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Catalog ID /Part #	Item	Lot #/Batch #
20099512	Deeplex® Myc-TB Combo IDP Kit	
GNSRUOTB01-48	Genoscreen Deeplex Myc-TB	251015DP51
GNSRUOTB01-48	Genoscreen Deeplex Myc-TB	251117DP52
20015826	ILMN DNA Prep, PCR + Buffers 24	21007506
20015827	ILMN DNA Prep, Tag Beads (M) 24	21007507
20049005	ILMN DNA Prep, IPB + Buffers 24	20994624
20083060	ILMN UD Indexes LT	20999301
20126565	MiSeq™ i100 Series 5M Rgt Kit 300 cyc	
20115217	MISEQ™ i100 SERIES 5M 300CYC DRY CART	21012188
20079638	MISEQ™ i100 SERIES, WET CARTRIDGE, A	21001596
FC-110-3001	PhiX CONTROL V3 KIT	
15017666	PhiX Control v3	21007683

Jan-05-2026

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Denature and Dilute Libraries for the MiSeq system

This guide explains how to denature and dilute prepared libraries for sequencing on the Illumina MiSeq system. It is intended to be used with the MiSeq Product Documentation. The MiSeq system pages on the Illumina support site provide additional resources, including training, compatible products, and other considerations. Always check the support site for the latest versions.

Before You Begin

Loading Volume and Concentration

This procedure denatures and dilutes libraries to a final volume of 600 µl. The recommended loading concentration varies depending on the version of MiSeq Reagent Kit used for the sequencing run. In practice, loading concentration can vary depending on library preparation and quantification methods. Refer to the protocol for additional information.

Chemistry	Recommended Final Loading Concentration
MiSeq Reagent Kit v3	Supports 6–20 pM loading concentration. Requires at least a 4 nM library before diluting and denaturing.
MiSeq Reagent Kit v2	Supports 6–10 pM loading concentration.

About Low Diversity Libraries

Low diversity libraries are libraries where a significant number of the reads have the same sequence. This lack of variation shifts the base composition because the reads are no longer random.

For example, low diversity can occur with some expression studies with > 25% of one type of transcript, low-plexity amplicon pools, adapter dimer, or bisulfite sequencing. A higher concentration spike-in of PhiX helps balance the overall lack of sequence diversity.

For low diversity libraries, dilute your PhiX control library to the same concentration as your denatured library.

Best Practices

- *Always* prepare freshly diluted NaOH to denature libraries for cluster generation. This step is essential to the denaturation process.
- To prevent small pipetting errors from affecting the final NaOH concentration, prepare at least 1 ml freshly diluted NaOH.
- For best results, begin thawing the reagent cartridge before denaturing and diluting libraries. For instructions, refer to the *MiSeq Product Documentation*.

Protocol

Introduction

Use this protocol to denature and dilute libraries that have been normalized using standard library quantification and quality control procedures recommended in the library prep documentation.

Follow the steps most appropriate for your library and the version of MiSeq reagent kit that you are using. Loading concentration can also vary depending on library type and quantification methods.

Chemistry	Compatible Denature and Dilute Steps
MiSeq Reagent Kit v3	**4 nM library**—Results in a 6–20 pM loading concentration.
MiSeq Reagent Kit v2	**4 nM library**—Results in a 6–20 pM loading concentration.
	2 nM library—Results in a 6–10 pM loading concentration.

The denaturation steps described here make sure that the concentration of NaOH is not more than 0.001 (1 mM) in the final solution after diluting with HT1. Higher concentrations of NaOH in the library inhibit library hybridization to the flow cell and decrease cluster density.

Consumables and Equipment

This protocol requires the following consumables and equipment.

Consumables

Consumable	Supplier
1 N NaOH, molecular biology grade	General lab supplier
HT1 (Hybridization Buffer), thawed and prechilled	Illumina, provided in the MiSeq Reagent Kit
Microcentrifuge tube, 1.7 ml	General lab supplier
Water, laboratory-grade	General lab supplier

The following additional consumables are required to prepare a PhiX control.

Consumables	Supplier
10 mM Tris-HCl, pH 8.5 with 0.1% Tween 20	General lab supplier
Illumina PhiX Control	Illumina, catalog # FC-110-3001

Equipment

Equipment	Supplier
Microcentrifuge	General lab supplier
Vortexer	General lab supplier

Prepare Reagents

Prepare a Fresh Dilution of NaOH

1. Combine the following volumes in a microcentrifuge tube.
   - Laboratory-grade water (800 µl)
   - Stock 1 N NaOH (200 µl)

   The result is 1 ml of 0.2 N NaOH.
2. Invert the tube several times to mix.

Use the fresh dilution within **12 hours**.

Prepare HT1

1. Remove HT1 from -25°C to -15°C storage and thaw at room temperature.
2. Store at 2°C to 8°C until you are ready to dilute denatured libraries.

Denature and Dilute, 4 nM Libraries

Denature a 4 nM Library

1. Combine the following volumes in a microcentrifuge tube.
   - 4 nM library (5 µl)
   - 0.2 N NaOH (5 µl)
2. Vortex briefly and then centrifuge at 280 × g for 1 minute.
3. Incubate at room temperature for 5 minutes.
4. Add 990 µl prechilled HT1 to the tube containing denatured library.

   The result is 1 ml of a 20 pM denatured library.

Dilute Denatured 20 pM Library

1. Dilute to the desired concentration using the following volumes.

Concentration	6 pM	8 pM	10 pM	12 pM	15 pM	20 pM
20 pM library	180 µl	240 µl	300 µl	360 µl	450 µl	600 µl
Prechilled HT1	420 µl	360 µl	300 µl	240 µl	150 µl	0 µl

2. Invert to mix and then pulse centrifuge.

Denature and Dilute, 2 nM Libraries

Denature a 2 nM Library

1. Combine the following volumes in a microcentrifuge tube.
   - 2 nM library (5 µl)
   - 0.2 N NaOH (5 µl)
2. Vortex briefly and then centrifuge at 280 × g for 1 minute.
3. Incubate at room temperature for 5 minutes.
4. Add 990 µl prechilled HT1 to the tube containing denatured library.

   The result is 1 ml of a 10 pM denatured library.

Dilute Denatured 10 pM Library

1. Dilute to the desired concentration using the following volumes.

Concentration	6 pM	8 pM	10 pM
10 pM library	360 µl	480 µl	600 µl
Prechilled HT1	240 µl	120 µl	0 µl

2. Invert to mix and then pulse centrifuge.

Next Steps

After denaturing and diluting the libraries and preparing the optional PhiX control, you are ready to load libraries onto the reagent cartridge and set up the sequencing run. Refer to the *MiSeq Product Documentation* for more information.

Denature and Dilute PhiX Control (Optional)

Introduction

Use the following procedure to denature and dilute a PhiX library for use as a sequencing control.

Follow the steps appropriate for the version of MiSeq reagent kit you are using.

Chemistry	Final PhiX Concentration
MiSeq Reagent Kit v3	Dilute the denatured PhiX control to 20 pM, which produces an optimal cluster density using v3 reagents.
MiSeq Reagent Kit v2	Dilute the denatured PhiX control to 12.5 pM, which produces an optimal cluster density using v2 reagents.

Prepare PhiX

Dilute PhiX to 4 nM

1. Combine the following volumes in a microcentrifuge tube.
   - 10 nM PhiX library (2 µl)
   - 10 mM Tris-HCl, pH 8.5 with 0.1% Tween 20 (3 µl)
2. If not prepared within the last **12 hours**, prepare a fresh dilution of 0.2 N NaOH.

Denature PhiX Control

1. Combine the following volumes in a microcentrifuge tube.
   - 4 nM PhiX library (5 µl)
   - 0.2 N NaOH (5 µl)
2. Vortex briefly to mix.
3. Centrifuge at 280 × g for 1 minute.
4. Incubate at room temperature for 5 minutes.

Dilute Denatured PhiX to Loading Concentration

Dilute Denatured PhiX to 20 pM

1. Add prechilled HT1 to the denatured PhiX library.
   - Denatured PhiX library (10 µl)
   - Prechilled HT1 (990 µl)

   The result is 1 ml of a 20 pM PhiX library.
2. Invert to mix.

You can store the denatured 20 pM PhiX library up to 3 weeks at -15°C to -25°C. After 3 weeks, cluster numbers tend to decrease.

Dilute Denatured PhiX to 12.5 pM

If you are using MiSeq Reagent Kit v3, no further dilution is required.

1. Add prechilled HT1 to the denatured PhiX library.
   - 20 pM denatured PhiX library (375 µl)
   - Prechilled HT1 (225 µl)

   The result is 600 µl of a 12.5 pM PhiX library.
2. Invert to mix.

Combine Library and PhiX Control

For most libraries, use a low-concentration PhiX control spike-in of 1% as a sequencing control. For low diversity libraries, increase the PhiX control spike-in to at least 5%.

1. Combine the following volumes of denatured PhiX control and denatured library.

	Most Libraries (1% Spike-In)	Low diversity Libraries (≥ 5% Spike-In)
Denatured and diluted PhiX	6 µl	30 µl
Denatured and diluted library	594 µl	570 µl

2. Set aside on ice until you are ready to load it onto the reagent cartridge.

Actual PhiX percentage varies depending upon the quality and quantity of the library pool.

Revision History

Revision History - Protocol for Standard Normalization

Document	Date	Description of Change
Document # 200047027 v00	January 2024	Initial release.

Revision History - PhiX Control

Document	Date	Description of Change
Document # 200047031 v00	January 2024	Initial release.

Revision History - MiSeq System

Document	Date	Description of Change
Document # 15039740 v11	January 2024	Converted to HTML format. Split out protocols and other information to separate documents and created a dynamic protocol generator with links to those documents.
Document # 15039740 v10	February 2019	Replaced Suggested Final Loading Concentration table in Protocol C with a single suggested concentration range.
Document # 15039740 v09	November 2018	Fixed AmpliSeq for Illumina Myeloid Panel pooling ratio in Protocol D.
Document # 15039740 v08	November 2018	Fixed AmpliSeq for Illumina Myeloid Panel pooling ratio in Protocol C. Added AmpliSeq for Illumina Childhood Cancer Research Assay Panel pooling ratio.
Document # 15039740 v07	October 2018	Added Protocol D for denaturing and diluting libraries prepared using the AmpliSeq Library Equalizer for Illumina workflow.
Document # 15039740 v06	July 2018	Added pooling ratio for AmpliSeq Myeloid Panel for Illumina.
Document # 15039740 v05	May 2018	Removed caution against using PhiX with Protocol C.
Document # 15039740 v04	April 2018	Added Protocol C for denaturing and diluting AmpliSeq for Illumina Panels.
Document # 15039740 v03	December 2017	Added recommendation in Protocol A to reference the Nextera DNA Flex Library Prep Reference Guide when working with the Nextera DNA Flex Library Prep Kit.
Document # 15039740 v02	February 2017	Added loading concentration recommendations for TruSeq Myeloid Sequencing Panel, TruSeq Custom Amplicon v1.5, and TruSeq Custom Amplicon Low Input Sequencing Kit.
Document # 15039740 v01	January 2016	Added procedure for denaturing and diluting libraries that have been normalized using a bead-based procedure. Organized procedures as Protocol A and Protocol B.
Part # 15039740 Rev. D	November 2013	Added recommendation for low diversity libraries to dilute PhiX control libraries to the same concentration as denatured sample libraries.
Part # 15039740 Rev. C	August 2013	Added recommendation to use molecular biology grade NaOH. Added recommended library denaturation and PhiX control protocols for use with MiSeq Reagent Kit v3. Removed loading samples library information. That information is now in the *MiSeq System User Guide (part # 15027617)*.
Part # 15039740 Rev. B	March 2013	Reduced PhiX recommendations for low diversity libraries from ≥ 25% to ≥ 5%. This change is possible when using RTA 1.17.28, or later, released with MCS v2.2. Corrected the resulting NaOH concentration for denatured 10 pM library to 1 mM. Updated instructions for combining prepared libraries and PhiX control to total 600 µl.
Part # 15039740 Rev. A	January 2013	Initial release.

DEEPLEX

Deeplex® Myc-TB

From clinical samples to drug resistance profile

A novel *Mycobacterium tuberculosis* drug resistance prediction assay, comprehensive, culture-free and based on deep sequencing

RESEARCH USE ONLY

GenoScreen
Innovative Genomics

A novel deep sequencing-based assay for antibiotic resistance prediction of *Mycobacterium tuberculosis* complex, with mycobacterial identification and genotyping

Highlights

- **Prediction of resistance to 15 anti-TB drugs**

  Easily visualise resistance associated mutations in (detected) *M. tuberculosis* complex (MTBC) gene targets, thanks to our Deeplex web app for automated analysis and interpretation of the sequencing data.

- **Genotyping and spoligotyping of MTBC strains**

  Get to know the lineage / sublineage and spoligotype of MTBC strains present in the sample. Detect mixed infection involving distinct MTBC lineages/sublineages.

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

  Identify mycobacteria including most species of clinical or veterinary relevance: MTBC, *M. kansasii, M. abscessus, M. intracellulare, M. avium complex,* and many more. Detect co-infection/co-colonization with distinct species.

- **Turnaround time of less than 48 hours**

  Save time using DNA from clinical samples*, prepare libraries, sequence and analyse results in the Deeplex web app for a total turnaround time of less than 48 hours.

- **High performances**

  Capture 97-99% of resistance phenotypes predicted by WGS, mean sensitivity of 95.2% and mean specificity of 97.1% vs phenotypic drug susceptibility testing**, **identify heteroresistance down to 1%** subpopulations and work with DNA loads down to 100 genomes.

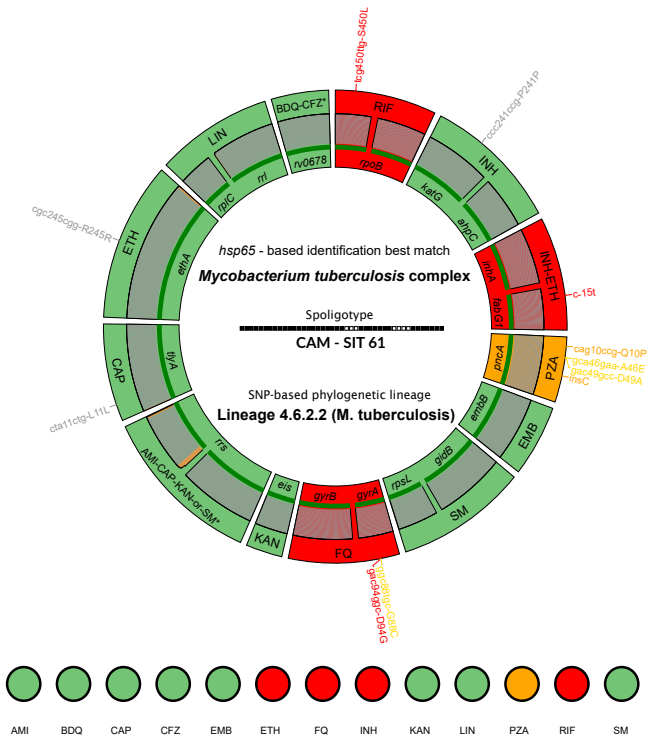


Figure 1. The Deeplex® web app (Top) **Deeplex® map** showing mutations associated (red and orange (heteroresistance)) or not associated (or synonym, grey) with antibiotic resistance of MTBC. Information on mycobacterial identification is shown in the center of the map. (Bottom) **Resistotype** of an identified MTBC strain showing its predicted resistance pattern to 15 anti-tuberculosis drugs. The Deeplex® map is a registred design.

Introduction

According to the World Health Organization, in 2020 there were more than half a million new cases of rifampicin (RR) or multi-drug (MDR) resistant forms, including more than 25,000 pre-extensively drug resistant (pre-XDR)*** or extensively drug resistant (XDR)**** forms[1]. Yet to treat tuberculosis efficiently, rapid and early detection of drug resistance is essential.

Advances in next-generation sequencing (NGS) technology, offer great potential for more efficient detection of drug resistant TB. Unfortunately today, routine clinical use of whole genome sequencing (WGS) requires time-consuming mycobacterial culturing and alternative molecular methods rely on a small set of common resistance associated mutations, limiting the detection spectrum[2,3].

Here, we present the **Deeplex® Myc-TB** assay which uses NGS-based targeted deep sequencing for the simultaneous prediction of (hetero)resistance to 15 anti-tuberculosis drugs/drug classes, MTBC genotyping and mycobacterial identification. This all-in-one assay is compatible with detection directly from clinical samples* and includes an automated analysis pipeline of the sequencing data in a secure web app with integrated databases (Figure1).

A comprehensive assay based on targeted sequencing

The **Deeplex® Myc-TB** assay starts with DNA extraction from either a (suspected) mycobacteria-containing clinical specimen or a mycobacteria-positive culture. A single multiplex PCR is then performed to amplify mycobacterial genome regions from 18 drug resistance associated MTBC genes, the *hsp65* gene (for mycobacterial speciation) and the DR/CRISPR locus of the MTBC (for spoligotyping).

The resulting PCR products are cleaned-up and libraries are prepared for sequencing. The obtained sequencing data are then uploaded to a secure web app for automated analysis, results can be viewed directly from the web app and exported in several formats (Figure 2).

The **Deeplex® Myc-TB** kit includes a master mix ready for multiplexed amplification, a positive and internal DNA control as well as an activation code to access the Deeplex® web app. Alternatively, the **Deeplex® Myc-TB** assay comes as a service (on demand). GenoScreen performs all steps, from DNA extraction (optional) to final generation of analysed data, made available to the user in the web app.

The assay has successfully been tested using the Nextera XT and Illumina DNA prep library kits on the iSeq 100, MiniSeq, MiSeq, and NextSeq 550 sequencing platforms (Illumina).

Prediction of resistance to 15 anti-TB drugs

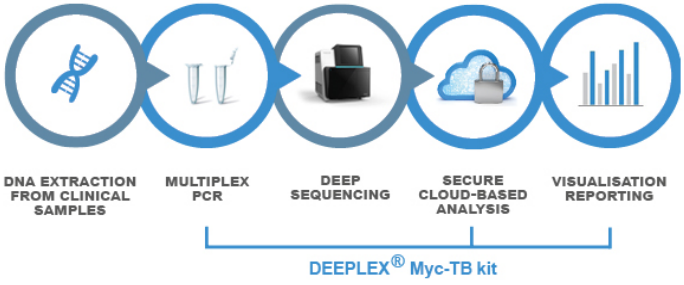


Figure 2. The Deeplex® Myc-TB workflow. From DNA extraction from clinical or culture samples to data analysis and result visualization. The assay comes as two options: the **Deeplex® kit** and the **Deeplex® service**. The kit includes a single PCR master mix ready for multiplex amplification of the mycobacterial targets, positive and internal control as well as an activation code to access the Deeplex® Web App. Service is performed at GenoScreen.

The **Deeplex® Myc-TB** assay relies on deep sequencing of 18 main MTBC gene targets associated with resistance to first and second line drugs (Figure 3). Based on the observed absence or presence of mutations in these loci and interrogation of reference databases*****, the MTBC strain present in the sample is predicted to be susceptible or resistant to each antibiotic, or with yet-to-be characterized mutations (Figure 1). Individual target positions and mutations can be easily visualized along with their sequence coverage depths. Information on reference literature describing the association of mutations with drug resistance can be accessed via hyperlinks. In total, the assay can predict resistance to 15 anti-tuberculosis drugs/ drug classes including the more recently introduced compounds such as bedaquiline and linezolid, making it the most exhaustive genotypic assay directly applicable on specimens available to date.

MTBC genotyping and spoligotyping

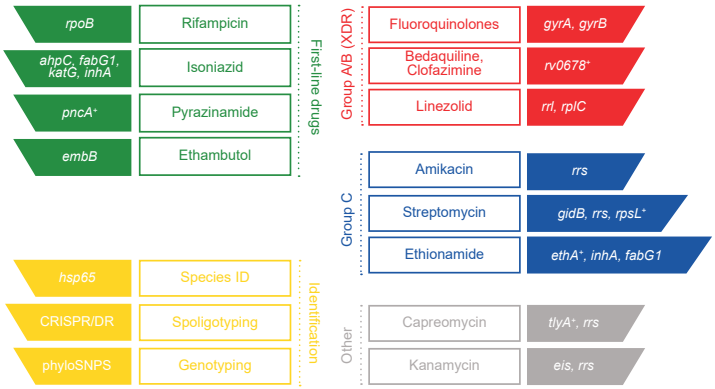


Figure 3. Genes or genes regions amplified and sequenced via the Deeplex® Myc-TB assay (+ : full genes).

In addition to antibiotic resistance prediction, the **Deeplex® Myc-TB** assay can be used to identify MTBC strain types present in the sample. When detected based on nucleotide identity of the *hsp65* gene, MTBC strains are spoligotyped and genotyped. This is achieved by detecting the presence-absence pattern of 43 spacers in the CRISPR locus and phylogenetic SNPs in the other gene targets, respectively. Mycobacterial species identification as well as MTBC spoligotyping and genotyping results can then easily be viewed on the Deeplex® web app, in the center of the Deeplex® map (Figure 1).

A highly sensitive assay

With the **Deeplex® Myc-TB** 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 for mutant/heteroresistant subpopulations representing as low as 1-3% of bacteria in the sample[4], inaccessible to other rapid molecular tests. Extracted DNA representing as low as 100 mycobacterial genomes, thus below the limit of detection by classical microscopy[4], can be characterized. The **Deeplex® Myc-TB** assay captures ***in silico*** 97-99% of resistance phenotypes predicted by WGS and has a mean sensitivity of 95.2% and a mean specificity of 97.1% vs phenotypic drug susceptibility testing**.

Drug	Sensitivity (%)	Specificity (%)
Rifampicin	99.4	98.8
Isoniazid	98.3	98.4
Pyrazinamide	85.7	100
Ethambutol	92.2	90.7
Streptomycin	90.7	98.9
Fluoroquinolones	91.7	99.2
Amikacin	100	100
Kanamycin	88.9	100
Capreomycin	93.8	97.4
Ethionamide	92.6	68
Linezolid	NA	100
Total	95.2	97.1

Table 1. Deeplex® Myc-TB phenotype predictions versus pDST
Comparaison of Deeplex® Myc-TB drug susceptibility and drug resistance predictions (excluding uncharacterized mutations) against pDST on a reference collection of 429 MTB isolates, including the WHO-TDR collection. The dataset used did not include any linezolid resistant strains (only linezolid susceptible strains, all correctly predicted as such by Deeplex®) and bedaquiline susceptibility was not tested. Deeplex® Myc-TB phenotype predictions obtained with Deeplex® Myc-TB web app version 1.4. **

Identification of more than 100 mycobacterial species

Based on nucleotide identity of the *hsp65* gene[5], the **Deeplex® Myc-TB** assay can not only indentify *M. tuberculosis* complex but also >100 other mycobacterial species, including most clinically relevant species such as *M. kansasii, M. intracellulare, M. avium* complex...

Turnaround time of less than 48 hours

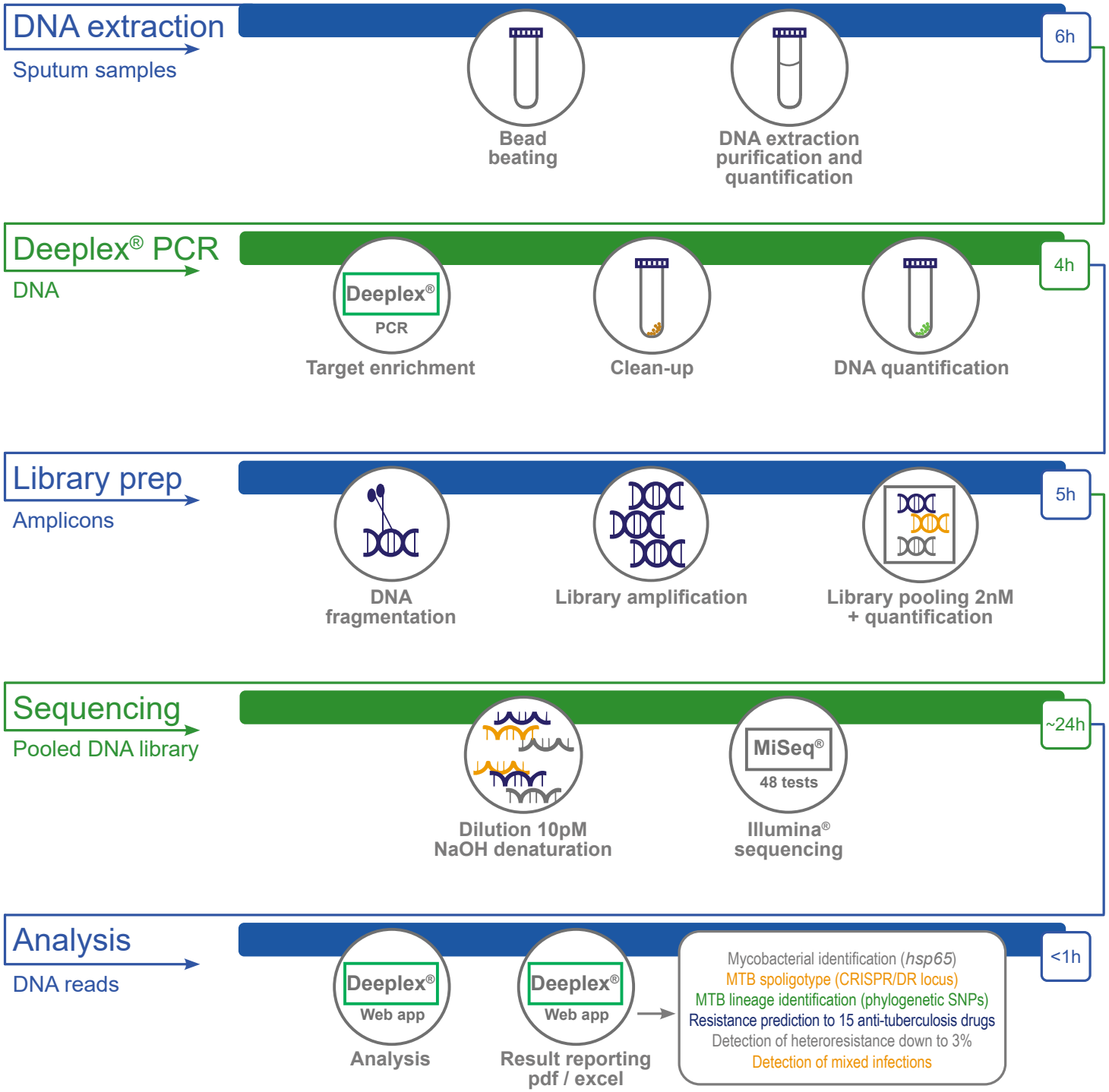
Mycobacterial cultures are not required for use with the **Deeplex® Myc-TB** and the assay can be used on clinical samples with minimal bacterial loads (see above). Deeplex® targets are amplified from extracted DNA with the ready-to-use master mix for multiplex PCR, purified and prepared to be sequenced. The total takes from 1 to 2 days (Table 2). Once targets are sequenced, output FASTQ (read) files are ready to be uploaded onto our secure web app, using the access code provided within the kit. Data will be analyzed with our fully parameterized Deeplex® pipeline in less than an hour and results can be easily visualized.

	Deeplex® Myc-TB
Input sample type	gDNA from clinical samples* (eg. sputum...) or culture
DNA input quantity	9 µl gDNA at 1 pg/µl mycobacterial DNA, 20 µL culture thermolysate, 200 µL sputum
Recommended library prep	Nextera® XT (Illumina®), Illumina® DNA prep
Recommended sequencing technologies	Illumina® iSeq 100 (13 samples), MiniSeq (21), MiSeq (45), NextSeq 550 (372)ᵗ
Turnaround time	iSeq 100: 1 day ; others sequencers: ≈2 days
Storage and shelf-life	-20°C for up to a year

Table 2. Specifications of the Deeplex® Myc-TB kit.
Turnaround time includes multiplex PCR, library preparation, sequencing and analysis.
ᵗNumber of effective samples – controls not included.

** with genome loads ≥ 100*
*** Results from Jouet A, Gaudin C, Badalato N, et al. Deep amplicon sequencing for culture6 free prediction of susceptibility or resistance to 13 anti-tuberculous drugs. Eur Respir J. 2021; 57(3):2002338. Results were obtained on 109 clinical specimens directly analyzed by Deeplex® Myc-TB versus WGS on culture and on 429 MTBC strains versus phenotypic DST.*
**** MDR additionally resistant to fluoroquinolones.*
***** MDR additionally resistant to fluoroquinolones and, at least one additional group A drug (bedaquiline, linezolid).*
****** © 2022 GenoScreen Mycobacterium tuberculosis variant database (all rights reserved) and © Catalogue of mutations in Mycobacterium tuberculosis complex and their association with drug resistance.*

Deeplex® Myc-TB workflow



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