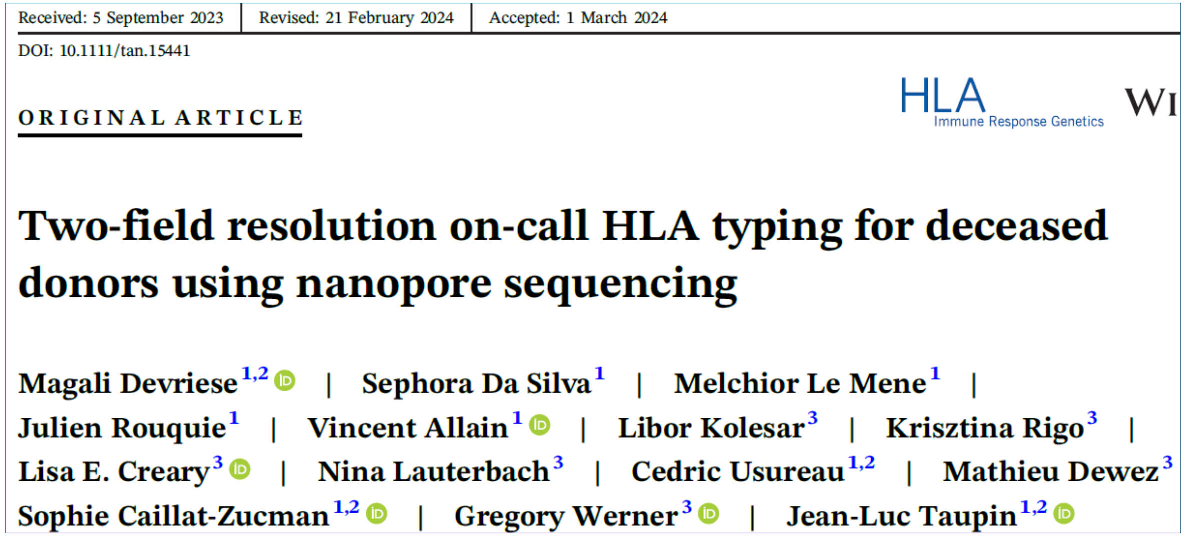
DOI: 10.1111/ajt.15938

AJT

ORIGINAL ARTICLE

Clinical importance of extended second field high-resolution HLA genotyping for kidney transplantation

Aleksandar Senev[1,2] | Marie-Paule Emonds[1,2] | Vicky Van Sandt[1] | Evelyne Lerut[3] | Maarten Coemans[2] | Ben Sprangers[2,4] | Dirk Kuypers[2,4] | Maarten Naesens[2,4]


Received: 5 September 2023 | Revised: 21 February 2024 | Accepted: 1 March 2024

DOI: 10.1111/tan.15441

HLA Immune Response Genetics

ORIGINAL ARTICLE

Two-field resolution on-call HLA typing for deceased donors using nanopore sequencing

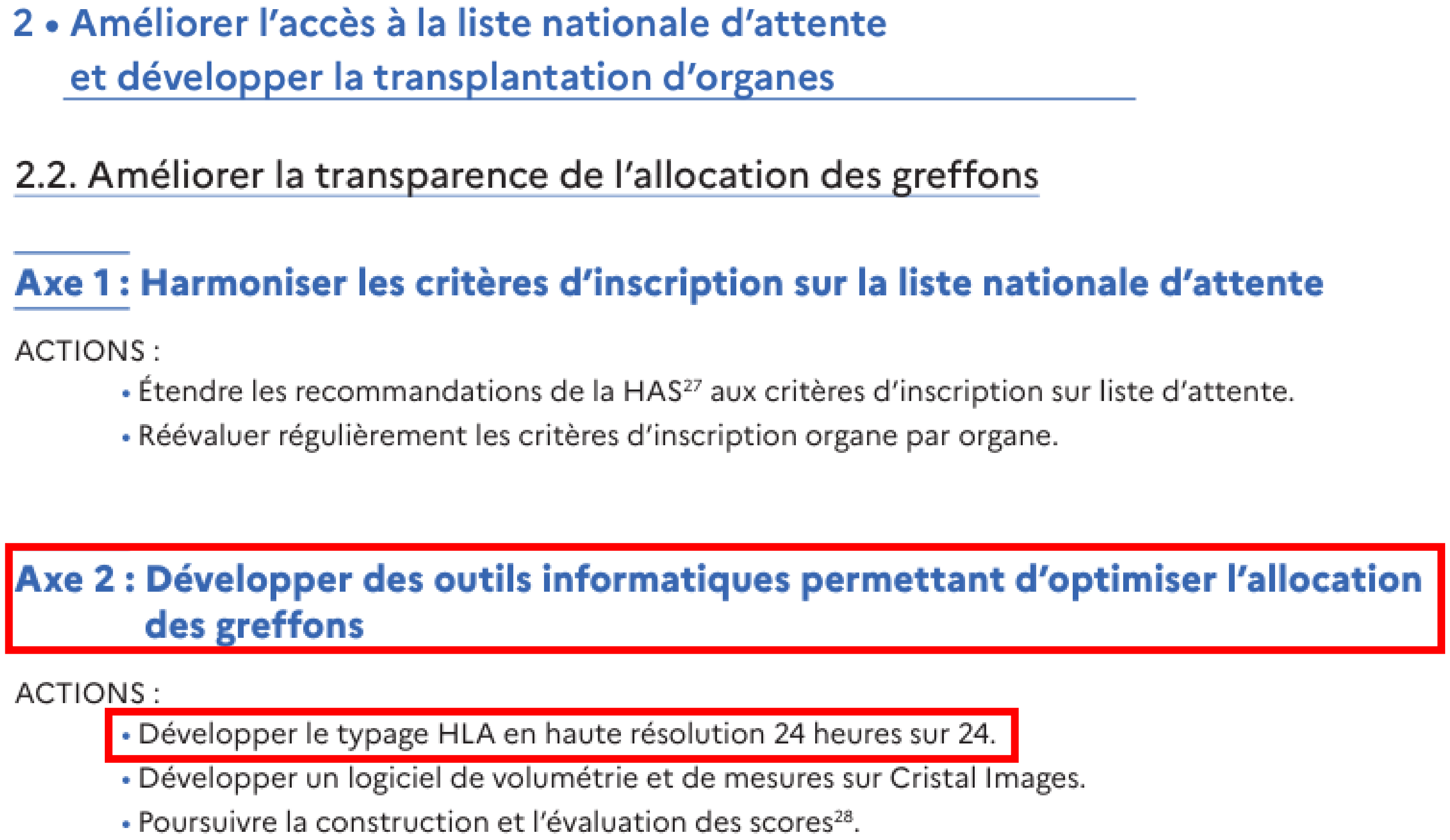
Magali Devriese[1,2] | Sephora Da Silva[1] | Melchior Le Mene[1] | Julien Rouquie[1] | Vincent Allain[1] | Libor Kolesar[3] | Krisztina Rigo[3] | Lisa E. Creary[3] | Nina Lauterbach[3] | Cedric Usureau[1,2] | Mathieu Dewez[3] | Sophie Caillat-Zucman[1,2] | Gregory Werner[3] | Jean-Luc Taupin[1,2]

Améliorer la compatibilité donneur/receveur

Typage HLA par séquençage nanopore

MINISTÈRE DES SOLIDARITÉS ET DE LA SANTÉ
Liberté
Égalité
Fraternité

PLAN MINISTÉRIEL
POUR LE PRÉLÈVEMENT
ET LA GREFFE D'ORGANES
ET DE TISSUS
2022-2026



2 • Améliorer l'accès à la liste nationale d'attente et développer la transplantation d'organes

2.2. Améliorer la transparence de l'allocation des greffons

Axe 1 : Harmoniser les critères d'inscription sur la liste nationale d'attente

ACTIONS :

- Étendre les recommandations de la HAS[27] aux critères d'inscription sur liste d'attente.
- Réévaluer régulièrement les critères d'inscription organe par organe.

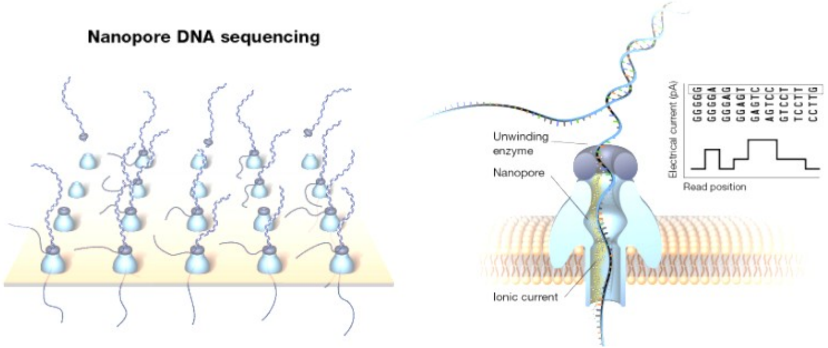
Axe 2 : Développer des outils informatiques permettant d'optimiser l'allocation des greffons

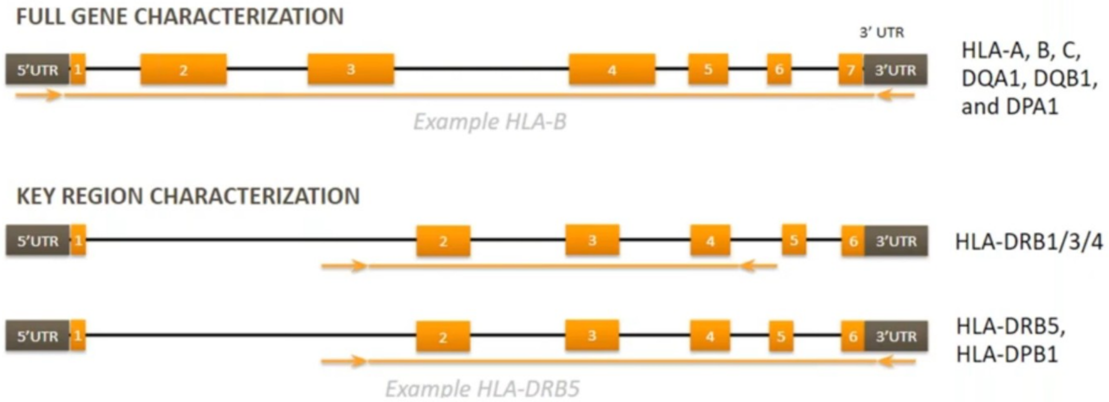
ACTIONS :

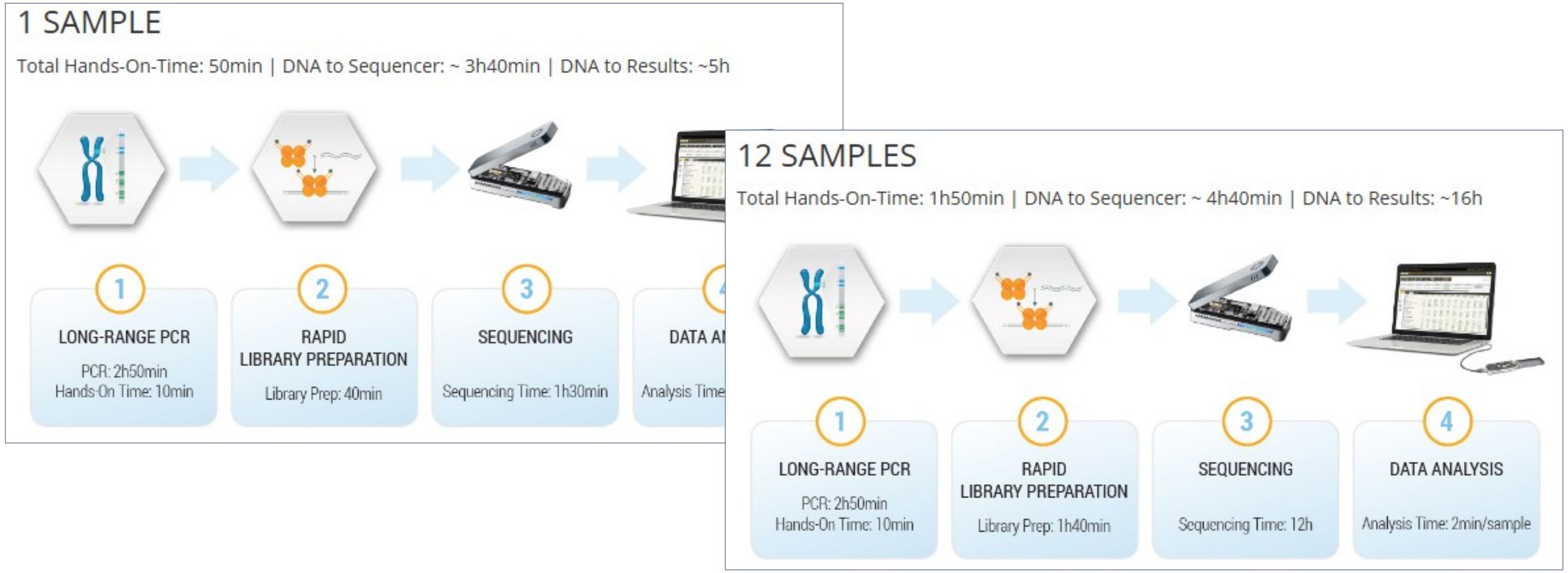
- Développer le typage HLA en haute résolution 24 heures sur 24.
- Développer un logiciel de volumétrie et de mesures sur Cristal Images.
- Poursuivre la construction et l'évaluation des scores[28].

Améliorer la compatibilité donneur/receveur

Typage HLA par séquençage nanopore







1 SAMPLE

Total Hands-On-Time: 50min | DNA to Sequencer: ~ 3h40min | DNA to Results: ~5h

1 LONG-RANGE PCR
PCR: 2h50min
Hands-On Time: 10min

2 RAPID LIBRARY PREPARATION
Library Prep: 40min

3 SEQUENCING
Sequencing Time: 1h30min

DATA A
Analysis Time

12 SAMPLES

Total Hands-On-Time: 1h50min | DNA to Sequencer: ~ 4h40min | DNA to Results: ~16h

1 LONG-RANGE PCR
PCR: 2h50min
Hands-On Time: 10min

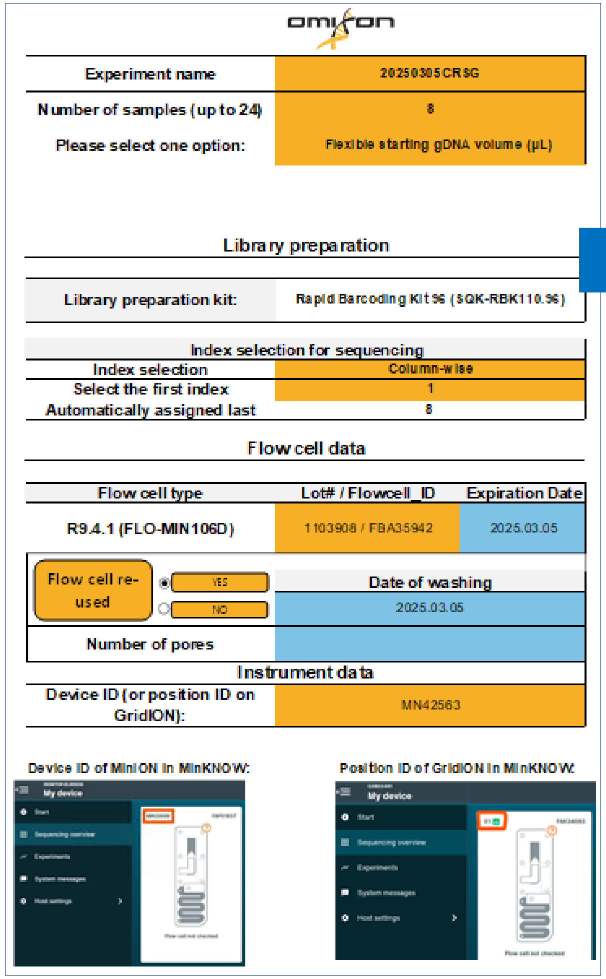
2 RAPID LIBRARY PREPARATION
Library Prep: 1h40min

3 SEQUENCING
Sequencing Time: 12h

4 DATA ANALYSIS
Analysis Time: 2min/sample

Typage HLA par séquençage nanopore
Workbook

Data input ▸ Genomic DNA preparation ▸ Amplicon quantitation ▸ Library preparation ▸ Sample sheet ▸ .JSON



Experiment name	20250305CRSG
Number of samples (up to 24)	8
Please select one option:	Flexible starting gDNA volume (µL)

Library preparation

Library preparation kit:	Rapid Barcoding Kit 96 (SQK-RBK110.96)

Index selection for sequencing

Index selection	Column-wise
Select the first index	1
Automatically assigned last	8

Flow cell data

Flow cell type	Lot# / Flowcell_ID	Expiration Date
R9.4.1 (FLO-MIN106D)	1103908 / FBA35942	2025.03.05

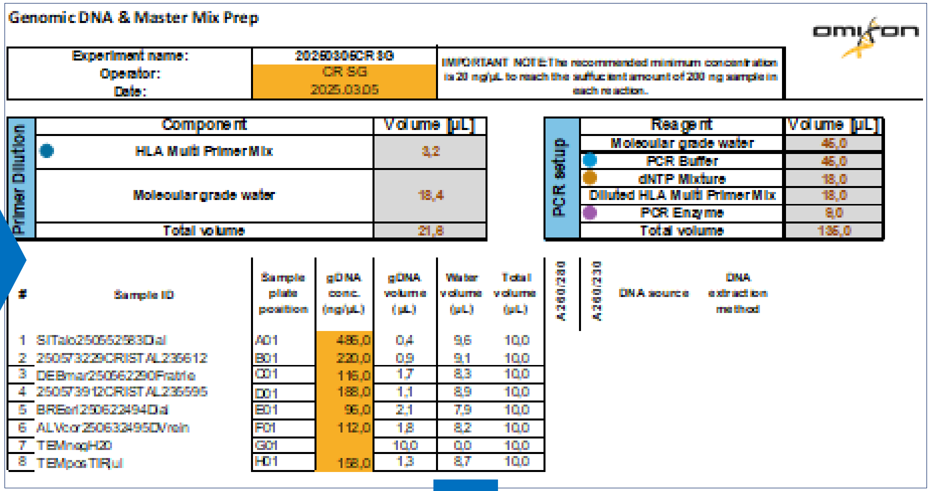
Flow cell re-used	● YES ○ NO	Date of washing 2025.03.05
Number of pores		

Instrument data

Device ID (or position ID on GridION):	MN42563

Device ID of MinION in MinKNOW:

Position ID of GridION in MinKNOW:



Genomic DNA & Master Mix Prep

Experiment name:	20250305CRSG
Operator:	CR SG
Date:	2025.03.05

IMPORTANT NOTE: The recommended minimum concentration is 20 ng/µL to reach the sufficient amount of 200 ng sample in each reaction.

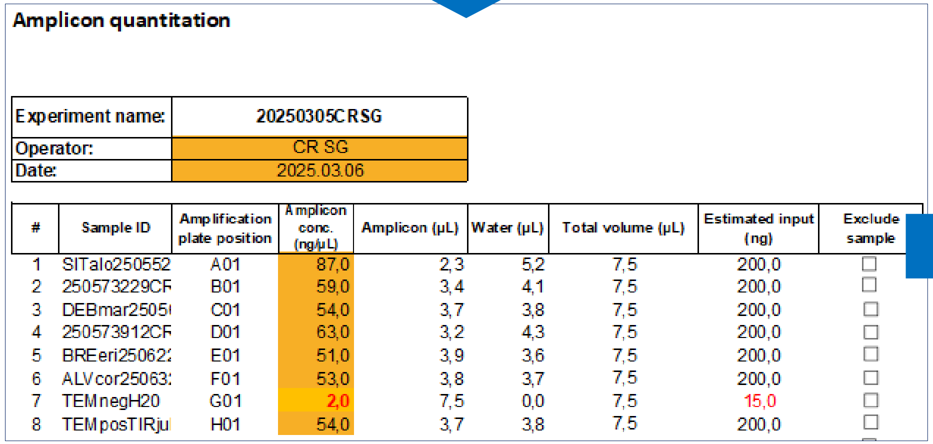
Primer Dilution

	Component	Volume [µL]
●	HLA Multi Primer Mix	3,2
	Molecular grade water	18,4
	Total volume	21,6

PCR setup

	Reagent	Volume [µL]
	Molecular grade water	45,0
●	PCR Buffer	45,0
●	dNTP Mixture	18,0
	Diluted HLA Multi Primer Mix	18,0
●	PCR Enzyme	9,0
	Total volume	135,0

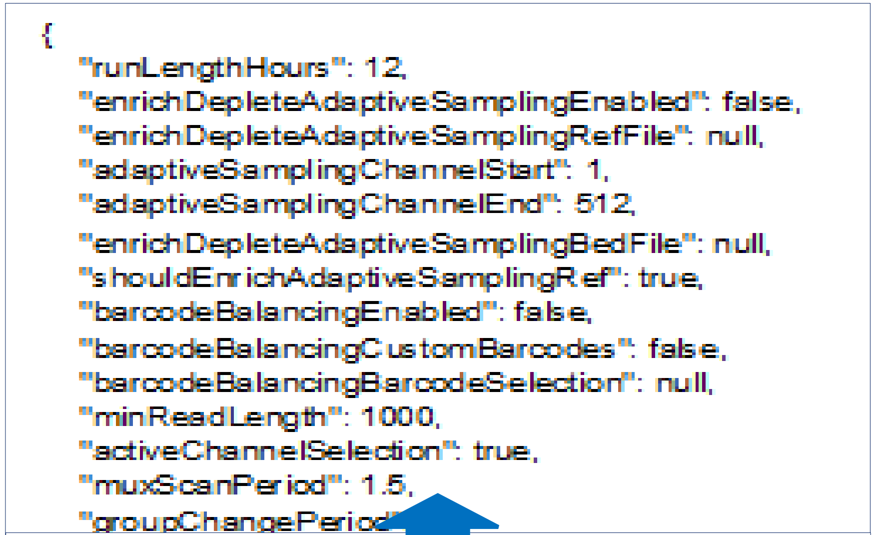
#	Sample ID	Sample plate position	gDNA conc. (ng/µL)	gDNA volume (µL)	Water volume (µL)	Total volume (µL)	A260/280	A260/230	DNA source	DNA extraction method
1	SITalo250552583Dial	A01	485,0	0,4	9,6	10,0				
2	250573229CRISTAL236612	B01	220,0	0,9	9,1	10,0				
3	DEBmar250562290Fratrie	C01	116,0	1,7	8,3	10,0				
4	250573912CRISTAL236595	D01	188,0	1,1	8,9	10,0				
5	BREeri250622494Dial	E01	96,0	2,1	7,9	10,0				
6	ALVcor250632495DVrein	F01	112,0	1,8	8,2	10,0				
7	TEMnegH20	G01		10,0	0,0	10,0				
8	TEMposTIRjul	H01	158,0	1,3	8,7	10,0				



Amplicon quantitation

Experiment name:	20250305CRSG
Operator:	CR SG
Date:	2025.03.06

#	Sample ID	Amplification plate position	Amplicon conc. (ng/µL)	Amplicon (µL)	Water (µL)	Total volume (µL)	Estimated input (ng)	Exclude sample
1	SITalo250552	A01	87,0	2,3	5,2	7,5	200,0	☐
2	250573229CR	B01	59,0	3,4	4,1	7,5	200,0	☐
3	DEBmar2505	C01	54,0	3,7	3,8	7,5	200,0	☐
4	250573912CR	D01	63,0	3,2	4,3	7,5	200,0	☐
5	BREeri25062	E01	51,0	3,9	3,6	7,5	200,0	☐
6	ALVcor25063	F01	53,0	3,8	3,7	7,5	200,0	☐
7	TEMnegH20	G01	2,0	7,5	0,0	7,5	15,0	☐
8	TEMposTIRjul	H01	54,0	3,7	3,8	7,5	200,0	☐



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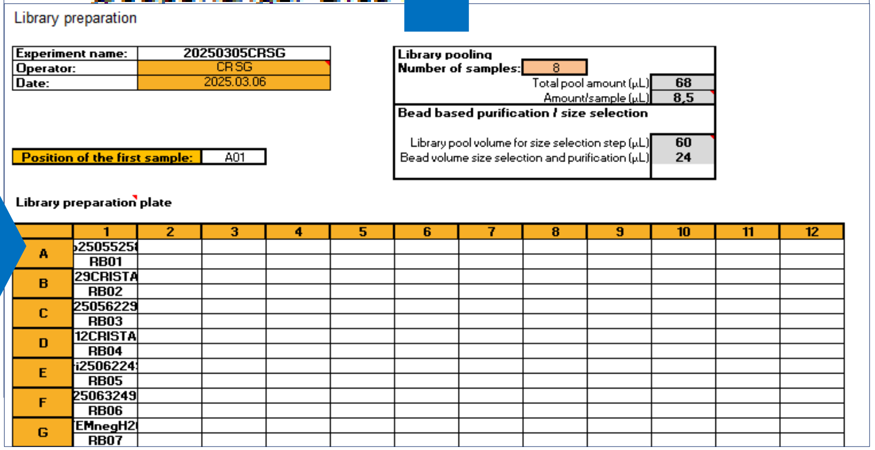


Library preparation

Experiment name:	20250305CRSG
Operator:	CR SG
Date:	2025.03.06

Library pooling

Number of samples:	8
Total pool amount (µL)	68
Amount/sample (µL)	8,5

Bead based purification / size selection

Library pool volume for size selection step (µL)	60
Bead volume size selection and purification (µL)	24

Position of the first sample:	A01

Library preparation plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	2505525 RB01											
B	29CRISTA RB02											
C	25056229 RB03											
D	12CRISTA RB04											
E	i2506224 RB05											
F	25063249 RB06											
G	EMnegH2 RB07											

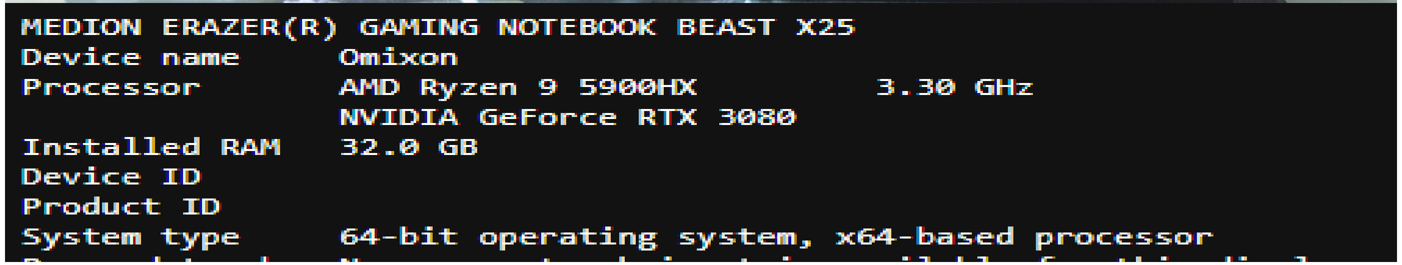
Typage HLA par séquençage nanopore
Paramètres

Generate Sample sheet and JSON files

- Run length

Number of samples	Run length (hours)
1	1.5
2	4
3	5
4-12	12
13-24	= sample number

- Minimum read length: 1000
- Time between pore scans: 0,75h/1,5h
- Basecalling: High Accuracy
- .Fast5: 4000 reads/file
- .FastQ: 4000 reads/file
- Filtering Qscore: 7



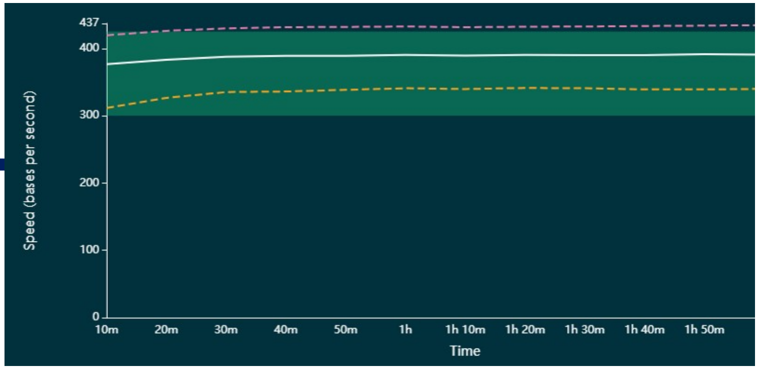
```
MEDION ERAZER(R) GAMING NOTEBOOK BEAST X25
Device name      Omixon
Processor        AMD Ryzen 9 5900HX         3.30 GHz
                 NVIDIA GeForce RTX 3080
Installed RAM    32.0 GB
Device ID
Product ID
System type      64-bit operating system, x64-based processor
```

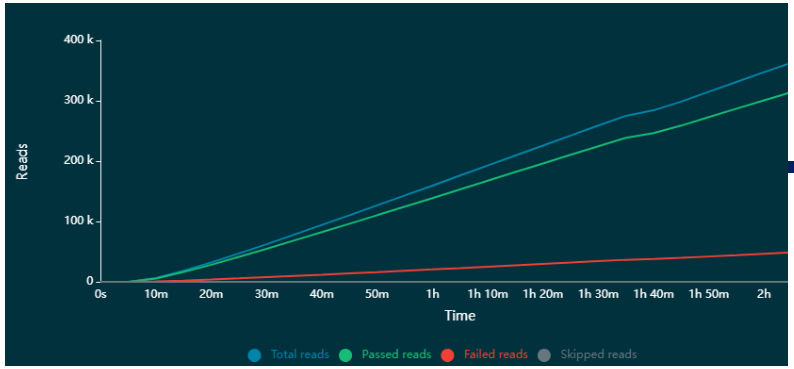
Typage HLA par séquençage nanopore

MinKnow: contrôle du MinIon

Oxford NANOPORE Technologies

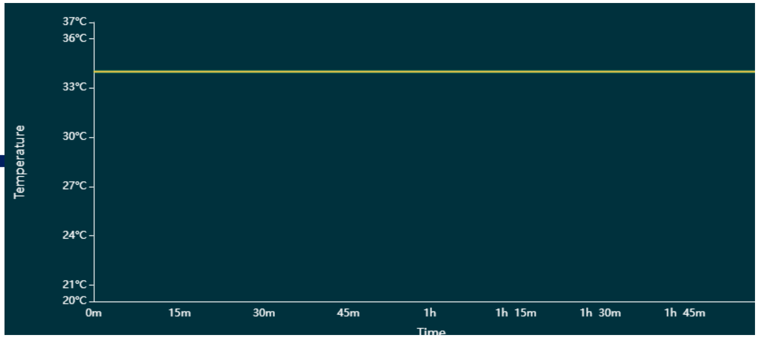
Température

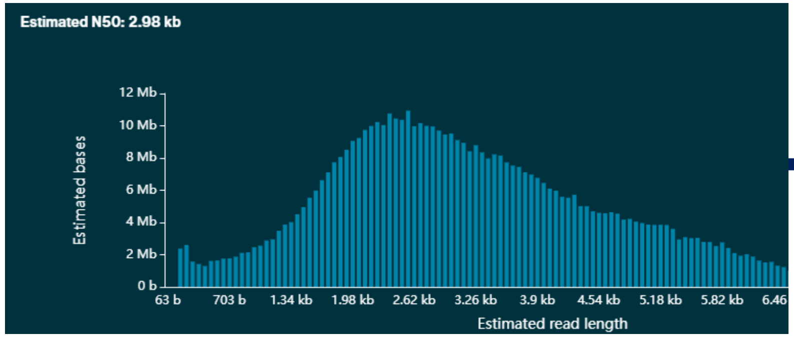




Nombre de reads générés

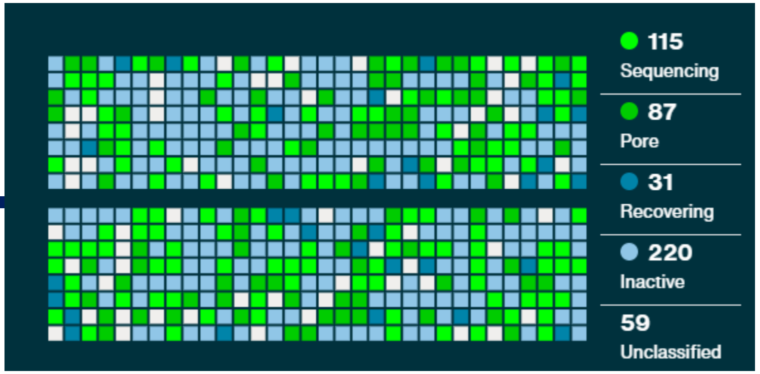
Vitesse de translocation




Estimated N50: 2.98 kb

Longueur des reads

Etat des canaux

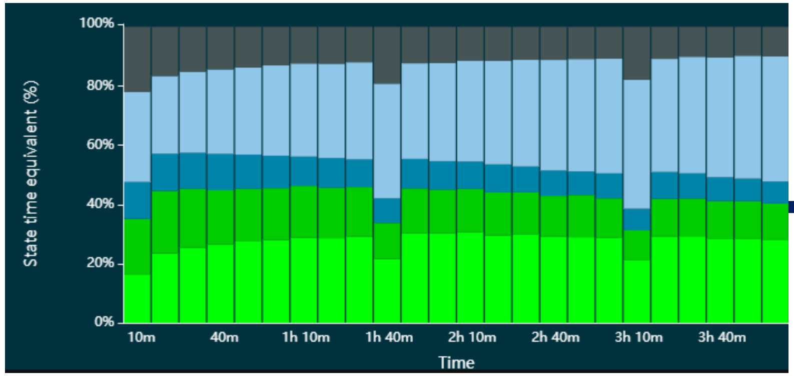

● 115 Sequencing

● 87 Pore

● 31 Recovering

● 220 Inactive

59 Unclassified

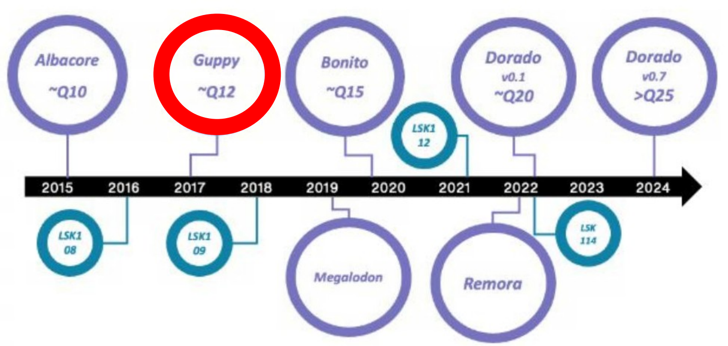


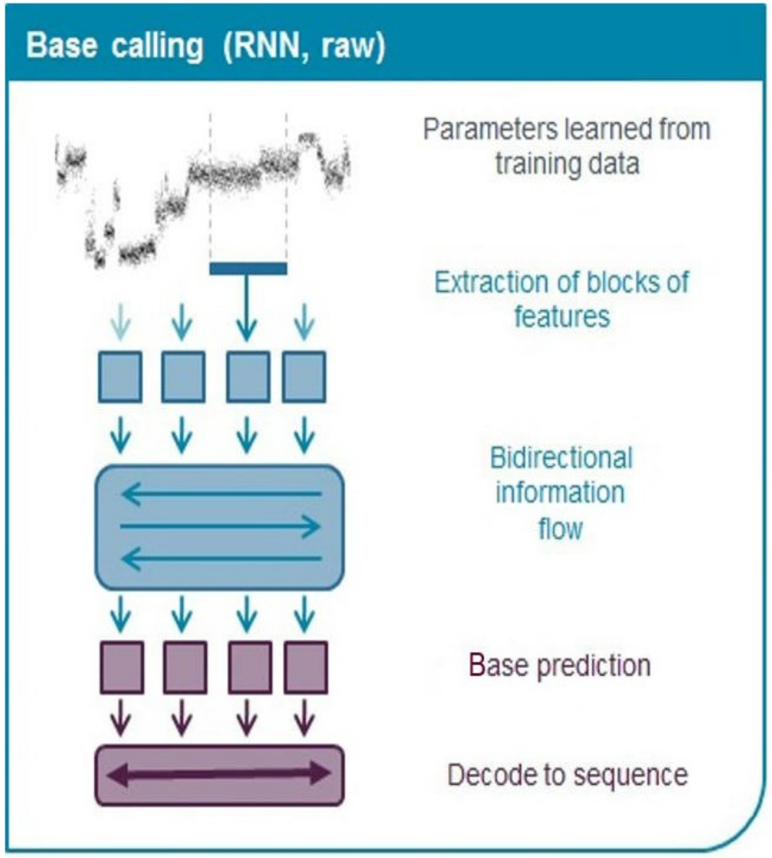
Activité des pores

Typage HLA par séquençage nanopore

Basecalling

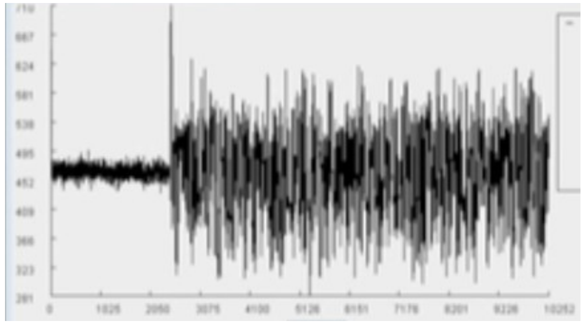
Guppy 6.1.5 (GPU version)





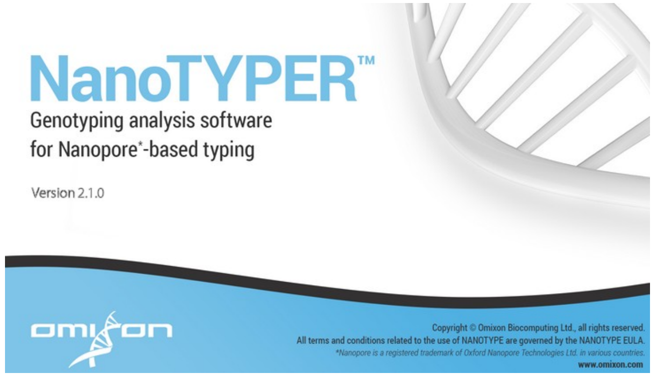
NANOPORE

Données brutes: .Fast5



Fichier output: .FastQ

Résultats analysés .htr



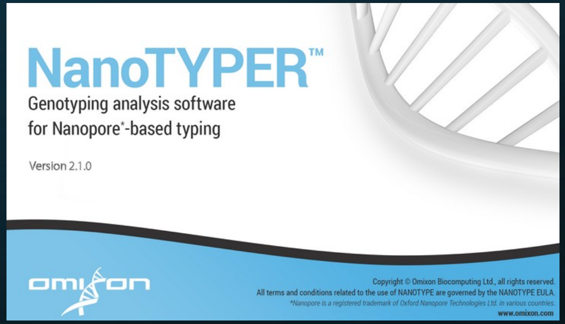
Genotyping analysis software
for Nanopore*-based typing

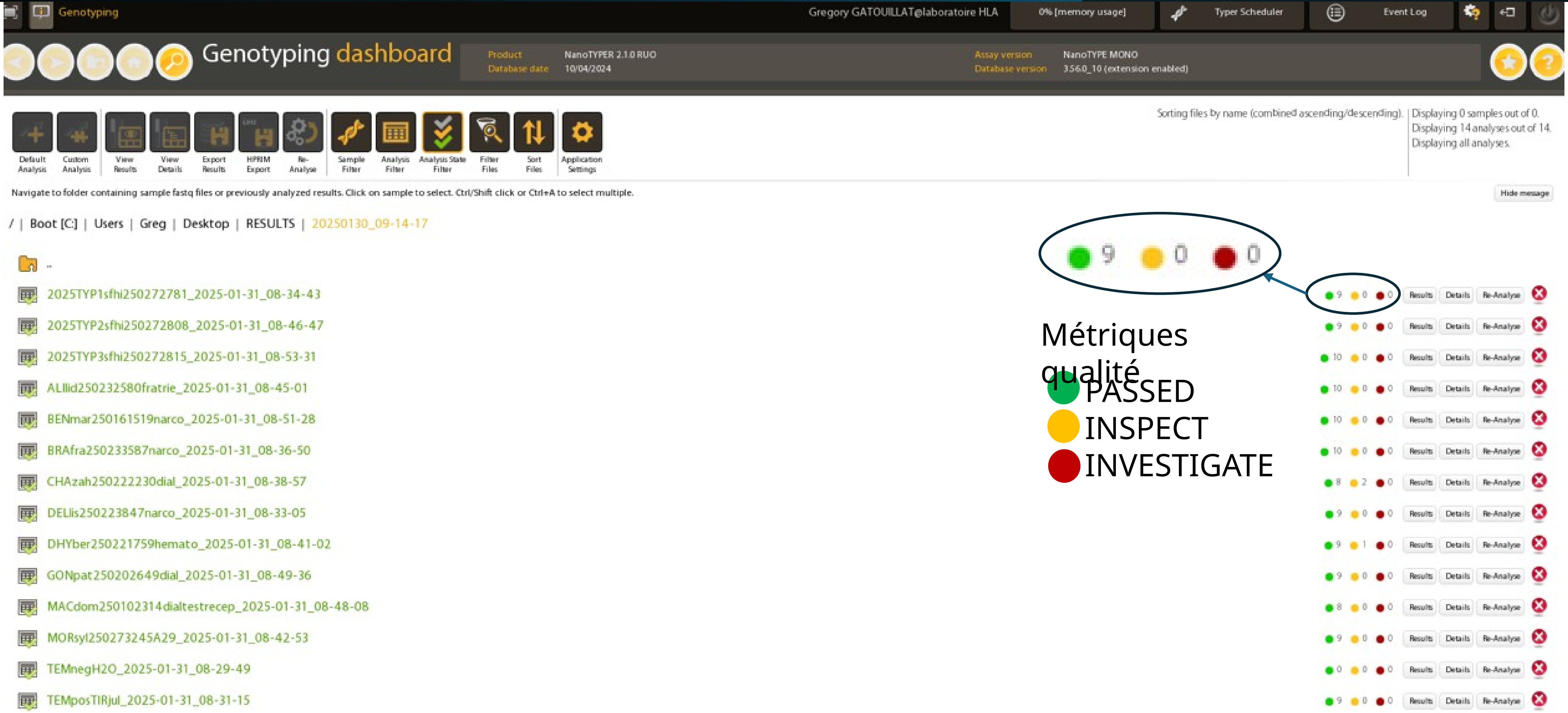
Version 2.1.0

Typage HLA par séquençage nanopore

Interprétation

Nanotyper™





Product: NanoTYPER 2.1.0 RUO
Database date: 10/04/2024
Assay version: NanoTYPE MONO
Database version: 3.56.0_10 (extension enabled)

Sorting files by name (combined ascending/descending).

Displaying 0 samples out of 0.
Displaying 14 analyses out of 14.
Displaying all analyses.

Navigate to folder containing sample fastq files or previously analyzed results. Click on sample to select. Ctrl/Shift click or Ctrl+A to select multiple.

/ | Boot [C:] | Users | Greg | Desktop | RESULTS | 20250130_09-14-17

..

	🟢	🟡	🔴
2025TYP1sfhi250272781_2025-01-31_08-34-43	9	0	0
2025TYP2sfhi250272808_2025-01-31_08-46-47	9	0	0
2025TYP3sfhi250272815_2025-01-31_08-53-31	10	0	0
ALIlid250232580fratrie_2025-01-31_08-45-01	10	0	0
BENmar250161519narco_2025-01-31_08-51-28	10	0	0
BRAfra250233587narco_2025-01-31_08-36-50	10	0	0
CHAzah250222230dial_2025-01-31_08-38-57	8	2	0
DELlis250223847narco_2025-01-31_08-33-05	9	0	0
DHYber250221759hemato_2025-01-31_08-41-02	9	1	0
GONpat250202649dial_2025-01-31_08-49-36	9	0	0
MACdom250102314dialtestrecep_2025-01-31_08-48-08	8	0	0
MORsyl250273245A29_2025-01-31_08-42-53	9	0	0
TEMnegH2O_2025-01-31_08-29-49	0	0	0
TEMposTIRjul_2025-01-31_08-31-15	9	0	0

🟢 9 🟡 0 🔴 0

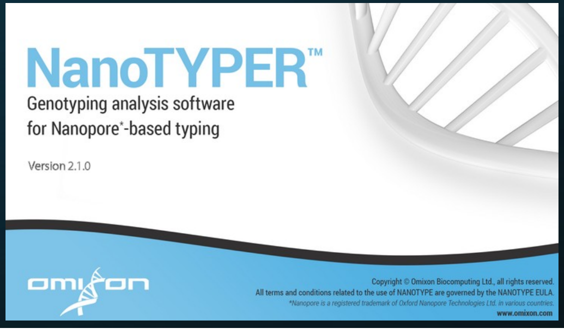
Métriques qualité

🟢 PASSED
🟡 INSPECT
🔴 INVESTIGATE

Typage HLA par séquençage nanopore

Interprétation

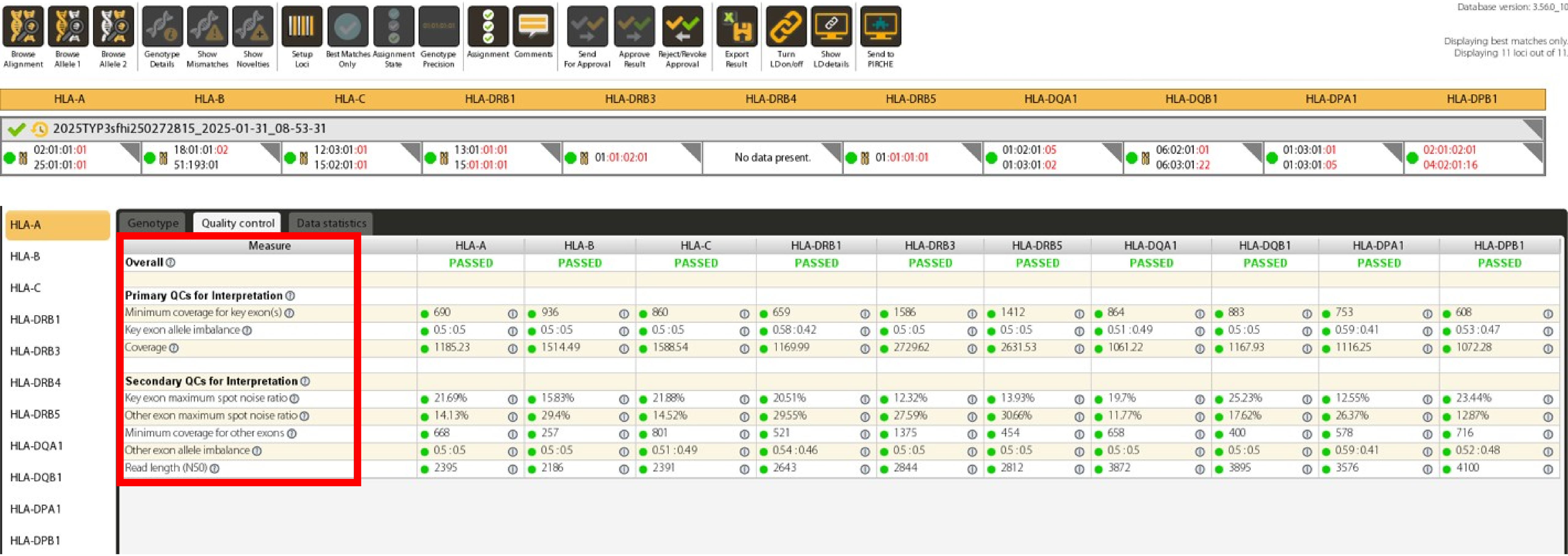
Nanotyper™



NanoTYPER™

Genotyping analysis software for Nanopore*-based typing

Version 2.1.0



Database version: 3.56.0_10

Displaying best matches only.
Displaying 11 loci out of 1

✓ 2025TYP3sfhi250272815_2025-01-31_08-53-31

HLA-A	HLA-B	HLA-C	HLA-DRB1	HLA-DRB3	HLA-DRB4	HLA-DRB5	HLA-DQA1	HLA-DQB1	HLA-DPA1	HLA-DPB1
● 02:01:01:01 25:01:01:01	● 18:01:01:02 51:193:01	● 12:03:01:01 15:02:01:01	● 13:01:01:01 15:01:01:01	● 01:01:02:01	No data present.	● 01:01:01:01	● 01:02:01:05 01:03:01:02	● 06:02:01:01 06:03:01:22	● 01:03:01:01 01:03:01:05	● 02:01:02:01 04:02:01:16

Measure	HLA-A	HLA-B	HLA-C	HLA-DRB1	HLA-DRB3	HLA-DRB5	HLA-DQA1	HLA-DQB1	HLA-DPA1	HLA-DPB1
Overall	PASSED	PASSED	PASSED	PASSED	PASSED	PASSED	PASSED	PASSED	PASSED	PASSED
Primary QCs for Interpretation										
Minimum coverage for key exon(s)	● 690	● 936	● 860	● 659	● 1586	● 1412	● 864	● 883	● 753	● 608
Key exon allele imbalance	● 0.5 : 0.5	● 0.5 : 0.5	● 0.5 : 0.5	● 0.58 : 0.42	● 0.5 : 0.5	● 0.5 : 0.5	● 0.51 : 0.49	● 0.5 : 0.5	● 0.59 : 0.41	● 0.53 : 0.47
Coverage	● 1185.23	● 1514.49	● 1588.54	● 1169.99	● 2729.62	● 2631.53	● 1061.22	● 1167.93	● 1116.25	● 1072.28
Secondary QCs for Interpretation										
Key exon maximum spot noise ratio	● 21.69%	● 15.83%	● 21.88%	● 20.51%	● 12.32%	● 13.93%	● 19.7%	● 25.23%	● 12.55%	● 23.44%
Other exon maximum spot noise ratio	● 14.13%	● 29.4%	● 14.52%	● 29.55%	● 27.59%	● 30.66%	● 11.77%	● 17.62%	● 26.37%	● 12.87%
Minimum coverage for other exons	● 668	● 257	● 801	● 521	● 1375	● 454	● 658	● 400	● 578	● 716
Other exon allele imbalance	● 0.5 : 0.5	● 0.5 : 0.5	● 0.51 : 0.49	● 0.54 : 0.46	● 0.5 : 0.5	● 0.5 : 0.5	● 0.5 : 0.5	● 0.5 : 0.5	● 0.59 : 0.41	● 0.52 : 0.48
Read length (N50)	● 2395	● 2186	● 2391	● 2643	● 2844	● 2812	● 3872	● 3895	● 3576	● 4100

Typage HLA par séquençage nanopore

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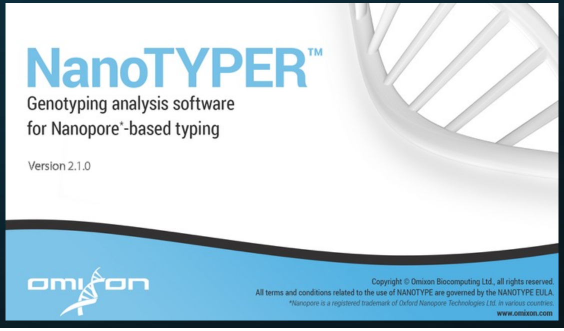
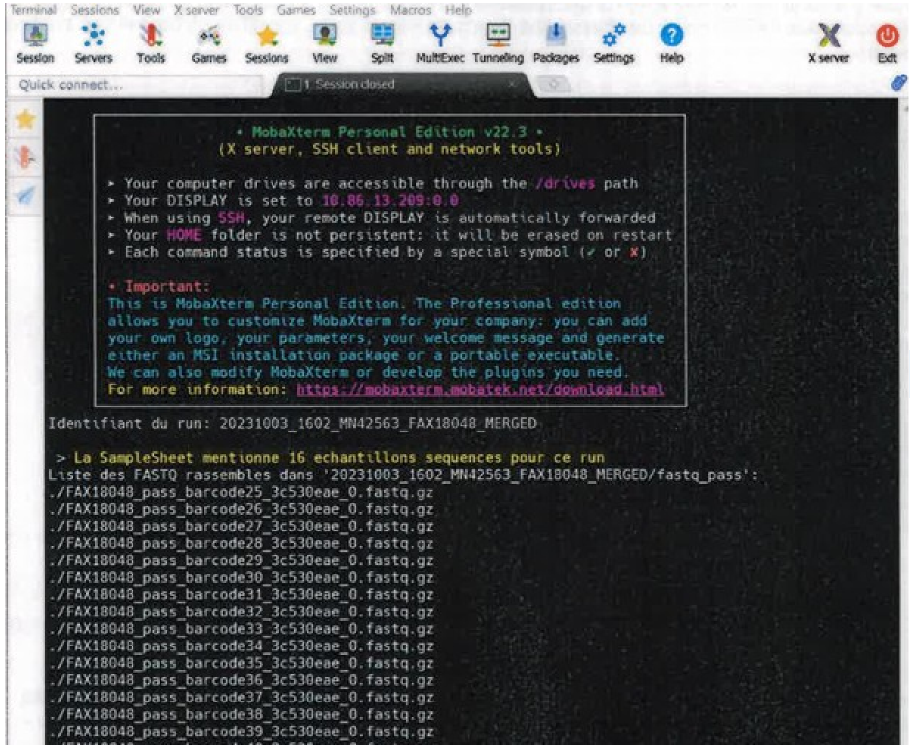


FastQ
Sample XX
Couverture/profondeur insuffisante

🟢 7 🟡 0 🔴 3

FastQ
Sample XX
Couverture/profondeur insuffisante

🟢 3 🟡 3 🔴 3



```
            • MobaXterm Personal Edition v22.3 •
          (X server, SSH client and network tools)

 ➤ Your computer drives are accessible through the /drives path
 ➤ Your DISPLAY is set to 10.86.13.209:0.0
 ➤ When using SSH, your remote DISPLAY is automatically forwarded
 ➤ Your HOME folder is not persistent: it will be erased on restart
 ➤ Each command status is specified by a special symbol (✓ or ✗)

 • Important:
 This is MobaXterm Personal Edition. The Professional edition
 allows you to customize MobaXterm for your company: you can add
 your own logo, your parameters, your welcome message and generate
 either an MSI installation package or a portable executable.
 We can also modify MobaXterm or develop the plugins you need.
 For more information: https://mobaxterm.mobatek.net/download.html

Identifiant du run: 20231003_1602_MN42563_FAX18048_MERGED

 > La SampleSheet mentionne 16 echantillons sequences pour ce run
Liste des FASTQ rassembles dans '20231003_1602_MN42563_FAX18048_MERGED/fastq_pass':
./FAX18048_pass_barcode25_3c530eae_0.fastq.gz
./FAX18048_pass_barcode26_3c530eae_0.fastq.gz
./FAX18048_pass_barcode27_3c530eae_0.fastq.gz
./FAX18048_pass_barcode28_3c530eae_0.fastq.gz
./FAX18048_pass_barcode29_3c530eae_0.fastq.gz
./FAX18048_pass_barcode30_3c530eae_0.fastq.gz
./FAX18048_pass_barcode31_3c530eae_0.fastq.gz
./FAX18048_pass_barcode32_3c530eae_0.fastq.gz
./FAX18048_pass_barcode33_3c530eae_0.fastq.gz
./FAX18048_pass_barcode34_3c530eae_0.fastq.gz
./FAX18048_pass_barcode35_3c530eae_0.fastq.gz
./FAX18048_pass_barcode36_3c530eae_0.fastq.gz
./FAX18048_pass_barcode37_3c530eae_0.fastq.gz
./FAX18048_pass_barcode38_3c530eae_0.fastq.gz
./FAX18048_pass_barcode39_3c530eae_0.fastq.gz
```

Merged FastQ
Sample XX
Couverture/profondeur OK

🟢 9 🟡 0 🔴 0

Typage HLA par séquençage nanopore

Interprétation

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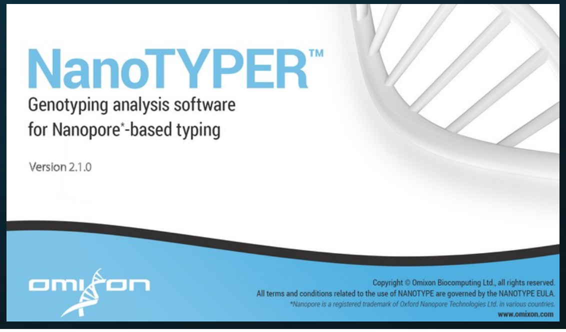

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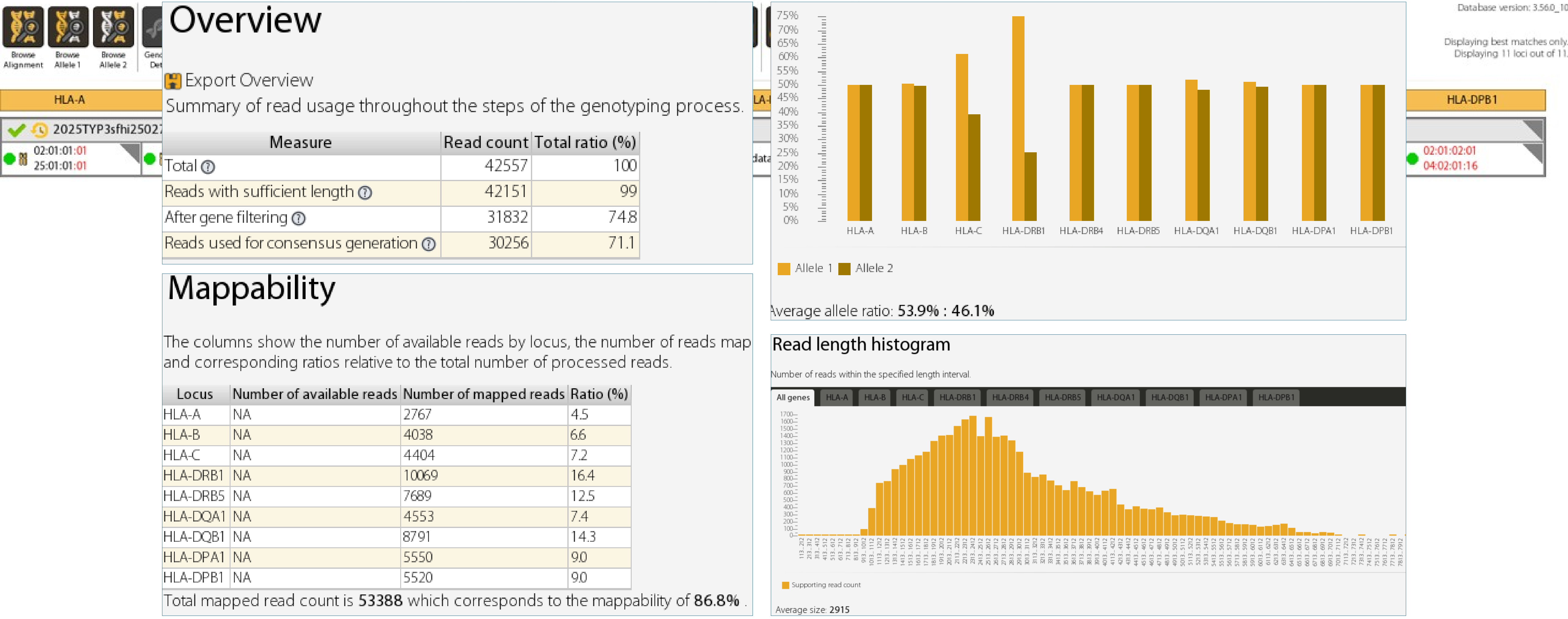
Version 2.1.0



Database version: 3.56.0_10

Displaying best matches only.
Displaying 11 loci out of 11.

Overview

Export Overview

Summary of read usage throughout the steps of the genotyping process.

Measure	Read count	Total ratio (%)
Total ⓘ	42557	100
Reads with sufficient length ⓘ	42151	99
After gene filtering ⓘ	31832	74.8
Reads used for consensus generation ⓘ	30256	71.1

Mappability

The columns show the number of available reads by locus, the number of reads map
and corresponding ratios relative to the total number of processed reads.

Locus	Number of available reads	Number of mapped reads	Ratio (%)
HLA-A	NA	2767	4.5
HLA-B	NA	4038	6.6
HLA-C	NA	4404	7.2
HLA-DRB1	NA	10069	16.4
HLA-DRB5	NA	7689	12.5
HLA-DQA1	NA	4553	7.4
HLA-DQB1	NA	8791	14.3
HLA-DPA1	NA	5550	9.0
HLA-DPB1	NA	5520	9.0

Total mapped read count is **53388** which corresponds to the mappability of **86.8%** .

verage allele ratio: **53.9% : 46.1%**

Read length histogram

Number of reads within the specified length interval.

Average size: 2915

Typage HLA par séquençage nanopore

Interprétation

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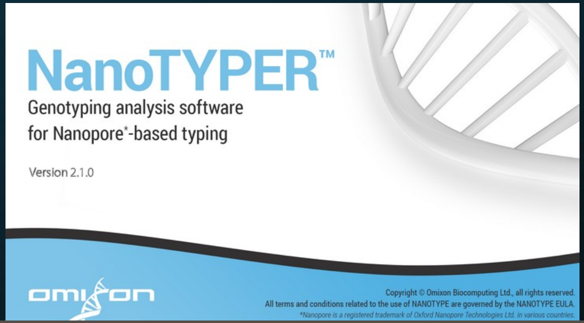
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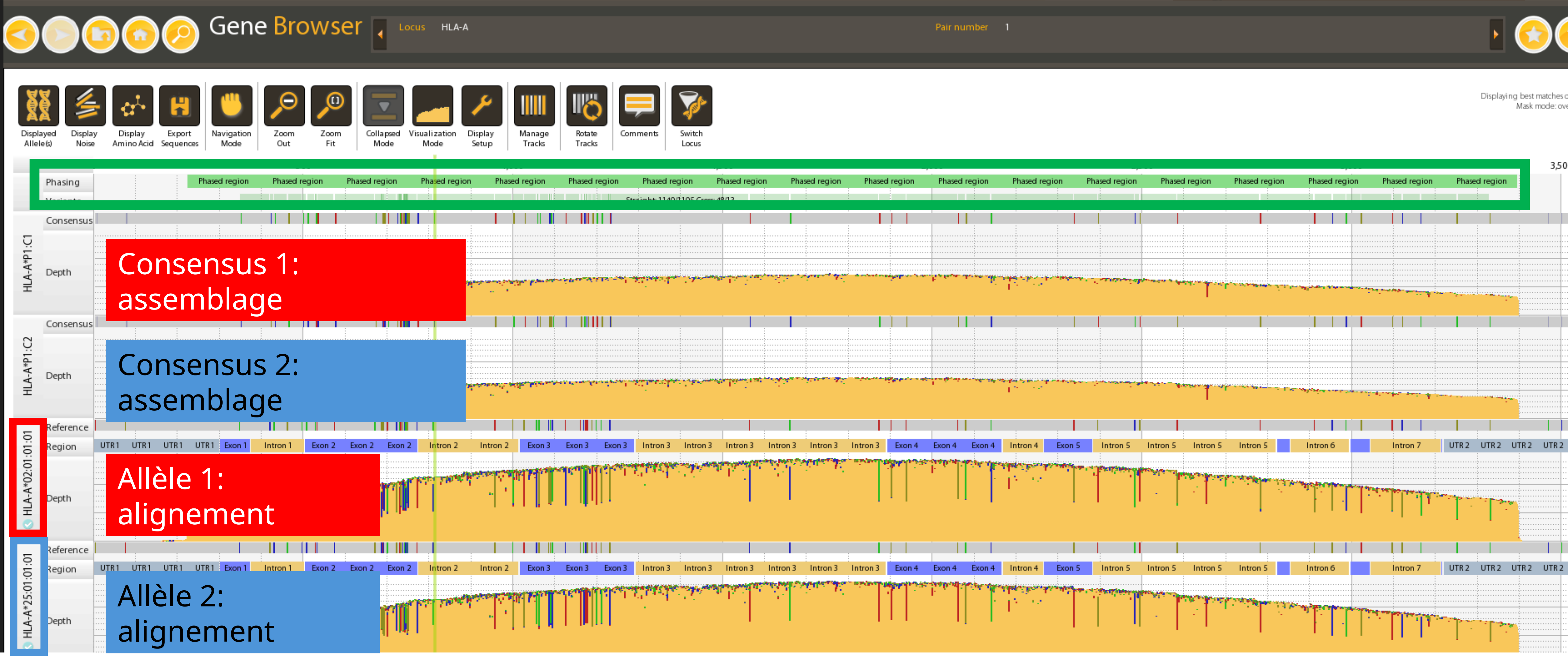

Consensus 1: assemblage

Consensus 2: assemblage

Allèle 1: alignement

Allèle 2: alignement

Typage HLA par séquençage nanopore

Interprétation

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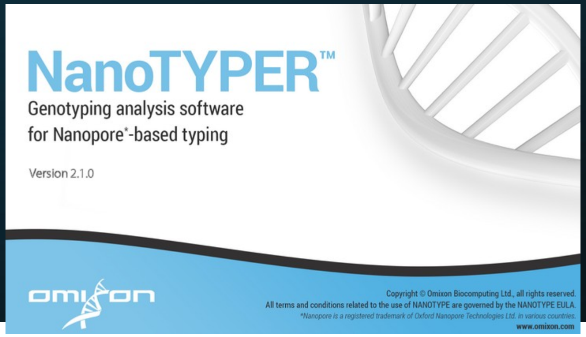

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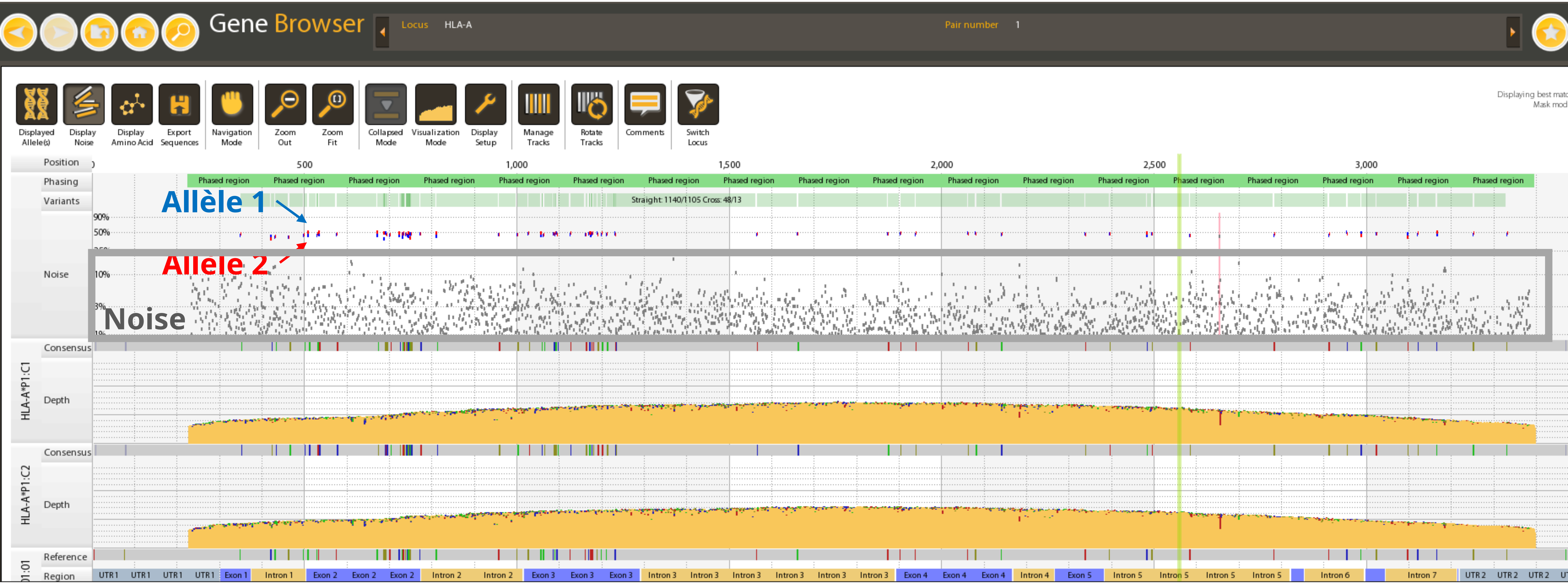

Typage HLA par séquençage nanopore

Interprétation

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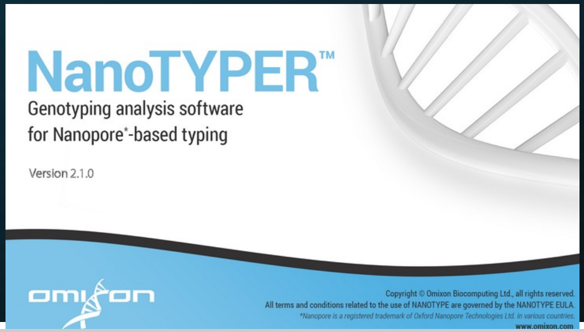
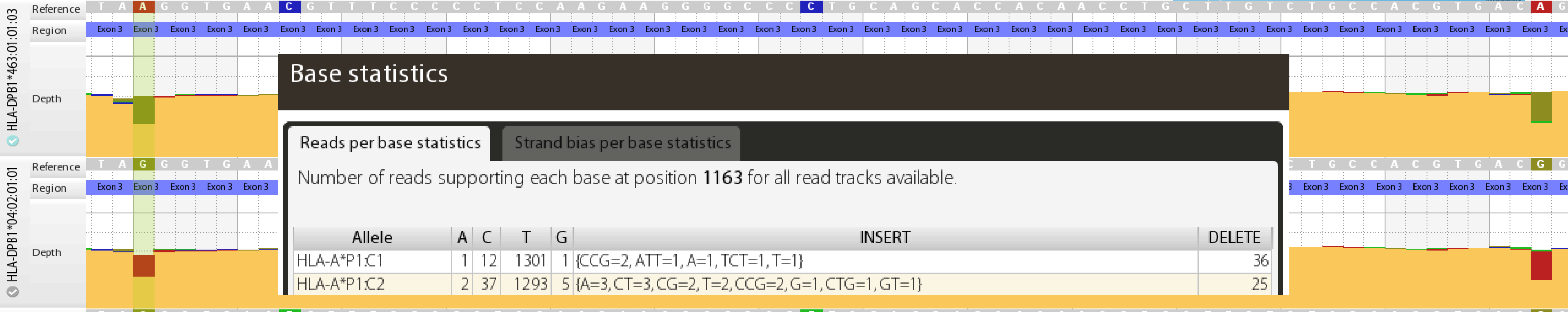
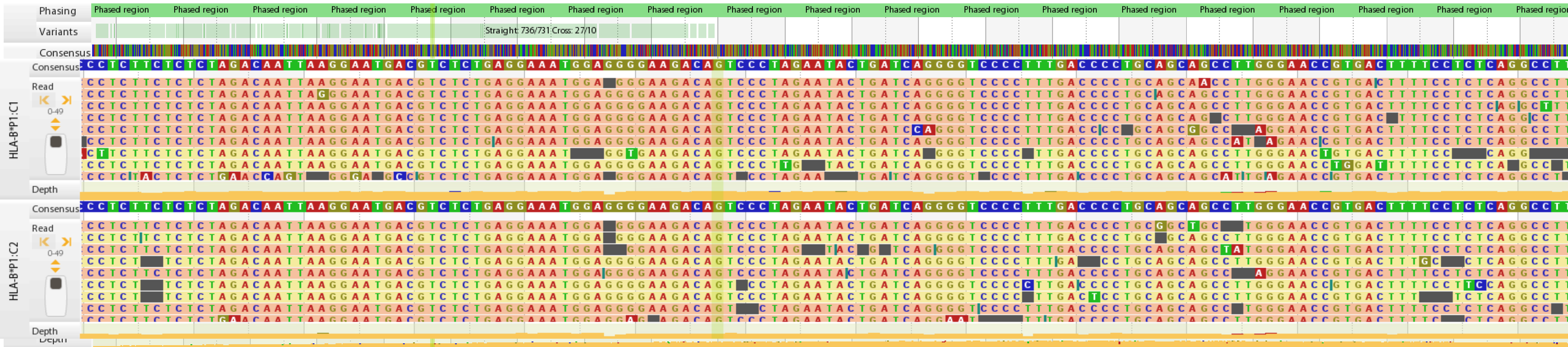




Number of reads supporting each base at position **1163** for all read tracks available.

Allele	A	C	T	G	INSERT	DELETE
HLA-A*P1:C1	1	12	1301	1	{CCG=2, ATT=1, A=1, TCT=1, T=1}	36
HLA-A*P1:C2	2	37	1293	5	{A=3, CT=3, CG=2, T=2, CCG=2, G=1, CTG=1, GT=1}	25



Typage HLA par séquençage nanopore

Interprétation

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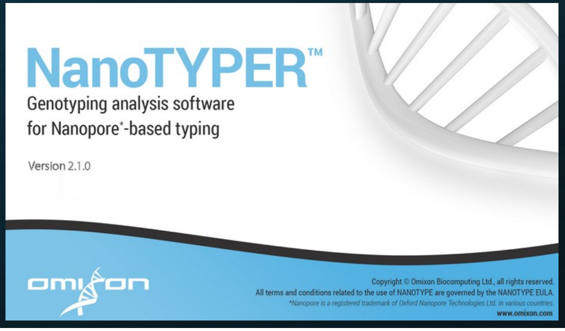

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■ Algorithme

Phasing

Filtre des reads

Comparaison à des groupes d'allèles de la base de données IMGT/HLA

Attribution des reads à l'allèle 1 ou à l'allèle 2

→ Consensus 1

→ Consensus 2

Comparaison à tous les allèles de la base de données IMGT/HLA

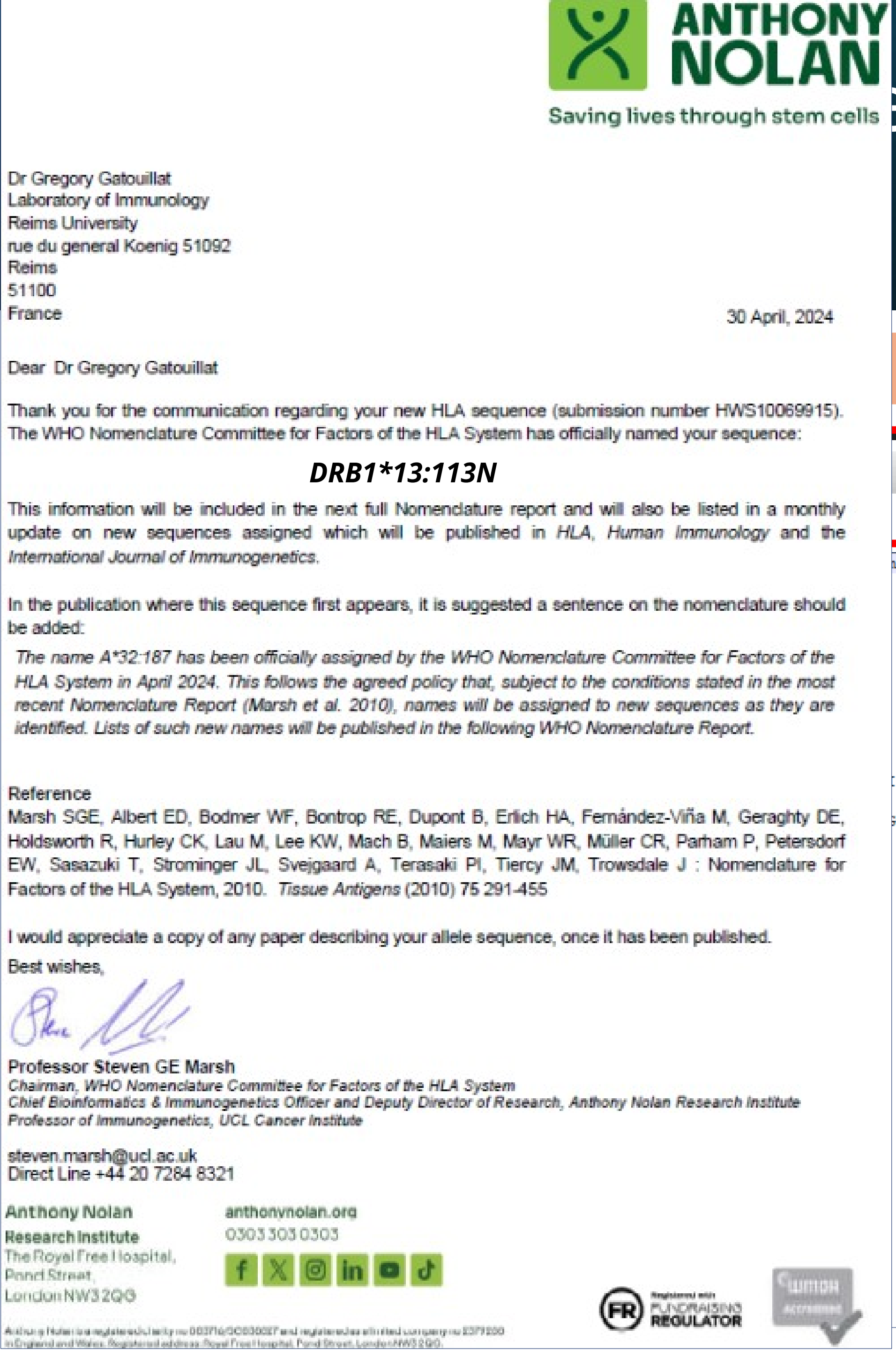
→ Typage

Reprise des reads et comparaison à la référence

Mapping

Alignement des reads à la référence

ençage nanopore



ANTHONY NOLAN
Saving lives through stem cells

Dr Gregory Gatouillat
Laboratory of Immunology
Reims University
rue du general Koenig 51092
Reims
51100
France

30 April, 2024

Dear Dr Gregory Gatouillat

Thank you for the communication regarding your new HLA sequence (submission number HWS10069915). The WHO Nomenclature Committee for Factors of the HLA System has officially named your sequence:

DRB1*13:113N

This information will be included in the next full Nomenclature report and will also be listed in a monthly update on new sequences assigned which will be published in *HLA*, *Human Immunology* and the *International Journal of Immunogenetics.*

In the publication where this sequence first appears, it is suggested a sentence on the nomenclature should be added:

> *The name A*32:187 has been officially assigned by the WHO Nomenclature Committee for Factors of the HLA System in April 2024. This follows the agreed policy that, subject to the conditions stated in the most recent Nomenclature Report (Marsh et al. 2010), names will be assigned to new sequences as they are identified. Lists of such new names will be published in the following WHO Nomenclature Report.*

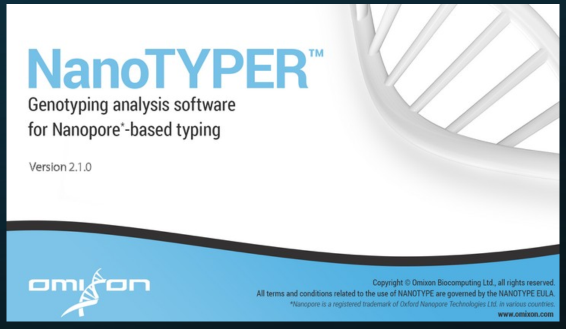
Reference
Marsh SGE, Albert ED, Bodmer WF, Bontrop RE, Dupont B, Erlich HA, Fernández-Viña M, Geraghty DE, Holdsworth R, Hurley CK, Lau M, Lee KW, Mach B, Maiers M, Mayr WR, Müller CR, Parham P, Petersdorf EW, Sasazuki T, Strominger JL, Svejgaard A, Terasaki PI, Tiercy JM, Trowsdale J : Nomenclature for Factors of the HLA System, 2010. *Tissue Antigens* (2010) 75 291-455

I would appreciate a copy of any paper describing your allele sequence, once it has been published.

Best wishes,

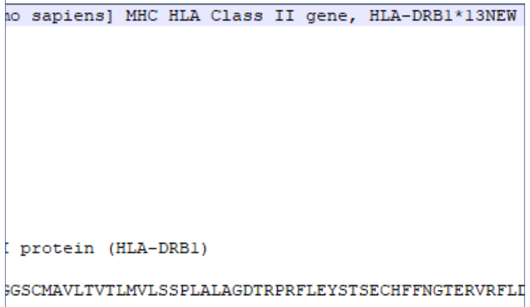
Professor Steven GE Marsh
Chairman, WHO Nomenclature Committee for Factors of the HLA System
Chief Bioinformatics & Immunogenetics Officer and Deputy Director of Research, Anthony Nolan Research Institute
Professor of Immunogenetics, UCL Cancer Institute

steven.marsh@ucl.ac.uk
Direct Line +44 20 7284 8321



allèle

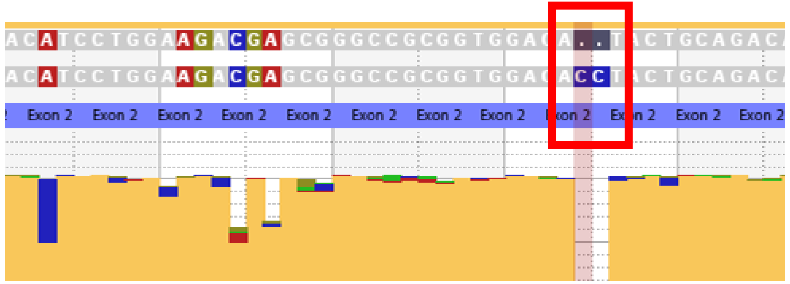
Region	Position	Type	Reference
ex2	217	DELETE	CC



```
o sapiens] MHC HLA Class II gene, HLA-DRB1*13NEW

 protein (HLA-DRB1)

GSCMAVLTVTLMVLSSPLALAGDTRPRFLEYSTSECHFFNGTERVRFLD
```



ENA European Nucleotide Archive
https://www.ebi.ac.uk/ena/browser/home

GenBank
https://submit.ncbi.nlm.nih.gov/about/bankit/

IPD-IMGT/HLA

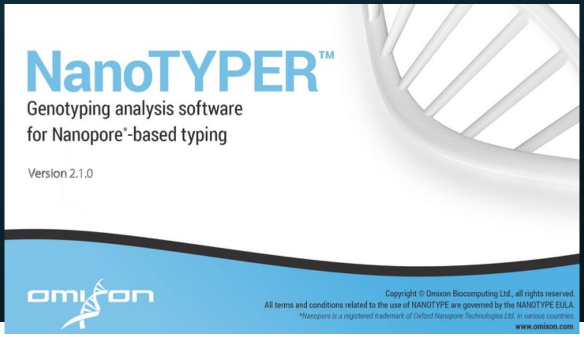
Submit a sequence to the IPD-IMGT/HLA Database ❯
https://www.ebi.ac.uk/ipd/imgt/hla/submission/

Table annotations

Typage HLA par séquençage nanopore

Interprétation

NanotyperTM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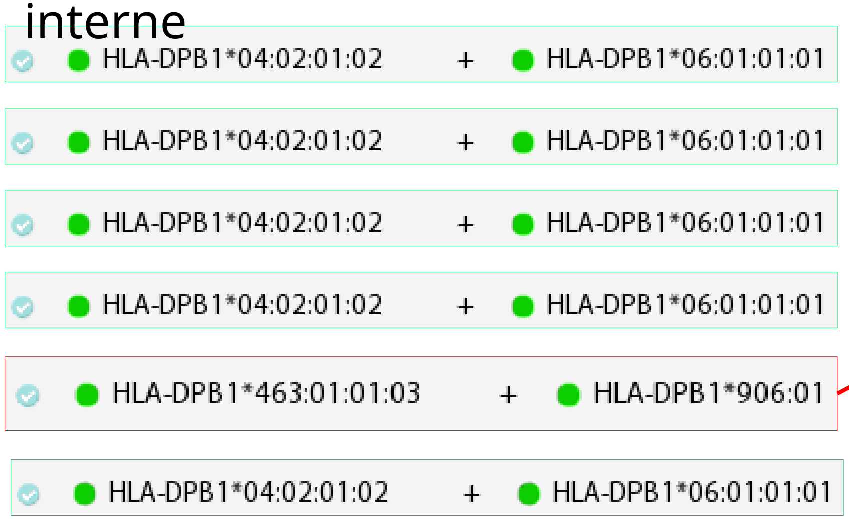
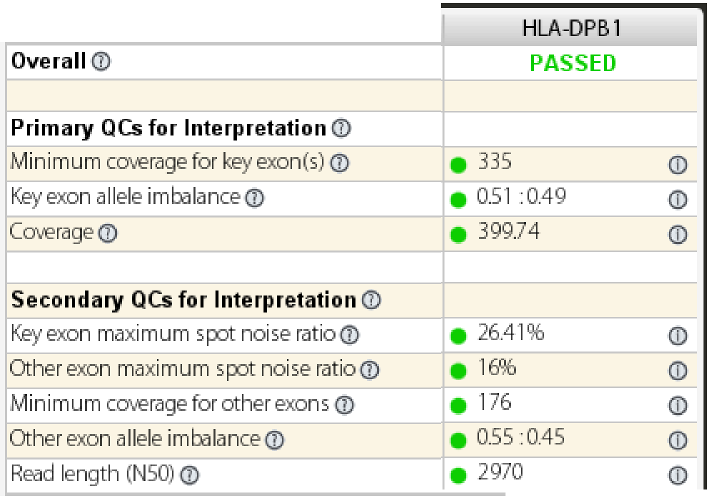
Version 2.1.0

Echantillon contrôle interne

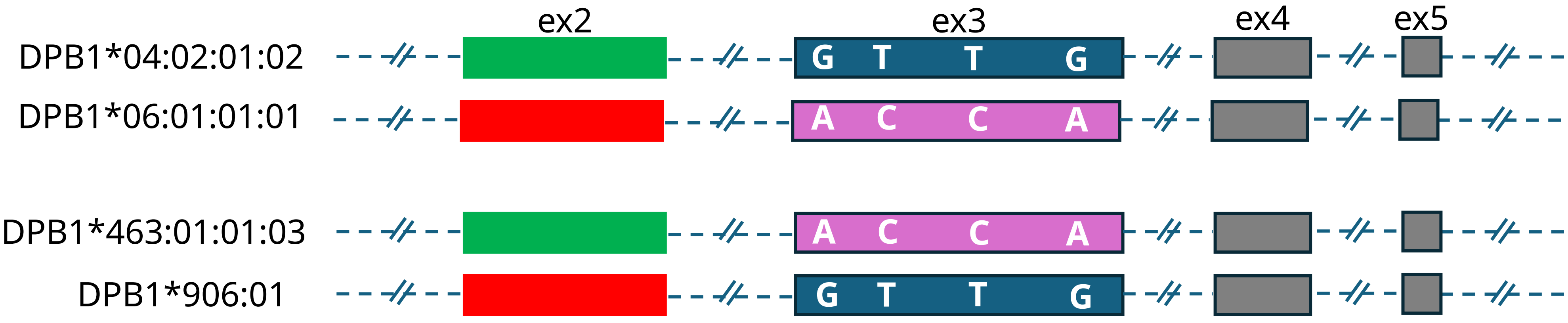

✓ ● HLA-DPB1*04:02:01:02 + ● HLA-DPB1*06:01:01:01
✓ ● HLA-DPB1*04:02:01:02 + ● HLA-DPB1*06:01:01:01
✓ ● HLA-DPB1*04:02:01:02 + ● HLA-DPB1*06:01:01:01
✓ ● HLA-DPB1*04:02:01:02 + ● HLA-DPB1*06:01:01:01
✓ ● HLA-DPB1*463:01:01:03 + ● HLA-DPB1*906:01
✓ ● HLA-DPB1*04:02:01:02 + ● HLA-DPB1*06:01:01:01

?



	HLA-DPB1
Overall ⓘ	**PASSED**
Primary QCs for Interpretation ⓘ	
Minimum coverage for key exon(s) ⓘ	● 335 ⓘ
Key exon allele imbalance ⓘ	● 0.51 : 0.49 ⓘ
Coverage ⓘ	● 399.74 ⓘ
Secondary QCs for Interpretation ⓘ	
Key exon maximum spot noise ratio ⓘ	● 26.41% ⓘ
Other exon maximum spot noise ratio ⓘ	● 16% ⓘ
Minimum coverage for other exons ⓘ	● 176 ⓘ
Other exon allele imbalance ⓘ	● 0.55 : 0.45 ⓘ
Read length (N50) ⓘ	● 2970 ⓘ

Déséquilibre du ratio reads forward/reverse ?



?

De la compatibilité antigénique….. …à la Compatibilité épitopique

RECEVEUR	DONNEUR
A2, A24	A2, A24
B7, B57	B8, B44
Cw7, Cw8	Cw4, Cw7
DR1, DR15	DR10, DR11
DQ5, DQ6	DQ6, DQ7
DP2, DP4	DP2, DP4

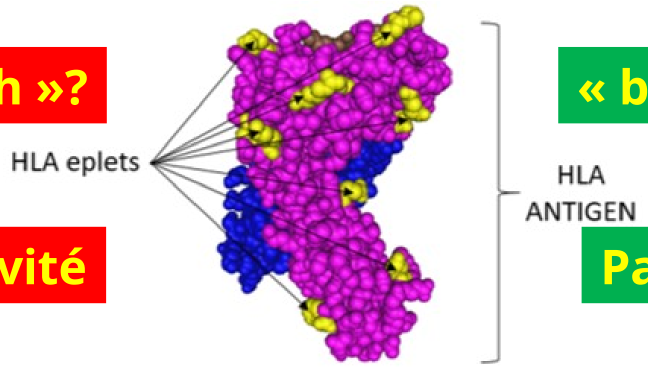
6 différences

RECEVEUR	DONNEUR
A*02:01, A*24:03	A*02:01, A*24:03
B*07:05, B*57:01	B*08:01, B*44:02
C*07:01, C*08:01	C*04:01, C*07:01
DRB1*01:01, DRB1*15:01	DRB1*10:01, DRB1*11:01
DQB1*05:02, DQB1*06:02	DQB1*03:01, DQB1*06:0[illegible]
DPB1*02:01, DPB1*04:02	DPB1*02:01, DPB1*04:02

Y-a-t-il des différences?

« mauvais mismatch »?

Risque d'allo-réactivité



Cn3D

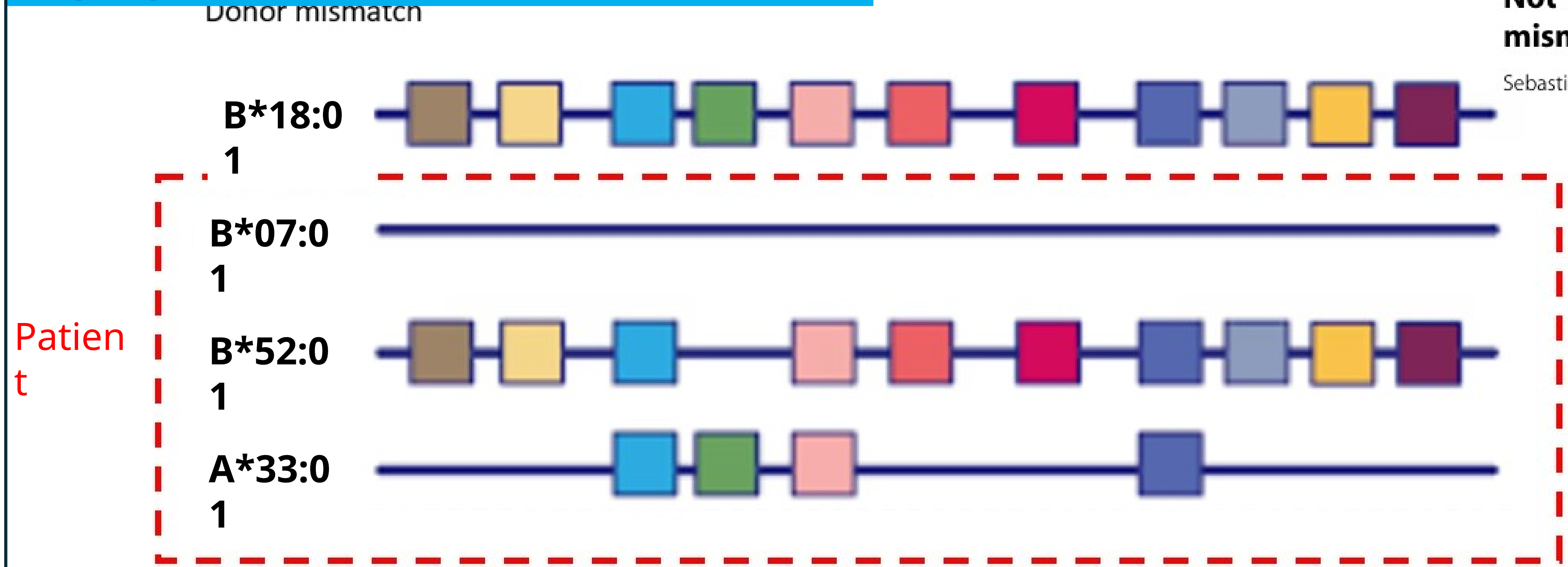
« bon mismatch »?

Pas de réactivité

De la compatibilité antigénique….. …à la Compatibilité épitopique

Molécule HLA = combinaison unique d'épitopes

Un épitope peut être commun à plusieurs molécules HLA



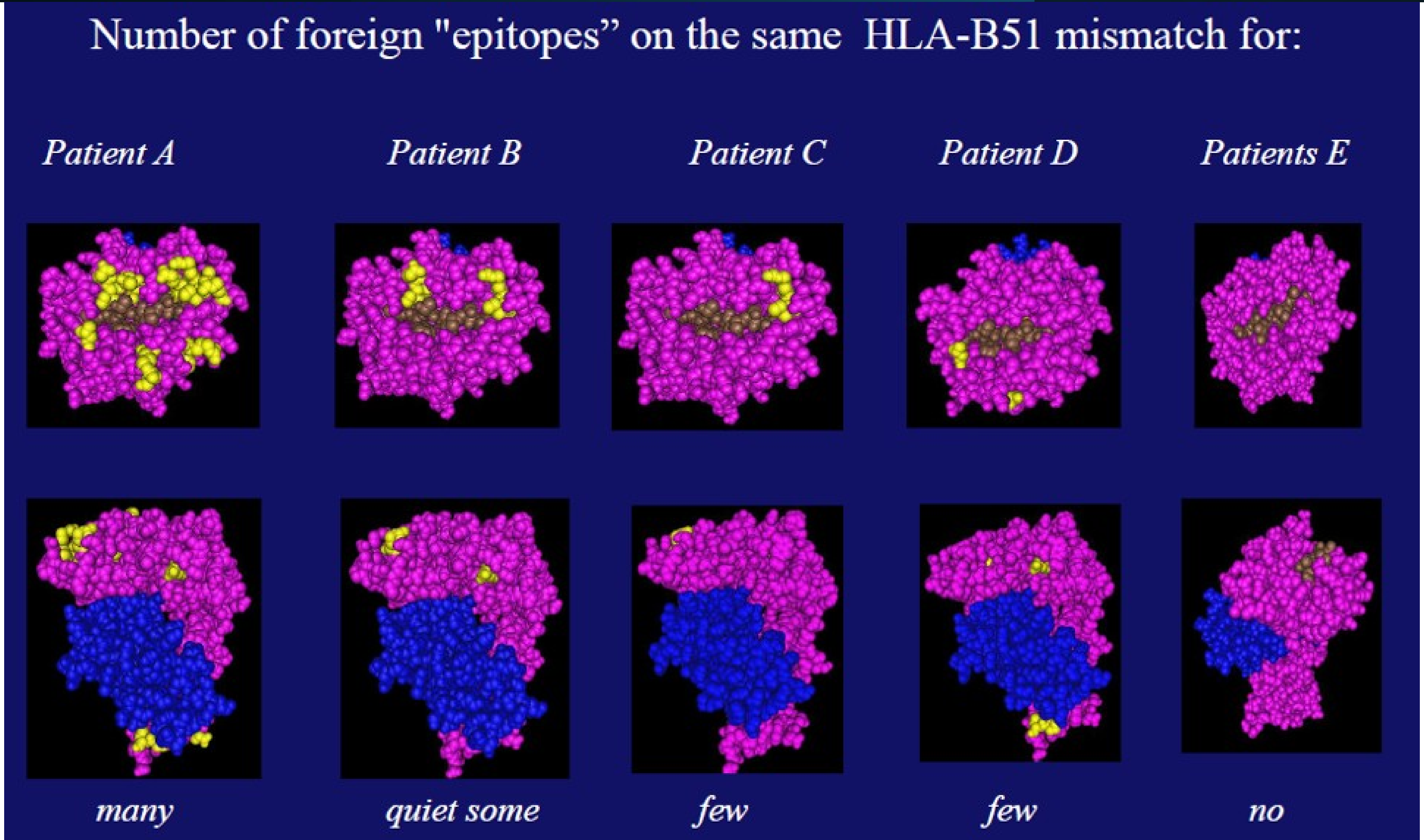
Not all HLA epitope mismatches are equal

Sebastiaan Heidt[1] and Frans H.J. Claas[1]

B*18:01 = « Bon mismatch »: Aucune différence aux yeux du système immunitaire du patient

→ Tous les mismatches ne sont pas égaux!

De la compatibilité antigénique….. **…à la Compatibilité épitopique**



Number of foreign "epitopes" on the same HLA-B51 mismatch for:

Patient A	*Patient B*	*Patient C*	*Patient D*	*Patients E*
many	*quiet some*	*few*	*few*	*no*

Pr F.J. Class

Séquençage Long reads

→ Rend possible le typage HLA haute résolution des donneurs d’organes 24h/24

Changement de concept de compatibilité donneur/receveur en transplantation

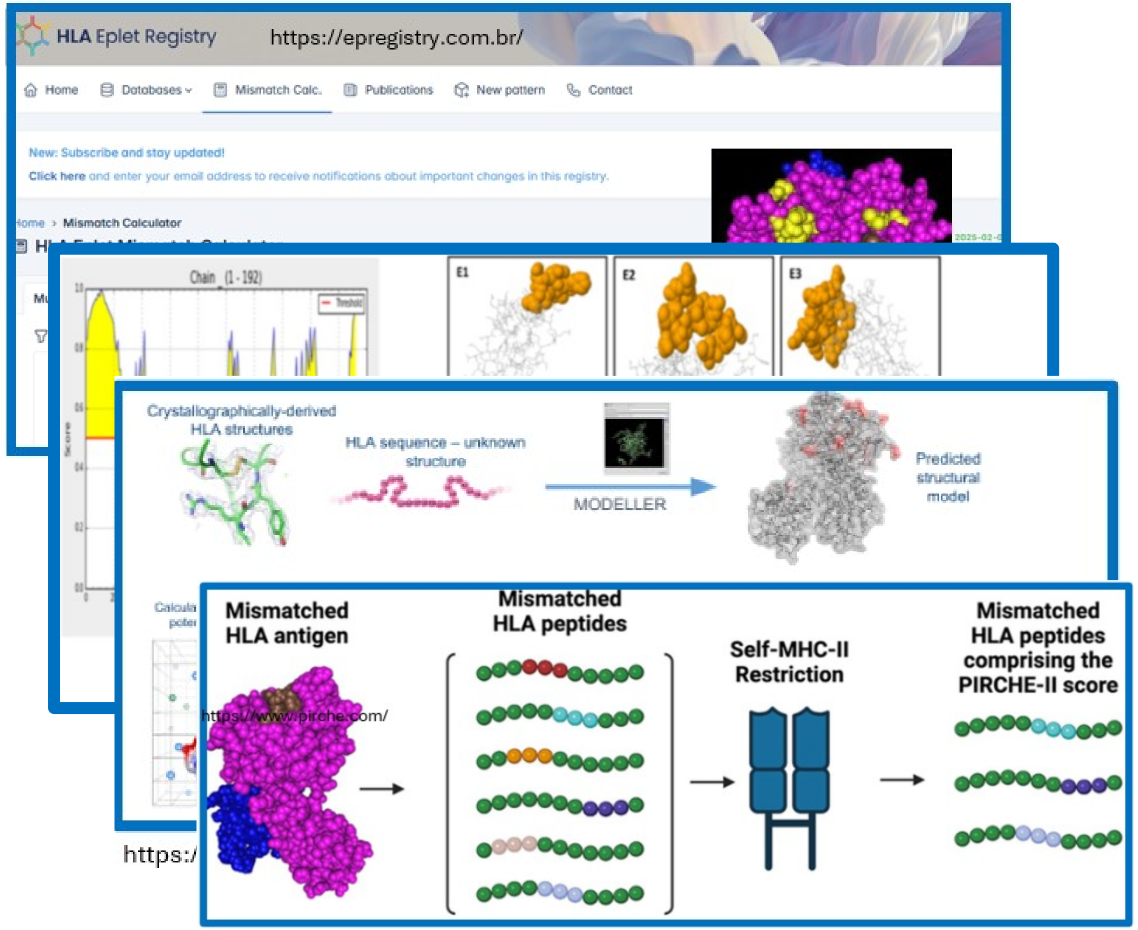
‣ Compatibilité épitopique

HLA MATCHMAKER
Prédiction d’une allo-réactivité
des lymphocytes B

EMS-3D
Mismatch électrostatique des épitopes

ELLIPRO
Exposition/ immunogénicité des épitopes

PIRCHE
Prédiction d’une allo-réactivité
des lymphocytes T



HLA Eplet Registry https://epregistry.com.br/

New: Subscribe and stay updated!

Click here and enter your email address to receive notifications about important changes in this registry.

Home › Mismatch Calculator

Mieux prédire un allo-réactivité → Mieux apparier les donneurs/receveurs → Prolonger la durée de vie des greffons

Merci