

Fluorescence In Situ Hybridization on DNA Halo Preparations to Reveal Whole Chromosomes, Telomeres and Gene Loci

Lauren S. Godwin¹, Joanna M. Bridger¹, Helen A. Foster²

¹Laboratory of Nuclear and Genomic Health, Centre for Genome Engineering and Maintenance, Division of Biosciences, Department of Life Sciences, College of Health, Medicine and Life Sciences, Brunel University London ²Biosciences, Department of Clinical, Pharmaceutical and Biological Science, School of Life and Medical Sciences, University of Hertfordshire

Corresponding Author

Joanna M. Bridger
joanna.bridger@brunel.ac.uk

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Materials

Name	Company	Catalog Number	Comments
10X PBS	Thermo Fisher Scientific	10388739	Used to create DNA halos
5-bromo-2'-deoxy-uridine	Sigma-Aldrich	B5002-100MG	Labelled nucleotide
5-Fluoro-2'-deoxyuridine	Sigma-Aldrich	F0503-100MG	Labelled nucleotide
Agar Technical	Thermo Fisher Scientific	15562141	DNA isolation of BAC clones
Agarose	Sigma-Aldrich	A939-50G	Check product size of DOP-PCR and nick translation
Atypical type 2 HGPS fibroblasts (AG08466)	Coriell Institute	AG08466	Cell line
Bacto tryptone	Thermo Fisher Scientific	16269751	DNA isolation of BAC clones
Biotin-16-dUTP	Roche Diagnostics	11093711103	Labelled nucleotides
Chloramphenicol	Sigma-Aldrich	C0378-25G	DNA isolation of BAC clones
Classical Hutchinson-Gilford progeria syndrome (HGPS) fibroblasts (AG06297)	Coriell Institute	AG0297	Cell line
Coplin jar	Thermo Fisher Scientific	12608596	Holds 5 slides or 8 slides back to back
Cot-1 DNA	Thermo Fisher Scientific	15279011	Block nonspecific hybridization in HALO FISH
DEPC-treated water	Sigma-Aldrich	693520-1L	DNA isolation of BAC clones
Dextran sulphate	Sigma-Aldrich	S4030	Hybridisation mixture
Digitonin	Sigma-Aldrich	D141	Component of extraction buffer
Digoxigenin-11-dUTP	Sigma-Aldrich	11093088910	Labelled nucleotides
Donkey anti-mouse Cy3	Jackson Laboratory	715-165-150	Secondary antibody
EDTA	Sigma-Aldrich	E6758	Component of extraction buffer
Ethanol			Component of extraction buffer
Ethanol	Sigma-Aldrich	443611	Probe precipitation and HALO FISH

Fetal bovine system	Thermo Fisher Scientific	26140079	Cell culture serum
Formamide	Thermo Fisher Scientific	10523525	2D FISH of DNA halos
Glass wool	Sigma-Aldrich	18421	Spin column
Herring sperm	Sigma-Aldrich	D7290	Probe precipitation
HXP™ Lamp (metal halide microscope lamp)	OSRAM	HXP-R120W45C VIS	Image capture of DNA halos
Hydrochloric acid	Thermo Fisher Scientific	10313680	Cleaning microscope slides
Isopropanol	Sigma-Aldrich	I9516-25ML	DNA isolation of BAC clones
KAPA HiFi PCR Kit	KAPA Biosystems	KK2103	PCR Kit
Leica DM4000 fluorescent microscope with DFC365 FX camera and LAS AF (Version: 4.5.0) image acquisition software.	Leica Microsystems		Image capture of DNA halos
Luria-Bertani agar	Thermo Fisher Scientific	13274843	DNA isolation of BAC clones
Magnesium chloride	Sigma-Aldrich	M8266	Component of CSK buffer
Methanol	Thermo Fisher Scientific	10284580	Cleaning and sterilizing microscope slides
Mouse anti-BrdU antibody	BD Pharmingen	B2531-100UL	BrdU visualisation
Newborn calf serum	Thermo Fisher Scientific	16010159	Cell culture serum and blocking reagent
Nick translation kit	Invitrogen		
PCR grade water	Sigma-Aldrich	693520-1L	PCR and DNA isolation of BAC clones
PCR Primers	Sigma-Aldrich		
PIPES	Sigma-Aldrich	P1851	Component of CSK and extraction buffers
Potassium acetate	Sigma-Aldrich	P1190-100G	DNA isolation of BAC clones
QuadriPERM® 4 X 12	SARSTEDT	94.6077.307	Square cell culture dish, polystyrene with four compartments. This has hydrophobic surface, is sterile, non-pyrogenic/endotoxin-free and non-cytotoxic.
Rabbit Anti-Ki67 antibody	Sigma-Aldrich	ZRB1007-25UL	Proliferation marker
Rnase A	Sigma-Aldrich	R6513	DNA isolation of BAC clones
Rubber cement	Halford's	101836	2D FISH of DNA halos
Sephadex G-50	Sigma-Aldrich	S6022-25G	Spin column
Sodium acetate	Sigma-Aldrich	S2889	Probe precipitation
Sodium chloride	Sigma-Aldrich	S5886	Component of CSK, extraction and SSC buffers
Sodium citrate	Sigma-Aldrich	C8532	Component of SSC buffer
Sodium dodecyl sulphate		L3771-100G	DNA isolation of BAC clones
Sodium hydroxide	Sigma-Aldrich	S8045-500G	DNA isolation of BAC clones
Spermidine	Sigma-Aldrich	S2626	Component of extraction buffer
Spermine	Sigma-Aldrich	S4264	Component of extraction buffer
Streptavidin-Cy3	Amersham Life Sciences Ltd, Scientific Laboratory Supplies	pa43001	Probe antibody
Sucrose	Sigma-Aldrich	S0389	Component of CSK buffer
Sucrose	Sigma-Aldrich	S0389	CSK buffer+A66:D68
SuperFrost™ microscope slides	Thermo Fisher Scientific	12372098	Microscope slides: 1 mm thickness, 76 mm length, 26 mm width. Uncoated.
Swine anti-rabbit TRITC	Dako		
TELO-PNA FISH KIT	Agilent Dako	K532511-8	Delineation of telomeres

Tris-HCl	Sigma-Aldrich	T3253-100G	Column buffer
Triton™ X-100	Sigma-Aldrich	T9284	Component of CSK buffer
Tryptone	Thermo Fisher Scientific	10158962	DNA isolation of BAC clones
Tween-20	Sigma-Aldrich	P9416- 100ML	Detergent
Vectashield mountant containing DAPI	Vector Laboratories	H-1200	2D FISH of DNA halos
Whole human chromosome probes	Calbiochem		2D FISH of DNA halos
Yeast extract	Thermo Fisher Scientific	10108202	DNA isolation of BAC clones