



Highest quality & performance

All Fermentas products are manufactured in class D clean room facilities, qualified and certified as per EU directives and ISPE guidelines. Fermentas quality assurance is carried out according to ISO9001 quality and ISO14001 environmental management systems, guaranteeing batch-to-batch reproducibility. Integration of our clean room and ISO systems ensures stability and the absence of contaminants in all of our products.

MOLECULAR LABELING & DETECTION

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Selection Guide

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7. MOLECULAR LABELING & DETECTION

Probe type	Labeling strategy	Radioactive	Non-radioactive	Product	Cat. #	Page	
DNA	Random primed labeling	✓		DecaLabel™ DNA Labeling Kit	K0621/2	376	
			✓	Biotin DecaLabel™ DNA Labeling Kit	K0651/2	375	
	Nick-translation		✓	✓	Klenow Fragment, exo ⁻	EP051/2/4	251
			✓	✓	DNA Polymerase I	EP0041/2	249
			✓	✓	DNase I, RNase-free	EN0521/3/5	256
			✓	✓	Taq DNA Polymerase	EP0281/2/3/4 EP0401/2/3/4/5/6	282
	PCR		✓	✓	DreamTaq™ DNA Polymerase	EP0701/2/3/4	280
			✓	✓	High Fidelity PCR Enzyme Mix	K0191/2	297
			✓	✓	Long PCR Enzyme Mix	K0181/2	298
			✓	✓	Pfu DNA Polymerase	EP0501/2 EP0571/2	296
	First strand cDNA synthesis		✓	✓	Maxima™ Reverse Transcriptase	EP0741/2/3	299
			✓	✓	RevertAid™ Premium Reverse Transcriptase	EP0731/2/3	301
			✓	✓	RevertAid™ H Minus Reverse Transcriptase	EP0451/2	303
			✓	✓	RevertAid™ Reverse Transcriptase	EP0441/2	305
			✓	✓	AMV Reverse Transcriptase	EP0641	309
			✓	✓	M-MuLV Reverse Transcriptase	EP0351/2	307
	3'-end labeling		✓	✓	Klenow Fragment	EP0051/2/4	250
			✓	✓	T4 DNA Polymerase	EP0061/2	252
			✓	✓	T7 DNA Polymerase	EP0081	253
			✓	✓	Terminal Deoxynucleotidyl Transferase	EP0161/2	254
5'-end labeling	✓		T4 Polynucleotide Kinase	EK0031/2	244		
RNA	<i>In vitro</i> transcription		✓	TranscriptAid™ T7 High Yield Transcription Kit	K0441	362	
		✓	✓	T7 RNA Polymerase	EP0111/2/3	363	
		✓	✓	SP6 RNA Polymerase	EP0131/3	365	
		✓	✓	T3 RNA Polymerase	EP0101/2/3	364	
	3'-end labeling	✓	✓	T4 RNA Ligase	EL0021	240	
Oligonucleotide	3'-end labeling	✓	✓	Terminal Deoxynucleotidyl Transferase	EP0161/2	254	
	5'-end labeling	✓		T4 Polynucleotide Kinase	EK0031/2	244	

Product group	Radioactive	Non-radioactive	Product	Cat. #	Page
Modified nucleotides		✓	Biotin-11-dUTP	R0081	471
		✓	Fluorescein-12-dUTP	R0101	470
		✓	Aminoallyl-dUTP	R0091/1101	471
		✓	Aminoallyl-UTP	R1091	471
dNTP	✓	✓	dNTP Set	R0181/2/6	466
	✓	✓	dNTP Mix, 2 mM each	R0241/2	466
	✓	✓	dNTP Mix, 10 mM each	R0191/2	466
	✓	✓	dNTP Mix, 25 mM each	R1121/2	466
NTP	✓	✓	NTP Set	R0481	468
Primers	✓	✓	Oligo(dT) ₁₈ Primer	S0131/2	474
	✓	✓	Random Hexamer Primer	S0142	474
	✓	✓	Exo-Resistant Random Primer	S0181	474
Water	✓	✓	Water, nuclease-free	R0581/2	476
	✓	✓	DEPC-treated Water	R0601/3	476
Detection kit		✓	Biotin Chromogenic Detection Kit	K0661/2	377
Chromogenic substrates		✓	BCIP-T	R0821/2	483
		✓	NBT	R0841/2	483
Hybridization	✓	✓	SensiBlot™ Plus Nylon Membrane	M1001/2	377



Products

Biotin DecaLabel™ DNA Labeling Kit

#K0651 for 10 reactions

#K0652 for 30 reactions

Related Products

• Biotin Chromogenic Detection Kit	p.377
• SensiBlot™ Plus Nylon Membrane	p.377
• BCIP-T	p.483
• NBT	p.483
• GeneJET™ Genomic DNA Purification Kit	p.351
• GeneJET™ PCR Purification Kit	p.348
• GeneJET™ Gel Extraction Kit	p.349
• GeneJET™ Plasmid Miniprep Kit	p.345
• Agarase	p.354

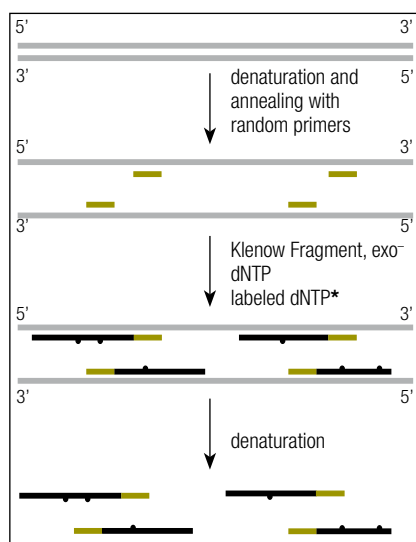
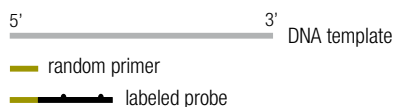


Figure 7.1. DNA labeling by the random primed method.

* [α -³²P]-dNTP, [α -³³P]-dNTP, biotin-dUTP, fluorescein-, aminoallyl- or DIG-dUTP can be used.



Description

The Biotin DecaLabel™ DNA Labeling Kit is an advanced system for the efficient synthesis of biotin-labeled DNA probes, based on an improved random-primed labeling method originally developed by Feinberg and Vogelstein (1, 2). The primary improvement over the traditional random-primed method involves the use of random decamers instead of hexamers to ensure more efficient annealing with DNA at 37°C. Klenow Fragment, exo⁻ is also included in the kit; this genetically engineered enzyme has no detectable exonuclease activity. Therefore, the enzyme does not degrade the labeled probe during reaction, which results in a high labeling yield even with low amounts of template. As a result, DNA fragments of any length can be uniformly labeled. Biotin-labeled DNA is detected with the Biotin Chromogenic Detection Kit (#K0661) or with conventional biotin-avidin or biotin-streptavidin detection systems.

Features

- Non-radioactive labeling of DNA.
- Efficient priming of labeling reactions with random decamers.
- High yields with Klenow Fragment, exo⁻: no degradation of the labeled probe during reaction.

Application

Generation of biotin-labeled DNA probes for a variety of non-radioactive hybridization experiments, including Southern and Northern blots, colony/plaque hybridizations, dot/slot blots and in situ hybridizations.

Principle

Random decamers are annealed to a denatured template DNA molecule and new strands are synthesized by Klenow Fragment, exo⁻ in the presence of biotin-dUTP. During this reaction, the biotinylated nucleotides are incorporated into the newly synthesized complementary DNA strand (see Fig. 7.1).

Quality Control

The kit is tested in a control labeling reaction. The biotin-labeled DNA probe is used in dot-blot. Low amounts of homologous DNA (0.1-0.03 µg) are detected after 16 hours of color development with Biotin Chromogenic Detection Kit (#K0661).

Components of the Kit

- Klenow Fragment, exo⁻
- Decanucleotides in Reaction Buffer
- Biotin Labeling Mix
- Control Template
- Biotin-labeled DNA
- Water, nuclease-free
- Detailed Protocol



Figure 7.2. Dot-blot hybridization with a biotin-labeled probe.

Lambda DNA/HindIII was biotin-labeled with the Biotin DecaLabel™ DNA Labeling Kit and used as a hybridization probe in a dot-blot of the homologous DNA on SensiBlot™ Plus Nylon Membrane (#M1002). The blot was developed with the Biotin Chromogenic Detection Kit (#K0661).

References

1. Feinberg, A.P., Vogelstein, B., A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity, *Biochem.*, 132, 6-13, 1983.
2. Feinberg, A.P., Vogelstein, B., A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity, *Addendum, Biochem.*, 137, 266-267, 1984.



Protocols and Recommendations

» 7.6. Southern Blotting

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DecaLabel™ DNA Labeling Kit

#K0621 for 10 reactions
#K0622 for 30 reactions

Related Products

- Genomic DNA Purification Kit p.352
- GeneJET™ PCR Purification Kit p.348
- GeneJET™ Gel Extraction Kit p.349
- GeneJET™ Plasmid Miniprep Kit p.345
- GeneJET™ Genomic DNA Purification Kit p.351
- Silica Bead DNA Gel Extraction Kit p.353
- SensiBlot™ Plus Nylon Membrane p.377
- Agarase p.354

Description

The DecaLabel™ DNA Labeling Kit is an advanced system for fast synthesis of radiolabeled DNA probes of high specific activity. The kit is based on the improved random-primed method developed by Feinberg and Vogelstein (1, 2).

The primary improvement over traditional random-primer kits involves the use of random decamers instead of hexamers to ensure more efficient annealing with DNA at 37°C. In addition, Klenow Fragment, exo⁻ has been genetically engineered to remove exonuclease activity. The improved enzyme does not degrade the labeled probe during reaction and allows high labeling yields even from low template amounts. DNA fragments of any length can be uniformly labeled. Two labeling mixes are provided for flexibility in using either radiolabeled dATP or dCTP.

Features

- High specific radioactivity of probes – $>1 \times 10^9$ dpm/μg DNA.
- Fast – 5 minutes at 37°C.
- High yields with Klenow Fragment, exo⁻: no degradation of the labeled probe during reaction.
- Flexible – suitable for either radiolabeled dATP or dCTP.

Application

Generation of radiolabeled DNA probes for use in a variety of hybridization experiments: Southern and Northern blots, colony/plaque hybridizations, dot/slot blots and *in situ* hybridizations.

Principle

Random decamers are annealed to a denatured template DNA, and radiolabeled dNTPs are then incorporated into new DNA strands by Klenow Fragment, exo⁻ (see Fig. 7.1 on p.375).

Quality Control

The kit is tested in a control labeling reaction. The typical specific radioactivity of the synthesized probe is $>1 \times 10^9$ dpm/μg DNA.

Components of the Kit

- Klenow Fragment, exo⁻
- Decanucleotides in Reaction Buffer
- Mix A (-dATP)
- Mix C (-dCTP)
- dNTP Mix
- Control Template
- Water, nuclease-free
- Detailed protocol

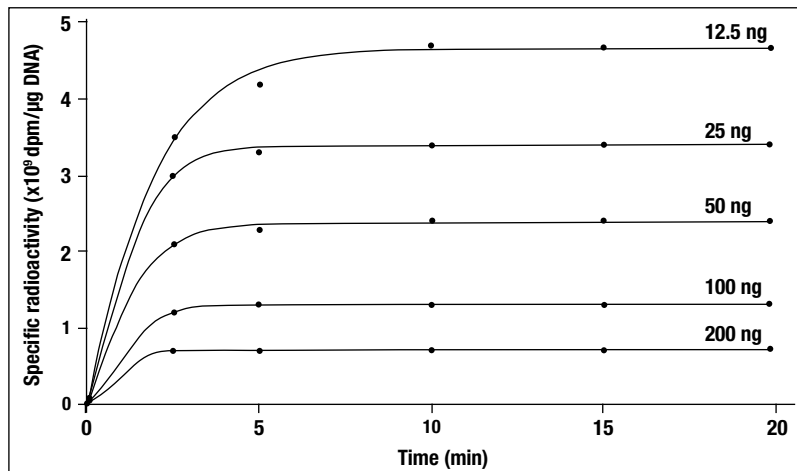


Figure 7.3. Effect of reaction time and amount of template on the specific radioactivity of a DNA probe. Varying amounts of λ DNA/HindIII fragments were labeled with 50 μCi [α-³²P]-dCTP (2500 Ci/mmol) according to the protocol supplied with the kit.



Protocols and Recommendations

- » 7.6. Southern Blotting p.382

References

1. Feinberg, A.P., Vogelstein, B., A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity, *Biochem.*, 132, 6-13, 1983.
2. Feinberg, A.P., Vogelstein, B., A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity, *Addendum, Biochem.*, 137, 266-267, 1984.

Biotin Chromogenic Detection Kit

#K0661 for 10 reactions
#K0662 for 30 reactions

Related Products

• Biotin DecaLabel™ DNA Labeling Kit	p.375
• Biotin-11-dUTP	p.471
• SensiBlot™ Plus Nylon Membrane	p.377
• NBT	p.483
• BCIP-T	p.483

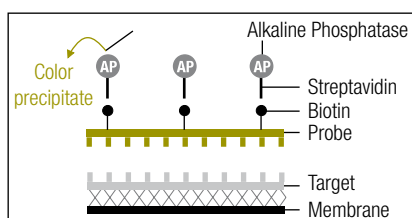


Figure 7.4. Chromogenic detection of a biotin-labeled probe.

Description

The Biotin Chromogenic Detection Kit is a convenient tool for chromogenic detection of biotinylated nucleic acid probes. The kit is optimized to reproducibly provide high sensitivity with low background in applications such as Southern, Northern, dot and slot blotting, as well as screening of viral plaques and bacterial colonies. Biotinylation of DNA and RNA probes is widely used as a safe and convenient alternative to radioactive labeling. Biotin can be incorporated into nucleic acids using various enzymatic or non-enzymatic methods including the Biotin DecaLabel™ DNA Labeling Kit (#K0651). Biotinylated probes are detected with streptavidin coupled to alkaline phosphatase (AP). Streptavidin-AP conjugates bind specifically and irreversibly to the biotin-labeled probes. The probes are then visualized using a chromogenic substrate for alkaline phosphatase -BCIP/NBT, which produces a blue-purple precipitate. Therefore, visualization does not require X-ray film or other specific equipment (see Fig. 7.4).

The Biotin Chromogenic Detection Kit includes Blocking reagent and Blocking/Washing buffers which are specifically designed to minimize background, which is often associated with biotin detection in conventional systems. The positively charged SensiBlot™ Plus Nylon Membrane (#M1001) is recommended for this detection system to ensure the highest detection sensitivity and the lowest background.

Features

- High sensitivity of detection – 0.03 pg of homologous DNA in dot blot hybridization with a biotin-labeled probe.
- Low background due to optimized washing and blocking procedures.
- Non-radioactive detection visualization does not require X-ray film or other specific equipment.
- Fast and convenient – ready-to-use components.

Applications

- Southern blots.
- Northern blots.
- dot/slot blots.
- Plaque or colony screening.

Quality Control

The kit is functionally tested in dot-blot hybridization with a biotin-labeled probe. 0.03 pg of homologous DNA is detected.

Components of the Kit

- Streptavidin-AP Conjugate
- BCIP/NBT Solution
- Blocking Reagent
- 10X Blocking/Washing Buffer
- 10X Detection Buffer
- Detailed protocol



Figure 7.5. Dot-blot hybridization with a biotin-labeled probe.

Lambda DNA/HindIII was biotin-labeled with the Biotin DecaLabel™ DNA Labeling Kit (#K0651) and used as a hybridization probe in a dot-blot of the homologous DNA on SensiBlot™ Plus Nylon Membrane (#M1002). The blot was developed with the Biotin Chromogenic Detection Kit.

SensiBlot™ Plus Nylon Membrane

#M1001 10 sheets (20 x 20 cm)
#M1002 1 roll (30 cm x 3 m)

Related Products

• Biotin DecaLabel™ DNA Labeling Kit	p.375
• DecaLabel™ DNA Labeling Kit	p.376
• Biotin Chromogenic Detection Kit	p.377
• Aminoallyl-dUTP	p.471
• Aminoallyl-UTP	p.471
• Biotin-11-dUTP	p.471
• Fluorescein-12-dUTP	p.470
• NBT	p.483
• BCIP-T	p.483

Description

The SensiBlot™ Plus Nylon Membrane is the hybridization membrane of choice for use with Fermentas radioactive and non-radioactive labeling and detection kits. This membrane ensures the highest sensitivity and lowest background in Southern blot and other blotting experiments.

The SensiBlot™ Plus Nylon Membrane is a nylon 6.6 microporous membrane with quaternary ammonium surface chemistry. Positive surface charge is maintained over a wide range of pH values.

Applications

- Southern blots.
- Northern blots.
- Dot/slot blots.
- Colony and plaque hybridizations.



Protocols and Recommendations

» 7.6. Southern Blotting

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Bulk quantities and custom formulations available upon request

Related Products by Application

DNA/RNA 5'-END LABELING	Cat. #	Size	Supplied with	Applications	Page
T4 Polynucleotide Kinase, 10 u/μl	EK0031	500 u	10X Reaction Buffer A 0.4 ml 10X Reaction Buffer B 0.2 ml 24% PEG Solution 0.2 ml	Radioactive labeling of 5'-termini of single and double-stranded DNA and RNA, such as: – DNA and RNA molecular weight markers, – sequencing primers, – PCR primers, – DNA and RNA oligonucleotides or linkers, – hybridization probes, – probes for transcript mapping.	244
	EK0032	2500 u	10X Reaction Buffer A 2 ml 10X Reaction Buffer B 1 ml 24% PEG Solution 1 ml		

Protocols and Recommendations

- » 7.1.1. DNA 5'-end labeling by T4 PNK in the exchange reaction p.380
- » 7.1.2. DNA/RNA 5'-end labeling by T4 PNK in the forward reaction p.380
- » 7.1.3. Radiolabeling of RNA Ladders by T4 PNK p.380

DNA 3'-END LABELING BY FILL-IN	Cat. #	Size	Supplied with	Applications	Page
Klenow Fragment, 10 u/μl	EP0051	300 u	10X Reaction Buffer 1 ml	DNA 3'-end labeling by fill-in of 5'-overhangs.	250
	EP0052	1500 u	10X Reaction Buffer 5x1 ml		
Klenow Fragment, 2 u/μl	EP0054	LC, 300 u	10X Reaction Buffer 1 ml		
T4 DNA Polymerase, 5 u/μl	EP0061	100 u	5X Reaction Buffer 0.35 ml		
	EP0062	500 u	5X Reaction Buffer 2x1 ml		
T7 DNA Polymerase, 10 u/μl	EP0081	300 u	10X Reaction Buffer 0.4 ml		253

Protocols and Recommendations

- » 7.1.4. DNA 3'-end labeling by fill-in of 5'-overhangs p.380

3'-END LABELING BY TAILING	Cat. #	Size	Supplied with	Applications	Page
Terminal Deoxynucleotidyl Transferase, 20 u/μl	EP0161	500 u	5X Reaction Buffer 0.4 ml	Oligodeoxyribonucleotide and DNA 3'-termini labeling by tailing.	254
	EP0162	2500 u	5X Reaction Buffer 2x1 ml		

Protocols and Recommendations

- » 7.1.5. DNA and oligonucleotide 3'-end labeling by tailing p.380

3'-END LABELING BY LIGATION	Cat. #	Size	Supplied with	Applications	Page
T4 RNA Ligase, 10 u/μl	EL0021	1000 u	5X Reaction Buffer 0.2 ml 10 mM ATP Solution 0.2 ml 1 mg/ml BSA Solution 0.2 ml	RNA and double-stranded DNA 3'-end labeling by ligation.	240

Protocols and Recommendations

- » 7.1.6. RNA 3'-end labeling by ligation p.381

RANDOM-PRIMED LABELING	Cat. #	Size	Supplied with	Applications	Page
Klenow Fragment, 10 u/μl	EP0051	300 u	10X Reaction Buffer 1 ml	Random-primed DNA labeling.	250
	EP0052	1500 u	10X Reaction Buffer 5x1 ml		
Klenow Fragment, 2 u/μl	EP0054	LC, 300 u	10X Reaction Buffer 1 ml		
Klenow Fragment, exo⁻, 5 u/μl	EP0421	300 u	10X Reaction Buffer 1 ml		
	EP0422	1500 u	10X Reaction Buffer 5x1 ml		

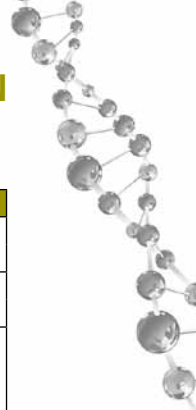
Protocols and Recommendations

- » 7.2. Random-primed labeling p.381

CHROMOGENIC SUBSTRATES	Cat. #	Size	Applications	Page
BCIP-T	R0821	1 g	Detection of alkaline phosphatase on Southern, Northern, dot/slot blots, plague or colony screening.	483
	R0822	5 g		
NBT	R0841	1 g		
	R0842	5 g		483

Protocols and Recommendations

- » 7.6. Southern Blotting p.382



NICK-TRANSLATION	Cat. #	Size	Supplied with	Applications	Page
DNA Polymerase I, 10 u/μl	EP0041	500 u	10X Reaction Buffer 1 ml	DNA labeling by nick-translation.	249
	EP0042	2500 u	10X Reaction Buffer 5x1 ml		
DNase I, RNase-free, 1 u/μl	EN0521	1000 u	10X Reaction Buffer with MgCl ₂ 1 ml		256
DNase I, RNase-free, 50 u/μl	EN0523	HC, 1000 u	25 mM EDTA 1 ml		
DNase I, RNase-free, 1 u/μl (supplied with MnCl ₂)	EN0525	HC, 1000 u	10X Reaction Buffer with MgCl ₂ 1 ml 10X Reaction Buffer w/o MnCl ₂ 1 ml 100 mM MnCl ₂ 1 ml 25 mM EDTA 1 ml		

Protocols and Recommendations

» 7.3. DNA labeling by nick-translation

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RNA LABELING BY <i>in vitro</i> TRANSCRIPTION	Cat. #	Size	Supplied with	Applications	Page
T7 RNA Polymerase, 20 u/μl	EP0111	5000 u	5X Transcription Buffer 1.25 ml	Synthesis of labeled RNA probes for: – blotting, – <i>in situ</i> hybridization, – microarray hybridization.	363
	EP0112	5x5000 u	5X Transcription Buffer 5x1.25 ml		
T7 RNA Polymerase, ≥100 u/μl	EP0113	HC, 25000 u			365
SP6 RNA Polymerase, 20 u/μl	EP0131	2000 u	5X Transcription Buffer 0.5 ml		
SP6 RNA Polymerase, ≥100 u/μl	EP0133	HC, 5000 u	5X Transcription Buffer 1.25 ml		
T3 RNA Polymerase, 20 u/μl	EP0101	2000 u	5X Transcription Buffer 0.5 ml		364
	EP0102	10000 u			
T3 RNA Polymerase, ≥100 u/μl	EP0103	HC, 10000 u	5X Transcription Buffer 2x1.25 ml		
TranscriptAid™ T7 High Yield Transcription Kit	K0441	50 reactions	–	Generation of non-radioactively labelled RNA probes.	362

Protocols and Recommendations

» 6.4. Synthesis of radiolabeled RNA probes of high specific activity

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cDNA LABELING BY REVERSE TRANSCRIPTION	Cat. #	Size	Supplied with	Applications	Page
Maxima™ Reverse Transcriptase	EP0741	2000 u	5X RT Buffer 1 ml	<ul style="list-style-type: none"> • First strand cDNA synthesis. • Real-time RT-PCR. • RT-PCR. 	299
	EP0742	10000 u	5X RT Buffer 1 ml		
	EP0743	4x10000 u	5X RT Buffer 2x1 ml		
RevertAid™ Premium Reverse Transcriptase, 200 u/μl	EP0731	2000 u	5X RT Buffer 1 ml	<ul style="list-style-type: none"> • First strand cDNA synthesis. • Construction of full length cDNA libraries. • Real-time RT-PCR. 	301
	EP0732	10 000 u	5X RT Buffer 1 ml		
	EP0733	40 000 u	5X RT Buffer 2x1 ml		
AMV Reverse Transcriptase, 20 u/μl	EP0641	1000 u	5X AMV RT Buffer 1 ml	Generation of labeled cDNA probes for hybridization experiments, such as microarray hybridization.	309
RevertAid™ H Minus Reverse Transcriptase, 200 u/μl	EP0451	10000 u	5X Reaction Buffer 1 ml		303
	EP0452	5x10000 u	5X Reaction Buffer 5x1 ml		
RevertAid™ Reverse Transcriptase, 200 u/μl	EP0441	10000 u	5X Reaction Buffer 1 ml		305
	EP0442	5x10000 u	5X Reaction Buffer 5x1 ml		
M-MuLV Reverse Transcriptase, 20 u/μl	EP0351	1000 u	5X Reaction Buffer 1 ml		307
	EP0352	5000 u	5X Reaction Buffer 5x1 ml		

Protocols and Recommendations

» 7.5.1. Synthesis of cDNA probes with high specific radioactivity

p.382

» 7.5.2. Synthesis of non-radioactively labeled cDNA

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Nucleotides for LABELING	Cat. #	Size	Applications	Page
Biotin-11-dUTP, 1 mM	R0081	50 nmol (50 μl)	All types of enzymatic labeling.	471
Fluorescein-12-dUTP, 1 mM	R0101	25 nmol (25 μl)		470
Aminoallyl-dUTP, 10 mM	R0091	1 μmol (100 μl)		471
Aminoallyl-dUTP, 50 mM	R1101	2.5 μmol (50 μl)		
Aminoallyl-UTP, 50 mM	R1091	2.5 μmol (50 μl)		
dNTP Set, 100 mM	R0181	(4x25 μmol) 4x0.25 ml		466
	R0182	(4x100 μmol) 4x1 ml		
	R0186	(4x500 μmol) 4x5 ml		

DNA LABELING BY PCR	Page
Thermophilic DNA polymerases can be used for label incorporation during PCR	245

Protocols and Recommendations

7.1. DNA/RNA end labeling

7.1.1. DNA 5'-end labeling by T4 PNK in the exchange reaction

All types of DNA ends can be successfully labeled with T4 Polynucleotide Kinase. However, the labeling efficiency is greatest for the 5'-protruding DNA ends, lower for blunt ends, and is the lowest for 5'-recessed DNA ends. This protocol is recommended for radiolabeling of DNA markers and ladders (ready-to-use versions with the loading dye pre-mixed are not suitable for labeling).

1. Prepare the following reaction mixture:

Linear DNA	1-20 pmol of 5'-termini
10X reaction buffer B for T4 PNK	2 μ l
$[\gamma\text{-}^{32}\text{P}]$ or $[\gamma\text{-}^{33}\text{P}]$ -ATP	40 pmol
24% (w/v) PEG 6000 solution	4 μ l
T4 Polynucleotide Kinase (#EK0031)	1 μ l (10 u)
Water, nuclease-free (#R0581)	to 20 μ l
Total volume	20 μl

- Incubate at 37°C for 30 min.
- Add 1 μ l 0.5 M EDTA, pH 8.0 (#R1021), incubate at 75°C for 10 min.
- Separate labeled DNA from unincorporated label by gel filtration on Sephadex G-50.

Note

- If an ethanol solution of $[\gamma\text{-}^{32}\text{P}]$ or $[\gamma\text{-}^{33}\text{P}]$ -ATP is used, vacuum-dry the required amount and dissolve in Water, nuclease-free (#R0581).
- The ATP concentration should be at least 2 μ M (1, 2).
- For calculation of pmol of DNA ends, see Appendix p.522 or www.fermentas.com/reviewer.

7.1.2. DNA/RNA 5'-end labeling by T4 PNK in the forward reaction

1. Prepare the following reaction mixture:

Dephosphorylated DNA or Oligonucleotide	1-20 pmol of 5'-termini 10-50 pmol
10X reaction buffer A for T4 Polynucleotide Kinase	2 μ l
$[\gamma\text{-}^{32}\text{P}]$ or $[\gamma\text{-}^{33}\text{P}]$ -ATP	20 pmol
T4 Polynucleotide Kinase (#EK0031)	1 μ l (10 u)
Water, nuclease-free (#R0581)	to 20 μ l
Total volume	20 μl

- Incubate at 37°C for 30 min.
- Add 1 μ l 0.5 M EDTA, pH 8.0 (#R1021), incubate at 75°C for 10 min.
- Separate labeled DNA from unincorporated label by gel filtration on Sephadex G-50.

Note

- If an ethanol solution of $[\gamma\text{-}^{32}\text{P}]$ or $[\gamma\text{-}^{33}\text{P}]$ -ATP is used, vacuum-dry the required amount and dissolve in Water, nuclease-free (#R0581).
- The ATP concentration should be at least 1 μ M (1, 2).
- For estimation of pmol of DNA ends, see Appendix p.522 or www.fermentas.com/reviewer.

7.1.3. Radiolabeling of RNA Ladders by T4 PNK

The ready-to-use versions of the RiboRuler™ RNA ladders can not be radiolabeled with T4 PNK. For efficient labeling of RNA ladders it is recommended to remove the 5'-phosphate groups from the RNA and then phosphorylate in the forward reaction using T4 Polynucleotide Kinase.

I. Dephosphorylation.

1. Prepare the following reaction mixture:

RiboRuler™ Low Range RNA Ladder (#SM1831) or RiboRuler™ High Range RNA Ladder (#SM1821)	8 μ l
RiboLock™ RNase Inhibitor (#EO0381)	0.5 μ l (20 u)
10X reaction buffer for alkaline phosphatase	2 μ l
FastAP™ Thermosensitive Alkaline Phosphatase (#EF0651) or Shrimp Alkaline Phosphatase (#EF0511)	2 μ l (2 u)
DEPC-treated Water (#R0603)	to 20 μ l
Total volume	20 μl

- Incubate at 37°C for 30 minutes.
- Remove the enzymes from the mixture with 20 μ l of Tris-saturated (pH 8.0) phenol chloroform mixture. Save the upper aqueous phase and extract it twice with 20 μ l of chloroform.
- Precipitate RNA by adding 1 μ l of 3 M Sodium Acetate Solution (#R1181) and 55 μ l of 96% ethanol. Store 15-30 min at -20°C. Centrifuge the mixture for 20 min at 10000-15000 rpm and 4°C.
- Rinse the pellet with 20 μ l of cold 75% ethanol. Centrifuge 10 min at 10000-15000 rpm, 4°C.
- Discard the supernatant and dissolve the air-dried pellet in 10 μ l of DEPC-treated Water (#R0603).

II. Labeling

1. Prepare the following reaction mixture:

Dephosphorylated RNA Ladder	1 μ l 2.5 μ l
$[\gamma\text{-}^{32}\text{P}]$ -ATP (5000 Ci/mmol, 10 μ Ci/ μ l)*	5 μ l (10 pmol)
RiboLock™ RNase Inhibitor (#EO0381)	0.25 μ l (10 u)
10X buffer A for forward reaction (supplied with T4 polynucleotide kinase)	1 μ l
T4 Polynucleotide Kinase (#EK0031)	1 μ l (10 u)
DEPC-treated Water (#R0603)	to 10 μ l
Total volume	10 μl

* If $[\gamma\text{-}^{32}\text{P}]$ -ATP with a high specific activity (higher than 5000 Ci/mmol) is used, the label can be diluted with cold ATP (#R0441). Total ATP concentration should be at least 1 μ M.

- Incubate at 37°C for 30 minutes.
- Stop the reaction by adding 1 μ l of 0.5 M EDTA, pH 8.0 (#R1021) and extract the mixture with an equal volume of chloroform.
- Determine the efficiency of label incorporation.

7.1.4. DNA 3'-end labeling by fill-in of 5'-overhangs

1. Prepare the following reaction mixture:

Linear DNA	0.1-4 μ g
10X reaction buffer for Klenow Fragment	2 μ l
$[\alpha\text{-}^{32}\text{P}]$ -dNTP, ~15-30 TBq/mmol (400-800 Ci/mmol) or $[\alpha\text{-}^{33}\text{P}]$ -dNTP, ~110 TBq/mmol (3000 Ci/mmol)	0.74 MBq (20 μ Ci) 2.96 MBq (80 μ Ci)
3 dNTP Mix, 2 mM each (without a labeled dNTP)	2.5 μ l (0.25 mM final concentration)
Klenow Fragment (#EP0051)	0.1 μ l (1 u)
Water, nuclease-free (#R0581)	to 20 μ l
Total volume	20 μl

- Incubate at 37°C for 15 min.
- Stop the reaction by heating at 75°C for 10 min.

Note

This protocol is suitable for labeling of the following Fermentas DNA markers, composed of DNA fragments with 5'-overhangs:

Lambda DNA EcoRI Marker, #SM028 (p.425)

Lambda DNA HindIII Marker, #SM0101 (p.425)

Lambda DNA EcoRI/HindIII Marker, #SM0191 (p.425)

Lambda DNA Eco911 Marker, #SM0111 (p.425)

Φ X174 DNA HinfI Marker, #SM0261 (p.427)

- The modified version of this protocol can be used for non-radioactive labeling of DNA markers. Substitute a part of dTTP with a modified nucleotide (e.g. Biotin-11-dUTP or Fluorescein-12-dUTP) at a molar ratio of 1:2.

- For estimation of pmol of DNA ends, see Appendix p.522 or www.fermentas.com/reviewer.

7.1.5. DNA and oligonucleotide 3'-end labeling by tailing

1. Prepare the following reaction mixture:

5X reaction buffer for TDT	10 μ l
Linear DNA	10 pmol
$[\alpha\text{-}^{32}\text{P}]$ -ddATP, ~110 TBq/mmol (3000 Ci/mmol)	1.85 MBq (50 μ Ci)
Terminal Deoxynucleotidyl Transferase (#EP0161)	2 μ l (40 u)
Water, nuclease-free (#R0581)	to 50 μ l
Total volume	50 μl

- Incubate at 37°C for 15 min.
- Stop the reaction by heating at 70°C for 10 min or by adding 5 μ l 0.5 M EDTA (#R1021).

Note

The efficiency of the reaction depends upon the type of 3'-OH termini of the DNA fragments. 3'-protruding ends are labeled with higher efficiency than recessed or blunt ends.

References

- Sambrook, J., Russell, D.W., Molecular Cloning: A Laboratory Manual, the Third edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001.
- Current Protocols in Molecular Biology, vol. 1 (Ausubel, F.M., et al., ed.), John Wiley & Sons, Inc., Brooklyn, New York, 3.10.2-3.10.5, 1994-2004.

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7.1.6. RNA 3'-end labeling by ligation

1. Prepare the following reaction mixture in a RNase-free microfuge tube:

10X ligation buffer for T4 RNA Ligase	2 µl
10 mM ATP	1 µl
RNA	50-100 pmol
[³² P]-pCp	50-100 pmol (equimolar amount)
T4 RNA Ligase (#ELO021)	1 µl (10 u)
DEPC-treated Water (#R0601)	to 20 µl
Total volume	20 µl

2. Incubate at 4°C for 10-12 hours (overnight).
3. Separate labeled RNA from unincorporated label by gel filtration on Sephadex G-50.

7.2. Random-primed labeling

7.2.1. Radioactive random-primed DNA labeling

1. Prepare the following reaction mixture:

DNA template	10 µl (100 ng)
10X reaction buffer for Klenow Fragment, exo ⁻	5 µl
6.0 A ₂₆₀ units/ml (100 µM) Random Hexamer Primer (#S0142)	12.5 µl
Water, nuclease-free (#R0581)	to 40 µl
Total volume	40 µl

2. Incubate the mixture in a boiling water bath for 5-10 minutes and then chill on ice.

3. Add:

3 dNTP Mix, 0.33 mM each (without a labeled dNTP)	3 µl (0.02 mM final concentration)
[α- ³² P]-dNTP, ~110 TBq/mmol (3000 Ci/mmol)	1.85 MBq (50 µCi)
Klenow Fragment, exo ⁻ (#EP0421)	1 µl (5 u)
Water, nuclease-free (#R0581)	to 50 µl
Total volume	50 µl

4. Incubate the reaction mixture for 10 minutes at 37°C.
5. Add 4 µl 0.25 mM dNTP mix and incubate at 37°C for 5 minutes.
6. Add 1 µl 0.5 M EDTA, pH 8.0 (#R1021) to stop the reaction.
7. Remove 1 µl of the reaction mixture and determine the percentage of incorporated label.
8. Purify by using Sephadex G-50 or Bio-Gel P-60.

7.2.2. Non-radioactive random-primed DNA labeling

1. Prepare the following reaction mixture:

DNA template	10 µl (100 – 1 µg)
10X reaction buffer for Klenow Fragment, exo ⁻	5 µl
6.0 A ₂₆₀ units/ml (100 µM) Random Hexamer Primer (#S0142)	12.5 µl
Water, nuclease-free (#R0581)	to 39 µl
Total volume	39 µl

2. Incubate the mixture in a boiling water bath for 5-10 minutes and then chill on ice.

3. Add:

3 dNTP Mix, 1 mM each (without the dTTP)	5 µl (0.1 mM final concentration)
dTTP, 1 mM*	3.25 µl (0.065 mM final conc.)
Biotin-11-dUTP**, 1 mM (#R0081)	1.75 µl
Klenow Fragment, exo ⁻ (#EP0421)	1 µl (5 u)
Total volume	50 µl

- * Prepare 1 mM dTTP solution by combining 1 µl of 100 mM ATP solution (#R0441) and 99 µl of water, nuclease-free.
- ** Fluorescein-12-dUTP (#R0101), DIG-dUTP or Aminoallyl-dUTP (#R0091) can also be used with the same protocol.

4. Incubate the reaction mixture at 37°C for 1 h.
5. Add 1 µl 0.5 M EDTA, pH 8.0 (#R1021) to stop the reaction.
6. Remove 1 µl of the reaction mixture and determine the percentage of incorporated label.
7. Optional: purify by using Sephadex G-50 or Bio-Gel P-60.

7.3. DNA labeling by nick-translation

7.3.1. Radioactive DNA labeling by nick-translation

1. Mix the following components:

10X reaction buffer for DNA Polymerase I	2.5 µl
Mixture of 3 dNTPs, 1mM* (without the labeled dNTP)	1.25 µl
[α- ³² P]-dNTP ~110 TBq/mmol (3000 Ci/mmol)	1.85-3.7 MBq (50-100 µCi)
DNase I, RNase-free (#EN0521) freshly diluted to 0.002 u/µl**	1 µl
DNA Polymerase I, <i>E. coli</i> (#EP0041)	0.5-1.5 µl (5-15 u)
Template DNA	0.25 µg
Water, nuclease-free (#R0581)	to 25 µl
Total volume	25 µl

2. Immediately incubate at 15°C for 15-60 min.
3. Terminate the reaction by adding 1 µl of 0.5 M EDTA, pH 8.0 (#R1021).
4. Take an aliquot (1 µl) to determine the efficiency of the label incorporation. A specific activity of at least 10⁸ cpm/µg DNA is expected.
5. If needed, the labeled DNA may be separated from the unincorporated radioactive precursors on Sephadex G-50 or Bio-Gel P-60 column.

Note

- * To prepare a mixture of three non-labeled dNTPs (1 mM of each), mix 1 µl aliquots of stock solutions of each dNTP (100 mM, from #R0181) with 97 µl of Water, nuclease-free (#R0581). These dNTP mixes can be stored at -20°C for further use.

- **DNase I, RNase-free (#EN0521) can be diluted with 1X reaction buffer for DNA Polymerase I (#EP0041).

- Radioactive DNA probes with higher specific activities can be prepared using two radioactively labeled dNTPs simultaneously. In this case, the composition of the unlabeled dNTP mix should be adjusted accordingly.

7.3.2. Non-radioactive DNA labeling by nick-translation

The protocol 7.3.1. can be used for non-radioactive labeling by Nick-translation using biotin-11-dUTP, fluorescein-12-dUTP, DIG-dUTP or aminoallyl-dUTP:

- normal dTTP is substituted for labeled-dUTP at a molar ratio of 1:3-1:5,
- reaction time is prolonged to 1-2 hours.

7.4. RNA labeling by *in vitro* transcription

7.4.1. Synthesis of radiolabeled RNA probes of high specific activity

1. Linearize template DNA with a restriction enzyme. Extract DNA with phenol/chloroform, then with chloroform/isoamyl alcohol, and precipitate with ethanol. Dissolve DNA in DEPC-treated Water (#R0601).
2. Combine the following reaction components at room temperature in the order given:

DEPC-treated Water (#R0601)	to 20 µl
5X transcription buffer	4 µl
3 NTP Mix, 10 mM each* (without labeled NTP)	1 µl (0.5 mM final concentration)
100 µM CTP (#R0451)	2.4 µl (12 µM final concentration)
[α- ³² P]-CTP, ~30 TBq/mmol (800 Ci/mmol)	1.85 MBq (50 µCi)
Linearized template DNA	0.2-1.0 µg
RiboLock™ RNase Inhibitor (#E00381)	0.5 µl (20 u)
T7 RNA Polymerase (#EP0111) or SP6 RNA Polymerase (#EP0131) or T3 RNA Polymerase (#EP0101)	1 µl (20 u)
Total volume	20 µl

3. Incubate at 37°C for 2 hours.
4. Stop the reaction by cooling at -20°C.
5. Determine the percentage of the label incorporated into RNA.

Note

- * To prepare a mix of three non-labeled NTPs, 10 mM each, combine 1 µl of all three NTPs, 100 mM, from the set (#R0481) with 7 µl of DEPC-treated Water (#R0601). Store the mix at -20°C for further use.
- Expect specific radioactivity of 3-5 x 10⁸ dpm/µg.
 - RNA can be radiolabeled with [³²P], [³⁵S] or [³H]-ribonucleotides. Recommended amounts of radiolabeled nucleotides in a 20 µl of reaction mixture are as follows:

Radiolabeled nucleoside	Amounts for 20 µl of reaction mixture	Specific activity
5'-[α- ³² P]-CTP	1.85 MBq (50 µCi)	~30 TBq/mmol (800 Ci/mmol)
5'-[α- ³⁵ S]-UTP	11.1 MBq (300 µCi)	>37 TBq/mmol (1000 Ci/mmol)
5,6-[³ H]-UTP	0.925 MBq (25 µCi)	1.1-2.2 TBq/mmol (30-60 Ci/mmol)

7.5. Synthesis of labeled cDNA

7.5.1. Synthesis of cDNA probes with high specific radioactivity

This protocol is provided for first strand cDNA synthesis using RevertAid™ H Minus Reverse Transcriptase (#EP0451). For specific reaction conditions using other enzymes, see Chapter 3. Mix and briefly centrifuge all components after thawing, keep on ice.

1. Add into sterile, nuclease-free tube on ice in the order given:

Template RNA	Total RNA <i>or</i>	up to 5 µg
	Poly(A) RNA <i>or</i>	up to 500 ng
	Specific RNA <i>or</i>	up to 500 ng
Primers	Oligo(dT) ₁₈ (#S0131) <i>or</i>	0.5 µg (100 pmol)
	Random Hexamer (#S0141) <i>or</i>	0.2 µg (100 pmol)
	Gene-specific	15-20 pmol
DEPC-treated Water (#R0601)		to 8.5 µl
Total volume		8.5 µl

2. Mix gently and briefly centrifuge.

Optional. Incubate at 65°C for 5 min, chill on ice and briefly centrifuge. Perform this step if RNA template is GC-rich or is known to contain secondary structures.

3. Place the tube with primer/template mix on ice and add the following components in the indicated order:

5X reaction buffer	4 µl
RiboLock™ RNase Inhibitor (#E00381)	0.5 µl (20 u)
dGTP, dCTP, dTTP mix, 10 mM each	1 µl
0.1 mM dATP	4 µl
[α- ³² P]-dATP, 3000 Ci/mmol	1 µl
RevertAid™ H Minus Reverse Transcriptase (#EP0451)	1 µl (200 u)
Total volume:	20 µl

4. Mix gently and briefly centrifuge drops.
5. If oligo(dT)₁₈ primer or a gene-specific primer is used, incubate at 42°C for 60 min. If random hexamers are used, incubate at 25°C for 10 min followed by 60 min at 42°C. For transcription of GC-rich RNA the reaction temperature can be increased to 45°C.
6. Stop the reaction by adding 5 µl of 0.5 M EDTA, pH 8.0 (#R1021).

Optional. Hydrolyze the RNA by the addition of an equal volume (25 µl) of 0.6 M NaOH and incubation at 70°C for 30 min.

7. Remove unincorporated dNTPs by chromatography on a Sephadex® G-50 column. Expect specific radioactivity of >10⁷ dpm/µg.

Note

To achieve higher specific activities (over 10⁸ dpm/µg), use up to 100 µCi of [α-³²P]-dATP in the labeling mixture. To keep the total reaction volume at 20 µl, vacuum-dry 10 µl of [α-³²P]-dATP (10 mCi/ml) to 1 µl in a separate tube.

7.5.2. Synthesis of non-radioactively labeled cDNA

The protocol 7.5.1. can be used for synthesis of non-radioactively labeled cDNA using biotin-11-dUTP, fluorescein-12-dUTP, DIG-dUTP or aminoallyl-dUTP:

- unlabeled dTTP is substituted for labeled-dUTP at a molar ratio of 1:3-1:4,
- reaction time is prolonged to 2-6 hours.

7.6. Southern Blotting

Required solutions (for preparation, see Appendix, p.524)

1. **Denaturation Solution:** 1.5 M NaCl, 0.5 M NaOH.
2. **Neutralization Solution:** 1.5 M NaCl, 0.5 M Tris-HCl (pH 7.2), 1 mM EDTA.
3. **20X SSC, pH 7.0 (blotting buffer):** 3 M NaCl, 0.3 M sodium citrate, 1 mM EDTA.
4. **100X Denhardt's solution:** 2% (w/v) BSA, 2% (w/v) Ficoll™, 2% (w/v) PVP (polyvinylpyrrolidone).
5. **Pre-hybridization Solution:** 6X SSC, 5X Denhardt's solution, 50% formamide, 0.5% SDS.

Electrophoresis

Load genomic DNA probes along with a DNA marker on a 0.7% agarose gel (20 cm length). Run for 18 hours at 3 V/cm in 1X TAE buffer.

Southern Blotting

1. Rinse the gel in deionized water, add Denaturation Solution and gently shake at room temperature for 30 min. Rinse the gel in deionized water and add Neutralization Solution. Shake at room temperature for 15 min. Repeat neutralization procedure.

Fill the glass dish with blotting buffer. Make a platform and cover it with a sheet of Whatman™ 3 mM filter paper, saturated with 20X SSC buffer (see Fig. 7.6).

2. Place the gel upside down on the filter and avoid trapping air bubbles beneath it.
3. Cut a sheet of SensiBlot™ Plus Nylon Membrane (#M1001) to match the size of the gel and place it on the top of the gel. Avoid trapping air bubbles beneath the membrane.
4. Cut 2-3 sheets of Whatman™ 3 mM filter paper to the size of the gel, wet with blotting buffer and place on the top of the membrane.
5. Place a stack of absorbent paper towels on top of the 3 mM paper, place a glass plate on the top of the paper towels and put a 0.5 kg weight on top.
6. Allow upward capillary transfer of DNA at room temperature for 18 hours.
7. Wash the membrane in 2X SSC buffer to remove any residual agarose, dry at room temperature and fix for 2 min under UV-light.

Generation of Labeled Probes

The labeled probes are prepared using Biotin DecaLabel™ DNA Labeling Kit (#K0651), DecaLabel™ DNA Labeling Kit (#K0621) or using the protocol on p.381 for random-primed labeling.

Hybridization

1. Prepare 30 ml of the pre-hybridization solution.
2. Denature sonicated salmon sperm DNA solution (10 mg/ml) by heating at 100°C for 5 min. Chill on ice and add to the pre-hybridization solution with a final concentration of 50-100 µg/ml.
3. Place the membrane into the hybridization container, add pre-hybridization solution with the denatured salmon sperm DNA (0.2 ml/cm² of membrane) and pre-hybridize at 42°C with shaking for 2 hours.
4. Prepare the hybridization solution:
 - denature the labeled probe by incubating at 100°C for 5 min and chill immediately on ice. Add the following amounts of the probe mixture to the pre-hybridization solution:
 - 10 ng/ml (1/5 of probe mix) if specific activity is 10⁸ dpm/µg,
 - 2 ng/ml (1/25 of probe mix) if specific activity is 10⁹ dpm/µg,
 - 25-100 ng/ml if non-radioactively labeled probes.
5. Discard the pre-hybridization solution (from step 3) and add the prepared hybridization solution to the hybridization container (60 µl/cm²). Incubate for at least 12 hours at 42°C.
6. Carry out the following washes of the membrane:
 - twice in 2X SSC + 0.1% SDS at room temperature for 10 min,
 - twice in 0.1X SSC + 0.1% SDS at 65°C for 10 min (for high stringency).
7. Dry the membrane using sheets of Whatman™ 3 mM paper.

Autoradiography

Wrap the dried membrane with Saran Wrap™ and expose to a phosphorimager or a film with an intensifying screen.

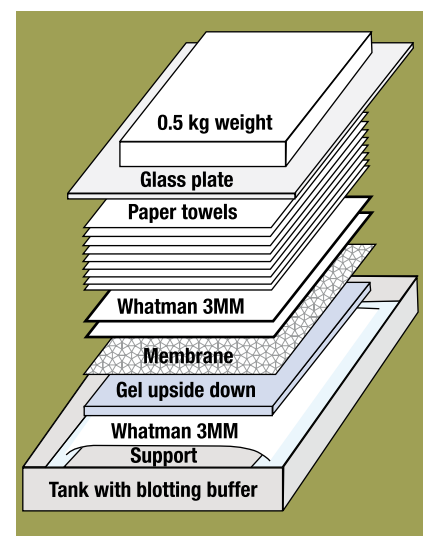


Figure 7.6. Upward capillary transfer of DNA from agarose gels.

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