

Rapid Sample Lysis in a Miniaturized Format

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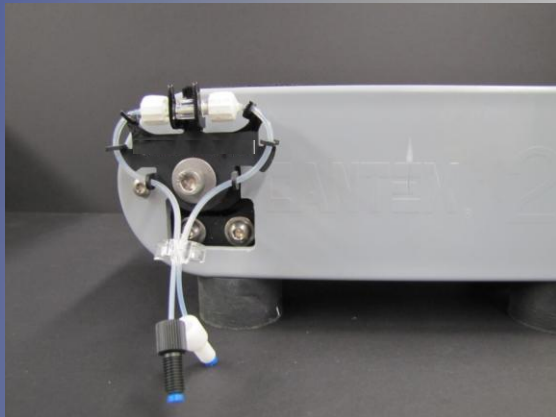
Sample Prep 2011

Rapid Nucleic Acid extraction



- **OmniLyse[®] Kit**- miniature disposable flow-through cell disruptor

- **PureLyse[®] Kit**- miniature disposable flow-through cell disruptor **plus** nucleic acid extraction



- **RapidLyser[™]** - benchtop flow-through instrument for mechanical cell lysis and nucleic acid extraction

Advantages of PureLyse[®]

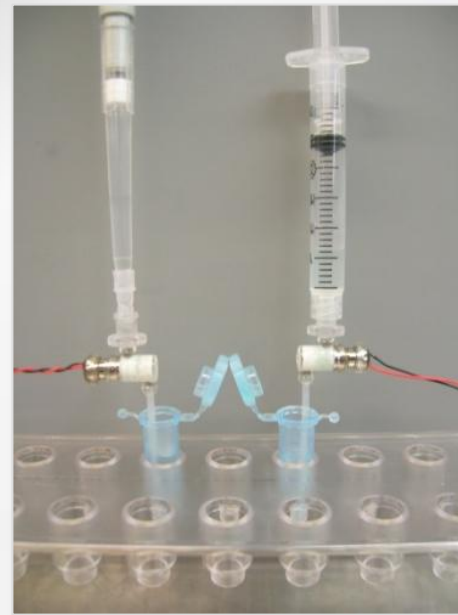
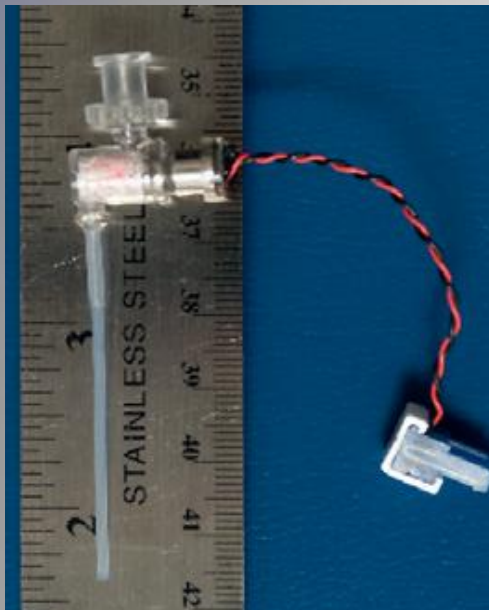
Sample Prep

Typical NA extractions from whole cells:

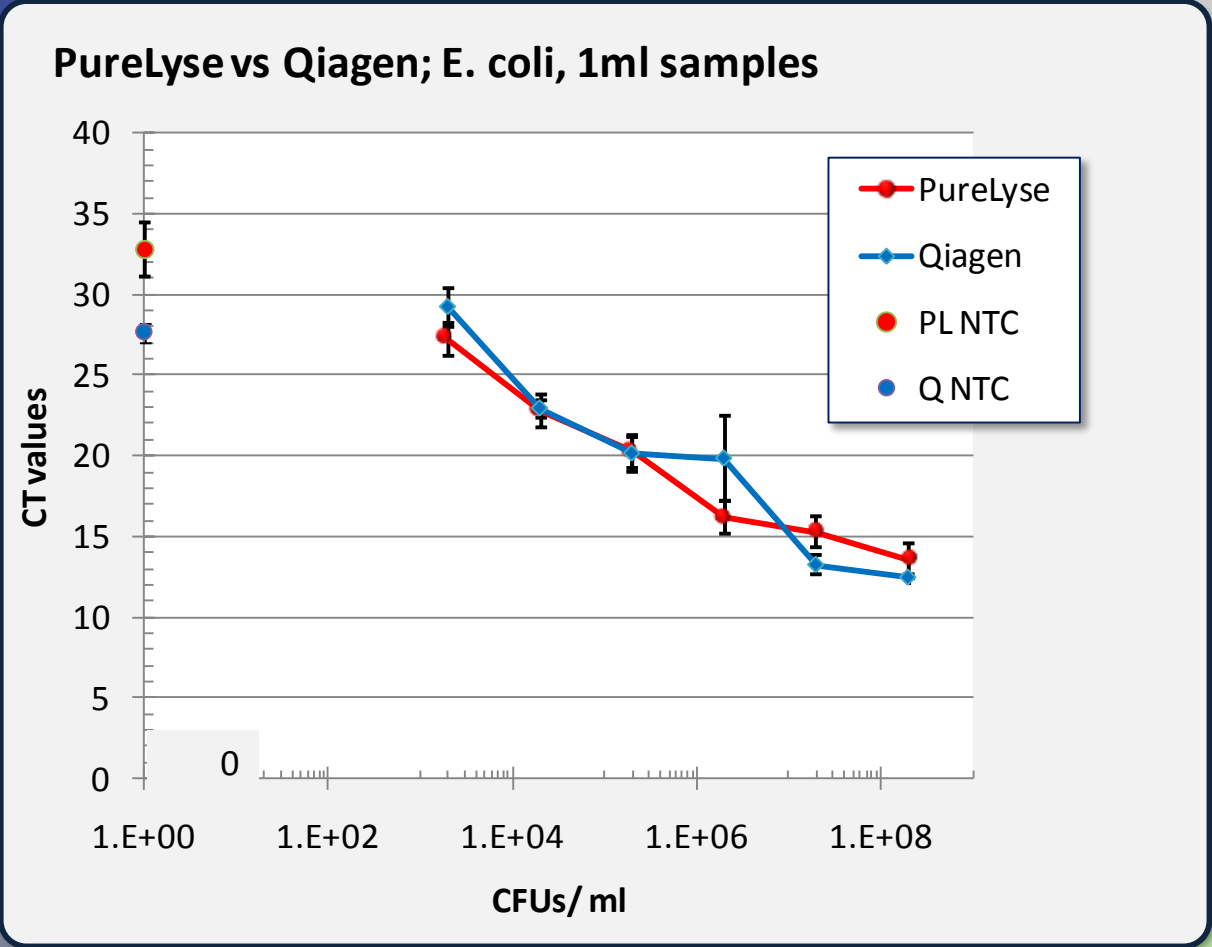
- 10 to 18 steps
- 1 to 2 hours

PureLyse[®] NA extraction:

- Lysis and extraction < 5 min
- Simple 2-step protocol
- Miniaturized



PureLyse[®] Prep vs. Qiagen DNeasy



CFUs in Sample	Copies in PCR
200,000,000	5,000,000
20,000,000	500,000
2,000,000	50,000
200,000	5,000
20,000	500
2,000	50

Sample: 1 ml of *E.coli* ,varying concentrations (n=3)
 Lysis 1 min, Elution 1 min. 200 µl, 2x, 5ul to PCR

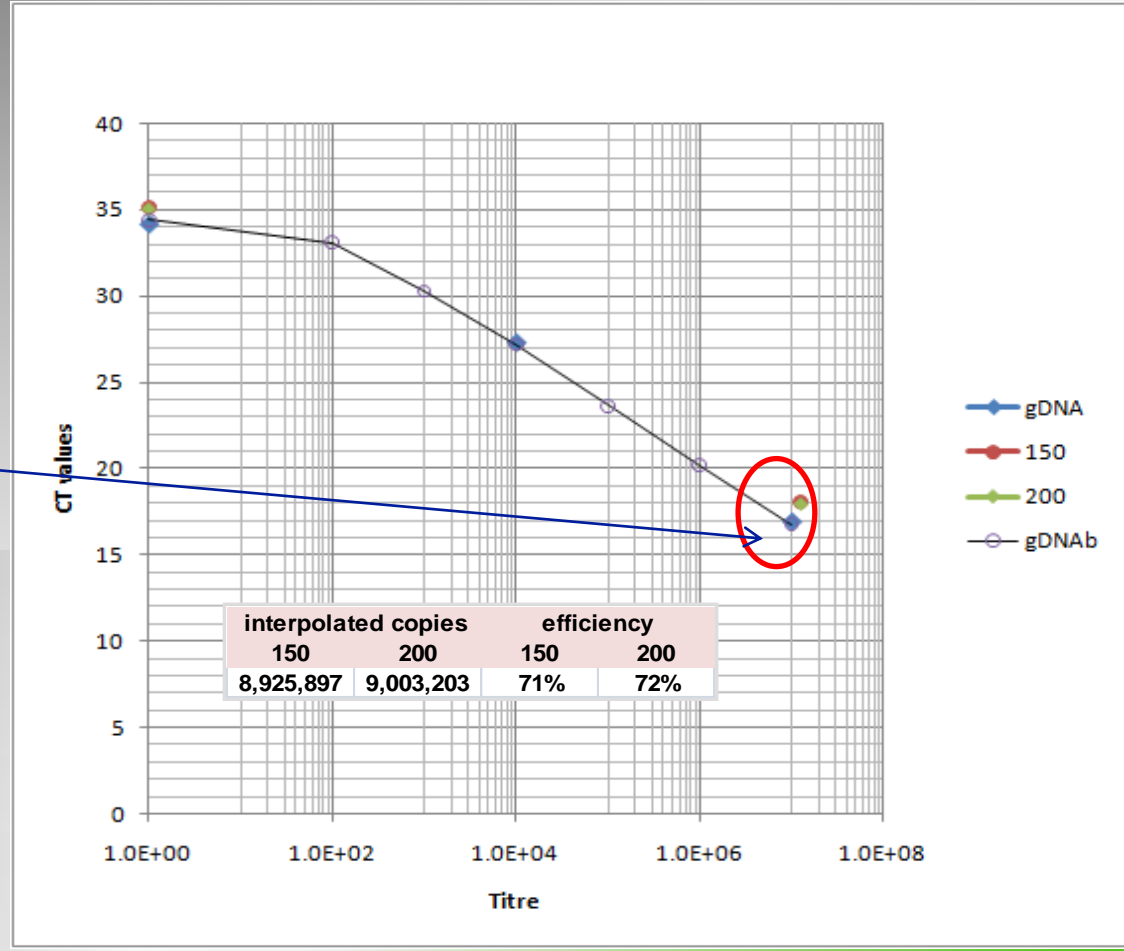
PureLyse[®] Prep 1x10⁸ CFU *E.coli*: PCR analysis

PureLyse[®] Prep - 1 mL sample

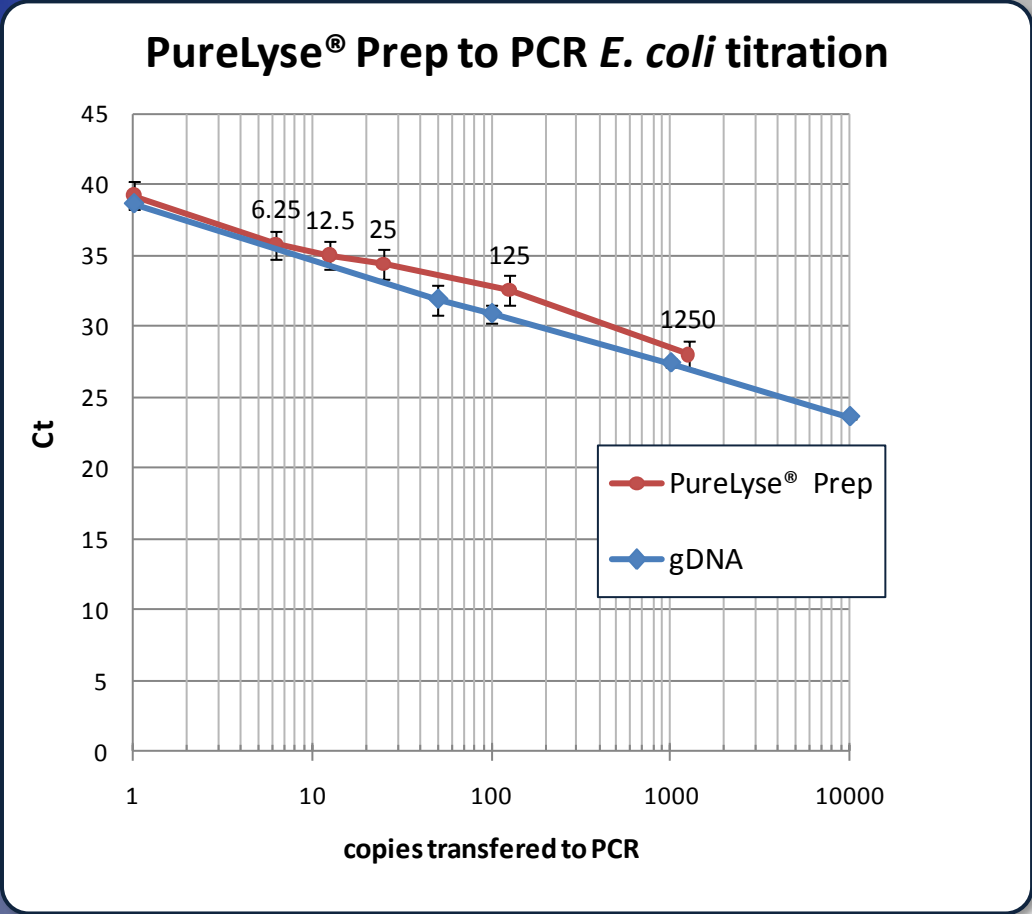
1x10⁸ CFUs *E.coli*
2 minute lysis
1 minute elution, 200 µl

Real-time PCR results
>70% extraction efficiency

Sample	replicates	CV
gDNA 10e7	2	0.1
PL 200	10	1.4
PL150	10	2.6
gDNA 10e4	2	0.0
PL 200 neg	2	1.1
PL150 neg	2	1.2
TE 8.0 neg	4	1.3
TE 8.8 neg	2	1.0



PureLyse® Prep Low Limit of Detection

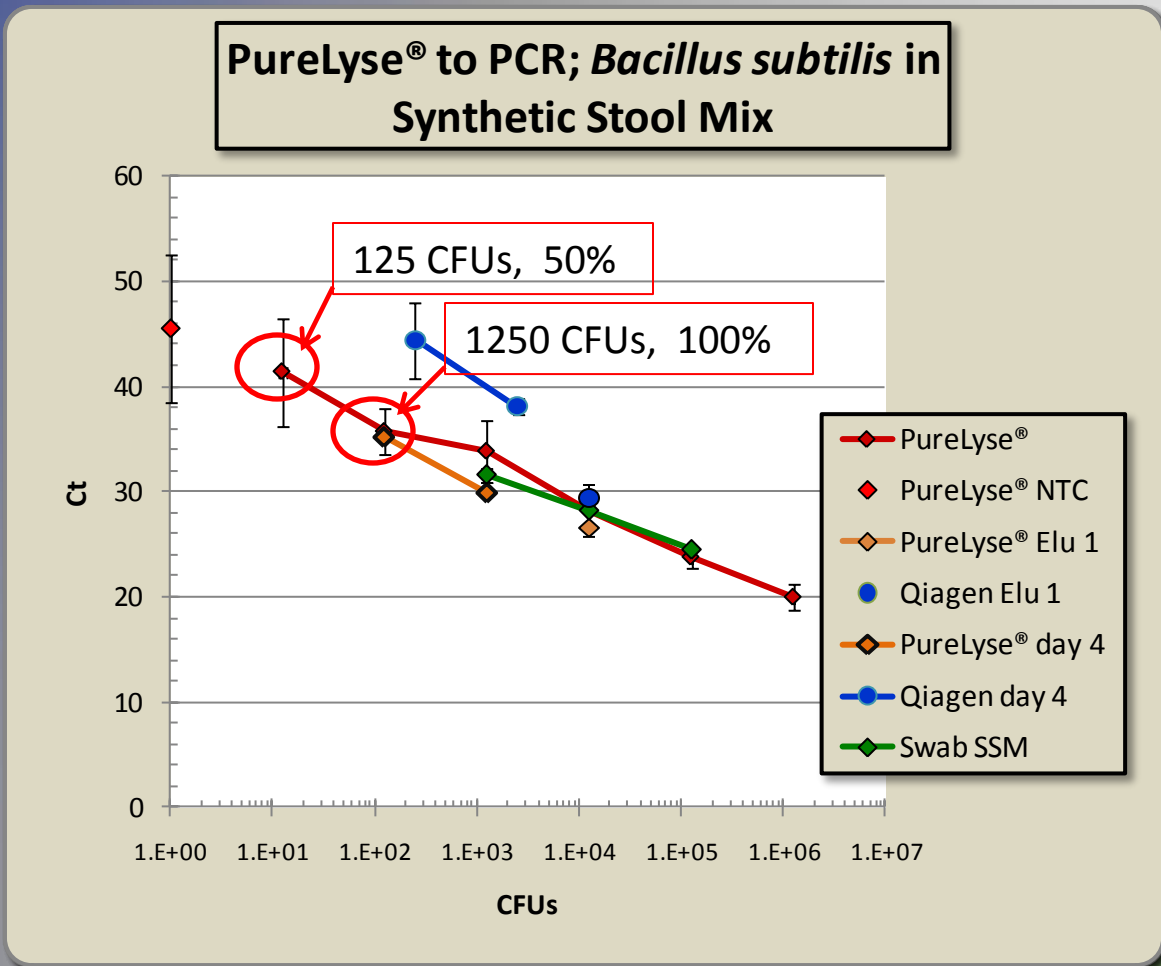


1 ml *E.coli*, 2.5 minute lysis
200 µl elution, 1 minute

Achievable detection:
50 CFUs (6.25 CFU/PCR reaction)

CFUs in PureLyse® prep	transferred to PCR	Efficiency
50	6.25	83%
100	12.5	67%
200	25	49%
1000	125	33%
10000	1250	55%

Detection of *Bacillus subtilis* in Synthetic Stool Mix: PureLyse[®] sample prep to PCR



Copies in PCR	CFUs in PureLyse [®] Prep	n
0	0	6
12.5	100	6
1.25E+02	1.E+03	6
1.25E+03	1.E+04	5
1.25E+04	1.E+05	5
1.25E+05	1.E+06	5
1.25E+06	1.E+07	5

Sample: 1 ml of *B. subtilis*, varying concentrations (n=3) Lysis 2 min, Elution 1 min. 200 µl, 2x, 25ul to PCR

PureLyse[®] Prep: *Mycobacterium bovis* BCG

PureLyse[®] samples:

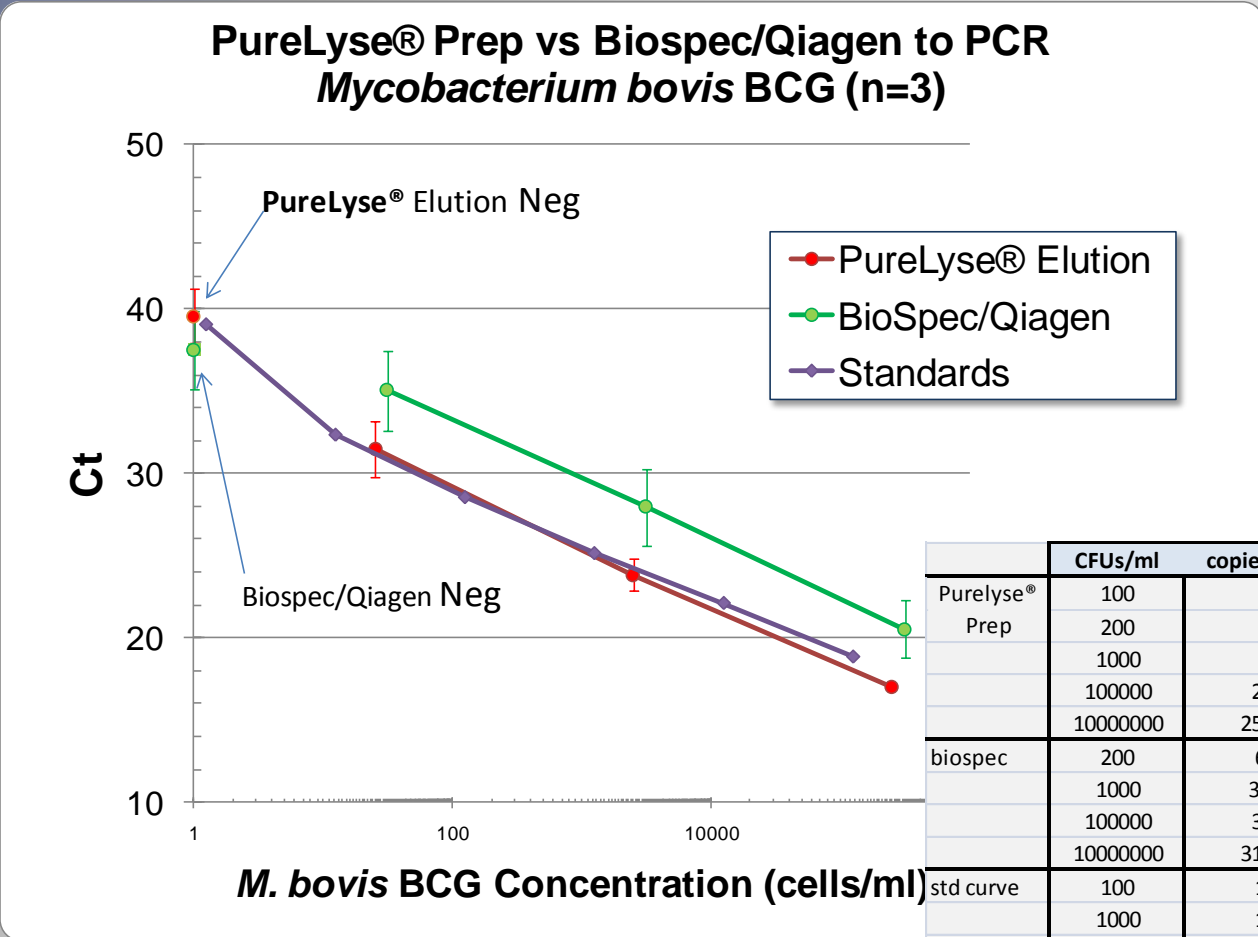
- 500 µl lysis and DNA capture – 3 minutes
- 250 µl elution

Biospec samples:

- 625 µl lysis Biospec bead beater - 3 minutes
- 500 µl processed via Qiagen DNeasy Kit
 - 250 µl elution

12.5 µl into PCR

Data provided by Peter Vandeventer and Angelika Niemz, Keck Graduate Institute



Detection of *Bacillus subtilis* in Synthetic Stool Mix

Synthetic Stool Mix

Dextran Sulfate
 Bile Salts
 Mucin, Bovine
 Human Serum Albumin
 Human gDNA
E. coli

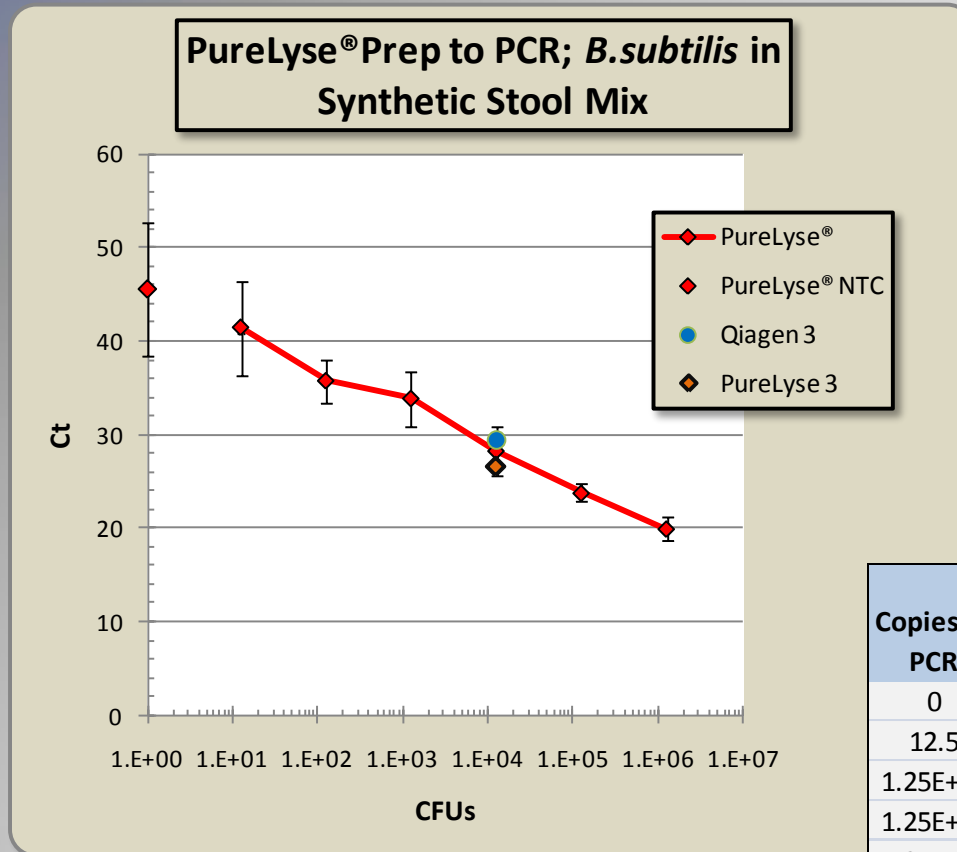
100 µl Synthetic stool samples brought up to 1 ml in Binding Buffer

- Lysis - 2 minutes
- Elution -1 minute, 200 µl
- 25 µl into PCR

100% negative - true neg.

50 % of the samples at 100 CFUs (12.5/PCR) amplified as true positives

Comparable to Qiagen Stool Kit



Copies in PCR	CFUs in PureLyse® Prep	n
0	0	6
12.5	100	6
1.25E+02	1.E+03	6
1.25E+03	1.E+04	5
1.25E+04	1.E+05	5
1.25E+05	1.E+06	5
1.25E+06	1.E+07	5

PureLyse[®] Efficiency

PureLyse[®] DNA extraction of 1ml Sample (*E. coli* 1 x 10⁸ cells)

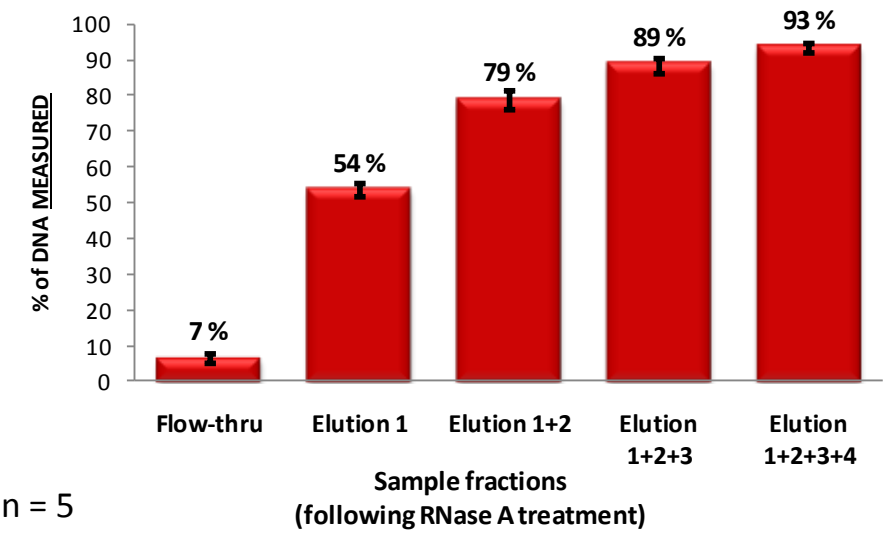


Figure 1. Pico Green determination of DNA yield (% predicted from cell count)

- flow-through and four 150 µl elutions
- 1 µg RNase A treatment
- Average yield of all fractions: 88%
- 82% of DNA in the sum of four elutions

PureLyse[®] DNA extraction of 1ml Sample (*E. coli* 1 x 10⁸ cells)

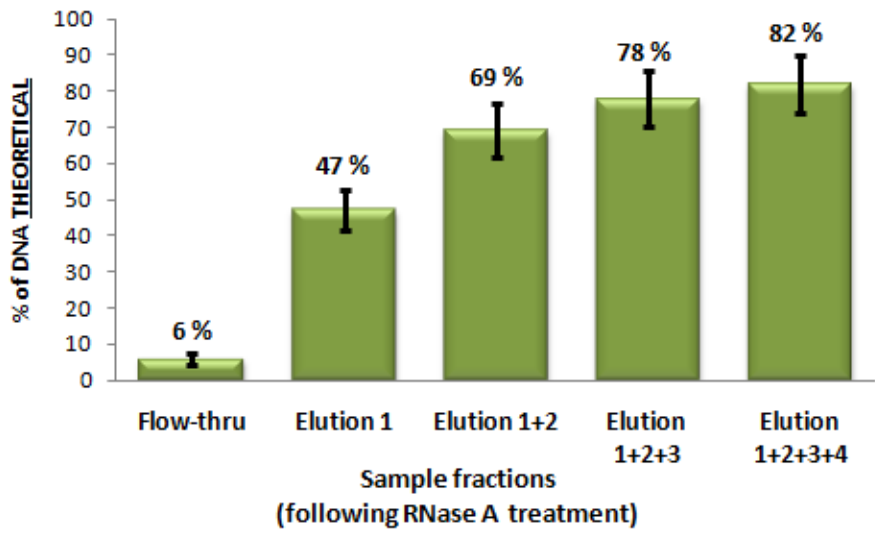


Figure 2. Pico Green measurement of NA in the flow through and the eluant before and after RNase A digestion

- Reference DNA (gold), predicted by cell count
- NA before RNase: 82%
- NA after RNase: 93%

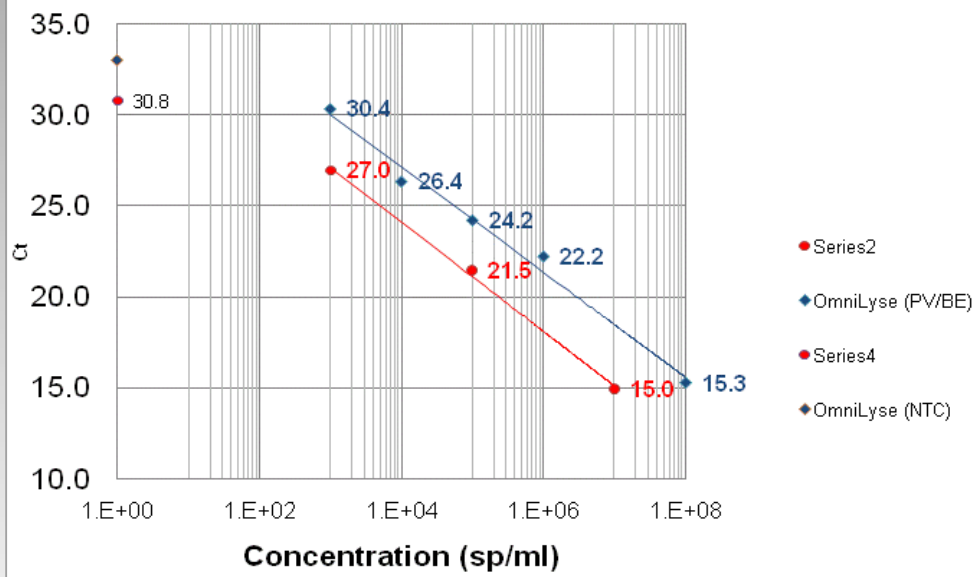
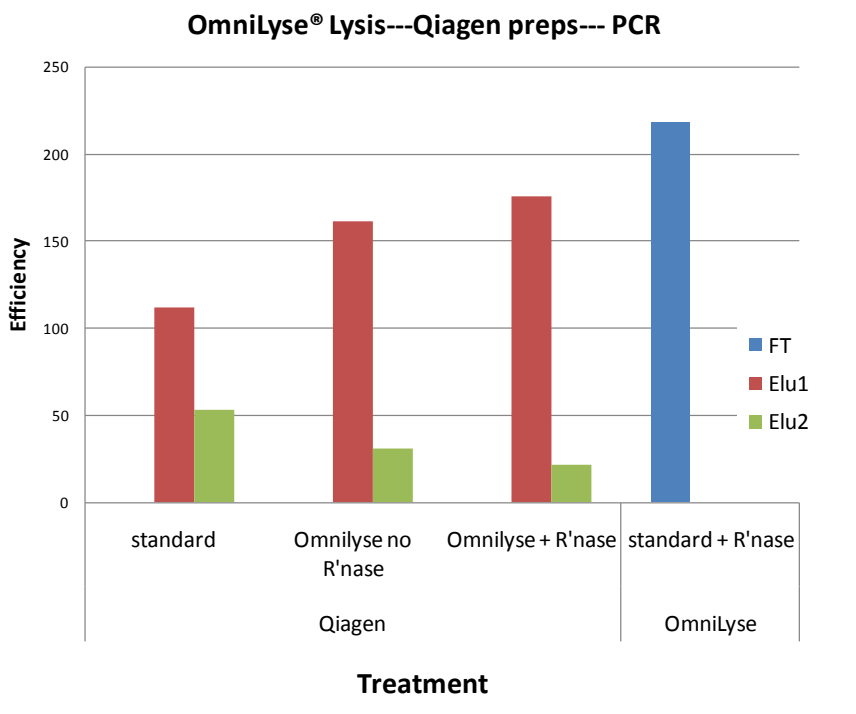
OmniLyse[®] Prep:

Lysis of bacterial cells and spores



E. coli cells

B. subtilis spores



OmniLyse[®] Prep: Lysis of *Bacillus subtilis* spores

- 500 μ l samples (12.5 μ l into PCR)
- Sample concentrations: 1,000, 100,000 and 100,000,000 spores

OmniLyse[®] Lysis Enhances Performance of Qiagen Kit, *E. coli*

de Boer RR. Improved detection of microbial DNA after bead-beating before DNA isolation. J Microbiol Methods 2010;80(2):209-11.

Rapid Protein Purification



OmniLyse[®] Sample Preparation: 50 mL Culture



Benefits of using OmniLyse[®] Prep

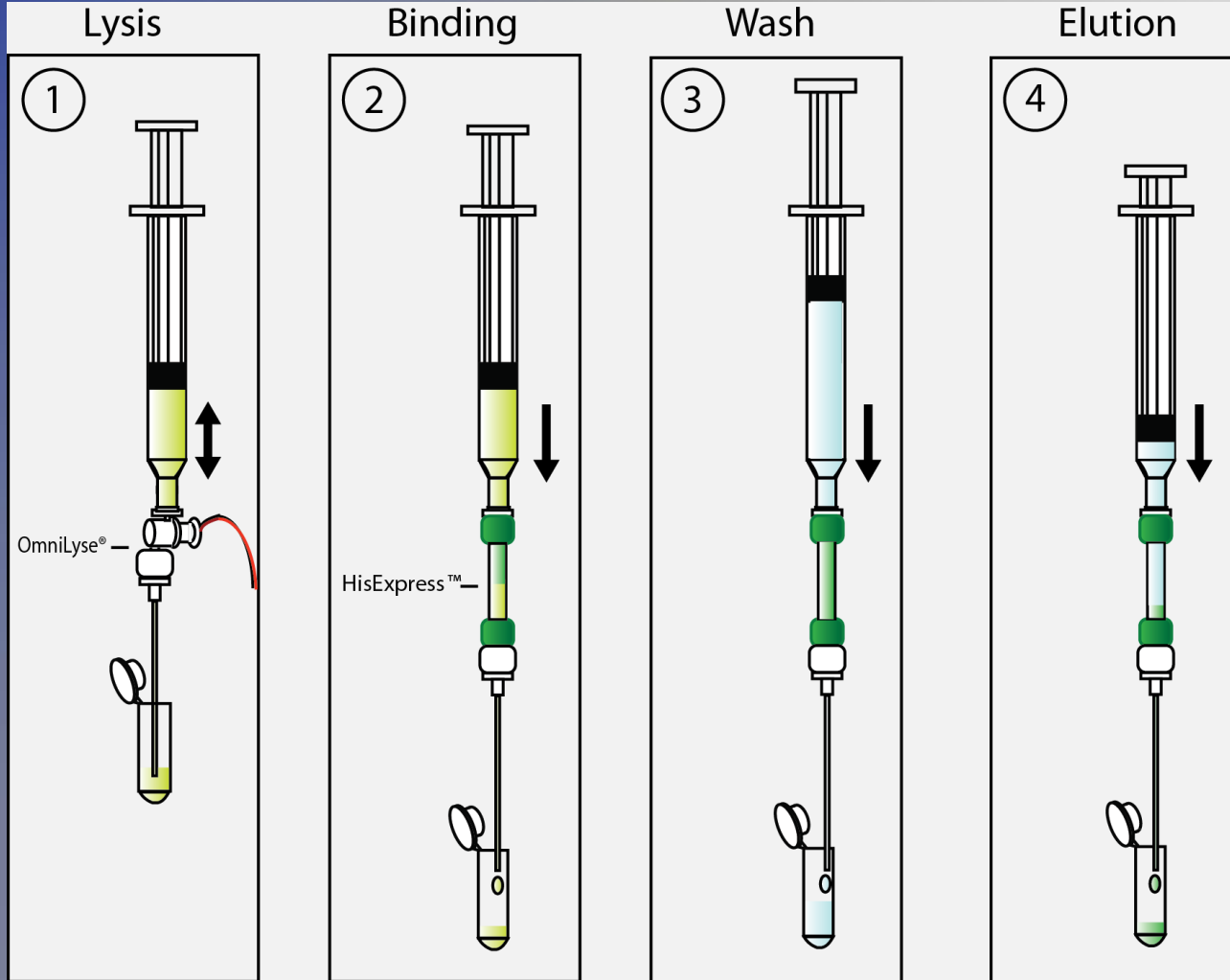
- Time savings - lysis to purification in <12 minutes
- Eliminates the need for lysing chemicals, lysozyme or DNase treatment
- Disposable
- Ideal for the lab bench, the hood, the cold room or in the field
- Easily adapted to variety of chromatography products
- Can process a bacterial pellet from 50 mL culture
- Lyses Yeast in 2 minutes

HisExpress™ Column: Rapid and Simple Purification of His-tagged Proteins

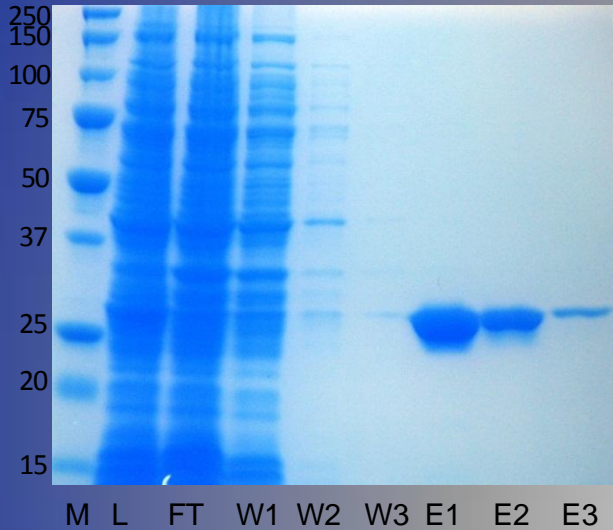
< 5 min

< 5 min

Pipette friendly



OmniLyse[®] Prep to HisExpress[™] Affinity tag Purification of GFP - His Tag



A pellet from 50 mL of *E. coli* expressing 6xHis-EmGFP was prepared using OmniLyse[®] prep for 3 min

6xHis-EmGFP (Invitrogen) was purified under native conditions using HisExpress[™] Column in <5 min.

Figure 2. OmniLyse[™] eliminates the need for a preclearing step. Bacteria expressing 6xHis-EmGFP were lysed using OmniLyse[™] and either directly applied to Ni-IDA resin (crude = CR) or clarified (CL) by centrifugation at 12,000xg for 15 minutes before applying to Ni-IDA resin. Protein was purified according to instructions. M = marker

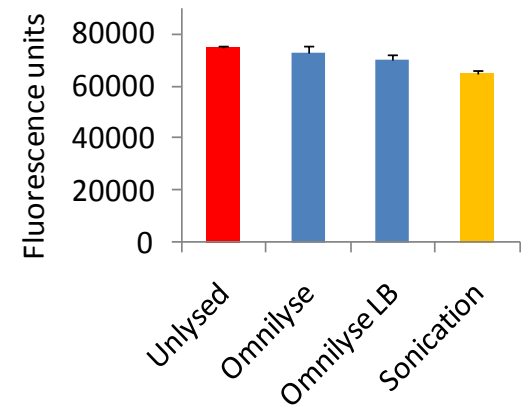
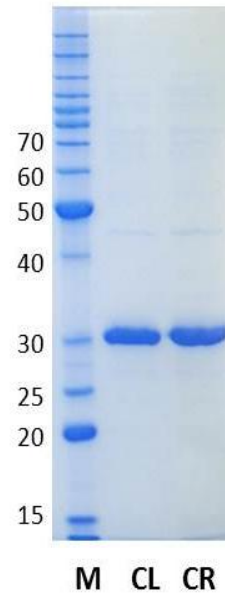
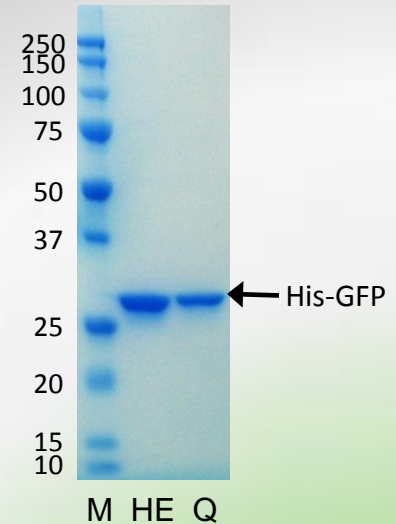


Figure 3. GFP fluorescence remains stable during lysis. 6xHis-EmGFP fluorescence was measured at 487 nm (ex) and 507 nm (em).

HisExpress™ Column out performs competitor in Yield, Purity, Time and Price

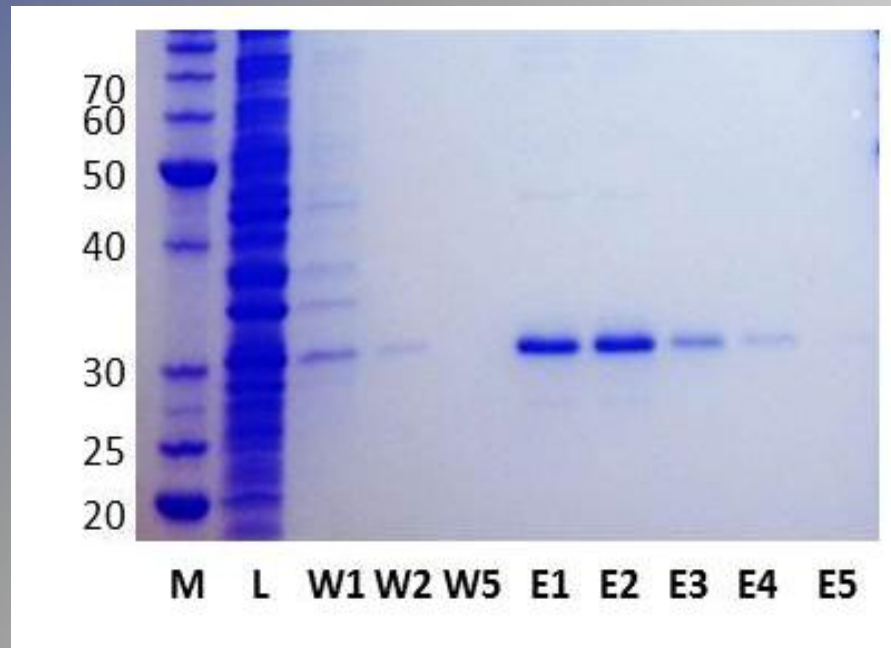
Purification of His-GFP using HisExpress™ Column or Qiagen Ni-NTA spin columns

- OmniLyse® Lysis preceded both methods
- OmniLyse® kit samples processed 3 minutes
- Qiagen samples purified as per manufacturer's instruction



Company	Total Yield (mg)	Purity %	Sample Prep Time	Purification Time
HisExpress™ Column	1.2	>95	<5 min	<5 min
Qiagen spin column	0.4	>95	Variable	1 hr

His-PureLyse™ Prep: Rapid lysis and purification of His-tagged protein in a single device



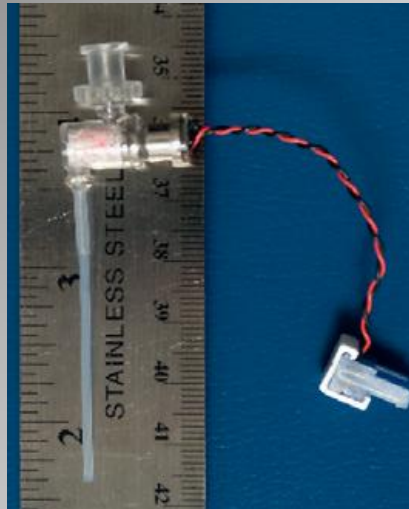
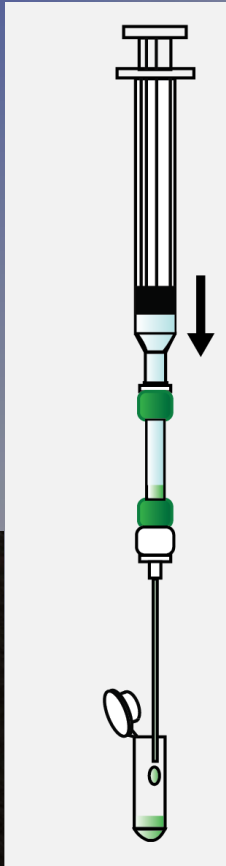
His-PureLyse™ sample preparation expedites lysing and purifying Hi-tagged proteins from a sample in less than 5 minutes.

- Bacteria expressing 6xHis-EmGFP were lysed for 3 minutes in the His-PureLyse™ Cartridge, followed by wash and elution steps.
- Fractions analyzed by SDS-PAGE and Coomassie staining:
M=marker, L=lysate, W=wash and E=elution.

Configurations



**HisExpress™
Column**



**HisPurLyse™
Prep**



HisExpress™ micro



**DNA express™
column**

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