Department of Cardiovascular Medicine
University of Oxford

Radiation Risk Assessment

READ THE NOTES ON COMPLETION BEFORE ATTEMPTING TO FILL IN THIS FORM. IF INSUFFICIENT SPACE IS AVAILABLE UNDER ANY SECTION, USE A SEPARATE PIECE OF PAPER AND ATTACH IT TO THE FORM.	FILE REF: DATE: 6th May 2011

DEPARTMENT:	Cardiovascular Medicine Wellcome Trust Centre for Human Genetics	PERSONS INVOLVED:	Bhattacharya Group See attached sheet	OTHERS AT RISK: None
LOCATION OF WORK:	Room 10/088 (Lab 3)			

DESCRIPTION OF PROCEDURE:

Hybridisation of Southern Blots with ^{32}P – see attached protocols

SUBSTANCES USED	QUANTITIES USED	FREQUENCY OF USE	HAZARDS IDENTIFIED	EXPOSURE ROUTE	DOSE PER PROCEDURE (μSv)
[α-P32]dCTP	1.85 MBq or 50μCi per reaction (taken from stock pot containing max 9.25 MBq)	Less than once per month	High energy β-emitter (1.71MeV). Skin dose rate from 1MBq point source at 30cm is 0.12mSv/h [Delacroix et al. *Radiat. Prot. Dosim.* **98**, 2002]	Skin (mostly fingers)	*Whole body:* Effectively zero *Extremities:* 300μSv per probe

COULD A LESS HAZARDOUS SUBSTANCE (OR FORM OF THE SUBSTANCE) BE USED INSTEAD? ~~Yes~~/No
JUSTIFY NOT USING IT:

COULD A LOWER ACTIVITY BE USED? ~~Yes~~ / No
JUSTIFY QUANTITY OF MATERIAL IN USE: Quantities used are specified in the probe labelling kit.

WHAT MEASURES HAVE YOU TAKEN TO CONTROL RISK?

ENGINEERING CONTROLS:
Perspex screen and radiation protection cabinet.
Stock pot shipped and stored encased in lead.
Tubes kept in Perspex racks with lids.
Hybridisation tubes containing labelled DNA carried from 10/088 to hybridisation oven in lidded Perspex box.
Reduce dosage by control of distance, time and shielding.

PPE:
Lab coat
Nitrile gloves
Safety goggles

MANAGEMENT MEASURES:
Follow the As Low As Reasonably Practicable (ALARP) rule
Adhere to local rules, EPR2010 and IRR99
Monitor work area before, during and after use
Lone working prohibited
Work only in designated Supervised areas
Radioactivity stock pot stored in locked refrigerator in Room 10/088
Adhere to limits of designated sink and bins
Adhere to storage time limits for bins [Green/yellow band bin - 6 months; Orange bin - 6 months]

CHECKS ON CONTROL MEASURES:
Regular monitoring and supervision
Checks on documentation – control of Monthly returns

Radiation monitor:	**EP15**	**TRAINING REQUIREMENTS:**	URPO lecture "Working with unsealed radioactive sources" and in-house training.
Is dosimetry required? **Type of dosimetry:**	~~Yes~~ / No ~~Extremity / Whole body / Biological~~		

EMERGENCY PROCEDURES:
Refer to Contingency Plans in Local Rules & University Policy Statement S8/05: Appendix 16.
Decontamination where necessary with Count-Off and/or Decon90 decontaminants as recommended. Dispose of contaminated materials in designated bins and sinks. For any incident beyond minor contamination of radioactive workspaces alert RPS (and SRPS/URPO if appropriate).

WASTE DISPOSAL:
Aqueous ~50% to designated sink
Solid ~50% to green-yellow band bin
Actual % amounts estimated after DNA labelling using EP15 or similar monitor

Are overall risk control measures adequate? Yes/~~No~~	**SIGNATURE**
NAME AND POSITION OF ASSESSOR:	**SIGNATURE:**
NAME OF RADIATION PROTECTION SUPERVISOR: Dr James Brown	**SIGNATURE:**

Date of routine review						
	DATE:	/ / 2012	/ / 2013	/ / 2014	/ / 2015	/ / 2016
	BY:					

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Radiation Risk Assessment Training Record

Title: Hybridisation of Southern Blots with ^{32}P

The undersigned have read the above radiation risk assessment and understand the safety arrangements required and their own obligations in ensuring compliance.

DATE	NAME & SIGNATURE	TRAINED BY (NAME & SIGNATURE)

Phosphorus - 32

$^{32}P_{15}$

Half life: 14.3 days
Specific activity: 1.06E+16 Bq.g^{-1}

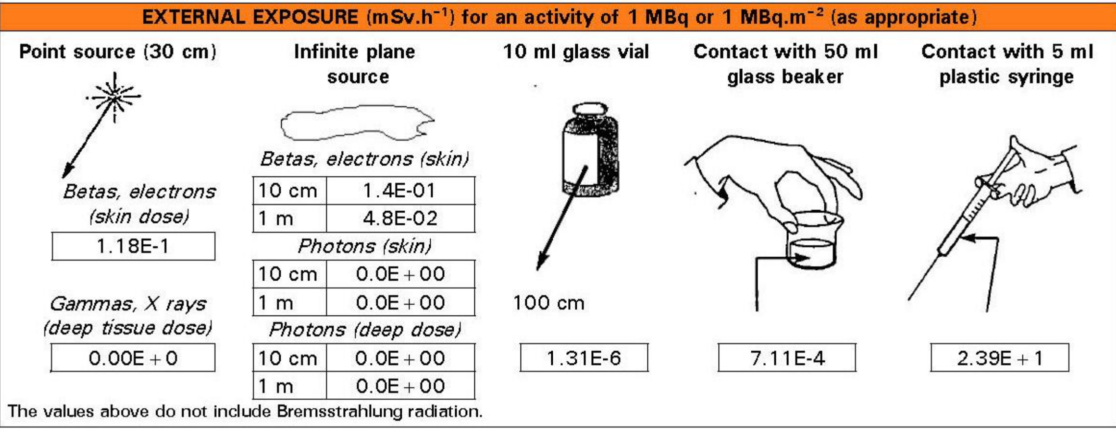
Risk group: 2
Risk colour: Orange

Main emissions (keV)	Gamma or X		Beta (Emax)		Electrons		Alpha	
	E	%	E	%	E	%	E	%
E1			1710	100				
E2								
E3								
% omitted				0				

Exemption levels	
Quantity (Bq)	1E+05
Concentration (Bq.g^{-1})	1E+03

Transport (TBq)	
IAEA ST1 A_1 value	5E-1
IAEA ST1 A_2 value	5E-1

EXTERNAL EXPOSURE (mSv.h^{-1}) for an activity of 1 MBq or 1 MBq.m^{-2} (as appropriate)



Point source (30 cm):

Betas, electrons (skin dose)	Gammas, X rays (deep tissue dose)
1.18E-1	0.00E+0

Infinite plane source:

Betas, electrons (skin)	
10 cm	1.4E-01
1 m	4.8E-02
Photons (skin)	
10 cm	0.0E+00
1 m	0.0E+00
Photons (deep dose)	
10 cm	0.0E+00
1 m	0.0E+00

10 ml glass vial (100 cm)	Contact with 50 ml glass beaker	Contact with 5 ml plastic syringe
1.31E-6	7.11E-4	2.39E+1

The values above do not include Bremsstrahlung radiation.

CONTAMINATION

Contamination skin dose (mSv.h^{-1})	
Uniform deposit (1kBq.cm^{-2})	1.89E+0
0.05 ml droplet (1 kBq)	1.33E+0

Uniform deposit — Droplet

Detection: Recommended probes*	
Alpha	
Beta	+ +
Gamma	
X rays	

Derived limits (Bq.cm^{-2})	
Removable contamination	5E+1
Fixed contamination	3E+2

* If no probes are indicated the recommended technique is to use a wipe test in association with a probe or liquid scintillation technique

SHIELDING (mm)

Betas and electrons (Total absorption)	
Glass	3.4
Plastic	6.3

Gamma and X rays (half and tenth value thickness)

	½	1/10
Lead	-	-
Steel	-	-

INTERNAL EXPOSURE FOR WORKERS

COMMITTED EFFECTIVE DOSE PER UNIT INTAKE (Sv.Bq^{-1})

Ingestion	f_1	
All compounds	0.800	2.4E-09

Inhalation		1 µm	5 µm
All unspec. compounds	F	8.0E-10	1.1E-09
Some phosphat. det. by comb. cation	M	3.2E-09	2.9E-09
	S		

Highest dose organ: Lungs — **20 mSv ALI$_{ingestion}$** 8.3E+06 (Bq) — **20 mSv ALI$_{inhalation}$** 6.3E+06 (Bq)

MAXIMUM RECOMMENDED ACTIVITIES IN LOW LEVEL OR INTERMEDIATE LEVEL LABORATORIES (Bq)

Subject to external exposure requirements which may be more restrictive

PHYSICOCHEMICAL STATE	Volatility factor (k)	Supervised area		Controlled area		
		Bench	Fume hood	Bench	Fume hood	Glove box
All compounds	0.01	5E+05	5E+06	2E+06	2E+07	2E+09

Dose estimation - Hybridisation of Southern Blots with ^{32}P

ALMOST ALL WORK WILL BE CARRIED OUT EITHER BEHIND A PERSPEX SHIELD OR USING PERSPEX TUBE RACKS SO THE DOSE SHOULD BE LIMITED TO FINGERS; WHOLE BODY DOSE WILL BE INSIGNIFICANT.

Handling stock pot	Maximum activity 9.25MBq
Radiolabel used per probe	Maximum activity of 1.85MBq
Assume 50% incorporation (varies between probes)	Maximum activity of 0.925MBq per labelled probe
Washing away unhybridised probe (progressively lower activity handled)	Maximum activity of 0.0148MBq per paper

Dose estimation **per probe** (some experiments may use up to 3 probes)

	For an activity of 1MBq, from Delacroix					
	High dose	**Syringe**		**Low dose**	**Point source (30cm)**	
	H		23.9 mSv/hr	L	0.12	mSv/hr
			0.398333333 mSv/min		0.002	mSv/min
			0.006638889 mSv/sec		3.33333E-05	mSv/sec
	dose rate enter H or L	*manipulation time (s)*	*manipulated amt (MBq)*	*dose (mSv)*		
Pipetting from stock pot into probe labelling reaction	H	3	9.25	0.1842		
Pipetting up and down	L	10	1.85	0.0006		
Closing lids and transfer to/from centrifuge	H	5	1.85	0.0614		
Adding probe to Quick Spin column for clean-up	L	5	1.85	0.0003		
Centrifugation & disposal of column	H	5	1.85	0.0614		
Adding to hybridisation buffer (assuming 50% incorporation)	L	10	0.925	0.0003		
Pouring off hybridisation buffer	L	10	0.925	0.0003		
Wash 1	L	20	0.185	0.0001		
Wash 2	L	20	0.0185	0.0000		
Wash 3 etc	L	20	0.00185	1.23333E-06		
			Total	0.3087	Total finger dose in mSev	

Southern Blotting Protocol

Prepare the gel
Digest DNA samples with appropriate restriction enzyme(s).

The amount of DNA per well for hemizygous transgenic mice should be 10µg. Prepare copy number standards of the transgene to construct a standard curve to determine transgene copy number from unknown samples.

Make an agarose gel. Use 1X TAE, and pour the gel no more than 0.7cm thick. Add loading dye to digests.

Load digested samples onto 0.8% TAE gel without EtBr *(EtBr can alter the mobility of DNA, confounding measurement, so stain afterwards)*:

Electrophorese relatively slowly until the dye passes off the end of the gel. *(Running the gel slowly results in thinner, sharper bands on the Southern).*

EtBr stain and photograph gel with ruler from bottom of wells

Wash the Gel
Rinse stained gel briefly in dH_20

DEPURINATION – Wash in 1L 0.12M HCl for 10 min on rocker, then rinse in dH_20

DENATURATION – 30 mins in 1L 1.5M NaCl/ 0.5M NaOH, then rinse in dH_20

Set up the transfer
Alkalyne transfer: DNA is transferred to Hybond XL membranes by capillary blotting in denaturation buffer.
Set up: Cling wrap, gel (turned over), Hybond XL membrane (parafilm the edges to prevent “short circuit”), 3-5 sheets of Whatman paper, approximately 2 inches of paper towels, flat plastic and weight down.
Transfer O/N

Disassemble the transfer pyramid
Recover the membrane & mark position of wells in pencil and ensure that the up-down and back-front orientations are recognisable.

Immobilise the DNA
Irradiate in UV crosslinker at 70,000 $\mu J/cm^2$
Store membrane between sheets of 3MM for later hybridisation
Use blunt forceps to handle membrane

PROTOCOL INVOLVING THE USE OF RADIOACTIVE ISOTOPES

PROCEDURE: Hybridisation of Southern blots with 32P

ISOTOPE: $P^{32}\alpha$-dCTP (Redivue)

STORAGE: Locked Box in +4°C refrigerator in room 10/088

QUANTITY: 1.85 MBq per reaction

Prehybridisation
Pre-warm *Modified Church & Gilbert Buffer* (Prehybridisation Buffer):
7% (w/v) SDS
0.5 M Phosphate buffer, pH 7.2 (Na_2HPO_4)
10 mM EDTA
1% BSA

Pre-wet membrane in 6xSSC

Prehybridise membrane in 15mL hybridisation buffer (DNA side facing in) for 1 hr at 65°C

Prepare Probe
Dilute 25ng probe in 45µL of 10/1 Tris/EDTA pH8.0
Denature the DNA at 95-100°C for 5 mins
Snap cool on ice for 5 minutes
Centrifuge briefly at max speed

Labelling Probe
Label using Amersham Rediprime II Random Prime Labelling system (GE Healthcare, RPN1633)
(In hot suite)
Add denatured probe to the reaction tube (do NOT mix at this stage)
Add 5µL $P^{32}\alpha$-dCTP to denatured probe and mix by pipetting up and down about 12 times, moving the pipette tip around in the solution.
Incubate at room temperature for at least 30 minutes.

Purification of probe using Sephadex G50 spin columns
Use Roche mini Quick Spin Columns #11 814 419 001
Preparing the column
1. Resuspend column matrix by flicking column sharply several times
2. Remove top cap, then snap off bottom tip, and place column into sterile microfuge tube.
3. Spin column (1000g x 1 min) to pack the column and remove residual buffer

Purifying the sample
Place prepared column in clean, sterile microfuge tube (preferably screw top)
Carefully apply sample to centre of column bed
Spin column (1000 g, 4 min)
Quickly check % incorporation by comparing counts in eluate vs column
Discard column

Hybridisation of labelled probe to the blot
Denature probe again at 95-100^{0}C for 5 mins, then snap cool on ice for 5 mins

Change the hybridization buffer in the tube. Allow to warm up to 65 degrees.

Add denatured probe directly to the hybridisation buffer already in the hybridisation tube.

Hybridise overnight at 65^{0}C

Next day. Washing the blot
Pour off liquid from hybridisation bottle straight down the designated sink and flush sink with tap water.

Carry out a series of washes, of differing stringency depending on the probe:

Rinse briefly in 2X SSC/0.1% SDS

Standard wash steps:

2X SSC/1% SDS, 65 degrees, 30 mins
0.5X SSC/0.1% SDS, 65 degrees, 15 mins
0.2X SSC/0.1% SDS, 65 degrees, 10 mins

Remove blots from the hybridisation tubes and rinse briefly in either low stringency buffer or 2XSSC

Wrap blot in Saran wrap and autoradiograph.

WASTE:
Depending on % incorporation

Liquid disposed activity:	% in probe (~50%)
Solid disposed activity:	% remaining in Sephadex column (~50)

- Record all solid and liquid waste on the appropriate disposal forms.
- Monitor work area, disposal sink and hybridisation oven for contamination.
- Fill in the relevant monitoring sheets.