DEEPLEX

Deeplex® Myc-Lep

Simultaneous identification, multidrug resistance prediction, and high-resolution genotyping of M. leprae

MAY 2023

A novel *Mycobacterium leprae* 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 *Mycobacterium leprae*, with mycobacterial identification and high-resolution genotyping

Highlights

- **Prediction of resistance to dapsone, rifampicin and fluoroquinolones**

Easily identify resistance-associated mutations in *M. leprae* gene targets, thanks to an automated analysis and reporting.

- **Identification of hypermutator strains**

Identify hypermutator strain genotypes associated with potential drug resistance and treatment failure, based on the detection of specific loss-of-function mutations.

- **High-resolution genotyping of *M. leprae* strains**

Get to know the strain (sub)type and VNTR profile of *M. leprae* present in the sample. Detect mixed infection involving distinct *M. leprae* (sub)types.

- **Identification of more than 100 mycobacterial species**

Identify leprosy-causing mycobacteria, including *M. leprae* and *M. lepromatosis*, and most species of clinical or veterinary relevance: *M. tuberculosis* complex, *M. ulcerans*, *M. intracellulare*, *M. abscessus* and many more. Detect co-infection/co-colonization with distinct species.

- **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 *M. leprae* DNA loads down to 100 genomes.

Introduction

Expansion of antimicrobial resistance monitoring and epidemiological surveillance are key components of the WHO strategy towards zero leprosy[1]. The inability to grow *M. leprae in vitro* precludes routine phenotypic drug susceptibility testing, and only limited molecular tests are available.

Here, we present the Deeplex® Myc-Lep assay which uses next generation sequencing-based targeted deep sequencing for simultaneous prediction of (hetero) resistance to three anti-leprosy drugs/drug classes, *M. leprae* high-resolution genotyping and mycobacterial identification, directly from clinical specimens*.

A comprehensive assay based on targeted sequencing

The Deeplex® Myc-Lep kit includes a ready-to-use master mix ready for multiplexed amplification, a positive and internal DNA controls. The Deeplex® Myc-Lep assay starts with DNA extraction from a (suspected) mycobacteria-containing clinical specimen (Figure 1). A single multiplex PCR is then performed to amplify genome regions from seven drug resistance-associated *M. leprae* genes, the mycobacterial *hsp65* gene (for mycobacterial identification), 18 canonical SNPs and 11 core VNTR markers (for high-resolution genotyping).

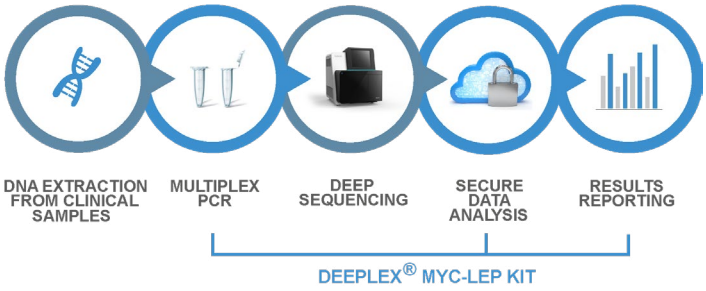


Figure 1. The Deeplex® Myc-Lep workflow. From DNA extraction from clinical samples to data analysis and result reporting. The assay comes as two options: the Deeplex® kit and the Deeplex® service. The kit includes a single PCR master mix ready for multiplexed amplification of the mycobacterial targets, positive and internal control, prior to deep sequencing using Illumina® kits and technology and analysis at GenoScreen. Service is performed at GenoScreen.

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, including synthetic visualisation (Deeplex® map) (Figure 2).

On demand, Deeplex® Myc-Lep testing on user's samples can also come as a service. 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 and Illumina® DNA prep library preparation kits on the MiSeq sequencing platform (Illumina®).

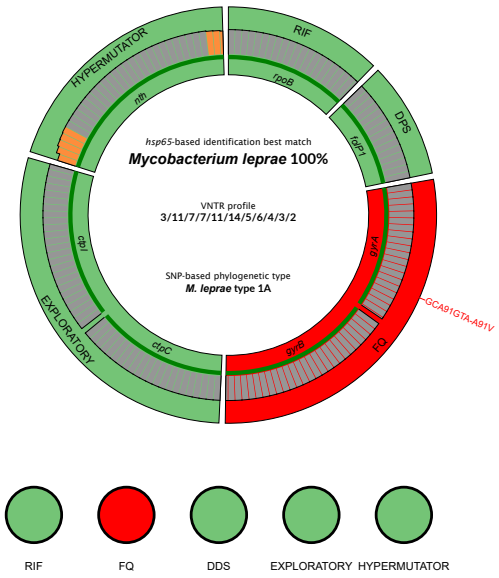


Figure 1. Deeplex Myc-Lep results identifying a *M. leprae* strain of SNP type 1A genotypically resistant to fluoroquinolones. Results are shown for a Thai53 strain derivative, mutated in gyrA (see Table S3). Information on hsp65 best match-based identification, VNTR allelic profile and SNP-based phylogenetic type is shown in the center of the circle. Information on predictions of drug susceptibility and drug resistance for anti-leprosy drugs/drug classes and on hypermutator genotype is as follows.

Target gene regions are grouped within sectors in a circular map according to the prediction feature (drug resistance, hypermutation) with which they are associated. Sectors in red and green indicate targets in which resistance- or hypermutation-associated mutations or no mutations are detected, resulting in predictions of resistant or susceptible phenotypes (for rpoB, folP1, gyrA, gyrB), or hypermutator strain (nth), respectively. The ctpC and ctpI sector (and their associated drug resistance or drug susceptibility predictions) are categorized as exploratory, based on previous work suggesting an association of missense mutations in these genes with resistance to rifampicin, observed in a single strain devoid of mutation in the rpoB DRDR (see text). 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 mutation) 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. Here, LOD is >10% at the extremities of the nth target only. In the VNTR profile, VNTR markers are ordered as follows: 6-3a, AC8a, AC8b, AC9, GTA9, GAA21, GGT5, 6-7, 12-5, 21-3, 23-3. *RIF: Rifampicin, DPS: Dapsone, FQ: Fluoroquinolones, VNTR, variable-number tandem-repeat, SNP, single nucleotide polymorphism.

Prediction resistance to 3 anti-leprosy drugs

The Deeplex® Myc-Lep assay relies on deep sequencing of seven *M. leprae* gene targets associated with resistance to anti-leprosy drugs (Figure 3). Based on the observed presence or absence of mutations in these loci and interrogation of the Deeplex® Myc-Lep database**, the *M. leprae* strain present in the sample is predicted to be resistant or susceptible to each antibiotic, or with yet-to-be characterized mutations. A hypermutator strain genotype associated with potential drug resistance and treatment failure is also predicted based on detection of loss-of-function mutations in a specific target. Individual target positions and mutations can be viewed in the report, along with their sequence coverage depths and read frequencies. Information on reference literature describing the association of mutations with drug resistance can also be viewed in the report. The assay can predict resistance to dapsone, rifampicin and fluoroquinolones.

Mycobacterium leprae genotyping

In addition to antibiotic resistance prediction, the Deeplex® Myc-Lep assay can be used to identify the *M. leprae* strain type detected in the sample. This is achieved by detecting and identifying the alleles of 18 canonical SNPs[2] and 11*** core plus two non-core variable-number tandem-repeat (VNTR) markers[3].

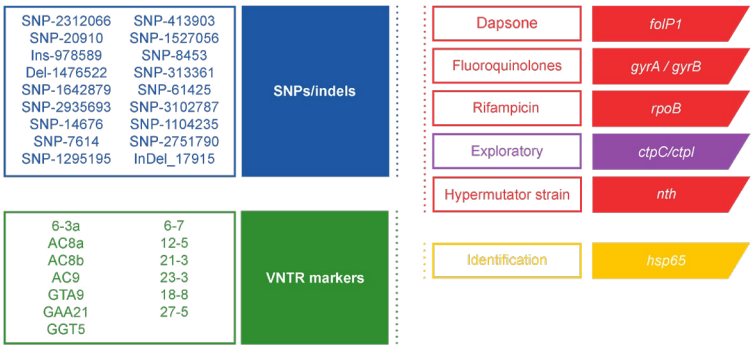


Figure 3. Gene regions amplified and sequenced using the Deeplex® Myc-Lep assay.

A highly sensitive assay

With the Deeplex® Myc-Lep assay, sequencing of mycobacterial gene targets can be achieved at high read depth which means that each sequence position is covered by many reads, enabling highly confident mutation calls including from mutant/heteroresistant subpopulations as low as 10% of bacteria in the sample, inaccessible to other rapid molecular tests. Extracted DNA representing as low as 100 mycobacterial genomes can be characterized.

Identification of more than 100 mycobacterial species

Based on nucleotide identity of the *hsp65* gene[4], the Deeplex® Myc-Lep assay can not only identify *M. leprae* but also >100 other mycobacterial species, including *M. lepromatosis* (the other causal agent of leprosy) as well as most clinically relevant species such as *M. tuberculosis* complex, *M. ulcerans*, *M. abscessus* and *M. intracellulare*.

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® Myc-Lep	
Input sample type	gDNA from clinical specimens* (eg. biopsies, slit skin smears…) or culture (mouse footpad model)
Minimal DNA input	30,000 RLEP copies, 1000 genomes
Recommended library prep	Nextera® XT (Illumina®), Illumina® DNA prep
Recommended sequencing platforms	Illumina® iSeq 100 (17 samples), MiniSeq (32/107), MiSeq (2/17/72), MiSeq*** (5/122)
Turnaround time	iSeq 100: 1 day; others sequencers: ≈2 days
Storage and shelf-life	-20°C for up to 6 months

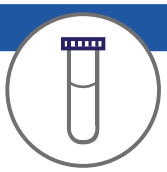
Table 1. Specifications of the Deeplex® Myc-Lep kit.
Turnaround includes multiplex PCR, library preparation and sequencing. Analysis is performed at GenoScreen. #Number of effective samples - controls not included. MiniSeq (Mid/High output), MiSeq 2x150bp (Nano/Micro/Full), MiSeq 2x250bp*** (Nano/Full).

** with genome loads ≥ 100-1000 (quantified e.g. by RLEP qPCR). If available, genomic DNA extracted from cultured M. leprae using the mouse footpad model can also be used.*
*** © 2022 GenoScreen Mycobacterium leprae variant database (All Rights Reserved).*
**** The number of repeats of non-core VNTR markers 18-8 and 27-5 can only be obtained using 250pb paired-end reads.*

Deeplex® Myc-Lep workflow

DNA extraction

Skin biopsies/
slit skin smears



DNA extraction
purification and
quantification

6h

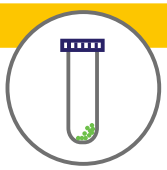
Deeplex® PCR

DNA

Deeplex®
PCR

Target enrichment

Clean-up



DNA quantification

4h

Library prep

Amplicons

DNA
fragmentation

Library amplification

Library pooling 2nM
+ quantification

5h

Sequencing

Pooled DNA library

Dilution 10pM
NaOH denaturation

MiSeq®
75 tests

Illumina®
sequencing

~24h

Analysis

DNA reads

Deeplex®
pipeline

Analysis

Deeplex®
pipeline

Report

Mycobacterial identification (hsp65)
M. leprae high resolution typing (18 canonical SNPs[2] and 11core plus /13 VNTRs)
Resistance prediction to 3 anti-leprosy drugs
Detection of heteroresistance down to 10%
Detection of mixed infections

<1h

References

1. World Health Organization. Towards zero leprosy. Global leprosy (Hansen's Disease) strategy 2021–2030 [Internet]. 2021. Available from: https://apps.who.int/iris/handle/10665/340774
2. Truman, R. W. et al. (2011). Probable zoonotic leprosy in the Southern United States. *New England Journal of Medicine*, 364 (17), 1626–1633.3.
3. Jensen RW, Rivest J, Li W, Vissa V. DNA fingerprinting of *Mycobacterium leprae* strains using Variable number tandem repeat (VNTR)- fragment length analysis (FLA). *Journal of Visualized Experiments*. 2011 (53)
4. Dai J, Chen Y, Lauzardo M. Web-accessible database of hsp65 sequences from *Mycobacterium* reference strains. *J Clin Microbiol*. 2011, 49 (6): 2296–303

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