Compatibilità e Alleli NEW

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IRCCS Ospedale Pediatrico Bambino Gesù

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OSPEDALE PEDIATRICO

Un alto livello di compatibilità diminuisce il rischio di aGvHD e di mortalità

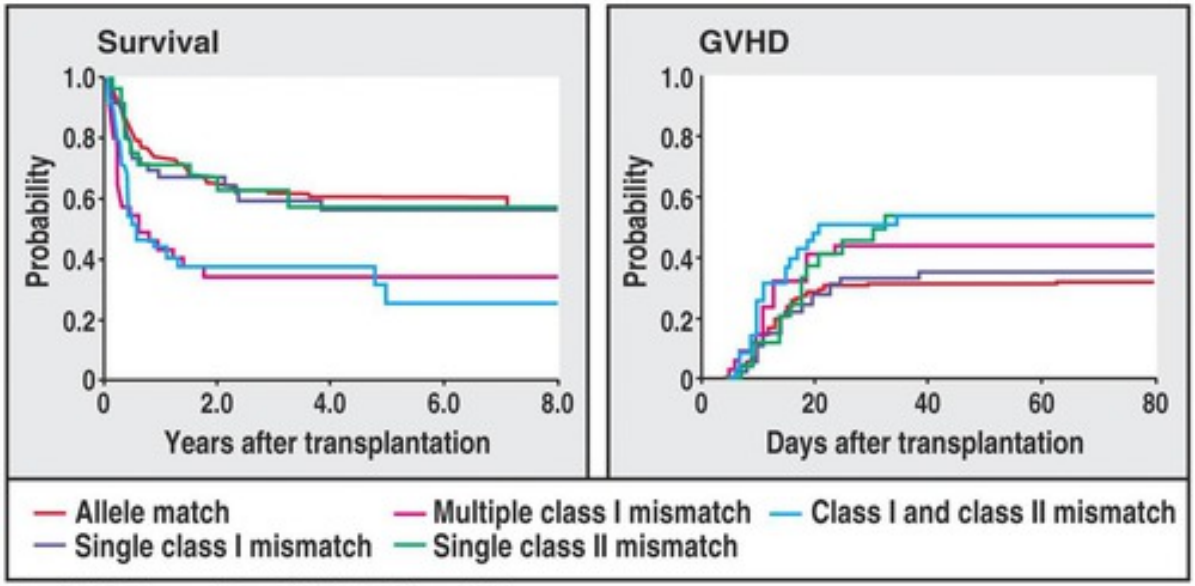

Figure 12-34 The Immune System, 2/e (© Garland Science 2005)

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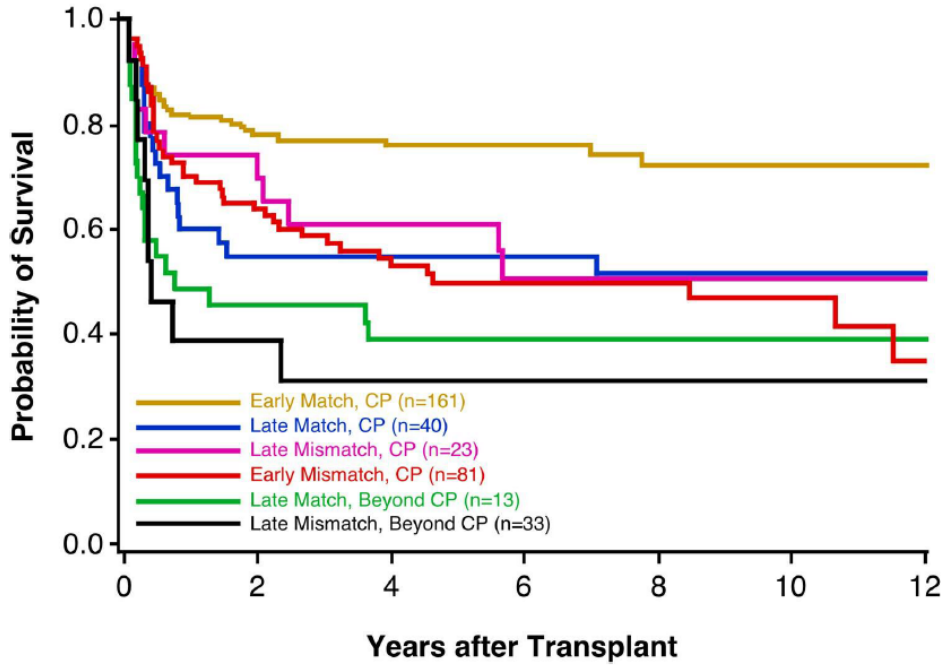
La possibilità di trovare un donatore compatibile entro breve tempo è cruciale per il successo di un trapianto allogenico di CSE



CML patients according to disease phase and presence or absence of a single HLA mismatch. EW Petersdorf Bllod, 2004Nov 1;104(9):2976-80.

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Categoria: Paziente

		Codice analisi	Data Analisi	
HLA-A	*01:NEW, *02:01;	1	30/08/2017	1,7
HLA-B	*08:01, *51:01P;	1	30/08/2017	1,7
HLA-C	*07:01P, *14:02;	1	25/08/2017	1,7
HLA-DRB1	*01:01, *03:01;	1	25/08/2017	1,7
HLA-DRB3	*01:01,	4	14/09/2017	
HLA-DRB4				
HLA-DRB5				
HLA-DQA1	*01:01P, *05:01;	1	15/09/2017	
HLA-DQB1	*02:01P, *05:01P;	1	25/08/2017	1
HLA-DPB1	*04:01P,	1	12/09/2017	

Step necessari per la determinazione e inserimento nel database di un new allele

- Accertarsi che sia nuovo
- Isolare la sequenza
- Inserimento in Bankit
- Inserimento in IMGT

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Step necessari per la determinazione e inserimento nel database di un new allele

- Accertarsi che sia nuovo
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- Inserimento in Bankit
- Inserimento in IMGT

ESEMPIO

2052-16 HLA-A*03 NEW

EX 2

GCTCCCACTCCATGAGGTATTTCTTCACATCCGTGTCCCGGCCCGGCCGCGGGGAGCCCCGCTTCATCGCCGTGGGCTA
CGTGGACGACACGCAGTTCGTGCGGTTCGACAGCGACGCCGCGAGCCAGAGGATGGAGCCGCGGGCGCCGTGAATAG
AGCAGGAGGGGCCGGAGTATTGGGACCAGGAGACACGGAATGTGAAGGCCCAGTCACAGACTGACCGAGTGGACCTG
GGGACCCTGCGCGGCTACTACAACCAGAGCGAGGCCG

INTR 2

NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNN

EX 3

GTTCTCACACCATCCAGATAATGTATGGCTGCGACGTGGGGTCGGACGGGCGCTTCCTCCGCGGGTACCGGCAGGACG
CCTACGACGGCAAGGATTACATCGCCCTGAACGAGGACCTGCGCTCTTGGACCGCGGCGGACATGGCGGCTCAGATCA
CCAAGCGCAAGTGGGAGGCGGCCCATGTGGCGGAGCAGCAGAGAGCCTACCTGGATGGCACGTGCGTGGAGTGGCTC
CGCAGATACCTGGAGAACGGGAAGGAGACGCTGCAGCGCACGG

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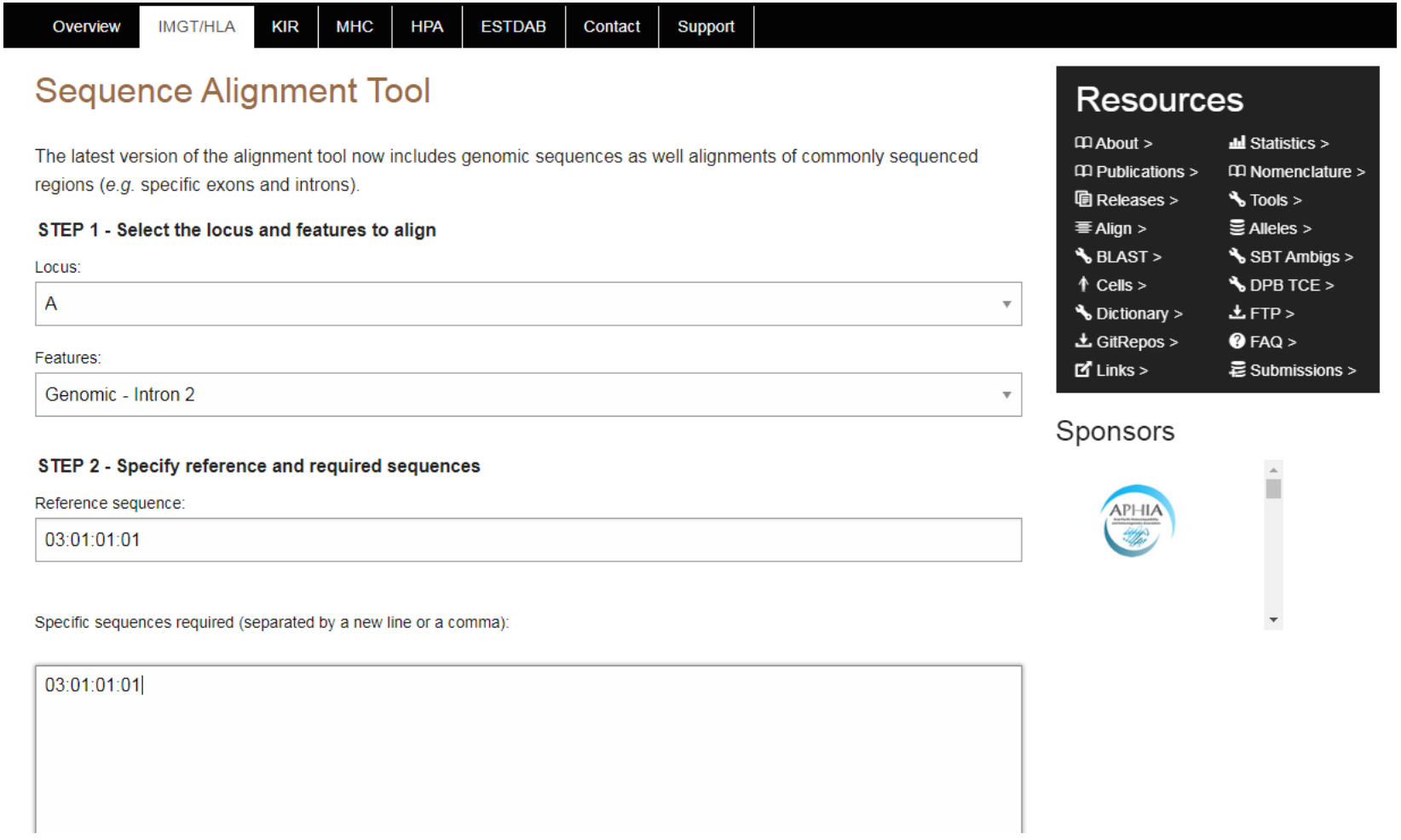
ESEMPIO



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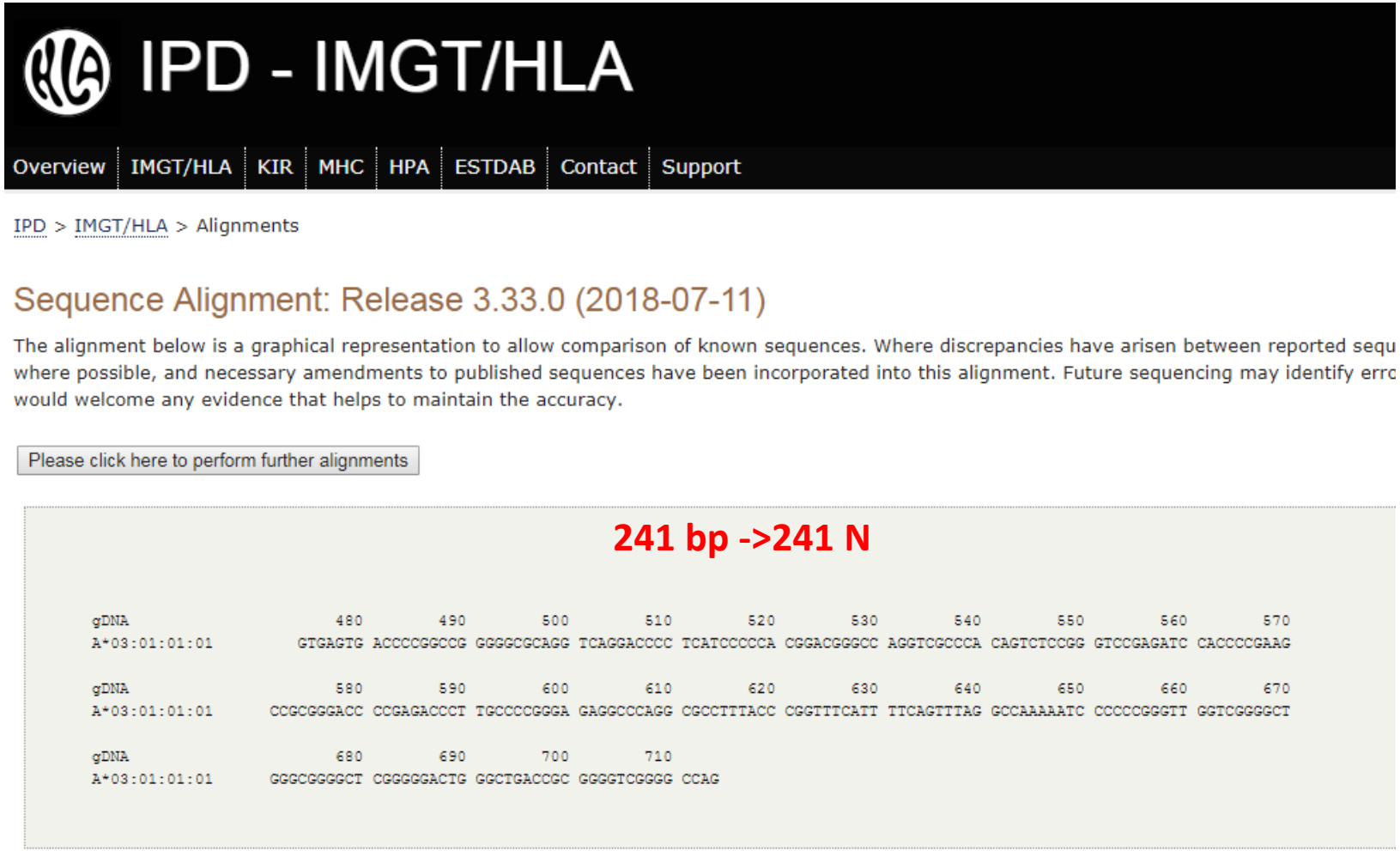

ESEMPIO

INTR 3

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NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN

EX 4

ACCCCCCCAAGACACATATGACCCACCACCCCATCTCTGACCATGAGGCCACCCTGAGGTGCTGGGCCCTGGGCTTCTA
CCCTGCGGAGATCACACTGACCTGGCAGCGGGATGGGGAGGACCAGACCCAGGACACGGAGCTCGTGGAGACCAGGC
CTGCAGGGGATGGAACCTTCCAGAAGTGGGCGGCTGTGGTGGTGCCTTCTGGAGAGGAGCAGAGATACACCTGCCATG
TGCAGCATGAGGGTCTGCCCAAGCCCCTCACCCTGAGATGGG

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OSPEDALE PEDIATRICO

ESEMPIO

```
EX 2:        270                 1 -  270 (EX2)
INTR2:       241               512 -  787 (EX3)
EX 3:        276              1366 - 1641 (EX4)
INTR3:       578
EX 4:        276
TOT:        1641
```

```
MMpos: 225  Base iniziale G ➡ A  Base mutata
```

AA: cod 51 cod iniziale **TGG** ➡ **TGA** cod mutato AA iniziale **W** ➡ **STOP** AA mutato

FASTA CON INTRONE

```
GCTCCCACTCCATGAGGTATTTCTTCACATCCGTGTCCCGGCCCGGCCGCGGGGAGCCCCGCTTCATCGCCGTGGGCTA
CGTGGACGACACGCAGTTCGTGCGGTTCGACAGCGACGCCGCGAGCCAGAGGATGGAGCCGCGGGCGCCGTGAATAG
AGCAGGAGGGGCCGGAGTATTGGGACCAGGAGACACGGAATGTGAAGGCCCAGTCACAGACTGACCGAGTGGACCTG
GGGACCCTGCGCGGCTACTACAACCAGAGCGAGGCCGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
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NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNGTTCTCACACCATCCAGATAAT
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ACCAGGCCTGCAGGGGATGGAACCTTCCAGAAGTGGGCGGCTGTGGTGGTGCCTTCTGGAGAGGAGCAGAGATACACC
TGCCATGTGCAGCATGAGGGTCTGCCCAAGCCCCTCACCCTGAGATGGG
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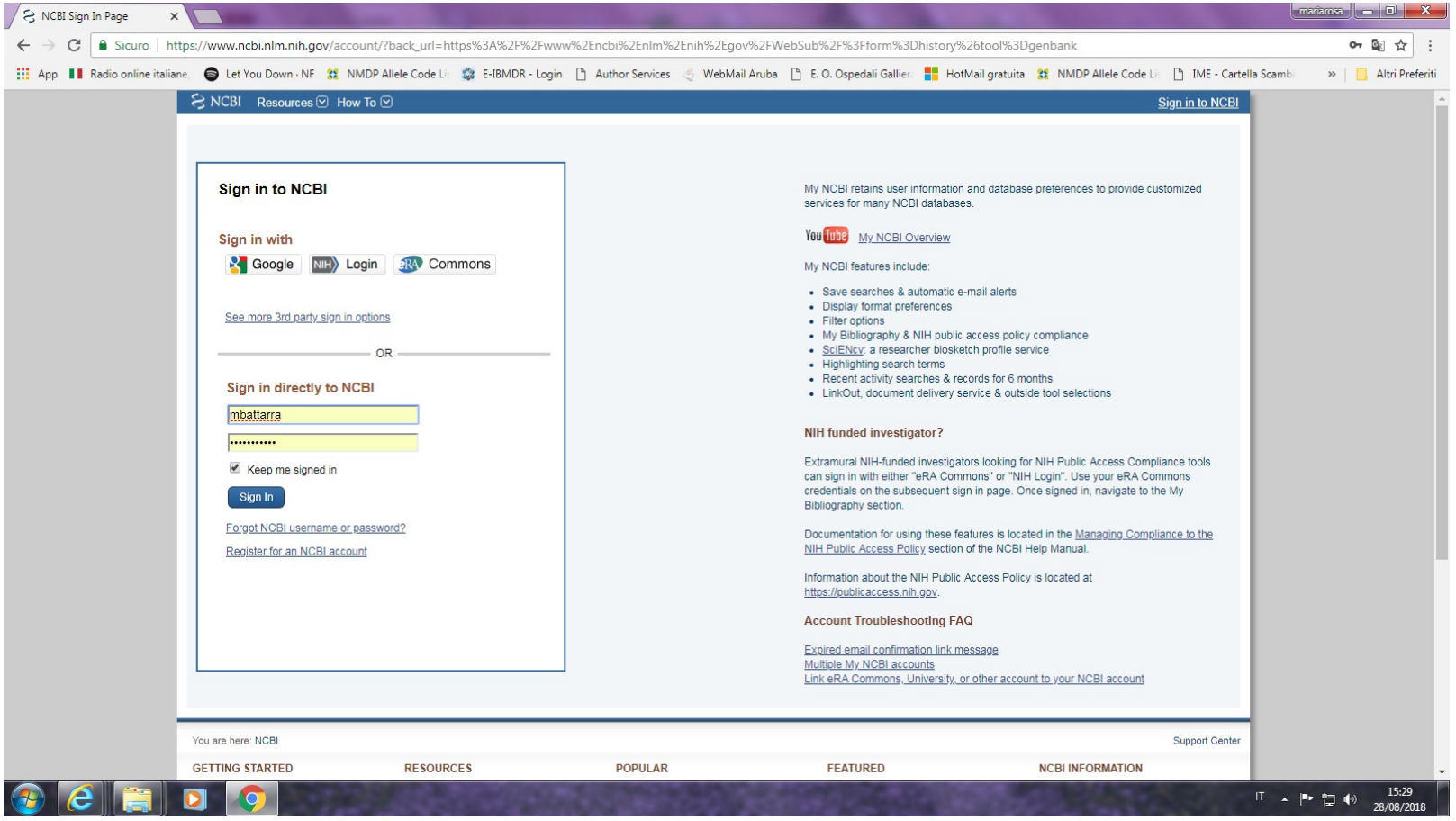
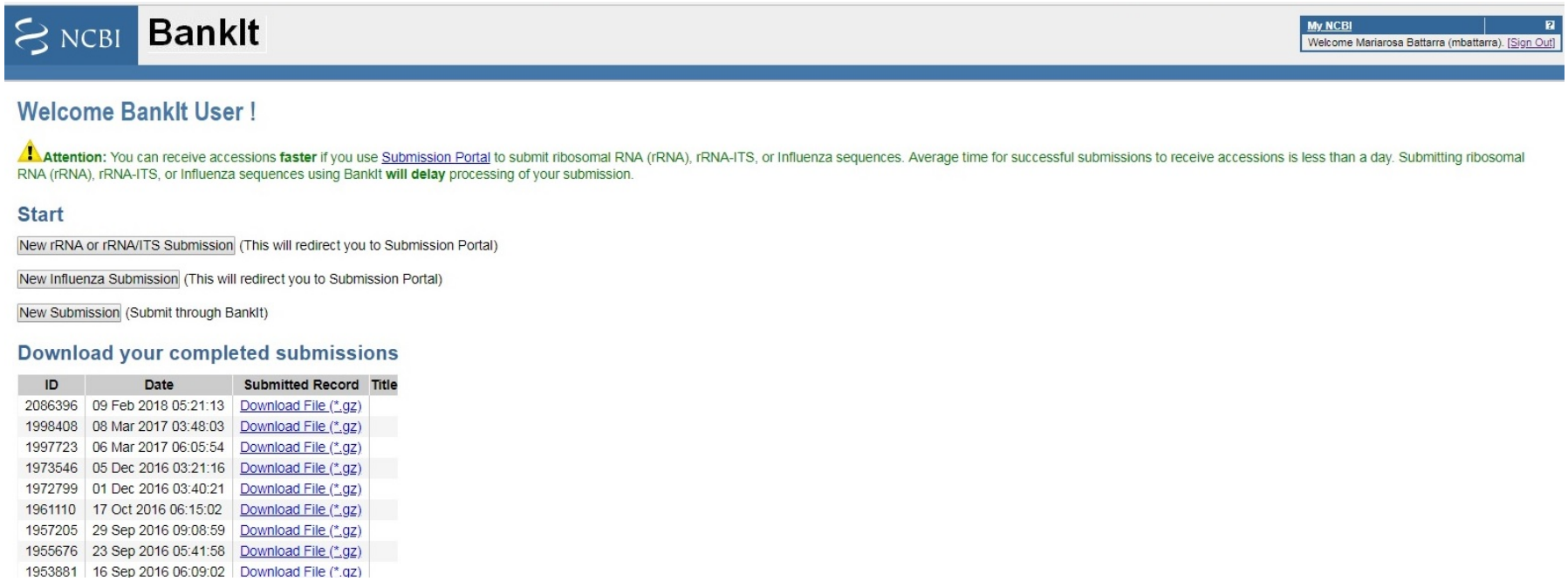
Inserimento in Bankit

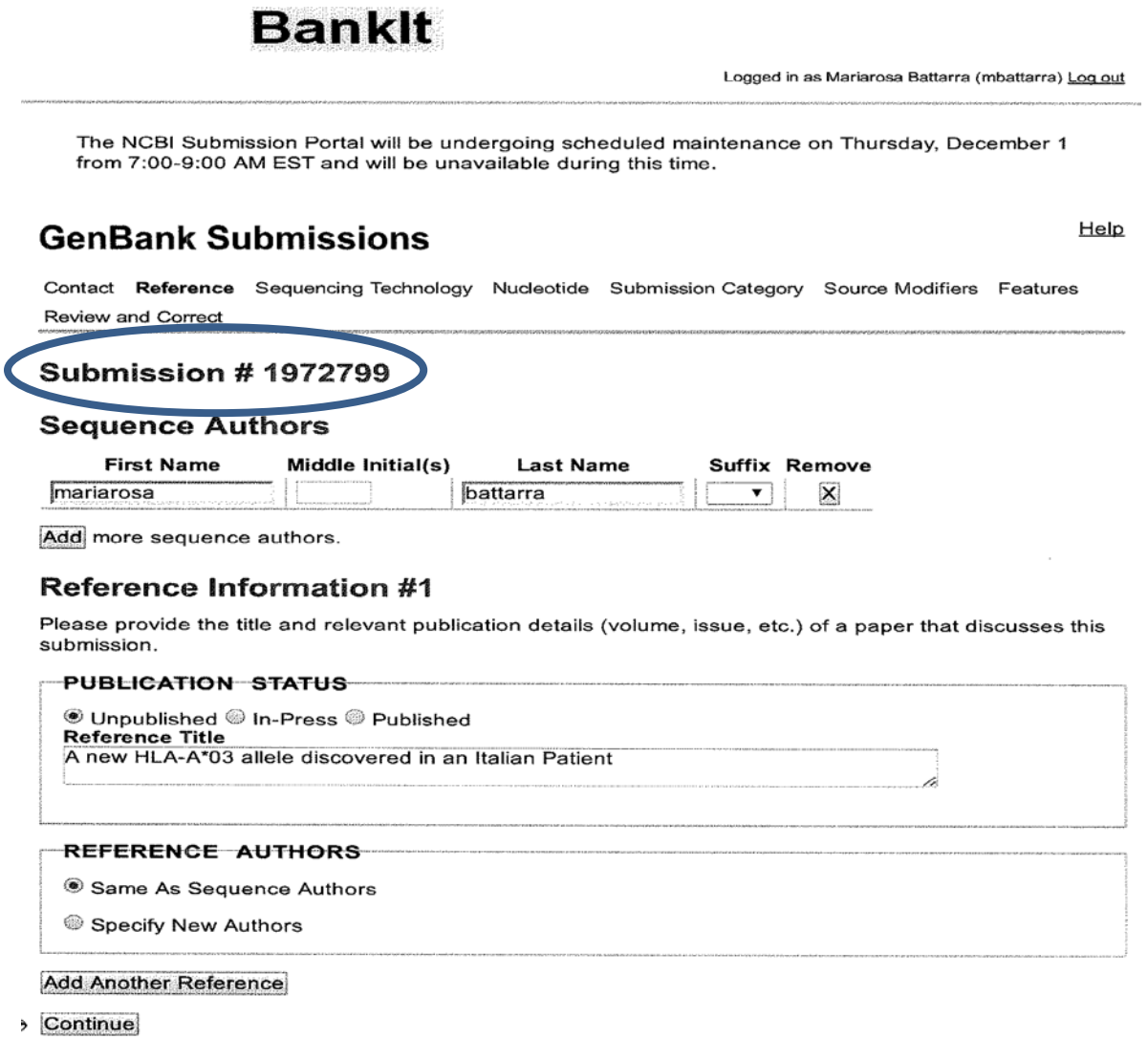
https://www.ncbi.nlm.nih.gov/account/



Inserimento in Bankit



Inserimento in Bankit



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The NCBI Submission Portal will be undergoing scheduled maintenance on Thursday, December 1 from 7:00-9:00 AM EST and will be unavailable during this time.

GenBank Submissions

Help

Contact **Reference** Sequencing Technology Nucleotide Submission Category Source Modifiers Features Review and Correct

Submission # 1972799

Sequence Authors

First Name	Middle Initial(s)	Last Name	Suffix	Remove
mariarosa		battarra		X

Add more sequence authors.

Reference Information #1

Please provide the title and relevant publication details (volume, issue, etc.) of a paper that discusses this submission.

PUBLICATION STATUS

Unpublished In-Press Published

Reference Title

A new HLA-A*03 allele discovered in an Italian Patient

REFERENCE AUTHORS

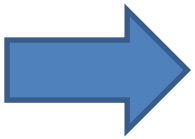
Same As Sequence Authors

Specify New Authors

Add Another Reference

Continue

Inserimento in Bankit



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GenBank Submissions Help

Contact Reference Sequencing Technology **Nucleotide** Submission Category Source Modifiers Features Review and Correct

Submission # 1972799

Submission Release Date

When may we release your sequence record?
Immediately After Processing
Release Date: Date format is 'DD-Mon-YYYY' (example: 20-Feb-2004)

16S rRNA submissions

Are the sequences in this submission ONLY 16S ribosomal RNA data? Yes No

Sequence(s) and Definition Line(s)

Molecule Type: genomic DNA
Topology: Linear
Genomic completeness: Unknown

Nucleotide Sequence(s) and Definition Lines

Sequences must be entered in the FASTA format, whether you are submitting a single sequence or multiple sequences.
Definition Lines which are used to describe each sequence, should be included in the FASTA format.

How many nucleotide sequences do you intend to send in this submission? 1

Paste Sequence(s) → INSERIRE FASTA CON N

NNACCCCCCCCAAGACACATATGACCCACCACCCCCATCTCTGACCATGAGGCCACC
CTGAGGTGCTGGGCCCTGGGCTTCTACCCTGCGGAGATCACACTGACCTGGCA
GCGGGATGGGGAGGACCAGACCCAGGACACGGAGCTCGTGGAGACCAGGCCT
GCAGGGGATGGAACCTTCCAGAAGTGGGCGGCTGTGGTGGTGCCTTCTGGAGA
GGAGCAGAGATACACCTGCCATGTGCAGCATGAGGGTCTGCCCAAGCCCCTCAC
CCTGAGATGGG

Example FASTA nucleotide format:

>Seq1 [organism=genus species] Definition Line for Seq1
aaccgatatagagagagga....

https://www.ncbi.nlm.nih.gov/WebSub/?form=orgseq&sid=1972799&tool=genbank

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OSPEDALE PEDIATRICO

ESEMPIO

```
EX 2:        270               1 -  270 (EX2)
INTR2:       241             512 -  787 (EX3)
EX 3:        276            1366 - 1641 (EX4)
INTR3:       578
EX 4:        276
TOT:        1641
```

MMpos: 225 Base iniziale G → A Base mutata
AA: cod 51 cod iniziale **TGG** → **TGA** cod mutato AA iniziale **W** → **STOP** AA mutato

FASTA CON INTRONE

```
GCTCCCACTCCATGAGGTATTTCTTCACATCCGTGTCCCGGCCCGGCCGCGGGGAGCCCCGCTTCATCGCCGTGGGCTA
CGTGGACGACACGCAGTTCGTGCGGTTCGACAGCGACGCCGCGAGCCAGAGGATGGAGCCGCGGGCGCCGTGAATAG
AGCAGGAGGGGCCGGAGTATTGGGACCAGGAGACACGGAATGTGAAGGCCCAGTCACAGACTGACCGAGTGGACCTG
GGGACCCTGCGCGGCTACTACAACCAGAGCGAGGCCGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNGTTCTCACACCATCCAGATAAT
GTATGGCTGCGACGTGGGGTCGGACGGGCGCTTCCTCCGCGGGTACCGGCAGGACGCCTACGACGGCAAGGATTACAT
CGCCCTGAACGAGGACCTGCGCTCTTGGACCGCGGCGGACATGGCGGCTCAGATCACCAAGCGCAAGTGGGAGGCGGC
CCATGTGGCGGAGCAGCAGAGAGCCTACCTGGATGGCACGTGCGTGGAGTGGCTCCGCAGATACCTGGAGAACGGGA
AGGAGACGCTGCAGCGCACGGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
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NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
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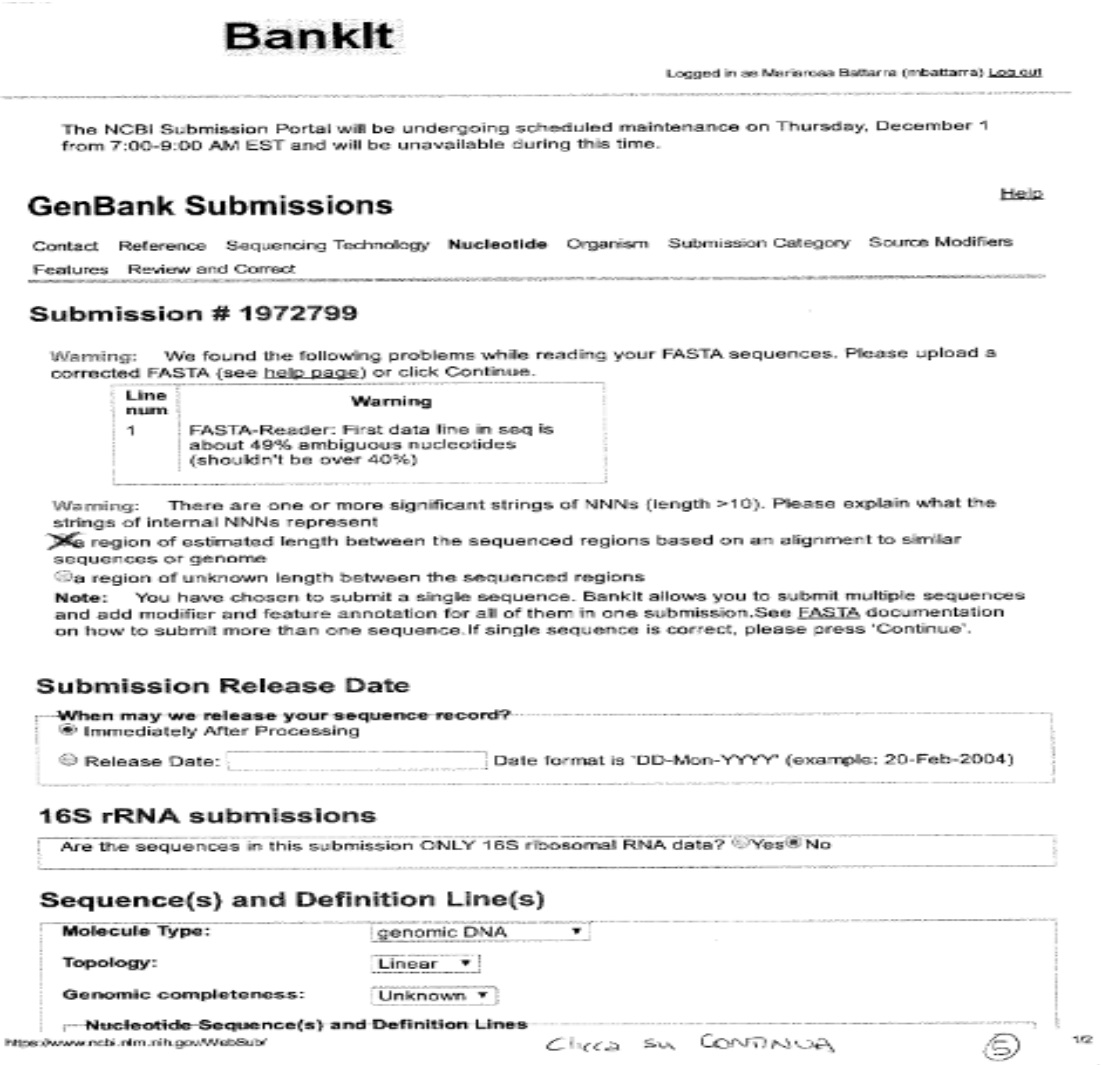
Inserimento in Bankit



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GenBank Submissions

Help

Contact Reference Sequencing Technology **Nucleotide** Organism Submission Category Source Modifiers Features Review and Correct

Submission # 1972799

Warning: We found the following problems while reading your FASTA sequences. Please upload a corrected FASTA (see help page) or click Continue.

Line num	Warning
1	FASTA-Reader: First data line in seq is about 49% ambiguous nucleotides (shouldn't be over 40%)

Warning: There are one or more significant strings of NNNs (length >10). Please explain what the strings of internal NNNs represent

- ✕ a region of estimated length between the sequenced regions based on an alignment to similar sequences or genome
- ○ a region of unknown length between the sequenced regions

Note: You have chosen to submit a single sequence. BankIt allows you to submit multiple sequences and add modifier and feature annotation for all of them in one submission.See FASTA documentation on how to submit more than one sequence.If single sequence is correct, please press 'Continue'.

Submission Release Date

When may we release your sequence record?

- ◉ Immediately After Processing
- ○ Release Date: Date format is "DD-Mon-YYYY" (example: 20-Feb-2004)

16S rRNA submissions

Are the sequences in this submission ONLY 16S ribosomal RNA data? ○ Yes ◉ No

Sequence(s) and Definition Line(s)

Molecule Type: genomic DNA

Topology: Linear

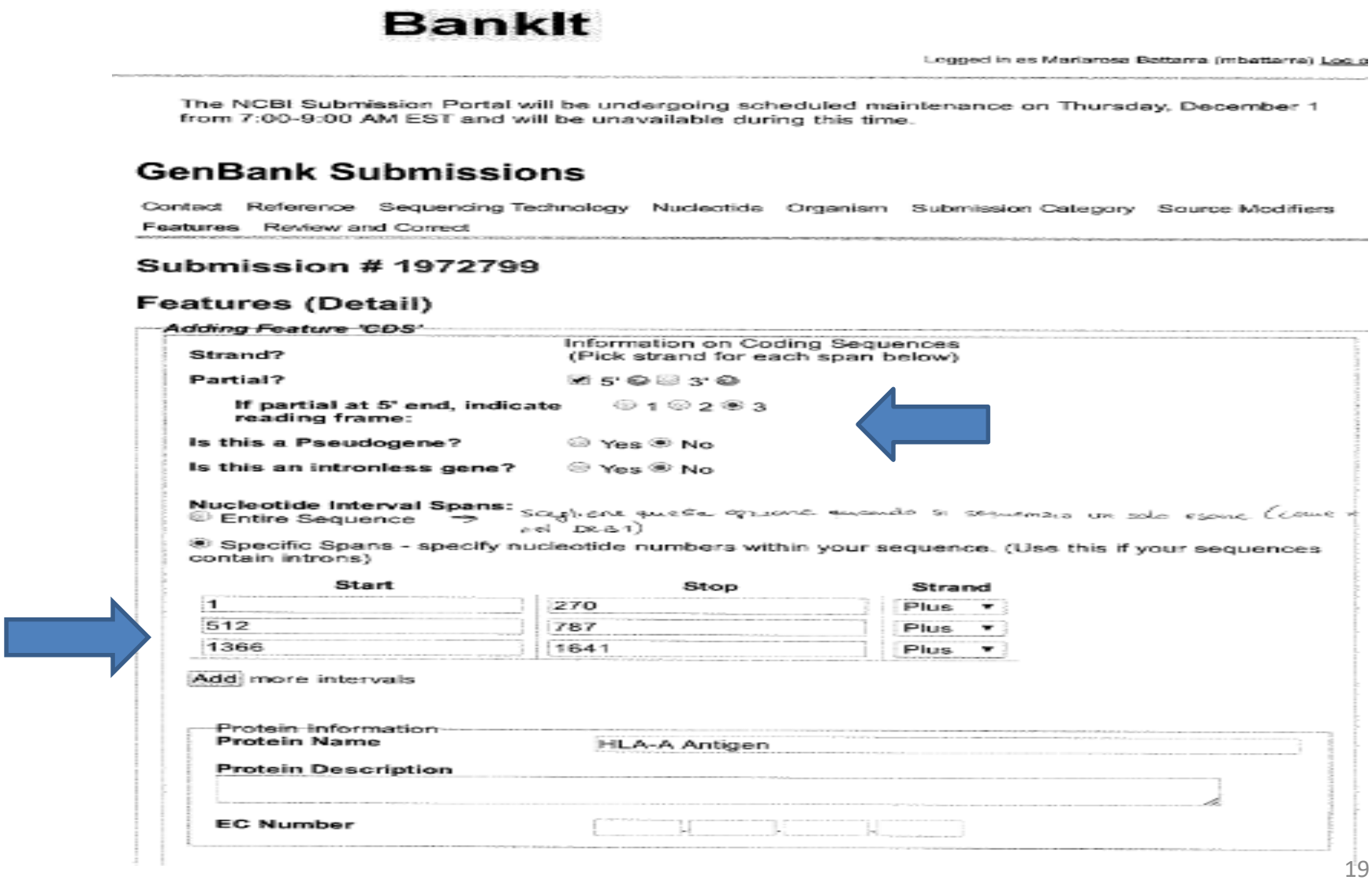
Genomic completeness: Unknown

Nucleotide Sequence(s) and Definition Lines

https://www.ncbi.nlm.nih.gov/WebSub/ 1/2

Clicca su CONTINUA (5)

Inserimento in Bankit



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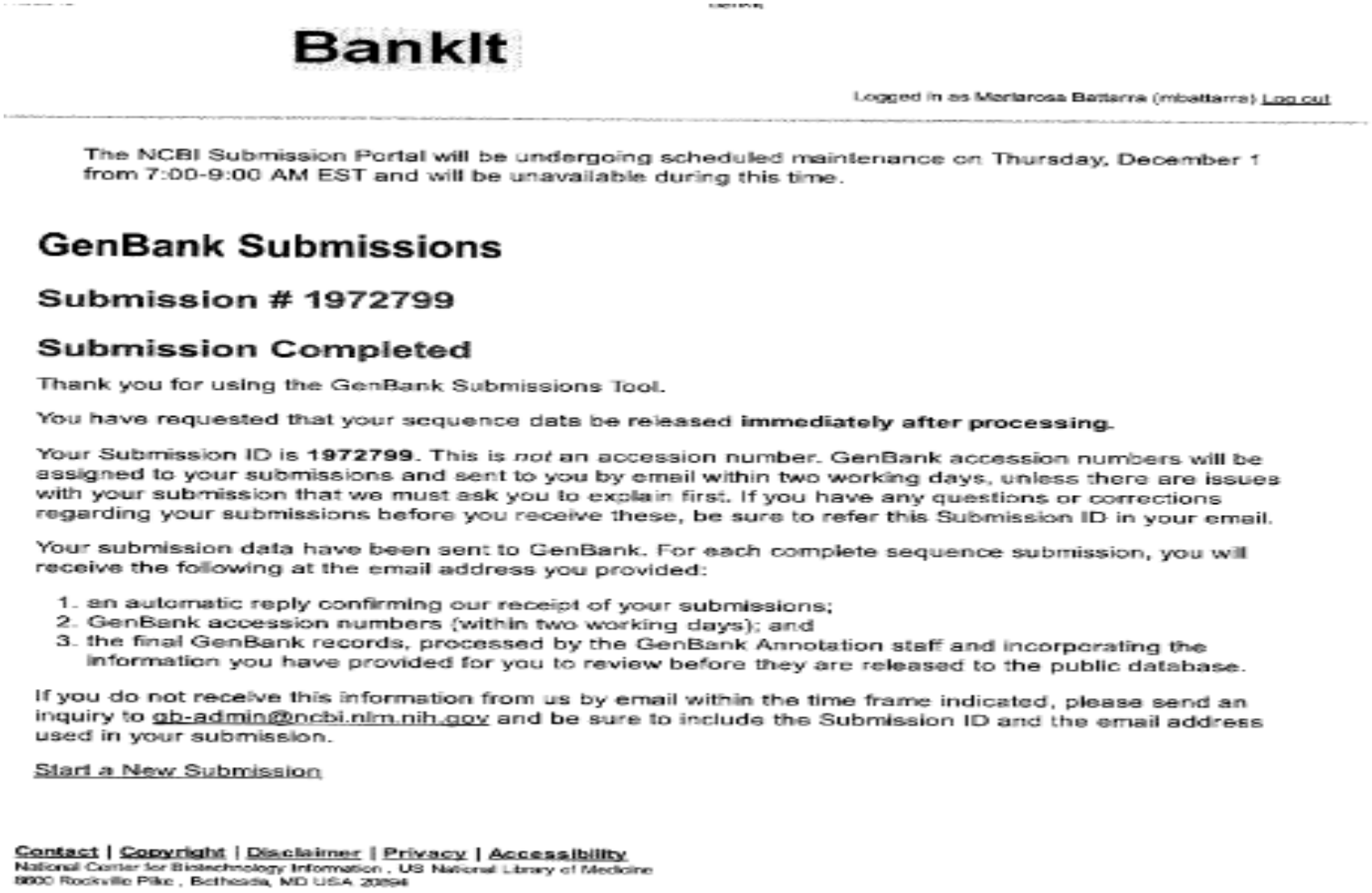

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GenBank Submissions

Submission # 1972799

Submission Completed

Thank you for using the GenBank Submissions Tool.

You have requested that your sequence data be released **immediately after processing.**

Your Submission ID is **1972799**. This is *not* an accession number. GenBank accession numbers will be assigned to your submissions and sent to you by email within two working days, unless there are issues with your submission that we must ask you to explain first. If you have any questions or corrections regarding your submissions before you receive these, be sure to refer this Submission ID in your email.

Your submission data have been sent to GenBank. For each complete sequence submission, you will receive the following at the email address you provided:

1. an automatic reply confirming our receipt of your submissions;
2. GenBank accession numbers (within two working days); and
3. the final GenBank records, processed by the GenBank Annotation staff and incorporating the information you have provided for you to review before they are released to the public database.

If you do not receive this information from us by email within the time frame indicated, please send an inquiry to gb-admin@ncbi.nlm.nih.gov and be sure to include the Submission ID and the email address used in your submission.

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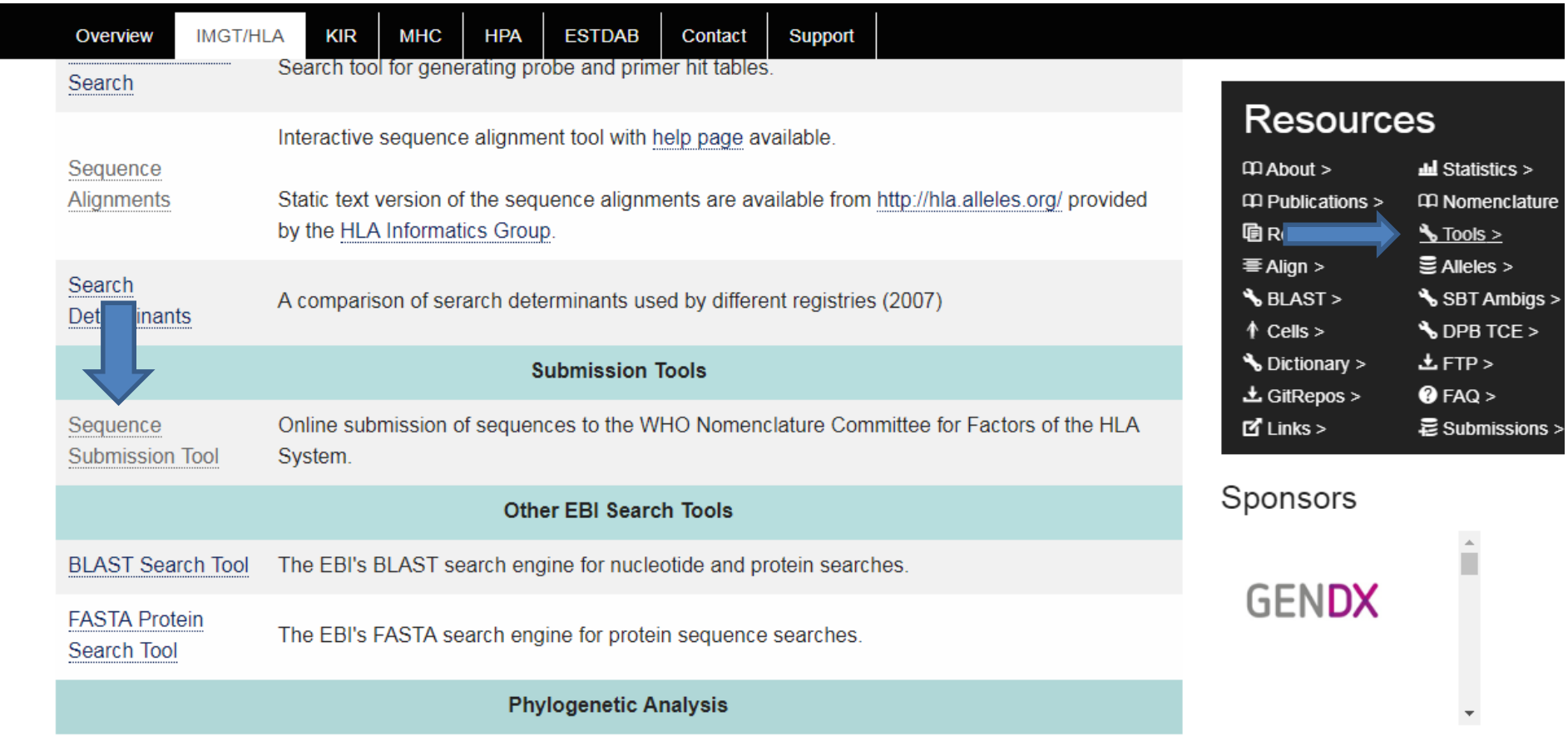
8600 Rockville Pike , Bethesda, MD USA 20894

Step necessari per la determinazione e inserimento nel database di un new allele

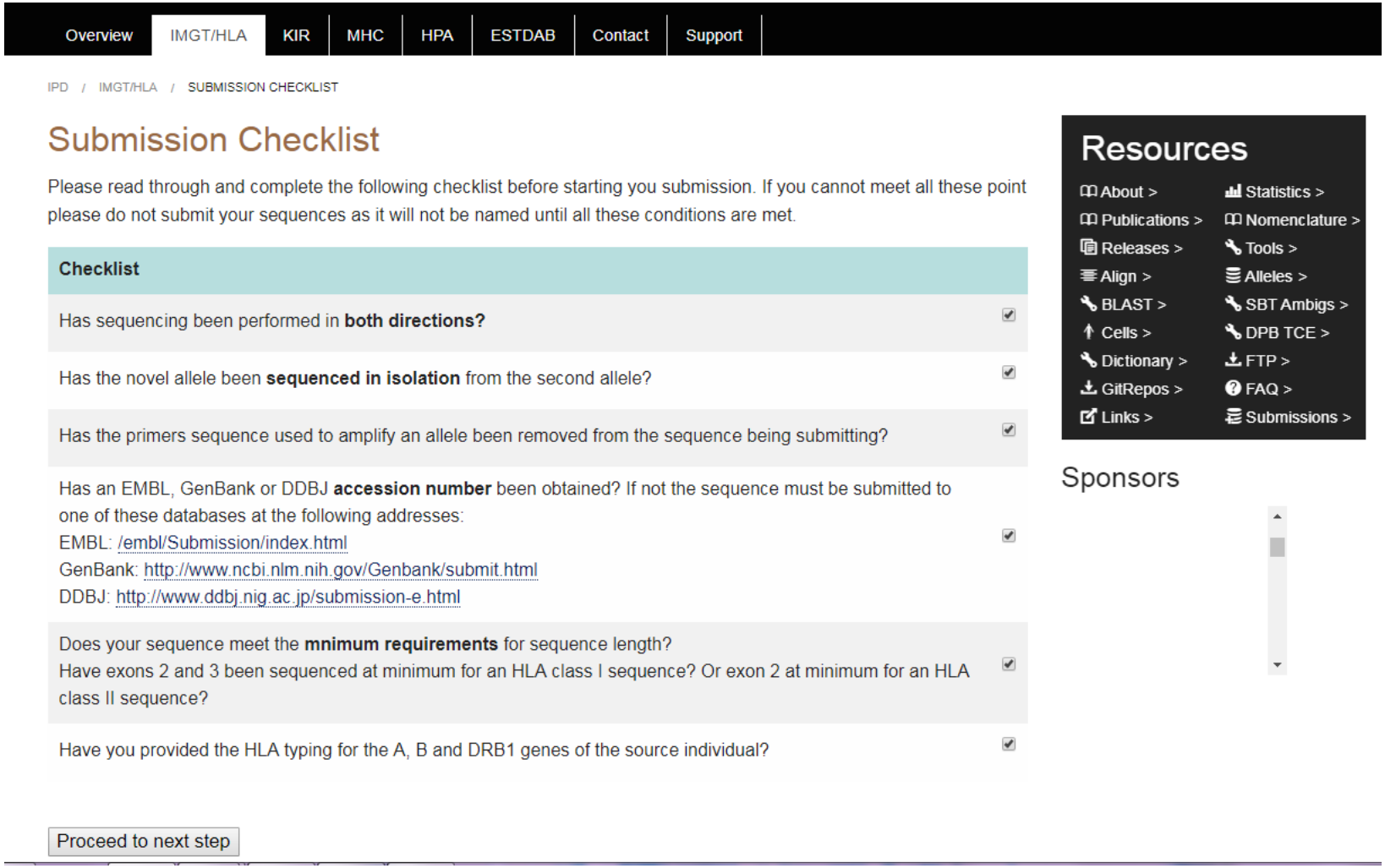
- Accertarsi che sia nuovo
- Isolare la sequenza
- Inserimento in Bankit
- Inserimento in IMGT

Inserimento in IMGT

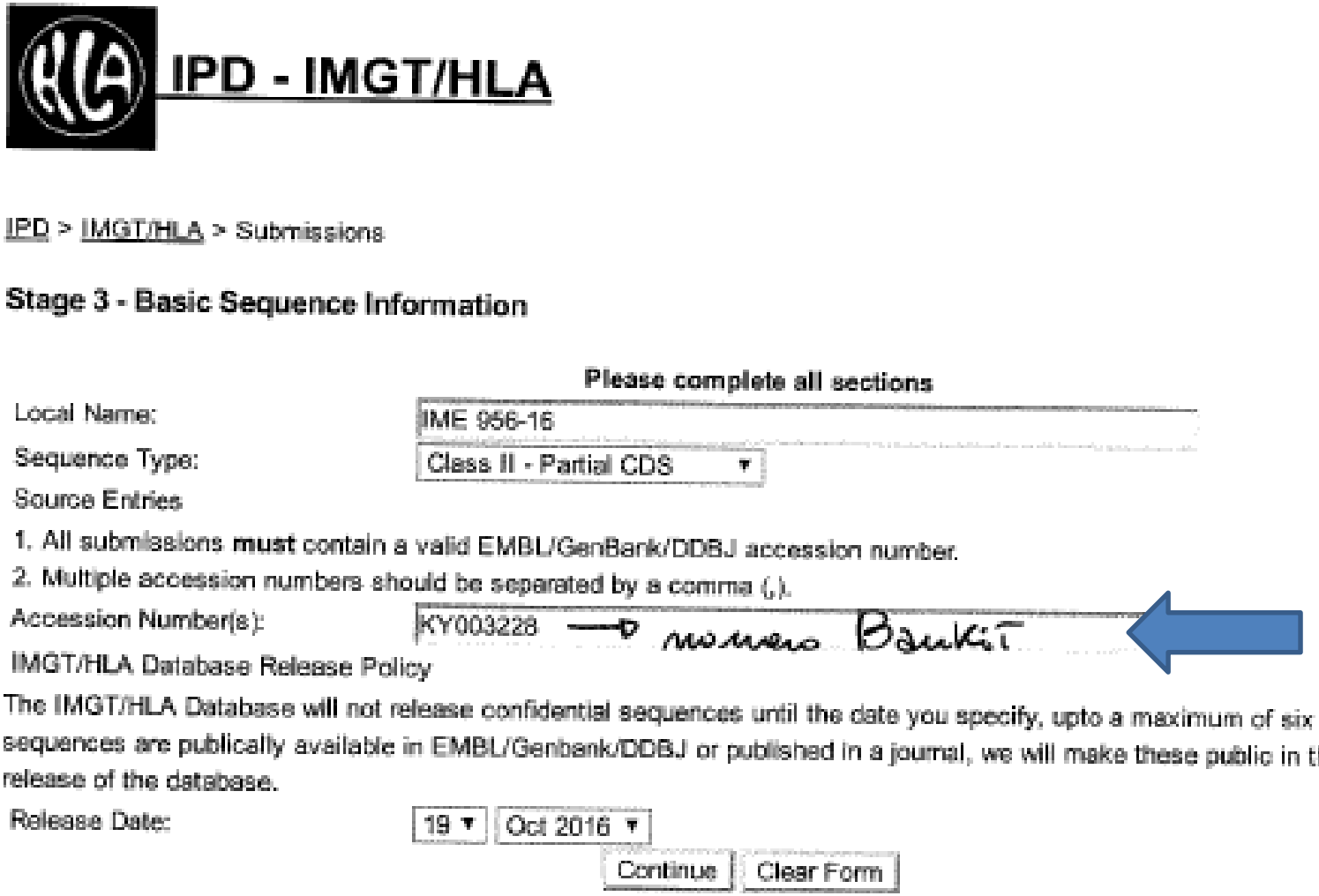
https://www.ebi.ac.uk/ipd/imgt/hla/access.html



Inserimento in IMGT



Inserimento in IMGT



IPD - IMGT/HLA

IPD > IMGT/HLA > Submissions

Stage 3 - Basic Sequence Information

Please complete all sections

Local Name: IME 956-16

Sequence Type: Class II - Partial CDS

Source Entries

1. All submissions **must** contain a valid EMBL/GenBank/DDBJ accession number.
2. Multiple accession numbers should be separated by a comma (,).

Accession Number(s): KY003228 → numero Bankit

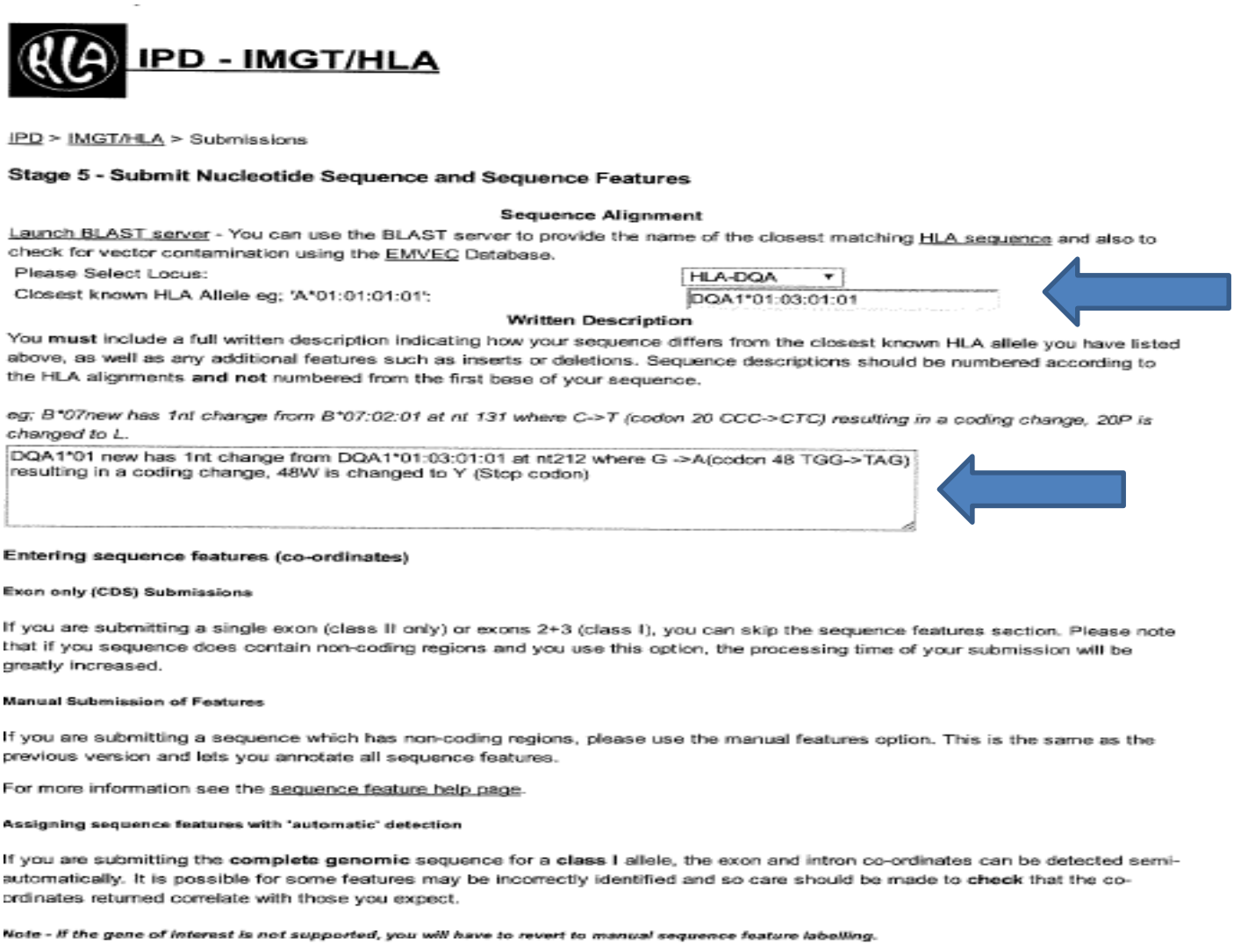
IMGT/HLA Database Release Policy

The IMGT/HLA Database will not release confidential sequences until the date you specify, upto a maximum of six months. If sequences are publically available in EMBL/Genbank/DDBJ or published in a journal, we will make these public in the following release of the database.

Release Date: 19 Oct 2016

Continue Clear Form

Inserimento in IMGT



IPD - IMGT/HLA

IPD > IMGT/HLA > Submissions

Stage 5 - Submit Nucleotide Sequence and Sequence Features

Sequence Alignment

Launch BLAST server - You can use the BLAST server to provide the name of the closest matching HLA sequence and also to check for vector contamination using the EMVEC Database.

Please Select Locus: HLA-DQA

Closest known HLA Allele eg; 'A*01:01:01:01': DQA1*01:03:01:01

Written Description

You **must** include a full written description indicating how your sequence differs from the closest known HLA allele you have listed above, as well as any additional features such as inserts or deletions. Sequence descriptions should be numbered according to the HLA alignments **and not** numbered from the first base of your sequence.

*eg; B*07new has 1nt change from B*07:02:01 at nt 131 where C->T (codon 20 CCC->CTC) resulting in a coding change, 20P is changed to L.*

DQA1*01 new has 1nt change from DQA1*01:03:01:01 at nt212 where G ->A(codon 48 TGG->TAG) resulting in a coding change, 48W is changed to Y (Stop codon)

Entering sequence features (co-ordinates)

Exon only (CDS) Submissions

If you are submitting a single exon (class II only) or exons 2+3 (class I), you can skip the sequence features section. Please note that if you sequence does contain non-coding regions and you use this option, the processing time of your submission will be greatly increased.

Manual Submission of Features

If you are submitting a sequence which has non-coding regions, please use the manual features option. This is the same as the previous version and lets you annotate all sequence features.

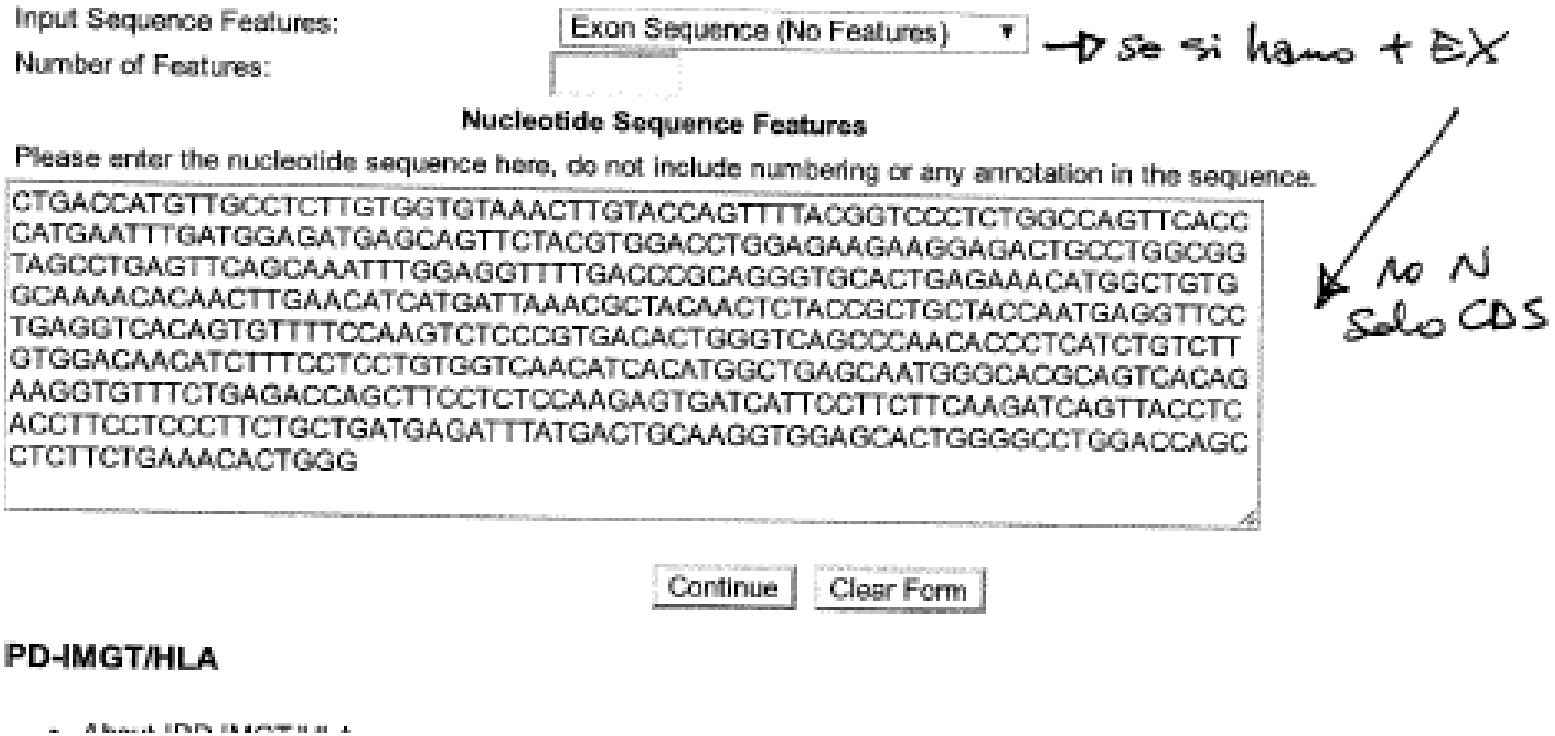
For more information see the sequence feature help page.

Assigning sequence features with 'automatic' detection

If you are submitting the **complete genomic** sequence for a **class I** allele, the exon and intron co-ordinates can be detected semi-automatically. It is possible for some features may be incorrectly identified and so care should be made to **check** that the co-ordinates returned correlate with those you expect.

Note - If the gene of interest is not supported, you will have to revert to manual sequence feature labelling.

Inserimento in IMGT



PD-IMGT/HLA

Inserimento in IMGT

IPD - IMGT/HLA

IPD > IMGT/HLA > Submissions

Stage 7 - Information on the Source Cell or Individual

This form allows you to enter details of the cell/individual from which the sequence originates. The IMGT/HLA Database requires details of the source cell/individual in order for the sequence to be submitted.

For help on any section of the cell details, click here to open the Cell Help Page in a separate window.

We would like to encourage all submittors to provide ethnic origin information when possible. This information is of great interest to the HLA community.

Cell/Individual Details

Cell/Individual ID/Code:	IME 956-16
	Important Note: Please do not use a person's name for this fields
Other names:	
Ethnic origin:	Caucasoid - Italy, Europe
Sex of donor:	Female
Consanguineous:	Unknown
Homozygous:	No
Comments:	
Contact Information	
Lab of Origin:	IME foundation
Lab Contact:	mariarosa battarra
Cell Availability	
Material Available:	DNA
Cell Bank;	Not Available
Cell Workshop Details	
IHW No:	
7th Workshop:	
8th Workshop:	
9th Workshop:	
10th Workshop:	
Other Workshops;	

Continue Clear Form

Inserimento in IMGT

IPD > IMGT/HLA > Submissions

Stage 8 - Source Sample Typing Profile

Information on the HLA typing or serology of the sample aids analysis and the eventual assignment of an official name. Whilst it is not a requirement to fully type a sample before submission, the IMGT/HLA database encourages submitters to provide as much information on the source of the sample as possible.

For each gene please enter any serological typing data and any DNA typing data in the appropriate box and separate serological subtypes and alleles by a comma. Whilst DNA typing is not mandatory the inclusion of HLA-A, B, C and DRB1 typing may speed up the processing of your submission.

For DNA-typing enter the allele names in the boxes provided.
e.g.: A cell DNA typed as HLA-A*02:01, 24:01 would be entered as 02:01, 24:01 in the HLA-A* box of the HLA DNA Typing section. Where possible do not use NMDP Ambiguity codes to replace allele names.

For serological data please enter the highest subtype specificity. If you have not performed serological typing, please do not infer serology typing from DNA typing methods, leave the boxes blank.
e.g.: A cell typed serologically as A2, A24(9) would be entered as 2, 24 in the HLA-A box.

Source Sample Profile - HLA DNA Typing

DNA typing data should be entered for each gene using the current nomenclature. DNA Typing includes entering your local allele designation in the appropriate box. HLA-A, B and DRB1 typing is mandatory.

HLA-A*	03, 24	HLA-DRB6*	
HLA-C*	04, 12	HLA-DRB7*	
HLA-B*	35, 38	HLA-DRB8*	
HLA-DRA*		HLA-DRB9*	
HLA-DRB1*	11, 13	HLA-DQA1*	01NEW, 05:05
HLA-DRB3*		HLA-DQB1*	03, 06
HLA-DRB4*		HLA-DPA1*	
HLA-DRB5*		HLA-DPB1*	

Source Sample Profile - Serology

If you have not performed serological typing, please do not infer serology typing from DNA typing methods, leave the boxes blank.

HLA-A		HLA-DR51	
HLA-C		HLA-DR52	
HLA-B		HLA-DR53	
HLA-Bw4/w6		HLA-DQ	
HLA-DR		HLA-DP	

Continue Clear Form

Inserimento in IMGT

IPD - IMGT/HLA

IPD > IMGT/HLA > Submissions

Return to Stage 7 - Submissions from Multiple Cells/Sources

If your sequence comes from multiple sources you may now add these to your submission. To add further sources please click the "New Source" button and repeat the procedure for entering a cell line. You may enter as many sources as you require. Once you have completed all entries please move to the section below.

New Source

Stage 9 - Methods

Once all cell entries are complete please enter method data below. Please complete all sections.

Methods

Primary Sequencing:	Direct sequencing of PCR product from DNA (SBT)
Secondary Sequencing:	Direct sequencing of PCR product from DNA (SBT)
Types of PCR primers:	Both allele and locus specific

Please confirm that your novel allele has been sequenced in isolation from any second allele of the same locus in a heterozygous DNA sample and that this allele has not only been determined by Sequencing Based Typing (SBT).

Sequenced in isolation: Yes

Primers Used

Please provide details of all primers used in the amplification and sequencing for this submission.
For each primer, provide its sequence, name and the location it binds.

Primer 1	GenDX locus HLA-DQA1
Primer 2	GenDX locus HLA-DQA1 EX2 DQA1*01
Primer 3	GenDX locus HLA-DQA1 EX2 DQA1*05
Primer 4	GenDX locus HLA-DQA1 EX3 FW
Primer 5	GenDX locus HLA-DQA1 EX3 Rv
Primer 6	
Primer 7	
Primer 8	
No. Clones sequenced:	
No. PCR reactions:	6

Sequencing Direction: Both

It is mandatory to sequence in both directions. If sequencing has been performed in only one direction, your submission will be held until such a time as the work is completed.

Confirmation Methods

☐ PCR-SSO	☐ PCR-SSP	☐ PCR-RFLP	
☐ Reverse-SSOP	☑ SBT	☐ NGS	☐ None

Other Comments i.e. analysis software,, phasing methods

Inserimento in IMGT

IPD - IMGT/HLA

IPD > IMGT/HLA > Submissions

FINE !

Submission Process Completed

Submission HWS10027015, by mariarosa battarra is now complete

Please write down the ID number for use in any further communications and print this page.

Your submission will now be processed and you will be contacted shortly regarding the official name or for further information. Every effort will be made to process your submission in a timely manner, however in the unlikely situation that you do not hear anything from us after three months, please contact us. Once processed your submission will be entered into the IMGT/HLA Database and also included in the next monthly update of HLA sequences published in *Tissue Antigens*, *Human Immunology* and the *International Journal of Immunogenetics*.

Your completed submission is shown below, please print this for your records;

Bambino Gesù
OSPEDALE PEDIATRICO

GRAZIE PER L'ATTENZIONE