DEEPLEX

Deeplex® HelP

From clinical samples to drug resistance profile

FEBRUARY 2024

A novel *Helicobacter pylori* drug resistance prediction and genotyping assay,

comprehensive, culture-free and based on deep sequencing

RESEARCH USE ONLY

GenoScreen

A novel deep sequencing-based assay for antibiotic resistance prediction of *Helicobacter pylori*, with virulence prediction and genotyping

Highlights

- **Prediction of resistance to clarithromycin, fluoroquinolones, tetracycline and rifampicin**

Easily identify resistance-associated variants in *Helicobacter pylori* (*H. pylori*) gene targets, thanks to an automated analysis and reporting.

- **Variants monitoring in metronidazole and amoxicillin-associated gene targets**

Detect variants in *frxA*/*rdxA* and *pbp1*, associated with metronidazole and amoxicillin resistance, respectively.

- **Genotyping of two *H. pylori* virulence factors**

Find whether the *H. pylori* strain present in the sample is CagA positive and if so, differentiate Western and East Asian types. Genotype *vacA* secretion signal, intermediate and middle regions.

- **Genotyping of *H. pylori* strains**

Get to know the allelic profile of the *H. pylori* strain present in the sample through multilocus sequence typing (MLST) analysis. Detect mixed infection involving distinct *H. pylori* strains.

- **Turnaround time of 48 hours**

Starting from extracted DNA from clinical specimens*, prepare libraries and sequence for a turnaround time of 48 hours. Analyse the data with our automated pipeline at GenoScreen.

- **High performances**

Identify heteroresistance down to 10% subpopulations and work with extracted *H. pylori* DNA loads down to 100 genomes.

Introduction

H. pylori infects around half of the world population and is responsible for diseases such as peptic ulcer disease, gastric cancer and MALT lymphoma (1-10% of cases)[1]. Since 2015, international guidelines have recommended the systematic eradication of *H. pylori* when diagnosed[2]. However, the success rate of eradication therapies has fallen below the acceptable 90% due (primarily) to the rise of antibiotic resistance[1]. Consequently, *H. pylori* has been listed by the World Health Organization among the 20 pathogens posing the greatest threat to human health[3].

Susceptibility testing can help guide therapy prior to treatment[4,5]. Unfortunately today, routine susceptibility testing either require time-consuming and fastidious culturing or rely on a small set of common resistance associated mutations, limiting the detection spectrum[6].

Here, we present the Deeplex® HelP assay which uses NGS-based targeted deep sequencing for the simultaneous prediction of (hetero)resistance to six** drugs/drug classes used in *H. pylori* eradication therapy, multilocus sequence typing and genotyping of CagA and VacA. The assay is directly applicable to clinical specimens* and includes an automated pipeline for sequencing data analysis (Figure 1).

A comprehensive assay based on targeted sequencing

The Deeplex® HelP assay starts with DNA extraction from either a (suspected) *H. pylori*-containing clinical specimen or a *H. pylori* positive culture (Figure 2). A single multiplex PCR is then performed to amplify genome regions from eight drug resistance-associated *H. pylori* genes, *cagA* and *vacA* (for virulence prediction), and seven housekeeping genes (for strain genotyping).

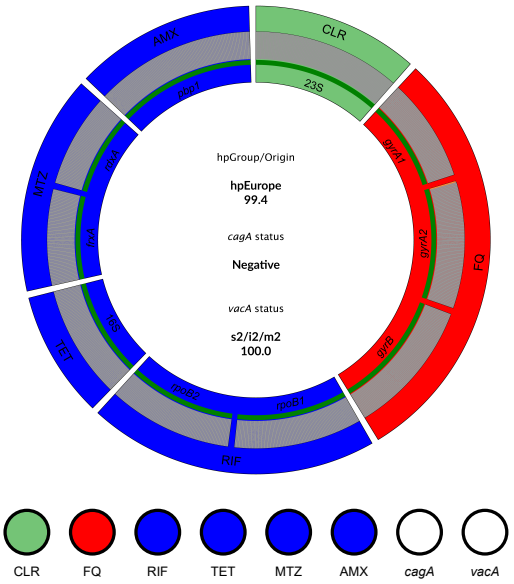


Figure 1. Deeplex HelP results identifying a *H. pylori* strain from the hpEurope group, genotypically resistant to fluoroquinolones. Results are shown for a strain mutated in *gyrA* (Top) Deeplex® map. The genotype of the *H. pylori* strain present in the sample is shown in the center of the circle, as well as *cagA* and *vacA* genotypes. Information on drug resistance predictions is as follows. Target gene regions are grouped within sectors in a circular map according to the prediction feature with which they are associated. Sectors in red and green indicate targets in which resistance-associated variants or no variants are detected, resulting in predictions of resistant or susceptible phenotypes. Sectors in blue indicate targets in which only variants uncharacterised by the Deeplex® HelP database are detected. Green lines above gene names represent the reference sequences with coverage breadth above 95%. Limit of detection (LOD) of minority variants (resulting from subpopulations of reads bearing a variant) depends on the read depth at each sequence position and is shown either as grey (LOD 10%) or orange zones (LOD >10%) above reference sequences. (Bottom) The resistotype summarizes the predictions shown in the Deeplex® map. The color white for *cagA* and *vacA* indicate that the strain is avirulent, with a lack of CagA (not being able to be translocated into host cells via the type IV secretion system) and a nontoxic vacA s2/i2/m2.*CLR: Clarithomycin, FQ: Fluoroquinolones, RIF: Rifampicin, TET: Tetracycline, MTZ: Metronidazole, AMX: Amoxicillin.

The resulting PCR products are cleaned-up and libraries are prepared for sequencing. The obtained sequencing data are then analysed using our automated pipeline at GenoScreen. Results can be viewed in tabular format and include synthetic visualisation (Deeplex® map and resistotype, Figure 1).

The Deeplex HelP kit includes a ready-to-use PCR master mix a positive and internal control as well as an access to our analysis pipeline. Alternatively, the Deeplex® HelP assay comes as a service (on demand). GenoScreen performs all steps, from DNA extraction (optional) to the reporting of results obtained from analysed data.

The assay has successfully been tested using the Nextera XT DNA library preparation kits on the MiSeq sequencing platform (Illumina®).

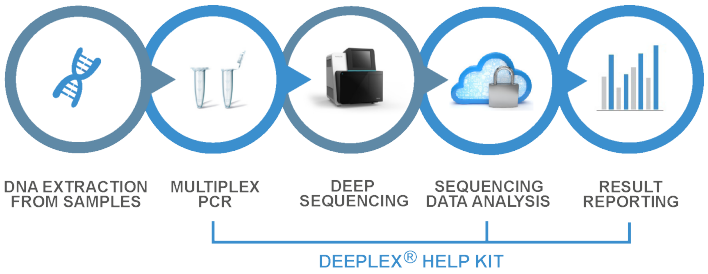


Figure 2. The Deeplex® HelP workflow. From DNA extraction from clinical or culture samples to data analysis and result reporting. The assay comes as two options: the Deeplex® kit and the Deeplex® service. The kit includes a single PCR master mix ready for multiplexed amplification of *H. pylori* targets, the positive and internal control, prior to deep sequencing using Illumina® kits and analysis at GenoScreen. Service is performed at GenoScreen.

Prediction of resistance to 6** antibiotics

The Deeplex® HelP assay relies on deep sequencing of eight *H. pylori* gene targets associated with antibiotic resistance (Figure 3). Based on the observed presence or absence of variants in these loci and interrogation of the Deeplex® HelP database***, the *H. pylori* strain present in the sample is predicted to be resistant or susceptible to each antibiotic, or with yet-to-be characterized variants. The assay can predict resistance to clarithromycin, fluoroquinolones, tetracycline and rifampicin. Information on reference literature describing the association of variants with drug resistance can be viewed in the results report. In addition, variants in *frxA*/*rdxA* and *pbp1*, associated with metronidazole and amoxicillin resistance are also reported. All variant descriptions include individual target positions, along with their sequence coverage depths and read frequencies.

Genotyping of 2 *H. pylori* virulence factors

In addition to antibiotic resistance prediction, the Deeplex® HelP assay can be used to genotype *cagA* and *vacA*, coding for two *H. pylori* virulence factors. This is achieved by detecting and identifying EPIYA[7] and CM[8] motifs in *cagA* as well as the signal secretion (s), intermediate (i) and middle (m) regions type of *vacA*[9,10].

Helicobacter pylori genotyping

The Deeplex® HelP assay can also be used to characterize the *H. pylori* strain detected in the sample. This is performed by sequence analysis of seven housekeeping genes: *atpA*, *efp*, *mutY*, *ppa*, *trpC*, *ureI* and *yphC*, using the PubMLST database[11] for best-match allele or strain identification number retrieval.

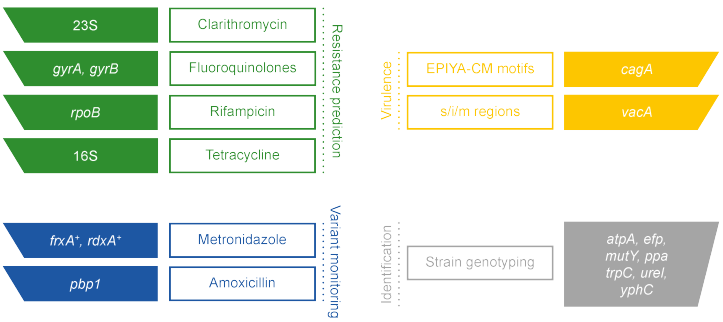


Figure 3. Gene regions amplified and sequenced using the Deeplex® HelP assay (+: full genes).

A highly sensitive assay

With the Deeplex® HelP assay, sequencing of *H. pylori* gene targets can be achieved at high read depth which means that each sequence position is covered by many reads, enabling highly confident variant calls including from mutant/ heteroresistant subpopulations as low as 10% of bacteria in the sample, inaccessible to other rapid molecular tests. The minimum recommended DNA input is 100 genomes but results can usually be obtained using 10 genomes.

Turn-around time of 48 hours

Starting from DNA extraction, sequencing results are obtained in 2 days (Table 1). Once targets are sequenced, output FASTQ (read) files are ready to be analysed with our fully parameterized Deeplex® pipeline at GenoScreen.

Deeplex® HelP	
Input sample type	gDNA from clinical specimens* (gastric biopsies) or culture
Recommended minimal DNA input	Minimum 100 genomes
Recommended library prep	Nextera® XT (Illumina®)
Recommended sequencing platforms#	Illumina® iSeq 100 (9 samples), MiniSeq (15/45), MiSeq (3/9/33)
Turnaround time	iSeq 100: 1 day; others sequencers: ≈2 days
Storage and shelf-life	-20°C for up to 6 months

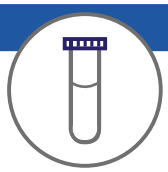
Table 1. Specifications of the Deeplex® HelP kit. Turnaround time includes multiplex PCR, library preparation and sequencing. Analysis is performed at GenoScreen.
Number of effective samples – controls not included. MiniSeq (Mid/ High output), MiSeq 2x150bp (Nano/Micro/Full).

** with genome loads ≥ 100 (quantified e.g. by qPCR). If available, genomic DNA extracted from cultured H. pylori can also be used.*
*** Amplification and analysis of eight H. pylori gene targets associated to resistance to six drugs/drug classes. Prediction of resistance possible for four drugs/drug classes through interrogation of the Deeplex® HelP database****
**** © 2022 GenoScreen Helicobacter pylori variant database (All Rights Reserved)*

Deeplex® HelP workflow

DNA extraction
Gastric biopsies

6h

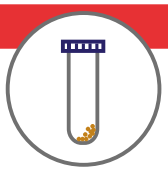


DNA extraction purification and quantification

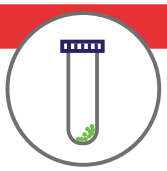
Deeplex® PCR
DNA

4h

Target enrichment



Clean-up



DNA quantification

Library prep
Amplicons

5h

DNA fragmentation

Library amplification

Library pooling 2nM + quantification

Sequencing
Pooled DNA library

~24h

Dilution 10pM NaOH denaturation

Illumina® sequencing

Analysis
DNA reads

<1h

Analysis

Result reporting Excel

H. pylori strain genotyping
(MLST analysis, 7 gene targets)
Resistance prediction to 6** antibiotics
Genotyping of two *H. pylori* virulence factors
Detection of heteroresistance down to 10%
Detection of mixed infections

References

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HEADQUARTERS:
1 rue du Pr. Calmette
59 000 Lille - FRANCE
Phone: +33(0) 362 263 777

CUSTOMER RELATIONS:
commercial.dept@genoscreen.fr
Phone: +33(0) 362 263 776

MORE INFORMATION:
www.genoscreen.com
www.deeplex.com

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