

QUALITATIVE MULTIPLEX PCR ASSAY FOR ASSESSING DNA QUALITY FROM FFPE TISSUES, AND OTHER SOURCES OF DAMAGED DNA

The assessment of DNA quality is a crucial first step in acquiring meaningful data from formalin-fixed paraffin-embedded (FFPE) tissues, and other sources of damaged DNA.¹ Formalin reacts with nucleic acids to cause irreversible damage resulting in DNA samples of poor quality that may not work in downstream processes²⁻³. To address this issue, we've developed a simple, qualitative, gel-based multiplex PCR assay that can be used to determine DNA quality prior to performing tedious and expensive downstream processes such as array-based comparative genomic hybridization (aCGH). The assay consists of five primer sets, derived from the NCBI UniSTS database, that amplify products ranging from 132 bp to 295 bp (see table below). Some or all of these products will fail to amplify as DNA sample quality fades, allowing the classification of genomic DNA quality based on the number and size of fragments amplified, with high quality genomic DNA producing all five amplicons (see figure 1). This assay cannot predict the usefulness of damaged DNA without prior validation work. By performing correlative experiments to empirically determine a quality threshold, one can ensure the repeatability of their downstream experiments. For example, some applications may require that all five amplicons be produced in order to predict a successful outcome, where as others, such as qPCR, may only require the presence of a single band. In this way, one can compare samples from different sources and repeatability of experiments is ensured.

Materials to be supplied by the User

- Water, molecular biology reagent (Cat. No. [W4502](#))
- 25 mM MgCl₂ (Cat. No. [M8787](#))
- 4% agarose gels (Cat. No. [P6097](#))
- 10X TBE buffer (Cat. No. [T4323](#))
- JumpStart™ REDTaq™ ReadyMix™ PCR reaction mix
- 0.2 μM final concentration each primer (see table below) (Cat. No. [P0982](#))

Primer sequences

UniSTS number	Forward Primer Sequence	Reverse Primer Sequence	Amplicon Size (bp)	Chr
STB39J12.SP6	GCAAAATCCATACCCCTTTCTGC	TCTTCCCTCTACAACCCTCTAACC	132	4
STSG50529	GCTGTTAGAGCTTTTATTGCAGC	CTAGAAATTTCTGCATAAACCAACC	150	22
CSNPHARP	CATGGCTCACTGGCTTACAA	TTGCCTCTACAGAGGAGCAG	196	2
SHGC147491	TTTGATGTTAGGACACGCTGAAA	AAAAACGGAAGAAGTCTCTTGGC	235	12
SHGC105883	GTCAGAAGACTGAAAACGAAGCC	GCTTGCCACACTCTTCTCAAGT	295	13

Multiplex PCR amplification of damaged DNA (e.g. FFPE tissue DNA)

JumpStart REDTaq ReadyMix PCR reaction mix (Cat. No. [P0982](#)) is recommended for this process. Reagents may be scaled proportionally if performing PCR reactions of smaller volume.

Reagent Name	Sigma Cat. No.	Final concentration	µl per reaction
Water	W4502 or equivalent	NA	16
JumpStart RedTaq ReadyMix	P0982	1X	25
25 mM MgCl ₂	M8787	3.5 mM*	3
10 µM MultiPlex Primer Mix	NA	0.2 µM each primer	1
Total			45

1. Prepare 10 µM MultiPlex Primer Mix by combining all ten primers in a suitable vessel at a final concentration of 10 µM each primer. Refer to the table above for primer sequences.
2. Combine the following reaction components in a suitable sized tube. Scale-up master mix appropriately for the number of reactions being performed. Make extra master mix to account for pipetting loss. **Note:** The final PCR reaction volume may be scaled up or down as long as reagent concentrations are unchanged.
 - * JumpStart RedTaq ReadyMix contains 2mM MgCl₂. The final MgCl₂ concentration is 3.5 mM after supplementing with 1.5 mM [M8787](#).
3. Add 45 µl of the resulting master mix to an appropriate PCR tube or plate.
4. Add 5 µl of genomic DNA and mix until homogenous. For best results, use between 10 and 100 ng of template DNA per reaction. **Note:** Alternatively, 5µl of undiluted WGA5 tissue lysate, or 100 ng of WGA amplicons generated with any of Sigma's GenomePlex products can be added instead of purified genomic DNA.
4. Place PCR tube(s) or plate in thermal cycler and amplify DNA using the following cycling parameters:
 - 94 °C for 2 minutes to denature
 - 35 cycles of:
 - o 94 °C for 1 minute
 - o 60 °C for 1 minute
 - o 72 °C for 1 minute
 - 72 °C for 7 minutes
 - 4 °C hold
5. Resolve 5 µl of resulting amplicons on a 4% agarose gel. **Note:** JumpStart RedTaq ReadyMix contains gel-loading solution, which allows immediate sample loading onto an agarose gel after PCR. All five PCR amplicons (132 bp, 150 bp, 196 bp, 235 bp and 295 bp) will be generated with high quality genomic DNA. Low quality DNA may fail to produce any amplicons, or may present as faint bands for some amplicons (See Fig 1).

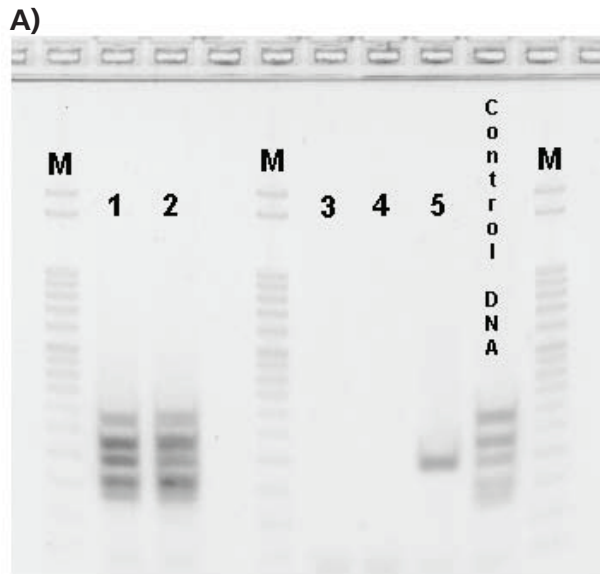
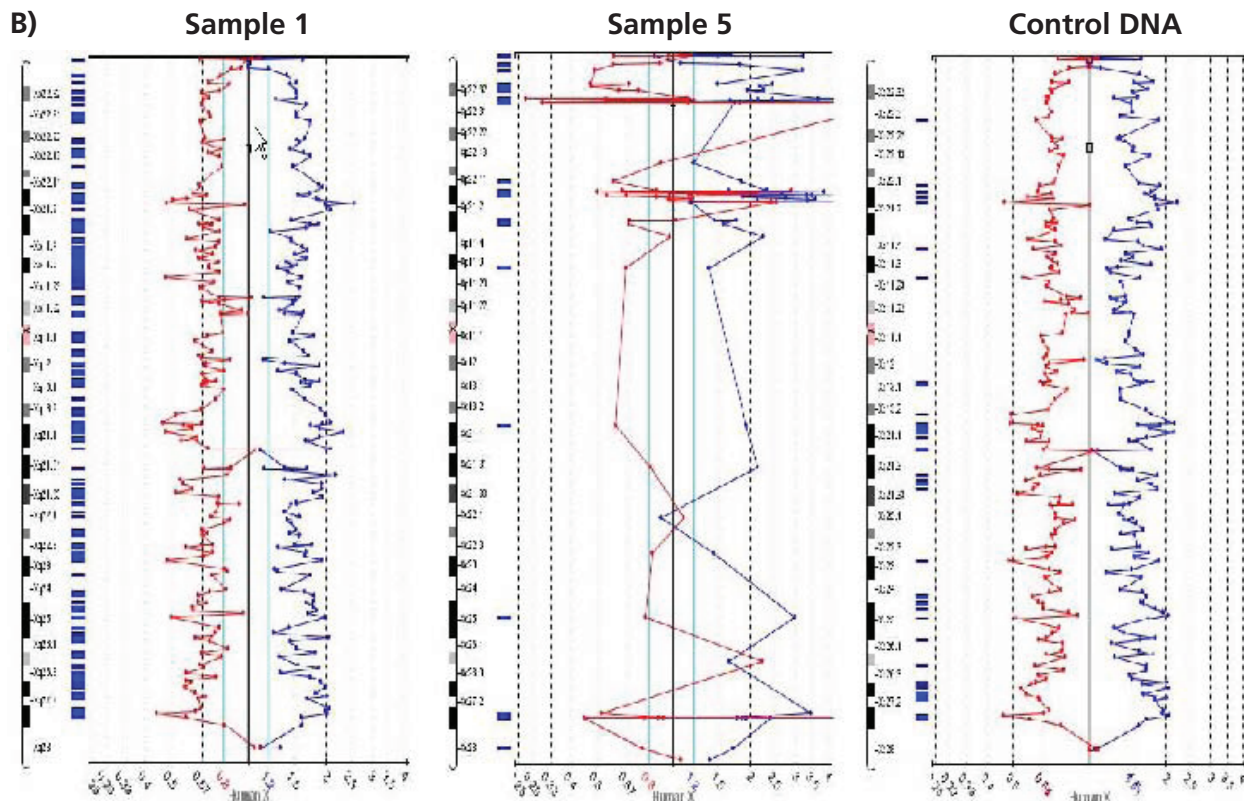


Figure 1: Approximately 1mg of tissue was collected from five FFPE tissue samples followed by processing with the GenomePlex Tissue Whole Genome Amplification Kit (Sigma Cat. No. WG45) as outlined in the technical bulletin. (A) 5 µl of undiluted WG45 tissue lysate was subjected to multiplex PCR amplification as outlined above, and 5 µl of each reaction was resolved on a 4% agarose gel (gel info here). The no-name ladder (company) was used as a size standard. All five bands were amplified in lanes 1 and 2 indicating that these FFPE tissue lysates contain high quality genomic DNA, where as lanes 3, 4 and 5 contain low quality DNA since all, or most, of the multiplex PCR fragments were not amplifiable. Similar results were observed when purified DNA or amplified WGA product derived from these FFPE tissues were used directly in the multiplex qPCR assay (data not shown). (B) aCGH was performed to demonstrate a correlation between the multiplex PCR results and aCGH performance. 1µg of WG45 products were used for BAC aCGH analysis using PerkinElmer’s Spectral Labeling Kit and SpectralChip™ 2600 array platform per manufacturer’s recommendations. The ideograms below are representative of the data obtained with this sample set. They were generated using PerkinElmer’s Spectralware™ BAC array analysis software. High quality array statistics and QC metrics were obtained with samples 1 & 2, where as samples 3, 4 and 5 produced irregular array statistics and poor QC metrics. Test and control hybridization samples are labeled in the figure below.



References

1. Van Beers E.H., et al. (2006) A multiplex PCR predictor for aCGH success of FFPE samples. *Br. J. Cancer* **94**: 333-337.
2. Schander C, Halanych, KM (2003) DNA, PCR and formalized animal tissue – a short review and protocols. *Org. Divers. Evol.* **3**: 195-205.
3. Srinivasan, M., et al. (2002) Effect of fixatives and tissue processing on the content and integrity of nucleic acids. *Am. J. Pathol.* **161**: 1961-1971.