Simplifying Progress

# PATfix® pDNA Platform and PCR Methods as Orthogonal Analytical Tools for Quantification of pDNA.

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## Introduction

Accurate characterisation and quantification of plasmid DNA (pDNA) is critical for gene therapy, biotechnology, and vaccine production. Ensuring pDNA quality and consistency is especially important for mRNA, AAV, and other vector-based modalities, where it directly impacts therapeutic efficacy and safety. pDNA exists in multiple topological isoforms: supercoiled (sc), open circular (oc), multimeric forms (multi), and linear (lin).

In this study, we evaluated three orthogonal analytical approaches across complex *Escherichia coli* lysates, process intermediates, and purified fractions using:

- PATfix® pDNA Platform,
- quantitative PCR (qPCR),
- and digital PCR (dPCR).

Sample matrices contained typical impurities, including host cell proteins (HCP), host cell RNA (hcRNA), other cellular components, and residual process chemicals. Selected samples were obtained from representative downstream process steps (Fig. 1) employing monolithic columns (DEAE, C4 HLD), and sample-preparation effects were assessed by comparing restriction-digested versus non-digested plasmids for PCR-based quantification.

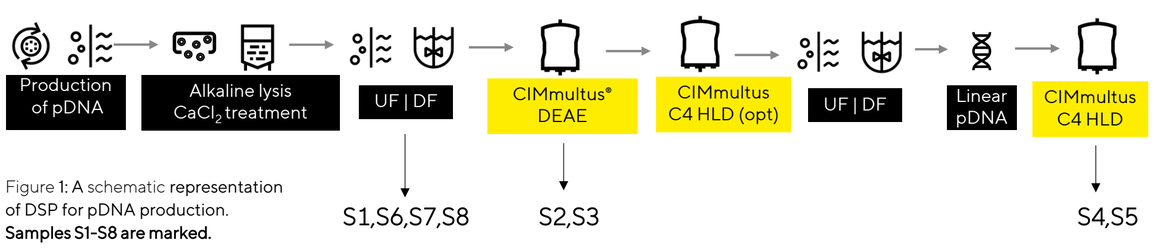


Figure 1: A schematic representation of DSP for pDNA production. **Samples S1-S8 are marked.**

## 1. Methods overview

### PATfix pDNA Platform

The PATfix system, utilizing the CIMac pDNA column, is an advanced analytical tool designed for gene therapy and biotechnology applications. It enables the separation and quantification of various pDNA isoforms and other biomolecules using developed methods.

Key features of the method using PATfix system:

- pDNA isoforms (%): sc, oc, lin, multi
- Total pDNA using calibration curve (LLQ 1 ng/µl)
- Selected impurities: free RNA

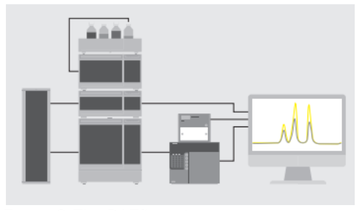

Figure 2: The PATfix system.

### Quantitative PCR (qPCR)

qPCR is a commonly used method for total pDNA by amplifying a defined target region and comparing sample Cq values against a calibration curve generated from known standards. qPCR generally requires linearisation of pDNA, particularly for supercoiled forms, to obtain reliable results.

Key features:

- Total pDNA quantification using calibration curve and specific primers with EvaGreen@ chemistry
- Cannot discriminate between pDNA isoforms and is sensitive to matrices inhibitors
- Quantification range: 15-30 Cq (LLQ is 3.9 ng/µl)

### Digital PCR (dPCR)

dPCR offers absolute quantification of pDNA, allowing for direct copy number determination without needing a standard curve. Linearisation may be required for but is generally less supseptible to PCR inhibitors in comparison to qPCR

Key features:

- Absolute copy number (copies/µL reaction) using specific primers and EvaGreen@ chemistry
- Conversion to mass concentration using plasmid data
- Quantification range: 40-5000 cp /µl (LLQ is 4.3 ng/µl)

## 2. Experimental setup

We analysed pDNA in complex matrices of typical gene therapy and biotechnology workflows (e.g., containing HCP, hcRNA, and residual process chemicals), including downstream process intermediates and purified fractions.

Samples were prepared in two pathways:

- Digested with the restriction enzyme *Not*I-HF (1 h at 37°C) to improve consistency of PCR-based quantification (qPCR/dPCR).
- Non-digested to assess the impact of plasmid isoforms on quantification and to enable isoform profiling by the PATfix® pDNA Platform.

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- **q/dPCR**

Dilution
Normalise 1 ng/µl → w/o Restriction *Not*I HF → Dilutions for quantification range of PCR

Total dilutions (600-1M)

Total time: 4-5 h for up to 15 samples

- **PATfix pDNA Platform**

Mob A: 100 mM TRIS, pH 7.9

Mob B: 100 mM TRIS, 1M NaCl pH 7.9

Dilutions:
50x Lysates
10x DEAE
4x C4 HLD

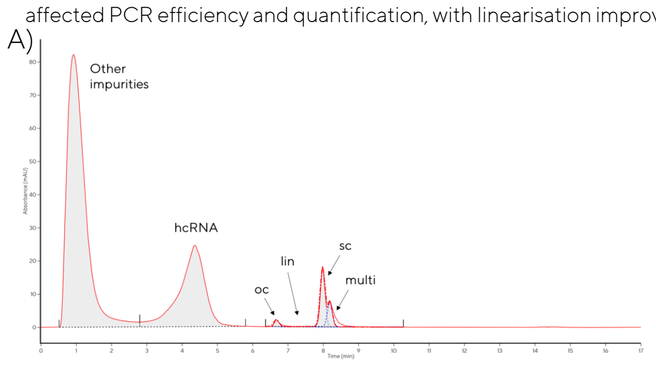
Total time: <20 min per sample



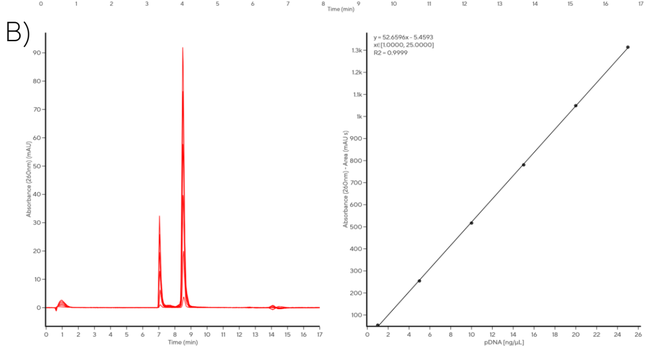
## 3. Results - Isoform quantity

- PATfix pDNA Platform enabled (%) isoform pDNA analysis, while qPCR/dPCR did not; nonetheless, plasmid topology affected PCR efficiency and quantification, with linearisation improving consistency.

A)




B)




C)

| DSP Sample | Ratio [%] oc | lin | sc | multi |
|---|---|---|---|---|
| S1 | 3 | 2 | 83 | 12 |
| S2 | 4 | 2 | 82 | 12 |
| S3 | 5 | 0 | 83 | 12 |
| S4 | 0 | 100 | 0 | 0 |
| S5 | 0 | 100 | 0 | 0 |
| S6 | 1 | 0 | 90 | 9 |
| S7 | 4 | 0 | 14 | 82 |
| S8 | 2 | 0 | 87 | 11 |

Figure 3: (A) Representative PATfix pDNA Platform chromatogram UV260 from the sample S1. (B) Aligned chromatograms for pFix5 and a calibration curve for pFix5 (1-25 ng/µl). (C) Table of calculated isoform ratios (%) from PATfix® pDNA Platform data.

## 4. Results - Quantification of pDNA

- Total pDNA was quantified using PATfix analytical system on native samples whereas linearising samples for qPCR/dPCR brought those results into closer agreement with PATfix system.

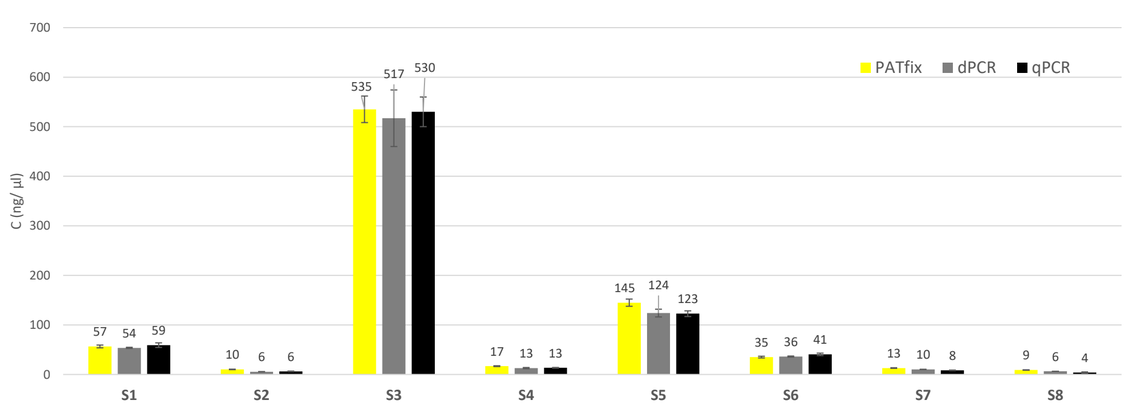



Figure 4: Comparison of quantification of pDNA using PATfix pDNA Platform and qPCR/dPCR methods

## 5. Discussion -Key findings

- Qualification criteria

  PATfix pDNA Platform meet suitability test (SST) criteria for each sequence, and total pDNA showed high linearity (R2 = 0.999) across the validated range with sample deviation (CV ≤ 10%, average 5.6 %).

  qPCR performance met MIQE-aligned acceptance criteria: standard curves showed linearity (R2 ≥ 0.998 across the working range) and high precision (CV ≤ 15%, average 9.5 %), melting-curve analysis confirmed specific amplification.

  dPCR met dMIQE-aligned performance criteria: sufficient partition numbers, recommended occupancy of positive partitions), clean no-template controls, and high precision (CV ≤ 10%, average 5.2 %).

- Isoform resolution

  The PATfix pDNA Platform was the only method separating and quantifying pDNA isoforms (sc, oc, lin, multi) and verified restriction-digest completeness (loss of sc peak upon linearisation)- including the detection of impurities such as hcRNA and HCP.

  Total pDNA quantification

  The PATfix pDNA Platform quantifies total pDNA directly, without sample pre-treatment, and provides automated calculations.

  In non-linearised samples, qPCR/dPCR underestimated total pDNA by approximately 25–50% due to plasmid topology effects; linearisation increased the measured concentrations.

  qPCR and dPCR yielded comparable values under matched conditions.

  PCR-based methods require knowledge of the exact target sequence for primer design.

- Application guidance

  Use PATfix system for detail isoform-resolved quality assessment and linearisation verification.

  Use dPCR for absolute quantification without standards but we need to know the exact sequence.

  Use qPCR for routine higher-throughput with validated calibration.

## 6. Conclusion

- PATfix system provides fast, isoform-resolved characterisation and reliable total pDNA quantification - use for isoform profiling and verification of restriction.
- dPCR delivers absolute copy-number quantification without external standards; copy number can be converted to mass when plasmid size/sequence is known - convenient when standards are unavailable.
- qPCR supports routine testing when assays are MIQE-compliant, and samples are linearised; A calibration curve is required for quantification.
- For consistent PCR-based quantification, linearisation is essential but no additional extraction is required for these matrices due to effective dilution strategies.
- PCR dilution series across several logs can introduce errors.
- PCR methods provide orthogonal quantification of total pDNA with some limitation, whereas chromatographic analysis using the PATfix pDNA Platform offers detailed sample insight - resolving isoforms and quantifying impurities across complex process matrices.