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Development of a quantitative PCR assay for residual mouse DNA and comparison of four sample purification methods for DNA isolation

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ABSTRACT

Reliable and sensitive assays are required to determine whether a pharmaceutical product meets current regulatory guidelines for residual host cell DNA. In this study, the sensitivity of the qPCR assay was significantly improved by targeting the repetitive elements of mouse genome. This improved method allowed for sensitive and accurate quantitation of mouse genomic DNA in the range of 1 to 10^6 pg/mL. In addition, four sample purification methods for DNA isolation (Wako DNA extractor kit, MasterPure™ DNA purification kit, PrepSEQ™ residual DNA sample preparation kit, and phenol–chloroform extraction method with addition of glycogen), each representing a different strategy for DNA isolation from proteinaceous solutions, were evaluated by isolating DNA from a mouse monoclonal IgG antibody. Among these methods, Wako DNA extractor kit and MasterPure™ DNA purification kit demonstrated superior DNA recovery, repeatability, and sensitivity, with quantitation limits of 1 pg/mL. To further evaluate these two DNA isolation methods, six replicates of an unspiked mouse polyclonal IgG antibody sample were tested by both methods, and both methods demonstrated a good degree of precision. Therefore, the residual mouse DNA quantitation methods described here represented rapid, accurate, precise, and sensitive procedures that can be used in quality control testing for regulatory compliance in the pharmaceutical industry.

1. Introduction

A wide variety of therapeutic proteins (e.g. antibodies, therapeutic proteins, and vaccines) are currently manufactured in bacterial hosts, yeast hosts, animal hosts, and continuous cell lines. Residual host cell DNA is therefore a potential contaminant in the final drug product. These residual host cell DNA, even though often present in minute amounts, are always a concern for drug product safety and there are regulatory recommendations to minimize residual host cell DNA contamination to below certain levels. The guidelines for the acceptable levels of residual host cell DNA specified by Food and Drug Administration (FDA) in 1997 was no more than 100 pg/dose in the final product [1]. Historically, the limit permitted by the World Health Organization (WHO) was 100 pg/dose or less for parenteral products [2], but this was modified to 10 ng/dose in 1998 [3]. The specification indicated by European Union (EU) in 2001 was also no more than 10 ng of residual host cell DNA per dose [4]. Although its effects in therapeutic proteins are largely unknown, host cell DNA may contain deleterious DNA fragments with infectivity (i.e. viral DNA) [5] or oncogenic activities (capable of cell transfection resulting in tumors) [6], making it essential to reduce such impurities to the minimum level possible in the drug products administrated to patients. The method for determination of DNA content should therefore be very sensitive in order to detect low levels of residual host cell DNA.

Three methods (hybridization, Threshold® assay, and quantitative PCR) have been recommended by regulatory agencies for residual host cell DNA quantitation [3,7]. The basic principle of hybridization-based assays is the binding of radioactive- or fluorescent-labeled DNA probes to immobilized and denatured host cell DNA. Signal detection is performed by phosphor- or fluorescent-imaging systems [8,9]. The Threshold® assay is an immuno-enzymatic assay based on binding of two proteins specific to single-stranded DNA (ssDNA) [10]. One protein, a single-stranded DNA binding protein, is biotinylated, and the other protein, an anti-ssDNA antibody, is conjugated to urease. In a Threshold® assay, both proteins bind to ssDNA and form a reaction complex, which is then concentrated by filtering through a biotinylated membrane [10]. The host cell DNA in the sample is subsequently quantified by measuring hydrolysis of urea by urease from the anti-ssDNA antibody [10]. By contrast, quantitative PCR (also called qPCR or real-time PCR) amplifies and simultaneously quantifies target DNA in a sample. It relies on the exonuclease activity of Taq polymerase, which results in degradation of a spe-

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cific probe annealed to the template [11,12]. The probe is linked to a fluorescent dye and quencher, and its degradation frees the fluorescent dye from the quencher resulting in a fluorescence emission proportional to the amount of template. The fluorescence signal after each PCR cycle is measured and used for DNA quantitation [11,12].

Differences between these residual DNA quantitation methods should be taken into account during experimental design and data interpretation. Firstly, both hybridization (when random sequences are used as probes) and Threshold® assay may be independent of source DNA, while the qPCR is specific to their target sequences. Secondly, hybridization often requires more than 2 days to complete; the time necessary for the Threshold® assay and qPCR is much shorter (4–6 h). Thirdly, the hybridization method and Threshold® assay are less sensitive than qPCR. A detection limit of 1 pg/mL can often be achieved by qPCR [11]. Overall, due to their sensitivity, accuracy, and precision, qPCR-based assays are increasingly being considered for residual host cell DNA quantitation.

Each proteinaceous solution to be analyzed for residual host cell DNA may contain differing amounts of formulation components, such as organic solvents, detergents, and salts, and the properties of the recombinant proteins may vary. Sample purification is necessary to reduce the protein and residual interference, and the purification method chosen for a particular biopharmaceutical product may be matrix-specific. To date, different DNA isolation methods, such as organic extraction, the sodium iodide method, protease treatment, and methods dependent on ion-exchange columns, have been developed and used. However, the literature comparing these different DNA isolation methods is limited.

In this study, a qPCR-based residual mouse DNA quantitation method was developed. The sensitivity of the qPCR assay was significantly improved by targeting the repetitive elements of the mouse genome. This improved method allowed for sensitive and accurate quantitation of mouse genomic DNA in the range of 1 to 10^6 pg/mL. In addition, four sample purification methods for DNA isolation, each representing a different strategy for DNA purification from proteinaceous solutions, were compared. Two methods demonstrated superior DNA recovery. These two methods were further evaluated for their precision and quantitation limits and were used for testing of a mouse polyclonal IgG antibody sample. The residual mouse DNA quantitation method described here represents an accurate, precise, sensitive, and robust method that can be used in quality control testing for regulatory compliance in the pharmaceutical industry.

2. Materials and methods

2.1. Mouse genomic DNA and standard curve

Mouse genomic DNA used for the standard curve was purchased from Promega (Madison, WI, USA). The initial concentration of mouse genomic DNA was 245 μg/mL. To prepare the DNA standard curve, serial dilutions of mouse genomic DNA were prepared using nuclease-free water (Ambion, Austin, TX, USA) over a range of 1 to 10^7 pg/mL.

2.2. Mouse monoclonal IgG antibody

Mouse monoclonal IgG antibody was used to evaluate the four sample purification methods. The mouse monoclonal IgG antibody (9.5 mg/mL) was purchased from ProMab (Richmond, CA, USA) and was diluted to 0.95 mg/mL using nuclease-free water immediately prior to the spiking experiments.

2.3. Spiked controls

To prepare spiked controls, a known amount of mouse genomic DNA was spiked into the diluted mouse monoclonal IgG antibody to a final concentration of 10, 100, 1000, and 10,000 pg/mL. At the same time, an unspiked control was analyzed to determine the inherent level of mouse genomic DNA in the mouse monoclonal IgG antibody. All spiked and unspiked controls were prepared in duplicate.

2.4. DNA extraction procedures

DNA from spiked and unspiked controls was isolated using the Wako DNA extractor kit (Wako Chemicals USA, Richmond, VA, USA), MasterPure™ DNA purification kit (EpiCentre, Madison, WI, USA), PrepSEQ™ residual DNA sample preparation kit (Applied Biosystems, Forster City, CA, USA), and phenol–chloroform extraction method with addition of glycogen. Among these methods, the Wako DNA extractor kit, MasterPure™ DNA purification kit, and PrepSEQ™ residual DNA sample preparation kit were used according to manufacturers' protocols with some modifications. These modifications involved the addition of glycogen (added for all the methods) and carrier tRNA (added for MasterPure™ DNA purification kit only) during sample purification, as well as resuspension of DNA in an equal volume of nuclease-free water rather than the buffer and volume suggested by the protocols.

To purify DNA using phenol–chloroform extraction method with addition of glycogen, the sample was treated with proteinase K (Invitrogen, Carlsbad, CA, USA) first, and then extracted twice with phenol/chloroform/isoamyl alcohol (25:24:1, v/v/v) (Sigma–Aldrich, St Louis, MO, USA) and once with chloroform (Sigma–Aldrich, St Louis, MO, USA). DNA was then precipitated by adding glycogen (Sigma–Aldrich, St Louis, MO, USA) and an equal volume of isopropanol (99.8% purity, Acros Organic, Pittsburgh, PA). The pellet was rinsed twice with 70% ethanol and air-dried before re-suspension in nuclease-free water for qPCR. To account for the sample volume loss during phenol–chloroform extraction, a correction factor was applied when calculating % DNA recovery.

2.5. Primer and probe design

The primer/probe set targeting the mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was obtained from Applied Biosystems (Forster City, CA, USA) and used following the manufacturer's protocol. The primer/probe sets targeting the L1 family of mouse repetitive element were designed using online software PrimerQuest™ [13] (Integrated DNA Technologies, IDT, Coralville, IA, USA). The amplification primers used were 5′-GTT ACA GAG ACG GAG TTT GGA G-3′ (forward) and 5′-CGT TTG GAT GCT GAT TAT GGG-3′ (reverse). This primer set defines a 93 bp fragment from the L1 family of mouse repetitive element. The probe used was 5′-TGT AGA GAC TGC CAT AGC CAG GGA-3′. The probe was labeled with a fluorescent reporter dye FAM at the 5′ end and a double quencher dye Zen/Iowa black FQ in the middle (Zen) and at the 3′ end (Iowa black FQ). Custom synthetic oligonucleotide primers and probe were obtained from IDT (Coralville, IA, USA). All primers and probes were HPLC-purified.

2.6. Negative control and quality control solutions

To exclude false results in the qPCR amplification and to control plate-to-plate variation, a negative control and three quality control solutions were included in each run. The negative control contained nuclease-free water in place of the DNA template in the qPCR reaction and the quality control solutions (50, 500, and 5000 pg/mL) were prepared by serial dilution of mouse genomic

DNA. The negative control and quality control solutions were analyzed in four replicate wells and run with the test samples on the same 96-well optical reaction plate (Applied Biosystems, Forster City, CA, USA).

2.7. qPCR amplification and data analysis

After DNA isolation, each sample was analyzed in duplicate by qPCR in a 96-well optical reaction plate. In the same plate, the DNA standard solutions for the standard curve, the quality control solutions, and negative control, were each analyzed in four replicate wells. Runs were deemed acceptable if (i) all 4 wells of negative control had undetermined C_T (threshold cycle) values or C_T values ≥38; (ii) the percent recovery of quality control solutions were within ±30% of their expected values; and (iii) the standard curve had a linear coefficient of determination (r^2) equal or greater than 0.99.

qPCR was performed with an Applied Biosystems 7500 Real-Time PCR system (Life Technologies, Forster City, CA, USA) using the following thermal cycling conditions: initial heat denaturation at 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles each of 95 °C for 15 s and 60 °C for 1 min. Four microliters of mouse genomic DNA were amplified in a total volume of 20 μL mixture containing 2× TaqMan Universal PCR Master Mix (Life Technologies, Carlsbad, CA, USA), and final concentrations of 250 nM probe and 500 nM of each primer.

Following amplification, data were analyzed using the 7500 Real-Time System Sequence Detection Software version 1.3 (Applied Biosystems, Forster City, CA, USA). The standard curve and DNA concentration were automatically determined by the software using manually input DNA standard concentrations. The standard curve was generated by plotting C_T against Log DNA concentration (pg/mL) of the DNA standard solutions. C_T is the cycle at which the fluorescence crosses the threshold value. Percent DNA recovery was calculated as:

$$\frac{\text{observed DNA concentration} - \text{inherent DNA concentration}}{\text{DNA spiking concentration}} \times 100$$

2.8. Repeatability and quantitation limit

The repeatability and quantitation limit of DNA recovery using Wako DNA extractor kit and MasterPure™ DNA purification kit were evaluated. The repeatability of the method was evaluated by preparing six replicates of spiked controls at 100 pg/mL mouse genomic DNA in mouse monoclonal IgG. Spiked controls were then analyzed in duplicate wells by qPCR.

The quantitation limit was determined by preparing spiked control in triplicate at 1 pg/mL and 5 pg/mL. An unspiked control was also analyzed in triplicate to determine the inherent DNA concentration of the sample. Purified samples were then analyzed in duplicate wells by qPCR. In this study, the quantitation limit was established as the lowest DNA concentration at which the mean $\log_{10}$ difference is equal to or less than 0.2. The $\log_{10}$ difference was calculated as:

$$\left| \log_{10} \left(\frac{\text{observed DNA concentration} - \text{inherent DNA concentration}}{\text{DNA spiking concentration}} \right) \right|$$

2.9. Mouse polyclonal IgG antibody and sample testing

To further evaluate the Wako DNA extractor kit and MasterPure™ DNA purification kit, a mouse polyclonal IgG sample was purified using both methods. Immediately prior to sample purification, the mouse polyclonal IgG sample was diluted using nuclease-free water. Six replicates of unspiked sample were prepared and purified. The purified samples were then analyzed in duplicate wells by qPCR.

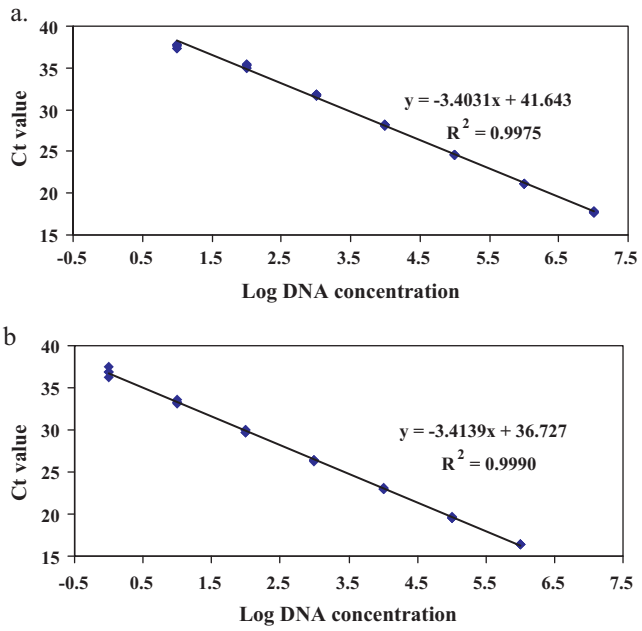

Fig. 1. Standard curves of mouse genomic DNA based on two sets of primers and probe. Set 1 (a) amplified the mouse GAPDH gene and set 2 (b) amplified the L1 family of mouse repetitive element. C_T (threshold cycle), which represented the PCR cycle at which fluorescence reaches threshold value, was plotted against Log DNA concentration (pg/mL) of DNA standard solutions.

3. Results and discussion

3.1. Selection of genomic target and improvement in sensitivity of qPCR assay

Quantitation of residual host cell DNA is technically demanding because of the high sensitivity required to quantitate trace amount of residual DNA present in a sample. In this study, the qPCR assay sensitivity was significantly improved by targeting the repetitive elements of mouse genome.

During method development, we first selected a region located within the mouse GAPDH gene as the target region for PCR. The mouse GAPDH gene was selected because the gene is highly conserved, which allows for the detection of the maximum amount of variants and strains possible. When serial 10-fold dilutions of mouse genomic DNA from 1 to 10^7 pg/mL were prepared for qPCR standard curve, a linear relationship was obtained between the C_T and the Log DNA concentration (r^2 = 0.9975, Fig. 1a). However, qPCR amplification was not sensitive enough to detect DNA at the 1 pg/mL level (C_T from all four PCR wells was undetermined). This low sensitivity was mainly due to the relatively low copy number of the target gene in the mouse genome. The GAPDH gene is present in ~10 copies on different chromosomes. In addition, the size of the mouse genome (~2.7 Bbp) is approximately 587 times larger than the *Escherichia coli* K12 genome (~4.6 Mbp) [14]. Thus when a region of the same copy number from *E. coli* K12 genome is amplified from the mouse genome, a reduction in detection sensitivity of >2 Log would be expected. To improve the sensitivity of qPCR assay, we then chose a high copy number region located within the L1 family of mouse repetitive elements as the target for PCR.

Repeated more than 80,000 times within the mouse genome, the L1 family of mouse repetitive element represents approximately 19% of the mouse genome [15]. Although the repetitive sequences

Table 1
Evaluation of four different DNA isolation methods.

DNA isolation method	Mean of % DNA recovery at 4 different spiking levels (pg/mL)[a,b]			
	10	100	1000	10,000
Wako DNA extractor kit	109	109	114	107
MasterPure™ DNA purification kit	116	103	125	108
PrepSEQ™ residual DNA kit	61	71	68	72
Phenol–chloroform with glycogen	71	44	39	62

[a] Mean of DNA concentration determined from sample preparation of spiked controls in duplicate followed by DNA quantitation in duplicate wells by qPCR.
[b] % DNA recovery was calculated as:

$$\frac{\text{observed DNA concentration} - \text{inherent DNA concentration}}{\text{DNA spiking concentration}} \times 100$$

can be less stable and can undergo significant changes in copy number during evolution [11], qPCR amplification targeting repetitive sequences often provides higher sensitivity. The optimized primer and probe set (optimization data not shown) targeting the L1 family repetitive element generated a linear relationship (Fig. 1b) between the C_T and the Log mouse DNA concentration ($r^2 = 0.9990$). Compared to the standard curve generated using GAPDH gene, the slope of the standard curve remained similar, but the *y*-intercept, which corresponds to the C_T value for a single copy of the target DNA, dropped from 41.643 to 36.727. Thus the sensitivity of the qPCR assay was significantly enhanced. It allowed for detection of mouse genomic DNA at 1 pg/mL or lower (detection limit not tested). This primer and probe set was then used for the residual mouse DNA quantitation in the subsequent experiments.

3.2. Evaluation of four DNA isolation methods

Four DNA isolation methods, each representing a different strategy for isolating DNA from proteinaceous solutions, were evaluated. The Wako DNA extractor kit uses sodium iodide (NaI) to solubilize the protein. The MasterPure™ DNA purification kit uses a nontoxic desalting method to precipitate the protein. The PrepSEQ™ residual DNA sample preparation kit uses magnetic particles to bind to the DNA. Finally, the phenol–chloroform extraction method with the addition of glycogen employs organic solvents to remove the protein from the solution. In addition, all four methods were suitable for isolating DNA molecules of various sizes, including genomic DNA.

To evaluate and compare these four DNA isolation methods, different levels of mouse genomic DNA (10, 100, 1000, and 10,000 pg/mL) were spiked into mouse monoclonal IgG. An unspiked control was also analyzed to determine the inherent DNA level of mouse monoclonal IgG. Samples were then purified by different DNA isolation methods, and DNA concentration was determined by the qPCR.

To exclude false results in the qPCR amplification and to control plate-to-plate variation, a negative control and three quality control solutions were included in each run. The negative control contained nuclease-free water in place of DNA template and the quality control solutions (50, 500, and 5000 pg/mL) were prepared by serial dilution of mouse genomic DNA. The quality control solutions were not purified. The quality control solutions were used to evaluate the accuracy of qPCR quantitation. Runs were deemed acceptable if (i) all 4 wells of negative control had undetermined C_T values or C_T values $\geq$38; (ii) the % recovery of quality control solutions were within ±30% of their expected values; and (iii) the standard curve had a linear coefficient of determination (r^2) equal or greater than 0.99. Data presented in this study met all these criteria.

3.2.1. DNA recovery

The four DNA isolation methods evaluated showed remarkable differences in % DNA recovery (Table 1 and Fig. 2). Overall, the percent DNA recovery of Wako DNA extractor kit and MasterPure™ DNA purification kit was significantly higher than PrepSEQ™ residual DNA sample preparation kit and phenol–chloroform extraction method. However, Wako DNA extractor kit and MasterPure™ DNA purification kit sometimes overestimated the quantity of residual DNA present in the sample. While the mechanism underlying this effect is unknown, it has been reported that addition of carrier RNA (added for MasterPure™ DNA purification kit), which was added to prevent non-specific absorption and loss of DNA, was associated with enhancement in DNA recovery [16,17]. In addition, possible interference by glycogen (added for all four methods) and other residuals from the sample matrix and purification kit with PCR amplification could not be ruled out. Nevertheless, an overestimation is preferable to underestimation from a regulatory standpoint, because overestimation increases the difficulty for a drug product to pass the guidelines on acceptance criteria for residual DNA. Future studies on the possible cause of overestimation in percent DNA recovery in these DNA isolation methods are required.

3.2.2. Specificity

Specificity of the qPCR assay was provided by the selective design of primers and probe to DNA sequences unique to the target genome. In this study, the specificity of the repetitive element primer and probe set designed for residual mouse DNA quantitation was evaluated using Chinese hamster ovary (CHO) and *E. coli*

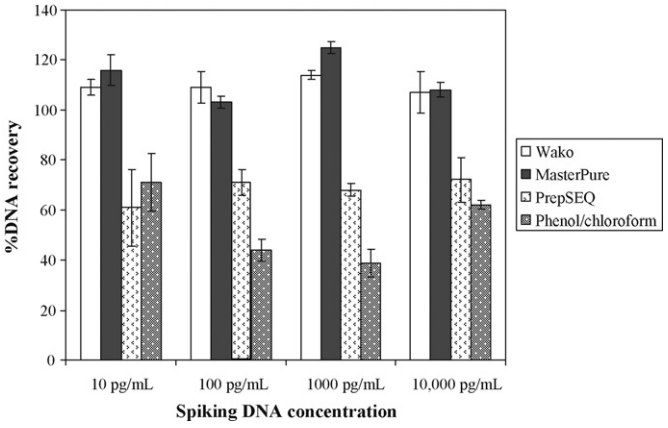

Fig. 2. Comparison of % DNA recovery at four spiking levels (10, 100, 1000, and 10,000 pg/mL) using four different DNA isolation methods (Wako DNA extractor kit, MasterPure™ DNA purification kit, PrepSEQ™ residual DNA sample preparation kit, and phenol–chloroform extraction method with addition of glycogen). Spiked controls were prepared by addition of a known amount of mouse genomic DNA to mouse monoclonal IgG. An unspiked control was also analyzed to determine the inherent DNA level of the sample. All the samples were purified in duplicate followed by DNA quantitation in duplicate wells by the qPCR. Percent DNA recovery was calculated as:

$$\frac{\text{observed DNA concentration} - \text{inherent DNA concentration}}{\text{DNA spiking concentration}} \times 100$$

Table 2
Repeatability of residual mouse DNA quantitation using Wako DNA extractor kit and MasterPure™ DNA purification kit at 100 pg/mL spiking concentration.

Replicate	Wako DNA extractor kit		MasterPure™ DNA purification kit	
	C_T value	% DNA recovery[a]	C_T value	% DNA recovery[a]
1	29.9	118	30.3	89
2	29.8	124	30.4	81
3	29.7	135	30.3	85
4	29.8	122	30.1	96
5	30.0	107	30.0	106
6	30.0	108	30.0	105
Mean	29.9	119	30.2	94
S.D.[b]	0.1	11	0.2	10
CV%[c]	0.4	9	0.6	11

[a] % DNA recovery was calculated as:

$$\frac{\text{observed DNA concentration} - \text{inherent DNA concentration}}{\text{DNA spiking concentration}} \times 100$$

[b] Standard deviation.
[c] Coefficient of variation.

genomic DNA. No amplification was detected after 40 cycles and no PCR products were observed on a 2% agarose gel (data not shown). Specificity was further demonstrated by performing sample purifications of nuclease-free water in place of sample, which were then analyzed in duplicate wells in qPCR. All four purification methods had DNA quantity undetermined, indicating no DNA was detected after 40 qPCR cycles.

3.2.3. Ease of execution

All the purification methods, except for the phenol/chloroform extraction method, were easy to perform. None of the methods required the use of expensive or unusual chemicals or reagents. The amount of time required for sample processing and cost per sample for each DNA extraction kit was determined. The time required to complete DNA purification with the MasterPure™ DNA purification kit was ~1 h. The Wako DNA extractor kit, PrepSEQ™ residual DNA sample preparation kit, and phenol/chloroform extraction method required ~2 h to complete. The MasterPure™ DNA purification kit and phenol/chloroform method were the least expensive on a per sample basis. The Wako DNA extractor kit and PrepSEQ™ residual DNA sample preparation kit were both approximately 5 times more expensive than the MasterPure™ DNA purification kit. Although the phenol/chloroform extraction method was one of the least expensive, it must be kept in mind that the percent DNA recovery with this method was the lowest (Table 1 and Fig. 1). In addition, due to the loss in sample volume during phenol–chloroform extraction, a correction factor has to be applied when calculate DNA recovery, which may not be recommended for development of assays for quality control or regulatory compliance.

Because of their superior percent DNA recovery, the Wako DNA extractor kit and MasterPure™ DNA purification kit were further evaluated for their repeatability and quantitation limit.

3.3. Repeatability and quantitation limit of DNA purification using Wako DNA extractor kit and MasterPure™ DNA purification kit

The repeatability and quantitation limit of DNA isolation using Wako DNA extractor kit and MasterPure™ DNA purification kit were evaluated. The repeatability of the method was evaluated by preparing six replicate spiked controls at 100 pg/mL of mouse genomic DNA in mouse monoclonal IgG. Spiked controls were then analyzed in duplicate wells by qPCR. As shown in Table 2, both methods demonstrated good repeatability. The standard deviation and coefficient of variation of the C_T values were 0.1 and 0.4, respectively, for the Wako DNA extractor kit, and 0.2 and 0.6, respectively, for the MasterPure™ DNA purification kit. In addition, the mean percent DNA recovery using the Wako DNA extractor kit and MasterPure™ DNA purification kit were 119% and 94%, respectively. The standard deviation and coefficient of variation for % DNA recovery were 11 and 9, respectively, for the Wako DNA extractor kit, and 10 and 11, respectively, for the MasterPure™ DNA purification kit.

The quantitation limit was determined by preparing spiked controls in triplicate at 1 pg/mL and 5 pg/mL. An unspiked control was

Table 3
Quantitation limit of residual mouse DNA purification using Wako DNA extractor kit and MasterPure™ DNA purification kit.

Spiking level	Replicate	Wako DNA extractor kit		MasterPure™ purification kit	
		Concentration (pg/mL)	Log_{10} difference[a]	Concentration (pg/mL)	Log_{10} difference[a]
5 pg/mL	1	6.6	0.1	4.2	0.1
	2	5.1	0.0	4.4	0.1
	3	3.1	0.2	4.5	0.0
	Mean	4.9	0.1	4.4	0.1
1 pg/mL	1	0.8	0.1	1.6	0.2
	2	1.1	0.1	1.0	0.0
	3	0.7	0.1	1.2	0.1
	Mean	0.9	0.1	1.3	0.1

[a] Log_{10} difference was calculated as:

$$\left| \log_{10} \left(\frac{\text{observed DNA concentration} - \text{inherent DNA concentration}}{\text{DNA spiking concentration}} \right) \right|$$

Table 4
Quantitation of residual DNA from a mouse polyclonal IgG sample using Wako DNA extractor kit and MasterPure™ DNA purification kit.

Replicate	Wako kit		MasterPure™ kit	
	C_T value	Quantity (pg/mL)	C_T value	Quantity (pg/mL)
1	33.3	12.4	34.1	10.3
2	33.5	11.2	33.5	14.4
3	33.5	11.1	34.1	10.1
4	33.2	13.4	33.6	13.7
5	33.2	13.2	33.5	14.5
6	33.2	13.2	33.8	12.0
Mean	33.3	12.4	33.8	12.5
S.D.[a]	0.1	1.0	0.3	2.0
CV%[b]	0.4	8.4	0.8	16.0

[a] Standard deviation.
[b] Coefficient of variation.

also analyzed to determine the inherent DNA level. Samples were then purified using Wako DNA extractor kit and MasterPure™ DNA purification kit and DNA concentration was determined by qPCR. In this study, the quantitation limit was established as the lowest DNA concentration at which the value for the mean $\log_{10}$ difference of the spiked control equal to or less than 0.2. As shown in Table 3, the mean $\log_{10}$ differences of the spiked control for both purification methods at 1 pg/mL and 5 pg/mL were 0.1. Therefore, both purification methods had quantitation limits of at least 1 pg/mL.

3.4. Sample testing using Wako DNA extractor kit and MasterPure™ DNA purification kit

To further evaluate the ability of the Wako DNA extractor kit and MasterPure™ DNA purification kit in residual DNA isolation, the DNA concentration of a mouse polyclonal IgG antibody sample was determined using both methods. Six replicates of unspiked samples were purified by both methods. DNA quantity was then determined by qPCR.

As shown in Table 4, despite the differences in DNA isolation strategies, DNA concentrations determined by both methods were in the range of 12–13 pg/mL. In addition, the standard deviation and coefficient of variation of the C_T values were 0.1 and 0.4, respectively, for Wako DNA extractor kit, and 0.3 and 0.8, respectively, for the MasterPure™ DNA purification kit. The standard deviation and coefficient of variation of the DNA quantity were 1.0 and 8.4, respectively, for Wako DNA extractor kit, and 2.0 and 16.0, respectively, for the MasterPure™ DNA purification kit. Overall, the Wako DNA extractor kit demonstrated better repeatability than the MasterPure™ DNA purification kit.

4. Conclusions

Reliable and sensitive assays are required to assess residual host cell DNA in a pharmaceutical product. In this study, a qPCR-based residual mouse DNA quantitation method was developed. The sensitivity of the qPCR assay was significantly improved by targeting the repetitive elements of mouse genome. This improved method allowed for sensitive and accurate quantitation of mouse genomic DNA in the range of 1 to 10^6 pg/mL. In addition, four sample purification methods for DNA isolation (Wako DNA extractor kit, MasterPure™ DNA purification kit, PrepSEQ™ residual DNA sample preparation kit, and phenol–chloroform extraction method with addition of glycogen), each representing a different strategy for DNA purification from proteinaceous solutions, were evaluated by isolating DNA from a mouse monoclonal IgG antibody. Among these methods, Wako DNA extractor kit and MasterPure™ DNA purification kit demonstrated superior DNA recovery, good repeatability, and had quantitation limits of 1 pg/mL. To further evaluate these two DNA isolation methods, six replicates of an unspiked mouse polyclonal IgG antibody sample were tested by both methods, and both methods demonstrated a high degree of precision, with Wako DNA extractor kit demonstrating even better precision.

In summary, the residual mouse DNA quantitation method described here represented an accurate, precise, sensitive and robust method that can be used in quality control testing for regulatory compliance in the pharmaceutical industry. The availability of two efficient and reliable but strategically different DNA isolation methods may allow for sample purification from a wide variety of proteinaceous solutions. We expect broad applicability of the assays with minor modifications to other biologics as well.

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