STORE

SOP Title:

Multiplex-PCR check of genomic DNA isolated from FFPE tissue for its usability in array CGH analysis

The STORE processing methods were shown to be fit-for purpose for DNA, RNA and protein extraction from FFPE material. The STORE characterisation methods were shown to be fit for-purpose for Quality Control of the extracted DNA, RNA and proteins.

HUMAN CANCER STUDIES GROUP

Imperial College London

Human Cancer Studies Group

SOP reference MB05

Standard Operating Procedure for

Multiplex-PCR check of genomic DNA isolated from FFPE tissue for its usability in array CGH analysis

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Summary
This protocol explains the procedure for testing the usability of genomic DNA in array CGH experiments, following use of SOP number MB01.

Safety Considerations

Protective clothing and gloves should be worn throughout.

A Preparing of PCR Primers

PCR Primers (Table 1) are purchase ordered at Metabion International GmbH in a 0,02 µmol scale, HPLC purified and lyophilized. The oligos are dissolved in nuclease-free water (Sigma-Aldrich) to 100 µM (stock solution) according to suppliers datasheet.

Table 1.: PCR Primers used for Multiplex PCR

	Name	Sequence	Number of bases
100bp-fragment*			
	100F	5'- gttccaatatgattccaccc -3'	20
	100R	5'- ctcctggaagatggtgatgg -3'	20
200bp-fragment*			
	200F	5'- aggtggagcgaggctagc -3'	18
	200R	5'- ttttgcggtggaaatgtcct -3'	20
300bp-fragment*			
	300F	5'- aggtgagacattcttgctgg -3'	20
	300R	5'- tccactaaccagtcagcgtc -3'	20
400bp-fragment*			
	400F	5'- acagtccatgccatcactgc -3'	20
	400R	5'- gcttgacaaagtggtcgttg -3'	20

* amplification products of non-overlapping fragments within human GDP-gene (chromosome 12), van Beers et al, BrJCanc. 2006, 30;94(2):333-7

For the preparation of a ready-to-use equimolar primer-solution, mix 10 µl of each of the 100 µmol primer stock-solutions and add 720 µl nuclease-free water (800 µl in total; 1,25 µM).

B Preparation of the PCR-Reaction

Although a hotstart Taq-polymerase is used, please prepare the reaction on ice!

Used Materials:

Amplitaq DNA Polymerase (5U/ µl)*
GeneAmp 10X PCR Buffer*
25 mM $MgCl_2$ Solution*
100 mM dNTP Mix (Applied Biosystems, Cat#: N8080007)
Nuclease-free water (Sigma, Cat#: W4502)
Human genomic DNA (Promega, male, Cat#: G1471 or female, Cat#: G1521)
* part of Amplitaq DNA Polymerase kit (Applied Biosystems, Cat#: 4311816)

Pipetting scheme:

1x reaction			5x reaction		
GeneAmp 10X PCR Buffer	3	µl	GeneAmp 10X PCR Buffer	15	µl
MgCl2 (25 mM)	1.8	µl	MgCl2 (25 mM)	9	µl
dNTP-mix (10 mM each)	0.6	µl	dNTP-mix (10 mM each)	3	µl
Primer-Mix (1.25 µM each)	3.2	µl	Primer-Mix (1.25 µM each)	16	µl
Taq (5 U/ul)	0.2	µl	Taq (5 U/ul)	1	µl
total	8.8	µl	total	44	µl

100ng genomic DNA to be tested is diluted with nuclease-free water to a volume of 21.2µl and mixed with 8.8µl master-mix.

For a positive control use male or female genomic DNA and for negative control use nuclease-free water.

Place PCR reaction tubes in the thermocycler and run the following PCR program:

Step 1: 96°C		9’
Step 2: 94°C		1’
Step 3: 56°C		1’
Step 4: 72°C		3’
Step 5:	Goto step 2 for additional 34 cycles	
Step 6: 72°C		7’
Step 7:	4°C	for ever

C Electrophoretic seperation of PCR amplification products

Used Materials:

10X Bluejuice gel loading buffer (Invitrogen, Cat#: 10816015)
GeneRuler 100 bp DNA length standard (Fermentas, Cat#: SM1143)

Agarose for molecular biology (Sigma-Aldrich, Cat#: A9539)
SYBR Safe DNA gel stain 10000x concentrate (Invitrogen, Cat#: S33102)
TAE buffer X50 (National Diagnostics, Cat#: EC-872)

Make 600ml X1 TAE buffer with SYBR Safe for gel and to fill tank:

X10 TAE buffer	12ml
dH2O	588ml
SYBR Safe	60µl

Place 1.5 g of Agarose into a small conical flask and add 100ml of X1 TAE buffer containing SYBR Safe. Heat in the microwave until the agaorse has fully dissolved (take care not to let the agarose boil over). Run cold water over the conical flask whilst swirling the gel to cool the gel down. The agarose should be approximately 50C before pouring (check this by placing the flask on the palm of your hand, if it is uncomfortable then it needs to be cooled more). Pour the cooled agarose into the gel tank apparatus and allow to set.

Once set, remove the combs and add enough TAE buffer to cover the gel.

Mix 10 ul of DNA with 2 ul of 10X loading buffer and load the samples into the gel.

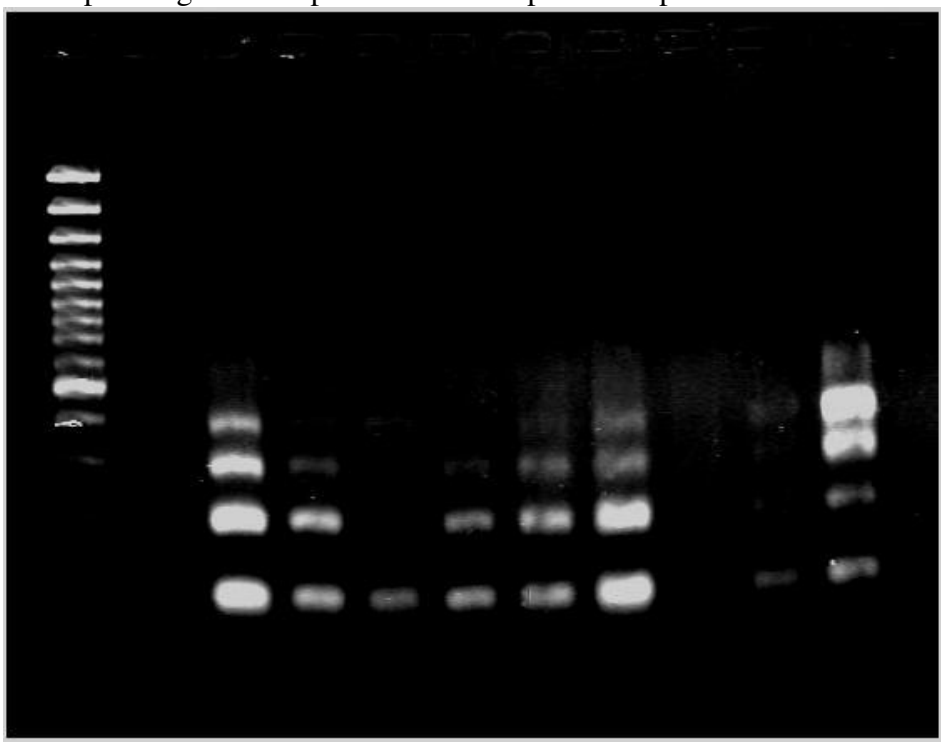
Run the gel at 150V for 30 minutes.

D Interpretation of Results

Place gel in a gel documenation system and make a picture. The number of visible bands represents integrity of the genomic DNA tested. The lane with the positive control should show 4 distinct bands (100 bp, 200 bp, 300 bp and 400 bp in length). No bands should be visible in the lane with the negative control.

DNA samples showing (Fig.1) at least 3 bands (100 bp, 200 bp and 300 bp) can be used for array CGH analysis. Samples showing bands smaller than 300 bp are not used for array CGH analysis.

Fig. 1: Example for gel electrophoresis of multiplex PCR products



lane1: 100 bp DNA standard; lane 2:negative control - no bands, negative;lane 3: 4 bands, positive; lane 4: 3 bands, positive; lane 5: 1 band, negative; lane 6: 3 bands, positive; lane 7: 3 bands, positive; lane 8: 4 bands, positive; lane 9: no bands, negative; lane 10: 1 band, negative; lane 11:positive control - 4 bands, positive;