

### Supplementary Information

## **Isothermal Recombinase polymerase amplification and Silver Nanoparticle Assay: A Sustainable Approach for Ultrasensitive Detection of *Klebsiella pneumoniae***

Naresh Patnaik<sup>a#</sup>, Nidhi Orekondey<sup>a#</sup>, Ruchi Jain Dey<sup>a,\*</sup>

<sup>a</sup>Department of Biological Sciences, BITS Pilani Hyderabad Campus, Telangana State – 500078, India

#Equal contribution as co-first authors

\*Corresponding author

Email for correspondence: [ruchij80@hyderabad.bits-pilani.ac.in](mailto:ruchij80@hyderabad.bits-pilani.ac.in)

**Running Title:** Sensitive, fast, accurate and equipment-free molecular detection of *Klebsiella pneumoniae*

## Table of Contents

Sl. No.	Description
1.	<b>Table S1.</b> Nucleotide BLAST analysis of <i>khe</i> and <i>uge</i> gene primers against pathogens of <i>Klebsiella pneumoniae</i> species complex (KpSC)
2.	<b>Table S2.</b> Genomic DNA amounts and their equivalent bacterial numbers for <i>Klebsiella pneumoniae</i>
3.	<b>Table S3.</b> Limit of detection of <i>Klebsiella pneumoniae</i> in urine sample using InstaDNA-PCR-AGE, InstaDNA-PCR-AgNP, InstaDNA-RPA-AGE and InstaDNA-RPA-AgNP
4.	<b>Figure S1.</b> Sensitivity of molecular detection of <i>Klebsiella pneumoniae</i> using PCR-AGE and primers specific to <i>uge</i> gene
5.	<b>Figure S2.</b> Sensitivity of molecular detection of urine sample spiked with gDNA of <i>Klebsiella pneumoniae</i> by InstaDNA-PCR-AgNP assay
6.	<b>Figure S3.</b> Sensitivity of molecular detection of urine sample spiked with gDNA of <i>Klebsiella pneumoniae</i> by InstaDNA-PCR-AgNP assay

**Table S1.** Nucleotide BLAST analysis of *khe* and *uge* gene primers against pathogens of *Klebsiella pneumoniae* species complex (KpSC)

Gene name	Primer	Organism name	Percentage identity	E value
<i>khe</i> gene	Forward primer	Kp1 - <i>K. pneumoniae</i>	100%	0.028
		Kp2 - <i>K. quasipneumoniae</i> subsp. <i>quasipneumoniae</i>	100%	0.078
		Kp4 - <i>K. quasipneumoniae</i> subsp. <i>similipneumoniae</i>	100%	1.2
		Kp3 - <i>K. variicola</i> subsp. <i>variicola</i>	100%	0.75
		Kp5 - <i>K. variicola</i> subsp. <i>tropica</i>	100%	0.25
		Kp6 - <i>K. quasivariicola</i>	100%	7e-05
		Kp7 - <i>K. africana</i>	100%	9e-05
	Reverse primer	Kp1 - <i>K. pneumoniae</i>	100%	0.001
		Kp2 - <i>K. quasipneumoniae</i> subsp. <i>quasipneumoniae</i>	100%	7e-06
		Kp4 - <i>K. quasipneumoniae</i> subsp. <i>similipneumoniae</i>	100%	7e-06
		Kp3 - <i>K. variicola</i> subsp. <i>variicola</i>	100%	5e-06
		Kp5 - <i>K. variicola</i> subsp. <i>tropica</i>	100%	2e-06
		Kp6 - <i>K. quasivariicola</i>	100%	2e-06
		Kp7 - <i>K. africana</i>	100%	2e-06
<i>uge</i> gene	Forward primer	Kp1 - <i>K. pneumoniae</i>	100%	0.001
		Kp2 - <i>K. quasipneumoniae</i> subsp. <i>quasipneumoniae</i>	100%	0.026
		Kp4 - <i>K. quasipneumoniae</i> subsp. <i>similipneumoniae</i>	100%	0.025
		Kp3 - <i>K. variicola</i> subsp. <i>variicola</i>	100%	0.018
		Kp5 - <i>K. variicola</i> subsp. <i>tropica</i>	100%	4e-04
		Kp6 - <i>K. quasivariicola</i>	100%	2e-06
		Kp7 - <i>K. africana</i>	100%	0.007
	Reverse primer	Kp1 - <i>K. pneumoniae</i>	100%	0.010
		Kp2 - <i>K. quasipneumoniae</i> subsp. <i>quasipneumoniae</i>	100%	9e-05
		Kp4 - <i>K. quasipneumoniae</i> subsp. <i>similipneumoniae</i>	100%	9e-05
		Kp3 - <i>K. variicola</i> subsp. <i>variicola</i>	100%	0.014
		Kp5 - <i>K. variicola</i> subsp. <i>tropica</i>	100%	0.005
		Kp6 - <i>K. quasivariicola</i>	100%	2e-05
		Kp7 - <i>K. africana</i>	100%	3e-05

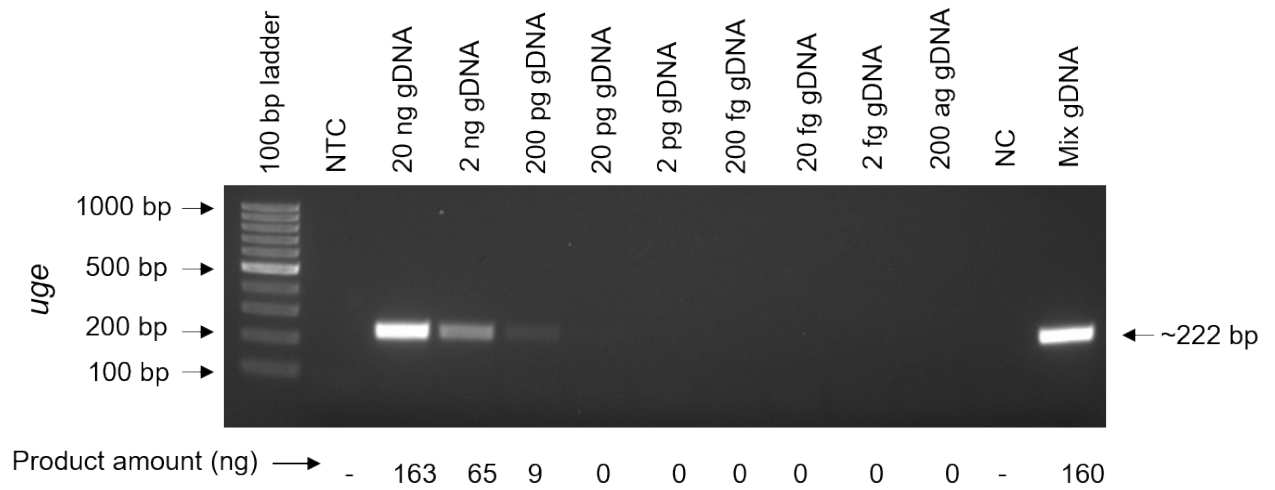
**Table S2.** Genomic DNA amounts and their equivalent bacterial numbers for *Klebsiella pneumoniae*

Amount of bacterial gDNA	Equivalent number of bacteria
20 ng	~33 X 10 <sup>5</sup> bacteria
2 ng	~33 X 10 <sup>4</sup> bacteria
200 pg	~33 X 10 <sup>3</sup> bacteria
20 pg	~33 X 10 <sup>2</sup> bacteria
2 pg	~33 X 10 <sup>1</sup> bacteria
200 fg	~33 bacteria
20 fg	~3 bacteria
2 fg	~3 X 10 <sup>-1</sup> bacteria
200 ag	~3 X 10 <sup>-2</sup> bacteria
* Amount of genomic DNA in one bacterial cell of <i>Klebsiella pneumoniae</i> is 5.92 fg, genome size is ~5.5 Mega base pair (Mbp)	

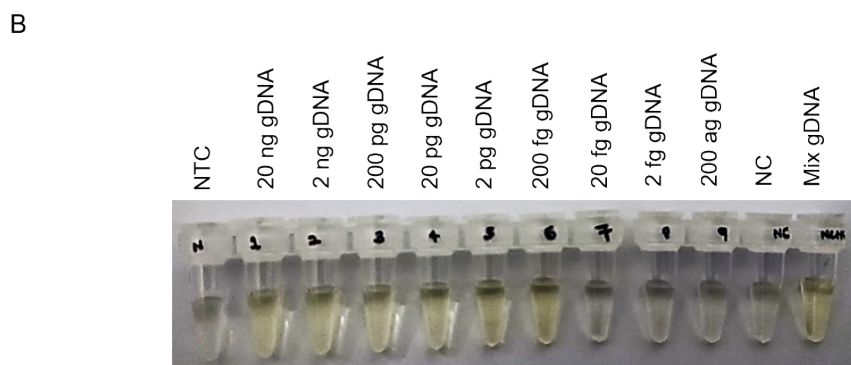
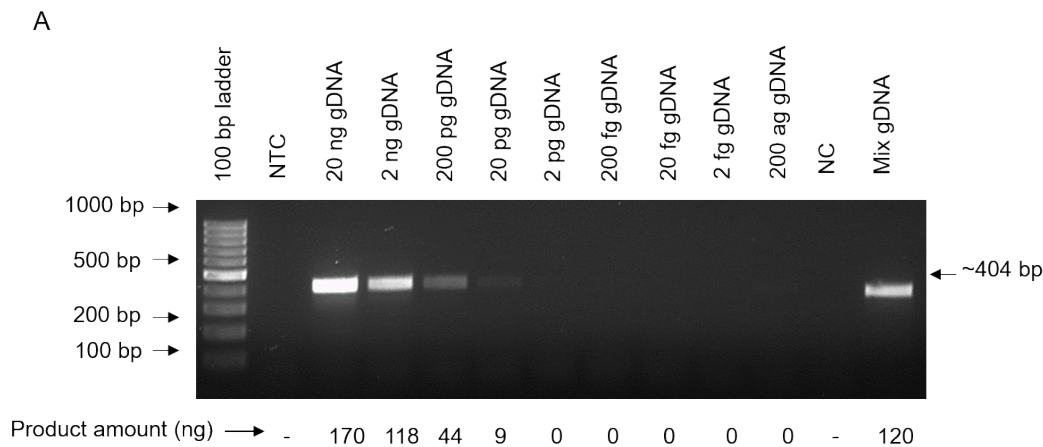
**Table S3:** Limit of detection of *Klebsiella pneumoniae* in urine sample using InstaDNA-PCR-AGE, InstaDNA-PCR-AgNP, InstaDNA-RPA-AGE and InstaDNA-RPA-AgNP

Sl. No	Input amount of DNA	Input number of bacteria	InstaDNA-PCR-AGE	InstaDNA-PCR-AgNP	InstaDNA-RPA-AGE	InstaDNA-RPA-AgNP
1	20 ng	~33 X 10 <sup>5</sup>	+	+	+	+
2	2 ng	~33 X 10 <sup>4</sup>	+	+	+	+
3	200 pg	~33 X 10 <sup>3</sup>	+	+	-	+
4	20 pg	~33 X 10 <sup>2</sup>	+	+	-	+
5	2 pg	~33 X 10 <sup>1</sup>	-	+	-	+
6	200 fg	~33	-	+	-	+
7	20 fg	~3	-	-	-	-
8	2 fg	~3 X 10 <sup>-1</sup>	-	-	-	-
9	200 ag	~3 X 10 <sup>-2</sup>	-	-	-	-

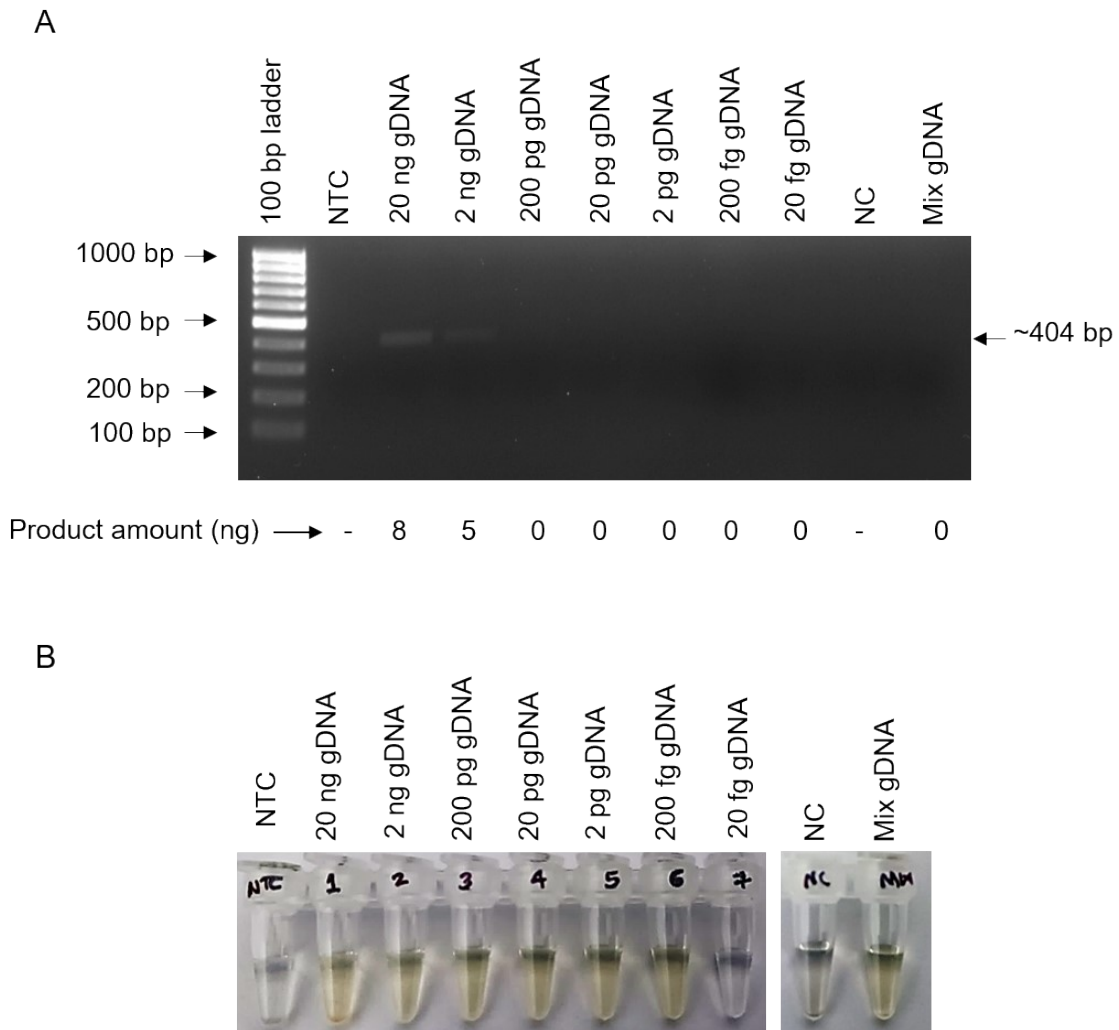
**Figure S1**



**Figure S1. Sensitivity of molecular detection of *Klebsiella pneumoniae* using PCR-AGE and primers specific to *uge* gene.** The figure depicts a sensitivity analysis of PCR-AGE-based molecular detection of *K. pneumoniae* using primers specific to the genomic region, *uge* gene. The labels on the top of the gel indicate the amount of genomic DNA (isolated using magnetic bead-based gDNA isolation) used per PCR reaction. The controls include- no template control (NTC) and negative control (NC) is a blend of gDNAs obtained from human and 11 different pathogens associated with the human host. Mix gDNA contains a mix of NC and genomic DNA of *K. pneumoniae*. The PCR-AGE for *uge* gene showed a detection sensitivity of  $\sim 33 \times 10^3$  bacteria. The gel images are representation of four experimental replicates. The numbers at the bottom of the image correspond to the relative quantification of the PCR amplicons in ng (with respect to the 100 bp ladder), done using ImageJ software as described in methods.



**Figure S2. Sensitivity of molecular detection of urine sample spiked with gDNA of *Klebsiella pneumoniae* by InstaDNA-PCR-AgNP assay.** AgNP assay is used for visual molecular detection of InstaDNA-PCR products obtained from PCR performed using *khe* gene primers. A. The InstaDNA-PCR-AGE assay shows a sensitivity of detection of  $\sim 33 \times 10^2$  bacteria of *K. pneumoniae*. The numbers at the bottom of the gel image, correspond to relative quantification of the PCR amplicons in ng (w.r.t to 100 bp ladder), done using ImageJ software as described in methods. B. The InstaDNA-PCR-AgNP assay displays a detection sensitivity of  $\sim 33$  bacteria for both the target genes. The images are representations of two experimental replicates. The controls include- no template control (NTC) which are blank InstaDNA discs blotted with sterile water. and negative control (NC), a blend of gDNAs obtained from human and 11 different pathogens associated with the human host. Mix gDNA contains a mix of NC and genomic DNA of *K. pneumoniae*.



**Figure S3. Sensitivity of molecular detection of urine sample spiked with gDNA of *K. pneumoniae* by InstaDNA-RPA-AgNP assay.** AgNP assay is used for visual molecular detection of InstaDNA-RPA products obtained from RPA performed using *khe* gene primers. A. The InstaDNA-RPA-AGE assay shows a sensitivity of detection of  $\sim 33 \times 10^4$  bacteria of *K. pneumoniae*. The numbers at the bottom of the gel image, correspond to the relative quantification of the RPA amplicons in ng (w.r.t to 100 bp ladder), done using ImageJ software as described in methods. B. The InstaDNA-RPA-AgNP assay displays a detection sensitivity of  $\sim 33$  bacteria. The images are representations of two experimental replicates. The controls include- no template control (NTC) which are blank InstaDNA discs blotted with sterile water. and negative control (NC), a blend of gDNAs obtained from human and 11 different pathogens associated with the human host. Mix gDNA contains a mix of NC and genomic DNA of *K. pneumoniae*.