

GENDX

NGSengine®

Next Generation Sequencing Based
Typing Software

Manual for KIR analysis

For Research Use Only

Edition 2, 2019/08

MAT 4302440

IMPORTANT NOTES AND UPDATES

Edition 2

- Section 'Ordering information' now includes both the company name 'Genome Diagnostics B.V.' and the trade name 'GenDx'.

CONTENTS

IMPORTANT NOTES AND UPDATES.....	2
CONTENTS	3
DISCLAIMER.....	3
1. KEY TO SYMBOLS	4
2. TECHNICAL ASSISTANCE	4
3. WARNING AND PRECAUTIONS	4
4. SYSTEM REQUIREMENTS	6
5. SOFTWARE AND LICENSE INSTALLATION INSTRUCTIONS	6
6. CHANGING A LICENSE AFTER INSTALLATION	8
7. NGSengine® HELP	9
8. Preparing NGSengine® for KIR analysis	10
9. NGSengine® WORKFLOW	10
10. UPDATING NGSengine®	12
11. PERFORMANCE CHARACTERISTICS.....	13
12. LIMITED LICENSE AGREEMENT	14

DISCLAIMER

GenDx has made every effort to ensure that this IFU is accurate. Information in this IFU is subject to change without notice. GenDx reserves the right to make improvements to this IFU and/or to the products described in this IFU, at any time without notice.

If you find information in this manual that is incorrect, misleading, or incomplete, we would appreciate your comments and suggestions. Please send them to info@gendx.com.

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1. KEY TO SYMBOLS



Material number



Consult Instructions For Use



Legal manufacturer

2. TECHNICAL ASSISTANCE

For technical assistance and more information:

Email: support@gendx.com
Website: www.GenDx.com
Phone: +31 (0) 30 252 3799

or contact your local GenDx distributor at www.gendx.com/company/distributors

3. WARNING AND PRECAUTIONS

Product Use Limitations

For **Research Use Only**. Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

- To ensure the best performance, use the NGSengine® software with the recommended system requirements (as mentioned in 'System requirements' chapter 4).
- Be aware that the incorrect use of settings within the NGSengine® software can lead to alterations in the generated typing results.
- NGSengine® has no influence on the quality of the input data.
- Data generated by NGSengine® does not provide a final conclusion or diagnosis in any form.
- Improper use of NGSengine® software including, but not limited to, unauthorized changes in the reference databases, use under false license etc. may lead to significant changes which GenDx does not support nor can be held accountable for. Changes may lead, but are not limited to, faulty data analysis, accreditation or regulatory issues and incomplete storage of data.
- Be aware that not all genes in the KIR database have been fully tested. See chapter 10 for the known performance characteristics of the KIR database.

Product Application

NGSEngine® is a software product designed for high resolution identification of KIR alleles from sequence data generated by a Next Generation Sequencing (NGS) platform.

Note:

Because the analysis of KIR data will be done with NGSEngine®, NGSEngine® will have an RUO status whenever the license for KIR typing is used. KIR typing results from NGSEngine® cannot be used for diagnostic purposes.

4. SYSTEM REQUIREMENTS

Minimum System Requirements

- Windows 7 (32-bit or 64-bit)
- Microsoft .NET Framework 4.6.1
- Dual core processor
- 4 GB RAM
- 3 GB free disk space*

Recommended System Requirements

- Windows 10
- Microsoft .NET Framework 4.6.1
- Quad core processor
- 8 GB RAM
- 3 GB free disk space*
- Internet connection

* hard disk space is for installation and running of NGSengine® only, so excluding space for data storage

5. SOFTWARE AND LICENSE INSTALLATION INSTRUCTIONS

1. The NGSengine® software can be downloaded onto your computer via www.gendx.com/NGSengine.
2. A valid license key is required to use the software, use the KIR license which has been provided.
3. After installation, please follow the on-screen instructions. Copy the key to the location indicated and click on [Activate]. NGSengine® will then automatically start.
4. Upon opening of NGSengine®, the next screen will appear:

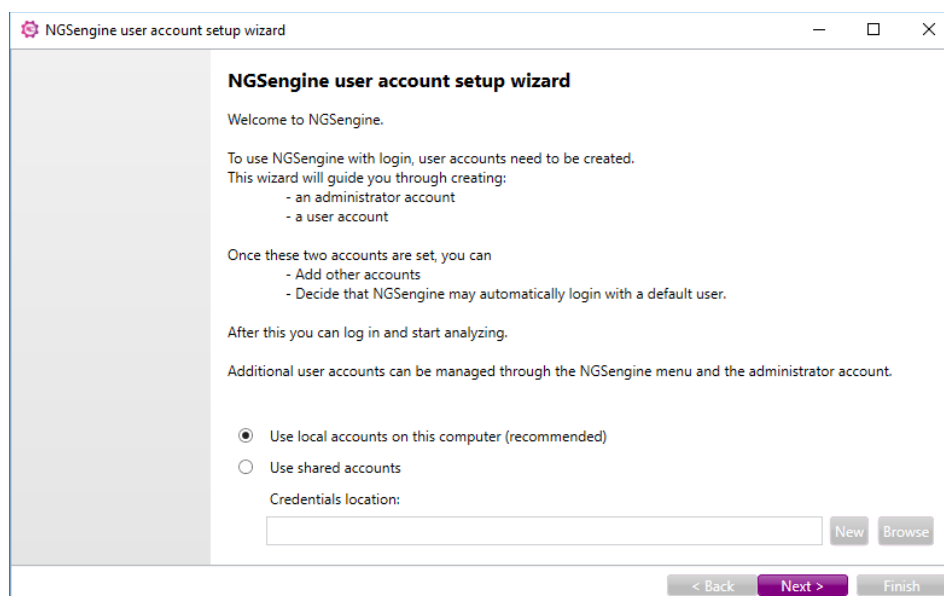


Figure 1. Opening screen NGSengine.

First you have to select whether you want to make local accounts or shared accounts.

Local accounts:

The user credentials will only be saved on the local computer. This is recommended for a user that uses NGSengine® on only that computer.

Shared accounts:

The user credentials are stored on a shared location that has to be indicated in the box "Credentials location". This is useful when an administrator has to make credentials for several computers: the administrator has to make the user credentials only once and then on each next computer the shared location can be used. It can also be useful for a user who uses NGSengine® on several different computers. The first time "shared accounts" is being used: click on "New" and then select a name and a location for the credentials file.

Next time, when you setup user accounts on a different computer: click on "Browse", go to the location where the file is stored and click on the file.

5. Click 'Next'
6. A password for the administrator account needs to be created and confirmed:

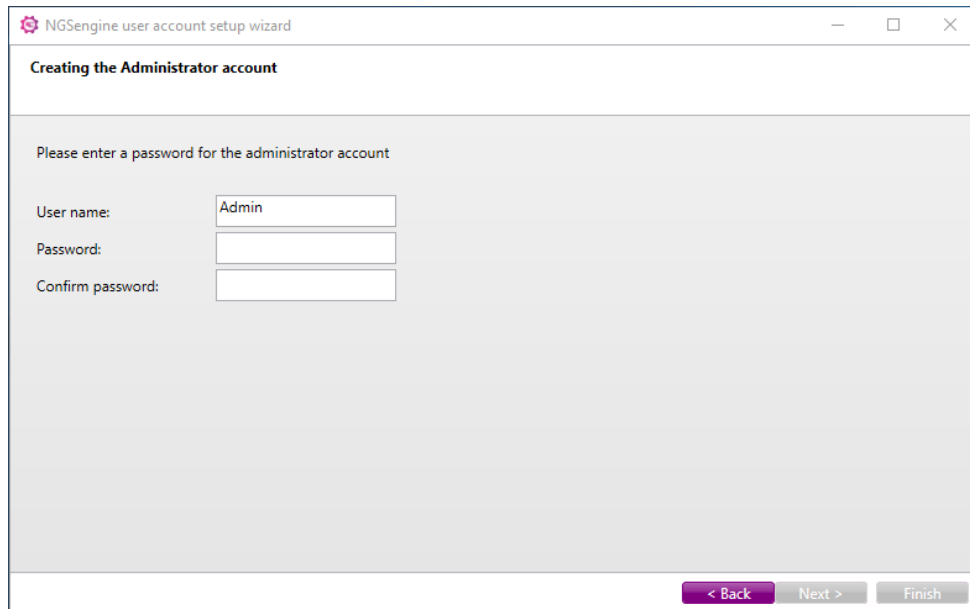


Figure 2. NGSengine® user account setup wizard, creating the administrator account.

NOTE: The Administrator is only responsible for managing users and their credentials, and cannot perform data analysis in NGSengine®.

7. When the password is confirmed, the 'Next' button is enabled.
8. Click 'Next'
9. At least one user account needs to be created:

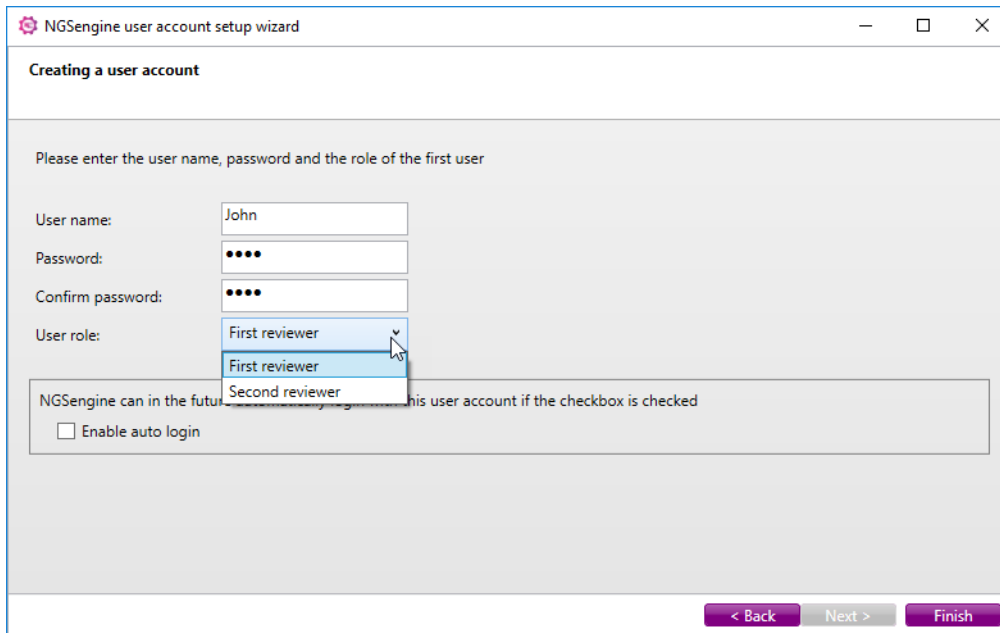


Figure 3. NGSengine® user account setup wizard, creating a user account.

10. In this screen, enter the user name, the password, and choose the role for this account.

NOTE: Reviewer1 can do the analysis, modify the settings, reanalyze and do the first approval. Reviewer2 can do the final approval by accepting or rejecting data that has been approved by Reviewer1. After approval by Reviewer2, Reviewer1 cannot modify the settings or reanalyze a sample but can load and inspect the data.

11. To automatically start NGSengine® without the need to login, check the 'Enable auto login' check box.

NOTE: If auto-login is activated, it can only be de-activated by an administrator.

12. Click 'Finish'.

6. CHANGING A LICENSE AFTER INSTALLATION

If the user already has NGSengine® installed, the user can change the existing license to a KIR license

1. Go to Help > Licensing

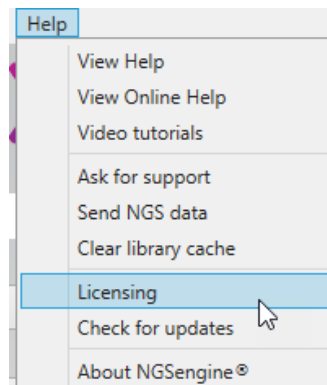


Figure 4. Licensing is accessible through the main menu.

2. Click Remove License; This action will close NGSengine, upon startup it will ask for a license. See Section 5, point 3.

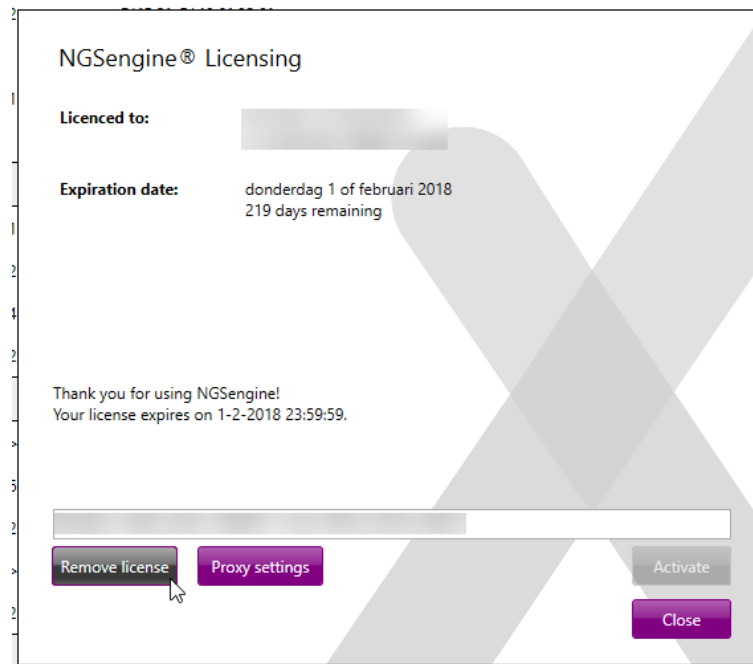


Figure 5. Removing a license

7. NGSengine® HELP

NGSengine® has an extensive help system with detailed information about the installation, login, the workflow, the analysis and the different menus. The user can reach this help system through the main menu.

Because of this extensive help system and the frequent updates to the help, both online and within the program, this document describes the different steps of the analysis in general.

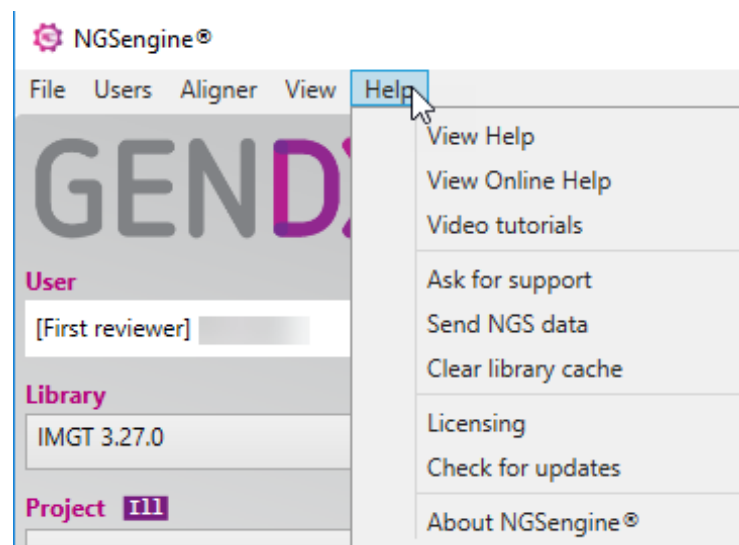


Figure 6. The Help is accessible through the main menu, from which the offline and online help as well as video tutorials and support can be reached.

8. Preparing NGSengine® for KIR analysis

The KIR allele libraries are not included in the NGSengine® installer. To enable KIR analysis, the KIR libraries should be copied to a proper folder. Typically, in the folder ‘%programdata%\NGSengine\Library’ a number of folders can be found, each representing a release of the IPD-IMGT/HLA database. Creating a folder ‘KIR’ and copying all KIR files into this folder enables KIR analysis.

9. NGSengine® WORKFLOW

NGSengine® is an NGS platform-independent software package that uses sequence files generated by an NGS platform as input.

The usage of NGSengine® is extensively described in the Help, accessible through the main menu (see Figure 4). The Help is updated upon each release and therefore is up to date.

Settings for KIR analysis

1. Open preferences through the main Menu.
2. In “General”: Browse to the folder where the KIR database is located. Select “KIR” in the “Library” section. Click “OK”

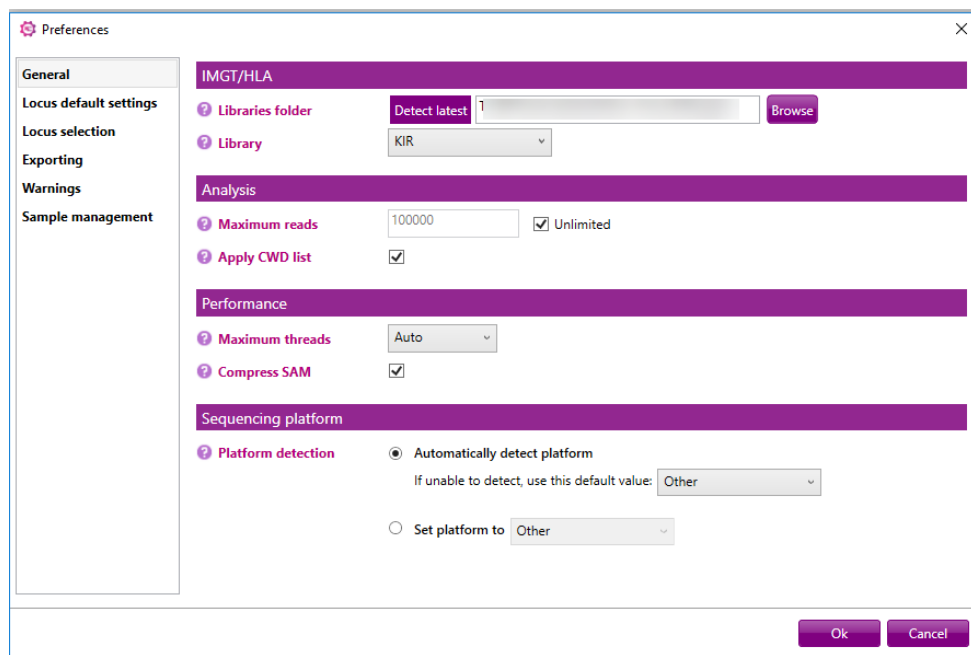


Figure 7. The General settings in which the KIR database can be selected.

3. Open preferences again
4. If necessary, change the settings in “Locus default settings”. This view shows HLA genes, as they are hardcoded in NGSengine® at the moment. KIR genes are indicate below the HLA genes.

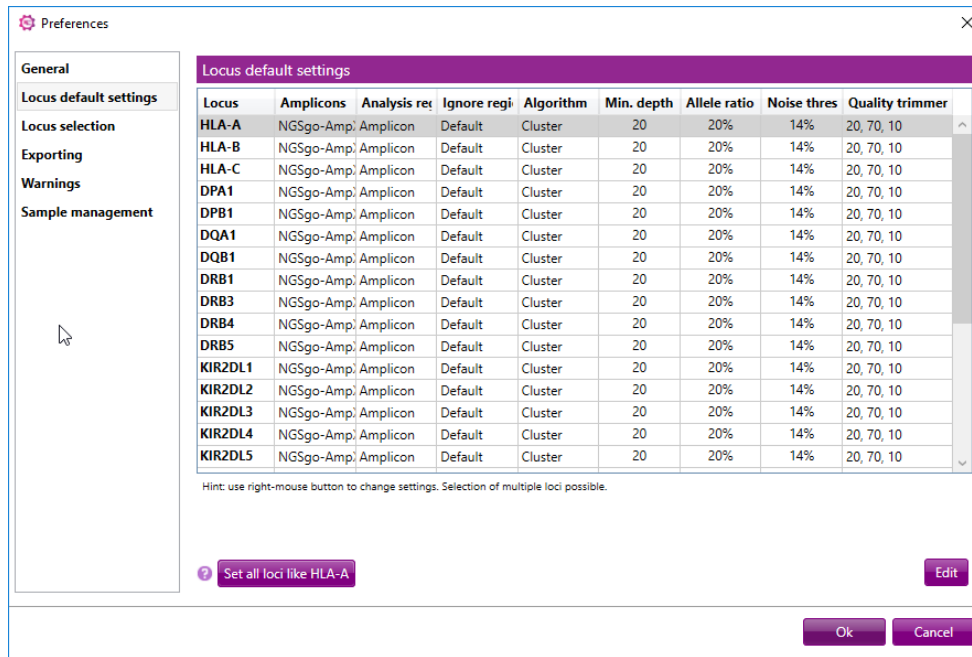


Figure 8. The Locus default settings

- Have a look at “Locus selection”. When the KIR genes are in the “Ignore” box, drag the genes of interest to the “Analysis” box. Either only analyze the KIR gene of interest, or let NGSengine® decide which genes will be analyzed in your sample (preferred for testing the KIR database).

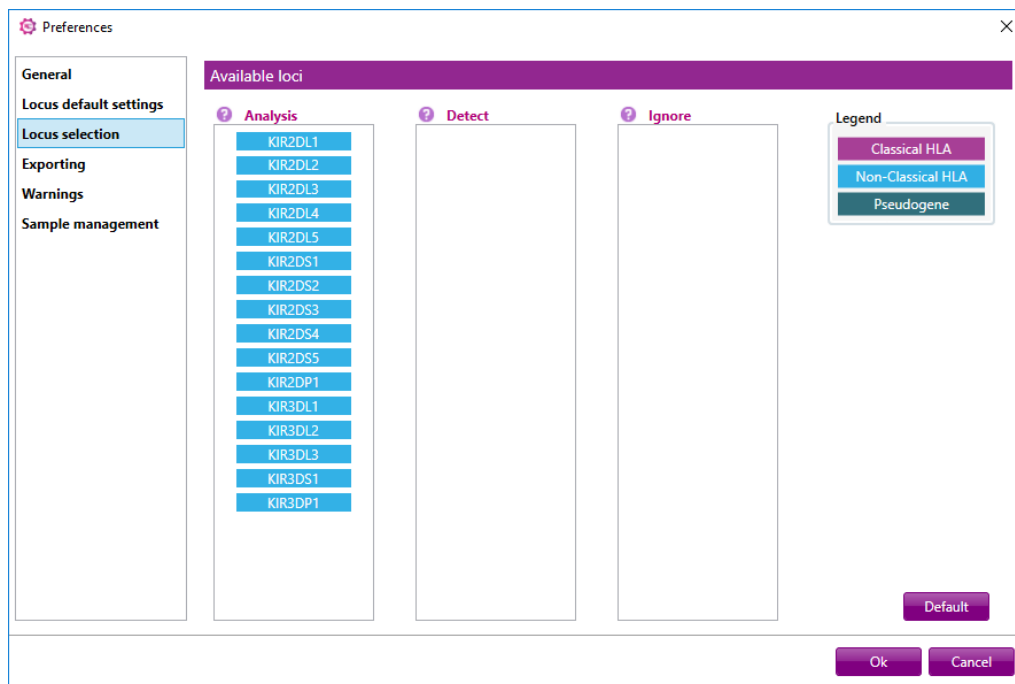


Figure 9. Locus Selection

- Click OK.
- Start the analysis

10. UPDATING NGSengine®

Upon the startup of NGSengine, the software checks whether the user is using the latest version of the software. If a new version is available, the following screen will appear:

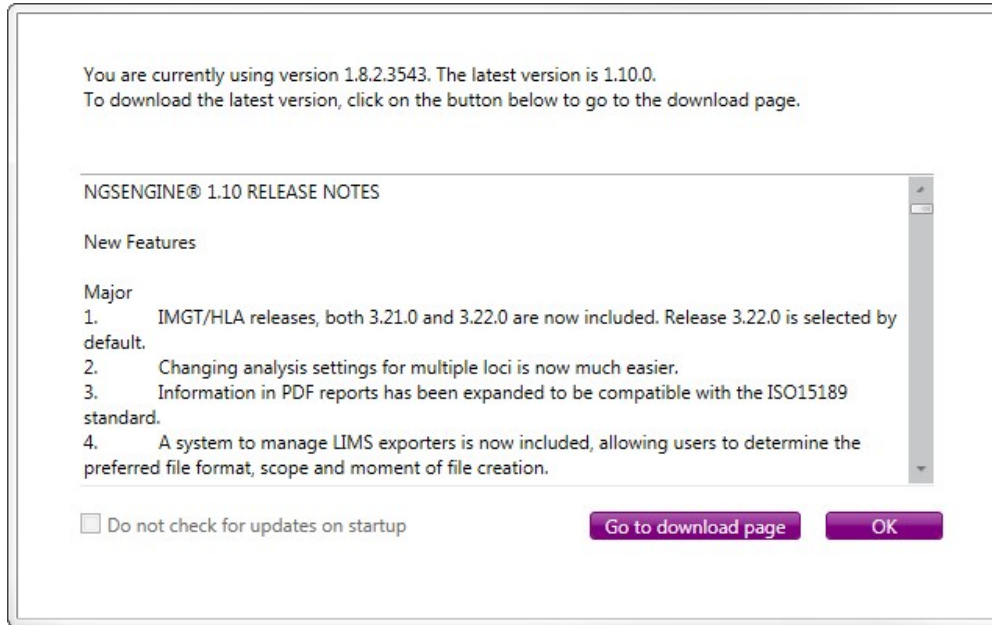


Figure 10. NGSengine® screen to show possible updates.

The user can check whether the current version of NGSengine® is up to date through "Help" → "Check for updates".

11. PERFORMANCE CHARACTERISTICS

The KIR database has been tested for most of the KIR genes. Table 1 gives an overview of the KIR genes that were tested. The genes are categorized as:

- 'not tested' since there was no data available
- 'worked directly', meaning that NGSengine® automatically identified the correct gene and performed a solid typing,
- 'Works after assigning correct gene', which means that NGSengine® could not uniquely identify the gene, but considered two genes because of the high homology. After manually assigning the proper gene, a solid typing was performed.

The genes in the database have been tested and found functional for amplicons from single gene amplification strategies.

Table 1. Overview of genes tested with KIR database in NGSengine.

KIR genes	Tested	Works directly	Works after assigning correct gene	Comments
KIR2DL1	+	yes		NGSengine® cannot differentiate between homologous short reads of KIR2DL1/KIR2DL2/KIR2DL3 when present together in one fastq file
KIR2DL2	+	yes		
KIR2DL3	+	yes		
KIR2DL4	+	yes		
KIR2DL5A/B	+	yes		NGSengine® can only determine presence of genes but cannot yet accurately type these genes*
KIR2DS1	+	yes		
KIR2DS2	+	yes		
KIR2DS3	+	yes		
KIR2DS4	+	yes		
KIR2DS5				Not yet tested
KIR2DP1				Not yet tested
KIR3DL1	+	no	yes	NGSengine® cannot differentiate between homologous short reads of KIR3DL1/KIR3DS1 when present together in one fastq file
KIR3DS1	+	yes		
KIR3DL2	+	yes		
KIR3DL3	+	yes		
KIR3DP1	+	yes		

NOTE*: The current version of NGSengine® considers a maximum of 2 alleles for a single gene. This means that samples containing more than 2 alleles, will probably not be analyzed correctly. Data of genes with more than 2 alleles is very valuable for GenDx to further development of the KIR database and software and data can be shared with GenDx, when possible.

12. LIMITED LICENSE AGREEMENT

Use of this product signifies the agreement of any purchaser or user of the NGSengine® for KIR typing with the following terms:

- NGSengine® software may be used solely in accordance with system requirements described in this Manual. GenDx grants no license under any of its intellectual property to use or incorporate the software with any other components, except when agreed with GenDx.
- Other than expressly stated licenses, GenDx makes no warranty that NGSengine® software and/or its user(s) do not infringe the rights of third-parties.
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