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APPLICATION NOTE

Antimicrobial resistance surveillance using the Urinary Pathogen ID/AMR Enrichment Kit

Broad, culture-free detection of antimicrobial resistance markers and associated pathogens with the MiSeq™ i100 Series

Target enrichment improves sensitivity to enable direct detection of > 3700 AMR markers

Fast sequencing delivers same-day results for efficient AMR marker profiling

High-quality sequencing data correlates with conventional phenotypic testing methods to provide culture-free AMR detection

Introduction

Antimicrobial resistance (AMR) is a clinical and public health threat that can influence patient outcomes regardless of underlying disease or microbiological etiology.[1] The slow development pipeline for new antimicrobials means that it is imperative to monitor mechanisms underlying known patterns of resistance and to detect evidence of emerging AMR rapidly in new populations.[2]

Common methods for AMR surveillance are based on monitoring key organisms identified as threats. It often relies on laboratory data from growth-based characterization of pathogens, which is an inherently reactive approach to surveillance.[3-4] Next-generation sequencing (NGS) has demonstrated promise for predicting phenotypic resistance in samples characterized by conventional microbiological methods, and offers the opportunity to interrogate the full spectrum of AMR markers in a sample without the limitations imposed by culture- and growth-based phenotyping.[5]

Sensitive detection and characterization of antimicrobial resistance genes (ARGs) and associated bacteria in primary samples using NGS can benefit from library preparation enrichment methods that can overcome the sequencing depth otherwise needed for samples without captured targets.[6-7] By increasing the relative abundance of genomic content of interest in the library, enrichment also unlocks the potential for sequencing libraries directly from samples on benchtop instruments.

This application note demonstrates detection and characterization of ARGs and associated bacterial pathogens in urine samples using an NGS workflow that integrates the Urinary Pathogen ID/AMR Enrichment Kit, the MiSeq i100 Series, and onboard DRAGEN™ secondary analysis (Figure 1). The MiSeq i100 Series delivers high-resolution microbial identification data for AMR surveillance.

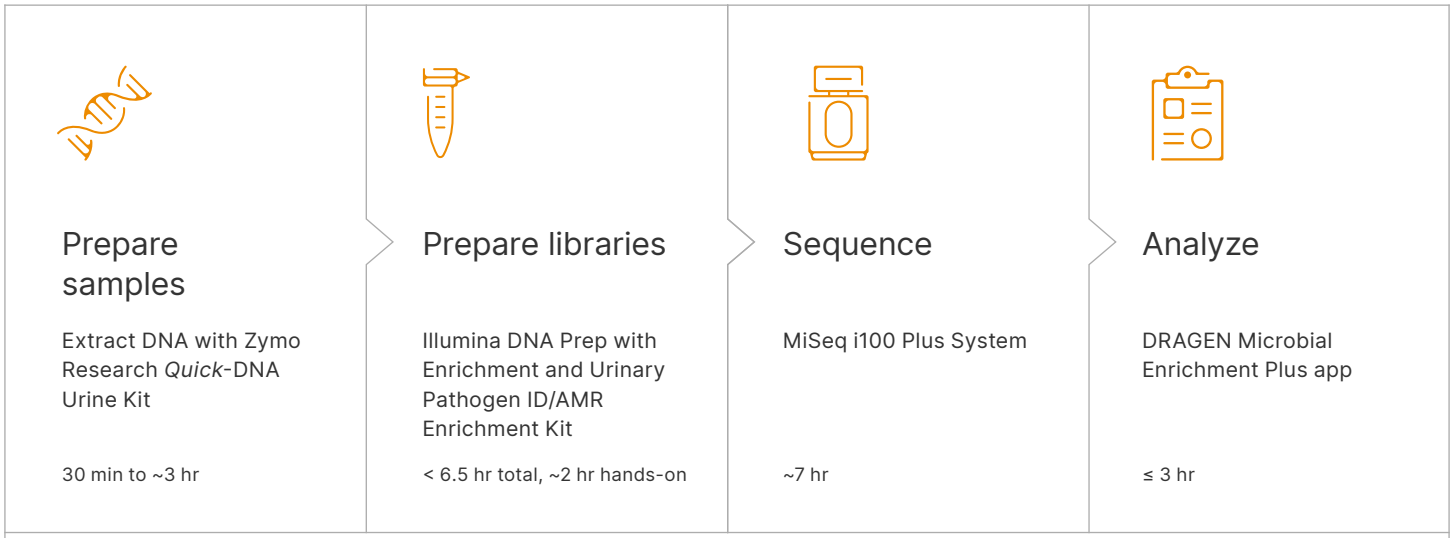

Figure 1: Comprehensive NGS workflow for AMR profiling in urine samples
In a streamlined, comprehensive workflow, libraries are prepared from urinary DNA extractions and enriched with the Urinary Pathogen ID/AMR Enrichment Kit, sequenced on the MiSeq i100 Plus System, and analyzed using the DRAGEN Microbial Enrichment Plus app v1.0.0 onboard the instrument or in BaseSpace Sequence Hub for user-defined microorganism detection, quantification, and bacterial AMR profiling.

Methods

Samples

Samples used for this study were obtained in collaboration with a large commercial diagnostic laboratory in the United States. These samples were remnant, deidentified specimens associated with an order for urine culture and stored with standard boric acid urine preservation. Sample source/collection was variable and included predominantly clean-capture and voided samples. A subset of 20 samples were selected for this application note based on microorganism identification and phenotypic testing results by conventional microbiological methods to include common phenotypic patterns in AMR. Reported quantification from conventional growth-based methods ranged from 10,000 to > 100,000 colony forming units/ml; isolated microorganisms included a range of Gram-negative and Gram-positive bacteria commonly associated with urinary tract infection.

Primary urine samples were stored at 4°C and shipped within 72 hours. DNA was extracted using the *Quick*-DNA Urine Kit (Zymo Research, Catalog No. D3061) following the manufacturer's instructions.

Library preparation

Sequencing-ready libraries were prepared from a maximum volume of 30 µl of extracted DNA from 1.8 ml of urine input using the Urinary Pathogen ID/AMR Enrichment Kit, Set A (RUO) (96 indexes, 96 samples) (Illumina, Catalog no. 20090308).

Sequencing

Prepared libraries (enriched and unenriched) were sequenced on the MiSeq i100 Plus System using a 25M flow cell with a run configuration of 2 × 151 bp.

Data analysis

After sequencing was complete, data were downsampled to 1M clusters/fragments per sample and analyzed using the DRAGEN Microbial Enrichment Plus app v1.0.0 onboard the MiSeq i100 Plus System. This app can also be accessed in the cloud in BaseSpace™ Sequence Hub.

Results

Improved detection of ARGs in urine

The targeted design of the Urinary Pathogen ID/AMR Enrichment Kit is highly sensitive, substantially improving the sequencing yield for direct detection of ARGs in urine, compared to unenriched libraries (Figure 2A). The Urinary Pathogen ID/AMR panel covers > 3700 genes and gene variants associated with AMR, enabling broad profiling of ARGs in urine samples. In this cohort of urine samples, various ARGs associated with multiple common mechanisms of resistance were detected with high coverage, ranging from 60–100% coverage of the genes shown (Figure 2B).

Co-detection of AMR markers with one or more bacterial pathogens

Analysis of Urinary Pathogen ID/AMR panel sequencing data with the DRAGEN Microbial Enrichment Plus app reported at least one AMR marker in all 20 samples analyzed. In this analysis, at least one relevant bacterial microorganism was co-reported in all samples (n = 246 unique AMR marker detections across the sample cohort). The majority of AMR marker detections (n = 212; 86%) were co-detected with only one or two associated microorganisms (Figure 3), facilitating interpretation of NGS results.

Correlation of detected ARGs with phenotypic resistance profiles of isolates derived from primary samples

The microorganisms and ARGs detected in the urine samples by sequencing with the Urinary Pathogen ID/ AMR Enrichment Kit were highly concordant with phenotypic testing results by conventional microbiological methods, carried out by the collaborating commercial laboratory (Table 1).

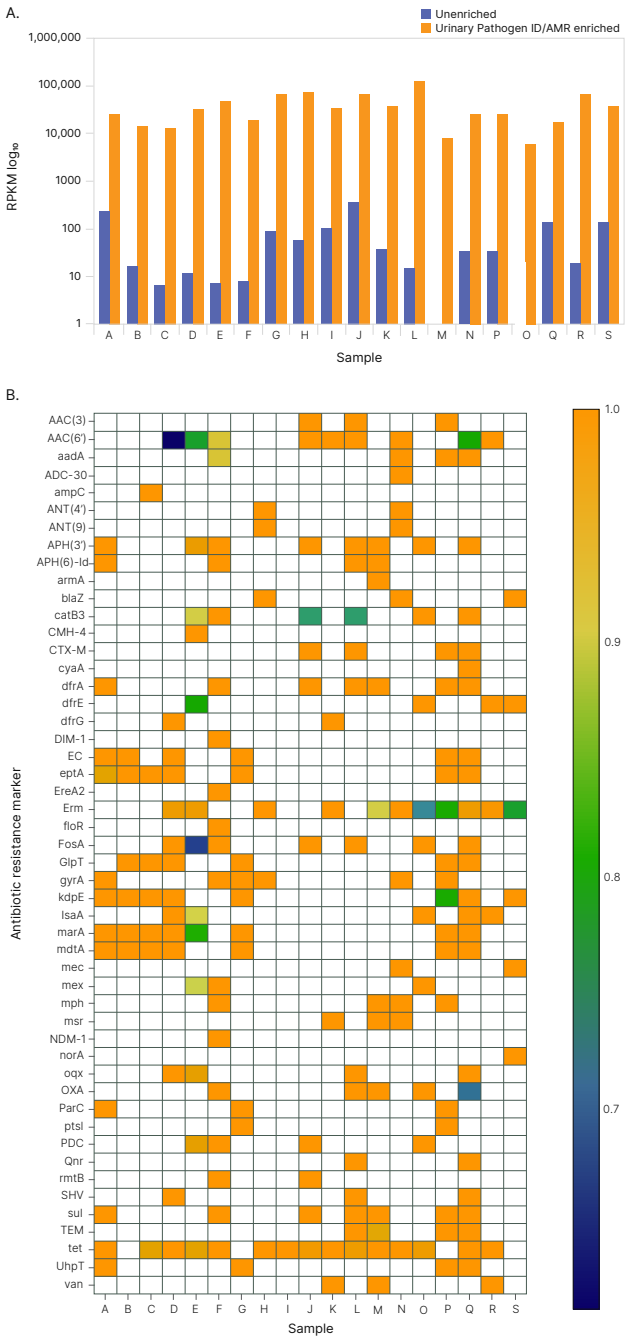

Figure 2: Improved yield for direct detection of ARGs in urine

Results showed (A) improved detection of relevant AMR markers with the Urinary Pathogen ID/AMR panel, compared to unenriched libraries, as measured by reads per kilobase of transcript per million mapped reads (RPKM) and (B) the coverage, expressed as a proportion, achieved for each targeted AMR marker in libraries enriched with the Urinary Pathogen ID/AMR panel, ie, 1 represents 100% coverage of the targeted genomic region.

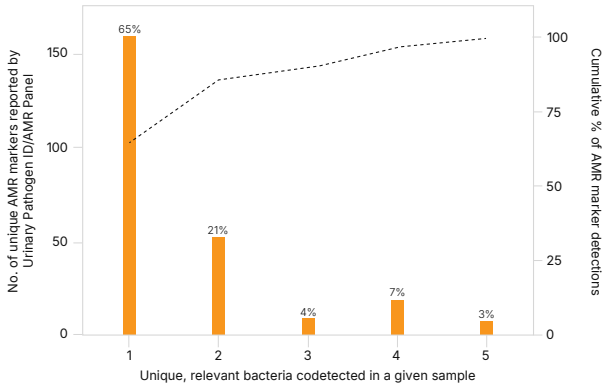

Figure 3: Co-detection of ARGs with associated bacteria

Results showed that at least one AMR marker was reported by the DRAGEN Microbial Enrichment Plus app in all 20 samples; at least one relevant bacterial microorganism was coreported in all samples (n = 246 unique AMR marker detections across the sample cohort). The majority of AMR marker detections (n = 212; 86% cumulative percentage of total AMR marker detections (dashed lines)) were codetected with only one or two associated microorganisms.

Table 1: Reported organism detections and ARGs are concordant with phenotypic resistance profile of isolates

Sample	ID by conventional methods	Microorganism identifications by Urinary Pathogen ID/AMR Panel (expected ID in bold)	Antimicrobial susceptibility testing (AST) phenotype	AMR markers reported by Urinary Pathogen ID/AMR Panel (known associations with tested and reported phenotypic resistance from isolates in bold)
A	*E. coli*	***E. coli***	Carbapenem- and nitrofurantoin-susceptible; resistant to TMP-SMX and fluoroquinolone	**dfrA17**; mdtA; tet(B); UhpT (*E. coli*); **EC-5**; marA; APH(3'')-Ib; **parC** (*E. coli*); **gyrA** (*E. coli*); APH(6)-Id; **sul2**; kdpE; eptA
B	*E. coli*	***E. coli***	Pan-susceptible	marA; **EC-5**; mdtA; GlpT (*E. coli*); eptA; kdpE
C	*E. coli*	***E. coli**, S. agalactiae*	Pan-susceptible	kdpE; marA; eptA; mdtA; GlpT (*E. coli*); ampC; tetM
D	*K. pneumoniae*	***K. pneumoniae**, E. faecalis, E. coli, B. breve, E. faecium*	Pan-susceptible	oqxA; eptA; SHV-11; FosA6; oqxB; marA; lsaA; GlpT (*E. coli*); mdtA; dfrG; tetM; kdpE; EC-15; ErmB; tet(L); AAC(6')-Ii
E	*E. cloacae*	***E. cloacae complex**, E. faecalis, P. aeruginosa, P. anaerobius, P. lymphophilum*	Carbapenem-susceptible; resistant to nitrofurantoin and cephalosporin	CMH-4; **oqxB**; ErmB; APH(3')-IIb; PDC-34; tetM; catB7; lsaA; MexB; **marA**; **dfrE**; AAC(6')-Ie-APH(2'')-Ia; fosA
F	*P. aeruginosa*	*V. parvula,* ***P. aeruginosa**, E. faecium*	Possible carbapenem-resistant *P. aeruginosa* (CRPA); incomplete AST	**QnrVC1**; PDC-19a; EreA2; **sul1**; mphA; DIM-1; catB7; APH(3'')-Ib; tetM; **dfrA5**; **fosA**; MexA; floR; APH(3')-IIb; APH(6)-Id; **NDM-1**; rmtB; **gyrA** (*P. aeruginosa*); **OXA-395**; aadA6/aadA10; AAC(6')-Ii
G	*E. coli*	***E. coli***	ESBL	mdtA; PtsI (*E. coli*); EC-5; **CTX-M-27**; gyrA (*E. coli*); UhpT (*E. coli*); marA; parC (*E. coli*); GlpT (*E. coli*); eptA; kdpE
H	*S. aureus*	***S. aureus**, P. lymphophilum, A. lactolyticus, F. hominis, A. schaalii*	MSSA, resistant to quinolone	blaZ; tetO; ErmA; ANT(4')-Ia; **gyrA** (*S. aureus*); ANT(9)-Ia
I	*S. aureus*	***S. aureus**, F. magna (P. magnus)*	MSSA	tet(38); tetM
J	*K. pneumoniae*	***K. pneumoniae***	ESBL	**TEM-1**; sul2; **SHV-11**; QnrB1; **CTX-M-15**; FosA6; AAC(6')-Ib-cr5; oqxA; oqxB; APH(3'')-Ib; APH(6)-Id; **OXA-1**; AAC(3)-IIe; dfrA14; tet(A); catB3
K	*E. faecium*	***E. faecium**, B. breve*	VRE	AAC(6')-Ii; **vanHA**; **vanRA**; msrC; tetM; dfrG; ErmB; tet(L)
L	*K. pneumoniae*	***K. pneumoniae***	ESBL	**TEM-1**; sul2; QnrB1; FosA6; oqxB; **CTX-M-15**; AAC(3)-IIe; AAC(6')-Ib-cr5; **SHV-28**; **OXA-1**; APH(6)-Id; dfrA14; APH(3'')-Ib; oqxA; tet(A); catB3
M	*A. baumannii/ calcoaceticus complex*	***A. baumannii**, E. faecium, F. magna (P. magnus)*	Resistant to carbapenems, quinolones, and other common antibiotics	**gyrA** (*A. baumannii*); vanRA; **sul2**; APH(6)-Id; AAC(6')-Ip; APH(3'')-Ib; aadA5; mphE; ADC-30; tetW; sul1; msrE; **OXA-72**; **OXA-66**; **dfrA17**; armA; tet(B); **TEM-192**; ErmX
N	*S. aureus*	***S. aureus**, P. aeruginosa*	MRSA	**mecI**; ANT(4')-Ia; ANT(9)-Ia; ErmA; **mecA**; mphC; msrA; **mecR1**; tet(38); gyrA (*S. aureus*); blaZ
O	*P. aeruginosa*	***P. aeruginosa**, E. faecalis, P. anaerobius, P. timonensis*	Pan-susceptible	APH(3')-IIb; dfrE; fosA; PDC-367; **OXA-494**; catB7; lsaA; MexA; tetM; ErmF
P	*E. coli*	***E. coli**, E. faecalis*	ESBL, pan-resistant to antibiotic panel tested	mphA; UhpT (*E. coli*); **gyrA** (*E. coli*); sul2; marA; **dfrA17**; aadA5; **sul1**; mdtA; **parC** (*E. coli*); **TEM-1**; GlpT (*E. coli*); AAC(3)-IId; PtsI (*E. coli*); **EC-5**; **CTX-M-14**; eptA; kdpE; ErmB; APH(3'')-Ib
Q	*E. coli*	***E. coli**, K. pneumoniae, E. faecalis, K. quasipneumoniae, F. magna (P. magnus)*	ESBL	catI; oqxA; APH(6)-Id; **SHV-120**; oqxB; dfrA17; GlpT (*E. coli*); FosA6; marA; **TEM-1**; QnrS1; UhpT (*E. coli*); APH(3'')-Ib; cyaA (*E. coli*); mphA; EC-19; tetM; tet(B); lsaA; **CTX-M-15**; sul1; mdtA; aadA5; kdpE; eptA; ErmB; ANT(4')-Ia; **OXA-116**
R	*E. faecium*	***E. faecium**, S. aureus*	VRE	dfrE; **vanRA**; **vanHA**; lsaA; AAC(6')-Ie-APH(2'')-Ia; tetM; ErmB; ANT(9)-Ia; ANT(4')-Ia
S	*S. aureus*	***S. aureus**, E. faecalis, S. epidermidis*	MRSA	blaZ; dfrE; **mecA**; norA; lsaA; ErmX
T	*S. aureus*	***S. aureus**; F. magna (P. magnus)*	MSSA	tet(38); tetM

Abbreviations: AST, antimicrobial susceptibility testing; ESBL, extended spectrum Beta-Lactamase; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*; TMP-SMX, trimethoprim/sulfamethoxazole; VRE, vancomycin-resistant Enterococci.

Summary

The MiSeq i100 Series enables detection and characterization of ARGs and associated bacterial pathogens. The results presented in this application note demonstrate the ability of enrichment-based sequencing with the Urinary Pathogen ID/AMR Enrichment Kit for broad ARG detection without the need for culture- and growth-based phenotyping, presenting an alternate approach for AMR surveillance as part of public health efforts.

Learn more

MiSeq i100 Series

Urinary Pathogen ID/AMR Enrichment Kit

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