SOLID PHASE PCR

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1. INTRODUCTION

The method of solid anchored PCR uses specific oligonucleotides coupled to a solid phase as primers for cDNA synthesis [1] and results in cDNA that is covalently linked to the solid phase such as agarose [1] , acrylamide [1] , magnetic [2-4] , or latex beads [5] . A solid phase with cDNA attached, generated using oligo (dT) as a primer, contains sequence information similar to a cDNA library, thus it represents a 'solid phase library' [1, 3, 4] . The cDNA that is attached to the solid phase can be used directly as a template for PCR reactions or can be modified enzymatically prior to the PCR reaction. Oligonucleotides that are attached to a solid phase can also serve for affinity purification of RNA [2] . RNA isolated this way can be directly reverse transcribed, using the primer that is coupled to the solid phase. Subsequent PCR reactions can employ this primer with or without additional internal primers. Since the cDNA is coupled to a solid phase, changing buffer conditions or primer composition is conveniently achieved by washing the solid phase and resuspending in a different PCR reaction mixture. The general principle of solid anchored PCR is shown in Figure 1.

PCR products immobilized on a solid phase have already been used in a wide variety of applications:

- for automated single strand sequencing of PCR products [6, 7] .
- for detection of PCR products in automated clinical assays [8-10]

- to generate single stranded DNA probes with high specific activity 11] .
- to construct enriched genomic and cDNA libraries [12-14]
- for hybrid selection of RNA [5]

Finally, nucleic acids that have been immobilized on nylon membranes have been successfully amplified [15]

In this chapter we will describe the attachment of specific oligonucleotides to a solid phase, the use of these oligonucleotides as primers for cDNA synthesis, the modification of this cDNA by homopolymer tailing, and the handling of this cDNA in PCR experiments.

Insert Figure 1 here

2. Coupling of Oligonucleotides to a solid phase

2.1. Introduction

In order to couple an oligonucleotide to a solid phase, the oligonucleotide has to contain a chemical side group that can be activated. Since no strong nucleophiles are present in ordinary oligonucleotides, the introduction of a strong nucleophile, such as a primary amine, allows coupling of the oligonucleotide. A primary amine can be introduced into the oligonucleotide using an aminolink during automated synthesis (Figure 2) [16] . The aminolink can be coupled to biotin ([17],(see also Chapter 2)) which leads to immobilization of the oligonucleotide on streptavidin-coated solid phases [7, 8], or it can be coupled directly to an activated solid phase. It is important that the buffer in which the coupling is done does not contain any amino groups such as Tris (Tris[hydroxymethyl]aminomethane), for example in TE buffer. We couple the aminolink group directly to a solid phase, because this leads to covalent binding between the oligonucleotide and the solid phase. Coupling of the oligonucleotide *via* the aminolink leaves the 3' end of the oligonucleotide accessible for enzymatic reactions such as priming cDNA synthesis.

Insert Figure 2 here

2.2 Choice of the solid phase

Several different solid matrices for performing solid phase PCR are commercially available. Aminolink activated oligonucleotides can be coupled to N-Hydroxysuccinimidyl activated agarose, polyacrylamide hydrazide particles or tosyl activated magnetic beads. The active groups that are present on these matrices can also be chemically introduced into other solid phases by standard methods [18] We found that agarose is best suited for reamplification experiments of coupled cDNA and polyacrylamide and magnetic beads are better suited for RNA affinity isolation from more viscous solutions. Magnetic beads are easier to see in a polypropylene tube, but we found that agarose beads stick better to the tube. Choosing the solid phase depends on the specific application and preference of the investigator.

2.3 The coupling reaction

The coupling conditions for several matrices are given in Protocol 1-3 and the structure of the reaction products is schematically shown in Figure 2.

Protocol 1. Coupling of oligonucleotides to tosyl activated beads

Reagents

- oligonucleotide with aminolink (100 nmol)
- tosyl activated Dynabead suspension (DYNAL, #M450)
- 500 mM Na borate buffer pH 9.5 (1.55 g boric acid in 50 ml of water, bring to pH 9.5 by adding approximately 2 g NaOH (the alkaline pH is important for coupling)

Method

1. Incubate 100 nmol oligonucleotide (modified with the aminolink) with 200 μl Dynabead suspension.

2. Add 1/10 vol. of 500 mM Na borate solution pH 9.5.

3. Incubate this mixture on a shaking platform or rotating wheel overnight at room temperature.

4. Wash the solid phase twice with water using 500 μl water each time and resuspend the beads in 100 μl water.

Store the coupled oligonucleotides at 4°C in the aqueous slurry. This has an approximate concentration of 0.5 nmol oligonucleotide/μl. Long term storage is recommended at -70°C in aliquots.

Protocol 2. Coupling of oligonucleotides to N-Hydroxysuccinimid groups

Reagents

- Oligonucleotide with aminolink (100 nmol)
- N-Hydroxysuccinimid coupled agarose (Sigma #H3512)
- 2 M $Na_2CO_3/NaHCO_3$, pH 9.5 (dissolve 16.8 g $NaHCO_3$ in water, adjust pH with 2M NaOH and bring volume up to 100 ml)
- cold saturated (3.3M) glycine solution

Method

1. Wash a 10-fold excess (1 mmol matrix for 100 nmol oligonucleotide) of matrix bound coupling groups (as specified by the manufacturer) twice by resuspending the gel particles containing the N-Hydroxysuccinimid groups in water, followed by a brief centrifugation to pellet the matrix.

2. Add 50 μl of 2 M $Na_2CO_3/NaHCO_3$ buffer to 100 nmol of oligonucleotide in a 500 μl reaction volume. Mix this solution with the washed matrix.

3. Perform coupling overnight at room temperature with agitation.

4. Stop the reaction by adding 60 μl of a 3.3 M glycine solution to the reaction mixture. Incubate for 2 h at room temperature.

5. Wash the solid phase twice with water and resuspend the beads in 100 μl water.

Store the coupled oligonucleotides at 4°C in the aqueous slurry. This has an approximate concentration of

0.5 nmol oligonucleotide/μl. Long term storage is recommended at -70°C in aliquots.

Protocol 3. Coupling of oligonucleotides to hydrazide groups

Reagents

- Oligonucleotide with aminolink (100 nmol)
- Hydrazide coupled to polyacrylamide powder (Sigma #P8885)
- 0.1 M amidosulfonic acid (H_3NO_3S)
- 0.1 M HCl
- 0.1 M $NaNO_2$
- 5 M $NH_4(OH)$

Method

1. Dissolve the dry polyacrylamide powder containing approximately 1-10 μmol hydrazide groups (typically 20 mg) in 250 μl water.

2. Activate the hydrazine groups by adding 250 μl 1.0 M HCl and 250 μl 0.1 M $NaNO_2$. Place the reaction mixture on ice for 20 minutes and wash twice with 0.1 M HCl and once with 0.1 M amidosulfonic acid, to remove NO.

3. Resuspend the activated matrix in 500 μl 0.2 M $Na_2CO_3/NaHCO_3$ buffer, pH 9.5 that contains the coupling oligonucleotide at a concentration of 100 nmol/500 μl.

4. Perform the coupling reaction at room temperature overnight.

5. Stop the reaction by adding 10 μl of 5 M $NH_4(OH)$.

6. After 1 h at room temperature wash the matrix twice with water and resuspend it in 100 μl of water.

Store the coupled oligonucleotides at 4°C in the aqueous slurry. This has an approximate concentration of 0.5 nmol oligonucleotide / μl. Long term storage is recommended at -70°C in aliquots.

3. Synthesis of solid phase coupled cDNA

3. 1 Reverse transcription of RNA using solid phase coupled oligonucleotides

Reverse transcription is performed in 10 μl reactions containing the solid phase with the coupled oligonucleotides as described in Protocol 4. The reverse transcription reaction is performed in the same tube in which the PCR reaction will be performed, since it is difficult to transfer small amounts of solid phase matrix. In order to optimize reaction conditions such as the Mg^{++} concentration, we usually prepare 6 tubes with identical reaction mixtures. One of the reaction is spiked with (α-^{32}P) dCTP to monitor cDNA synthesis (see section 3.2).

Protocol 4. Reverse transcription reaction using solid phase
oligonucleotides coupled to a solid phase

Reagents

- RT mixture (7 μl of the RT mix contain: 2 μl 5x reverse transcription buffer (250 mM Tris-HCl, pH 8.3, 15 mM $MgCl_2$, 375 mM KCl), 1 μl 100 mM DTT, and 1 μl of 10 mM of each dNTP solution. The RT mix is made up in a large volume and stored in 70 μl aliquots at -20°C.
- RNAse Inhibitor (Boehringer #799025)
- MMLV H^- reverse transcriptase (Superscript, BRL #8053SA)
- Solid phase coupled oligonucleotide resuspended in water (contains usually about 0.5 nmol of coupled oligonucleotide; see Protocols 1-3)

Method

1. Into a 500 μl polypropylene PCR reaction tube, pipette the following:

RT mixture	7	μl
solid phase coupled oligonucleotide	1	μl

total RNA (1μg/μl)	1	μl
RNAse Inhibitor (20 U)	0.5	μl
MMLV H^- reverse Transcriptase (100 U)	0.5	μl

2. Incubate the reaction at 37°C for 1 h.
3. Wash the cDNA coupled to the solid phase twice with water.
4. Store the solid phase cDNA at -20°C or use it in a PCR experiment (see Protocol 7). The PCR experiment is performed in the same tube in which the cDNA was synthesized.

3.2. Monitoring of cDNA synthesis

The amount of cDNA created by reverse transcription can be estimated by the incorporation of [α-^{32}P] dCTP into cDNA (Protocol 5). The final dCTP concentration in the reverse transcription reaction is 1 mM dCTP. Since the reaction volume is 10 μl, this equals 10 nmol of dCTP. The amount of cDNA synthesized can be estimated from the dCTP incorporation. The average formula weight of one nucleotide is 330 g/mol. Since DNA is composed of 4 nucleotides, for each mole of dCTP one mol of nucleic acid equalling 330x4 = 1320 g is incorporated. The amount of cDNA synthesized is then given by the formula in step 7 of Protocol 5. A typical [α-^{32}P] dCTP spiked reverse transcription reaction with 1 μg of total RNA emits about 2.1 x 10^6 cpm. After three wash steps, about 800 cpm are usually still bound to the solid phase, representing an incorporation of about 0.04%, or 5 ng cDNA. Since we used total RNA instead of poly(A^+) RNA, this first strand synthesis is lower than the cDNA synthesis usually observed in making cDNAs in solution. A typical washing pattern of solid phase coupled cDNA is shown in Figure 3. As evidenced in this figure, most of the unincorporated nucleotides are removed after two washes, indicating the high washing efficiency of this method.

Protocol 5. Estimation of amount of cDNA synthesized by [α-^{32}P] dCTP incorporation.

Reagents

- [α-^{32}P] dCTP (3000 Ci/mmol)
- all other reagents indicated in Protocol 4

Method

1. Perform a reverse transcription reaction as in Protocol 4, but in addition add 0.5 μl [α-^{32}P] dCTP, (3000 Ci/mmol) to the reaction mixture.
2. Count the radioactivity in the reaction tube.
3. Add 100 μl water to the tube, vortex, and spin for 5 min. in a microcentrifuge, at maximum speed.
4. Remove all the liquid from the tube.
5. Repeat the washing procedure two more times.
6. Count the tube after the three wash steps.
7. Calculate the approximate amount of cDNA in g by:

$$\text{cDNA}[\text{g}] = \frac{\text{counts after wash}}{\text{counts before wash}} \times 10 \times 10^{-9}[\text{mol}] \times 1320\ [\text{g/mol}]$$

Insert Figure 3 here

As an example, with the typical incorporation data given in the text, we obtain about:

$$\frac{800}{2.1 \times 10^{6}} \times 10 \times 10^{-9} \times 1320 = 5.03 \times 10^{-12}\ \text{g cDNA}$$

3.3 Addition of homopolymers to the coupled oligonucleotides

Using terminal deoxynucleotidyl transferase, nucleotides can be added to the free 3' end of the coupled oligonucleotides and to coupled cDNA [4]. This introduces a homopolymer tail, which is attached to the cDNA or the oligonucleotide. When added to oligonucleotides, the homopolymer tail is useful to determine whether the oligonucleotides have coupled to the solid phase. By using radioactive nucleotides in the terminal transferase reaction, radioactive labeled homopolymers are generated. Since the exact length of the homopolymer is hard to control, the amount of incorporation is only a rough

indication of the coupling efficiency. However, the absence of incorporation indicates that the oligonucleotides were not coupled to the solid support. Furthermore, homopolyer tails attached to cDNA can be used for RACE experiments [19 .

Protocol 6. Terminal transferase reaction with oligonucleotides coupled to a solid phase

Reagents

- Solid phase with attached oligonucleotide resuspended in water
- 5x Terminal transferase buffer (1 M potassium cacodylate, 125 mM Tris-HCl, pH 7.2)
- [α-^{32}P] α dATP (3000 Ci/mmol)[a]
- terminal deoxynucleotidyl transferase (50 U, Boehringer)

Method

1. Add the following reagents in order into a 500 μl PCR tube:

solid phase with attached oligonucleotide	1 μl
5x terminal transferase buffer	2 μl
[α-^{32}P] α dATP 3000 Ci/mmol	1 μl
water	4 μl
terminal deoxynucleotidyl transferase	1 μl

2. Incubate for 30 minutes at 37°C.

3. Remove the unincorporated label by resuspending the solid phase in 100 μl of water followed by a 5 minute centrifugation step.

4. Repeat the wash twice more.

[a] For the addition of non-labelled homopolymer tails, add 2.5 mM dNTP instead of labelled dATP. The dNTP used depends on which tail is to be synthesized.

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4. Use of solid phase coupled cDNA in PCR experiments

The cDNA that is created with oligonucleotides coupled to a solid phase can be repeatedly used in PCR experiments

using different primer combinations. Protocol 7 describes the procedures used. An example of such an experiment is shown in figure 4. In this experiment 1 μg of total rat brain RNA is reverse transcribed using primer SS020 (see Table 1, footnote a) coupled to agarose beads.

Protocol 7: Use of solid phase coupled cDNA in PCR experiments

Reagents

- cDNA coupled to a solid phase from Protocol 4.
- PCR mixture (16.6 mM $(NH_4)_2SO_4$, 170 μg/ml BSA, (Fraction V, Sigma #A7906) 67 mM Tris-HCl, pH 8.8 at 20°C, 1.5 mM $MgCl_2$, 200 μM of each dNTP and 7.8 μg/ml of each primer [a])
- mineral oil

Method

A. Removal of the reverse transcription reaction components

1. After reverse transcription (Protocol 4.), add 100 μl of water to the entire reaction, vortex and centrifuge for 10 min.

2. Remove and discard the aqueous phase.

3. Repeat the washing step in step 1 above two more times.

B. Use of the cDNA in a PCR reaction

1. Remove all the fluid from the tube and overlay the solid phase with 50 μl of PCR mixture.

2. Overlay the aqueous phase with mineral oil, centrifuge the tubes briefly, and perform the PCR reaction.

3. After the PCR reaction, centrifuge for 10 min.

4. Remove the oil. Recover the aqueous phase and analyze the PCR products in the aqueous phase as usual. Do not disturb the pellets; leave about 5 μl fluid in the tubes.

C. Reamplification of the cDNA

1. After the PCR reaction and the removal of the product, add 100 μl of water to the tube, vortex and centrifuge for 10 min. to wash the solid phase.

2. Repeat the washing step two more times.

3. Go back to step B, using a new primer combination.

[a] The exact primer and $MgCl_2$ concentration have to be optimized: the concentrations given here are only a starting point.

The fact that the same beads can be reused several times shows that

Insert Figure 4 and Table 1 here

(i) the cDNA is covalently linked to the beads;

(ii) the cDNA is chemically stable enough to be intact after being exposed to heat and alkaline conditions in numerous PCR cycles.

5. Isolation and reverse transcription of RNA using solid phase coupled primers

Oligonucleotides coupled to a solid phase can be used for direct affinity isolation of RNA [2]. Using the solid phase coupled primer, this RNA can be directly reverse transcribed and amplified. Since any oligonucleotide can be coupled to a solid phase using the aminolink, special RNA sequences can be enriched by this isolation procedure. By coupling specific oligonucleotides to a solid phase, one is no longer limited to the use of commercially available oligo(dT) containing matrices. A common modification is, for example, the introduction of adapter sequences [20] next to the oligo(dT) that can be used in the PCR amplification. The use of adaptor primers during the amplification step eliminates the need to employ oligo(dT) primer which may be a source of mispriming (Figure 1 F). Adaptor primers are furthermore useful in introducing restriction sites for cloning purposes.

A typical application of this direct isolation and amplification method is shown in Figure 5.

Protocol 8: One-step procedure to isolate RNA and reverse transcribe it on a solid phase

Equipment and reagents

- PBS (0.14 M NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.0)
- 18 cm Cell scraper (Falcon #3085)
- Lysis buffer (10 mM Tris-HCl, 0.14 M NaCl,5 mM KCl and 1% NP40 [Nonidet P-40, Sigma #N0896])
- 2x Binding buffer (20 mM Tris-HCl, pH 8.0, 1 M LiCl, 2 mM EDTA, 1% SDS, 10 mM DTT)
- Oligonucleotide coupled to a solid phase
- Washing buffer (10 mM Tris-HCl, pH 8.0, 0.15 M LiCl, 1 mM EDTA, 0.3 % SDS)

Method

1. Wash the cells twice with ice-cold PBS [a].

2. Harvest the cells by scraping them off the plate using a disposable 18 cm cell scraper (Falcon #3085).

3. Pellet the cells in a PCR microtube for 1 min. and resuspend the pellet in 50 μl lysis buffer. Keep on ice for 1 min. .

4. Add 50 μl 2x binding buffer, 10 μl solid phase coupled oligonucleotide and allow to bind for 5 min. at room temperature with gentle agitation on a shaking platform.

5. Pellet the solid phase at maximum speed in a microcentrifuge for 5 min. .

6. Add 100 μl ice cold washing buffer, vortex, and spin again for 5 min. .

7. Repeat the washing step (step 6) two more times.

8. Remove all the liquid and reverse transcribe the solid phase bound RNA as in Protocol 4.

9. Perform the PCR reaction as in Protocol 7.

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[a] This step is optional for cells in small culture volumes or serum free grown cells

6. Inclusion of an oligonucleotide with an aminolink does not interfere with the PCR reaction

Various primer combinations with an aminolink at the 5' end of an oligonuclotide used for PCR were found not to interfere with the amplification efficiency or the electrophoretic properties of the product. The DNA generated using PCR with a primer containing an aminolink can be coupled in the same manner as an oligonucleotide to a solid phase. Oligonucleotides can be chemically synthesized only to a limited size of about 100 bases. In contrast, generating a PCR product in which one primer contains an aminolink has virtually no size limitations. This allows, for example, the synthesis of defined DNA pieces that can be coupled to solid phases to generate affinity purification matrices [21].

Insert Table 2 here

7. Precautions and troubleshooting

As with other PCR applications, contamination is the most serious problem in working with immobilized cDNAs. We therefore routinely use aerosol filter tips, aliquot all our solutions (including small aliquots of water used for washing) and use, whenever possible, different locations for RNA isolation and cDNA synthesis. Possible problems and their solutions are listed in Table 2.

8. Discussion

Insert Figure 5 here

The major advantage of solid phase PCR is that, by binding any kind of DNA to a solid phase, the DNA is concentrated in a small volume and can be easily manipulated in different buffer systems. Furthermore, this DNA can be reused in a whole series of PCR experiments. A

portion of cDNA attached to a solid phase has the same information as a cDNA library.

By using different solid phase coupled oligonucleotides first in an affinity step during RNA isolation, and subsequently as primers for cDNA synthesis, specific solid phase 'libraries' can be constructed. The reuse of these solid phase 'libraries', in combination with the option to isolate RNA from small amounts of tissue or cells, makes solid phase PCR a useful tool for identifying new genes and analyzing gene expression.

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Figures and Tables

Figure 1. The principle of solid phase PCR. The solid phase matrix is represented as a checkered sphere on which the oligonucleotide is attached. In this example oligo(dT) is attached to an adapter primer shown as a hatched rectangle (A). Reverse transcription results in cDNA that is covalently linked to the solid phase (B). This cDNA can be tailed (C) and amplified using the adapter primers without (D) or in combination with internal primers (E,F) or nested internal primers (G).

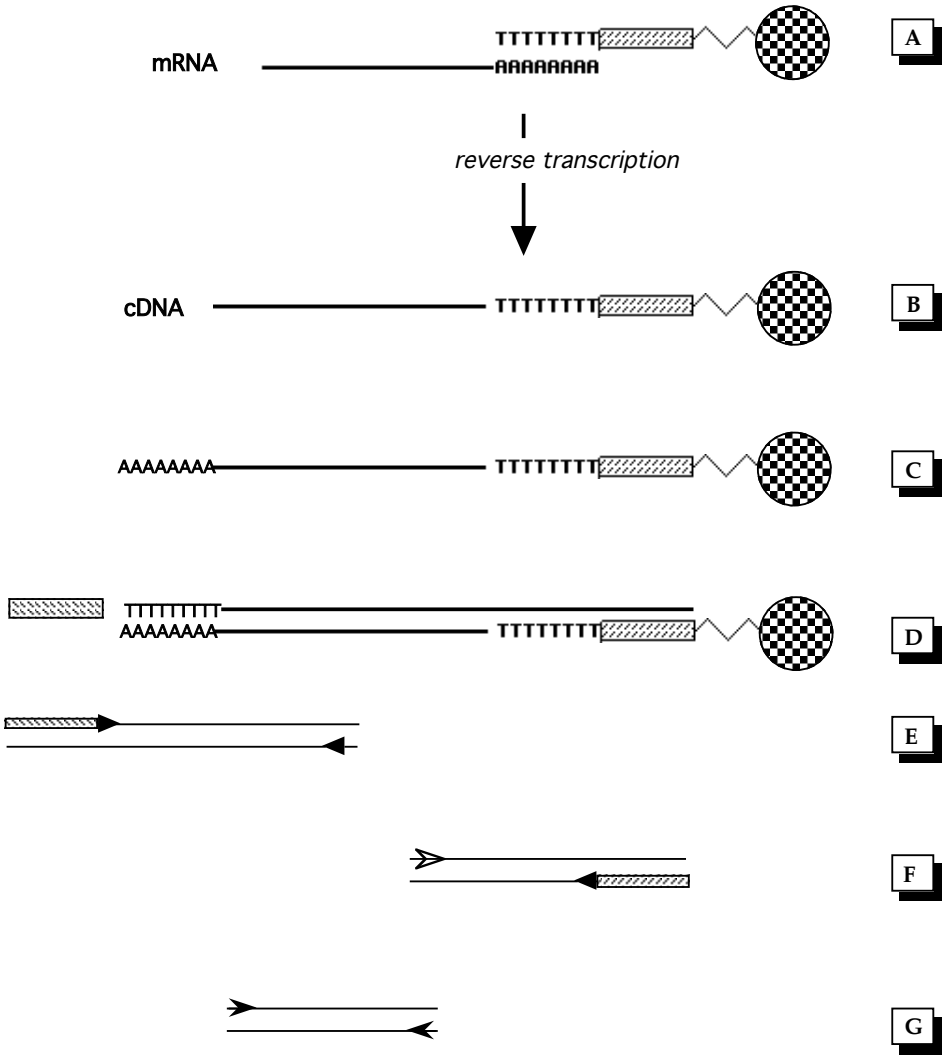
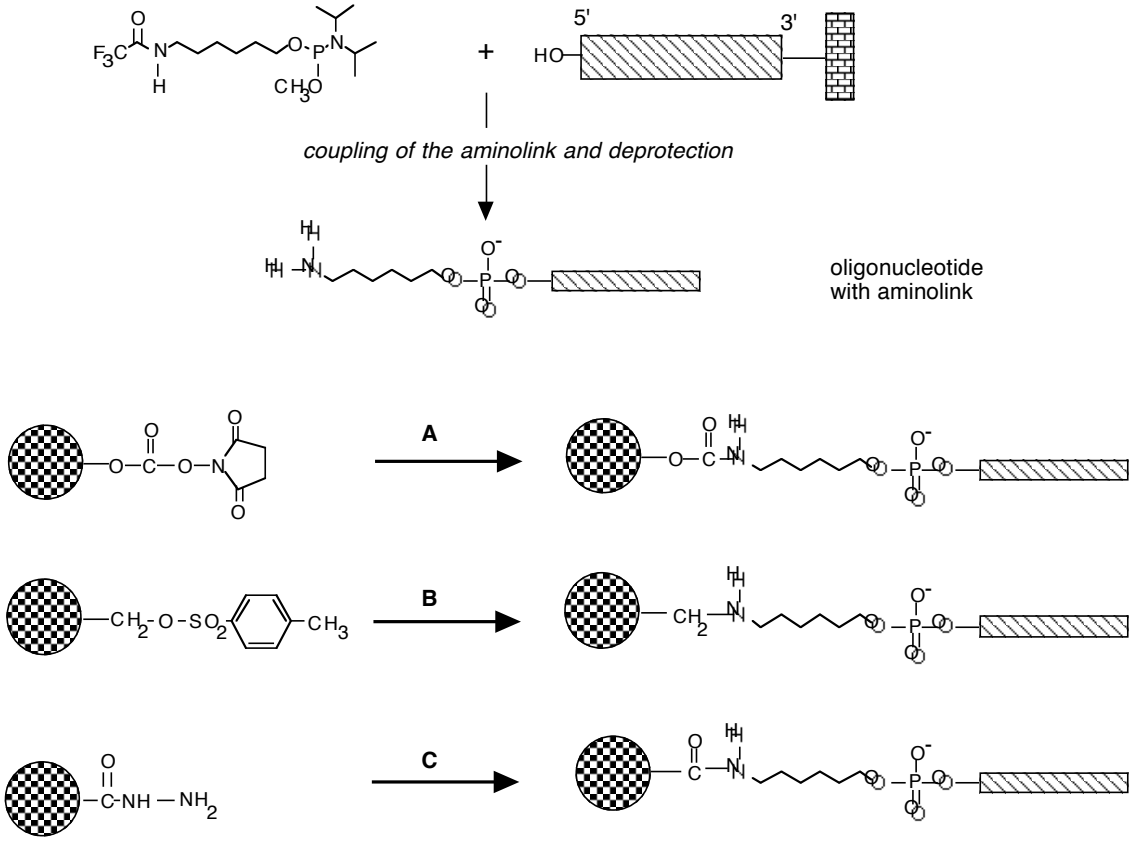
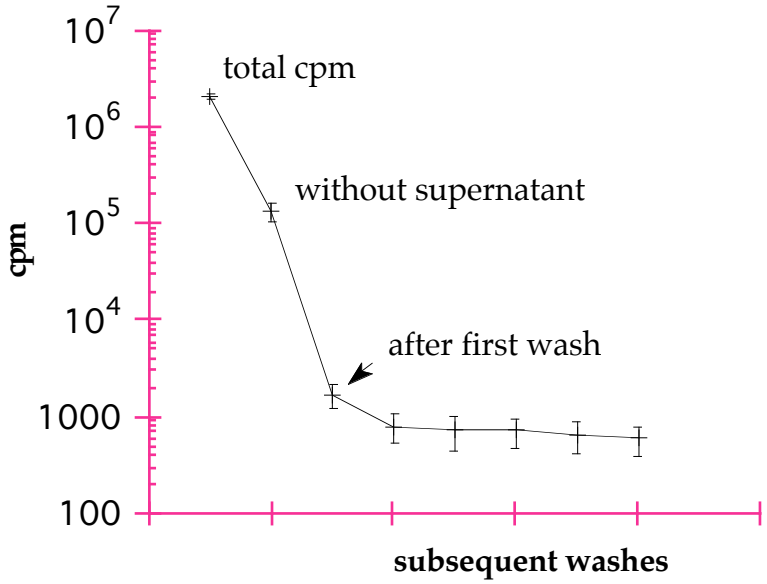


Figure 2. Coupling of an oligonucleotide using an aminolink. The aminolink is introduced to the 5' end of the oligonucleotide prior to cleavage of the oligonucleotide from the synthesizer support. The synthesizer support is indicated as a box with bricks. The NH_2 group of the aminolink can be coupled to N-Hydroxysuccinimid (A), tosyl (B) and hydrazine groups (C)



C

Figure 3. Washing behaviour of cDNA coupled to agarose beads. 1 μg of total rat brain RNA was reverse transcribed in the presence of [α-^{32}P] dCTP with primer SS020 (see Table 1, footnote [a]) attached to agarose. The beads were spun down and counted after removal of the supernatant ('without supernatant' in the figure). The beads were subsequently resuspended in 500 μl water and repelleted ('after first wash' in the figure). After one wash, virtually all of the unincorporated label was removed. Standard errors from five experiments are indicated. Note that the scale is logarithmic.



D

Figure 4. Re-use of cDNA attached to agarose beads in PCR experiments. 1 μg total liver RNA was reverse transcribed with primer SS020 (see Table 1, footnote [a]) attached to agarose beads and amplified with the PCR mixes and reaction conditions specified in Table 1. Denaturation was for 30 min. at 94°C and 30 cycles were used. The buffer contained 16.6 mM $(NH_4)_2SO_4$, 67 mM Tris-HCl, pH 8.8 and the $MgCl_2$ concentration indicated. The accuracy of the thermocycler was controlled with an external thermocouple

. For analysis, 1/5 of each reaction was loaded an a 2% TBE agarose gel. It is

interesting, that the 700 bp artifact band showing up in Lane A disappears in lane C due to different PCR conditions. This further indicates that the washing effectively removes products of prior reactions. The formation of a product using the amplification primer SS015 in lane D and F indicates that the amplification primer has access to the anchor primer close to agarose bead structures. Lanes indicated '+' represent complete reactions, lanes indicated '-' indicate control reactions performed using beads and RNA but without the reverse transcription step. Size markers were electrophoresed in the lane on the right hand side of lane F; the corresponding sizes are indicated alongside this lane. E

F

Figure 5. Direct RNA isolation using solid phase coupled oligonucleotides. RNA from 10^5 α T3-1 cells was isolated and reverse transcribed using SS020. The cDNA was then used in a PCR reaction containing primers SS003/SS004 (A) and subsequently reused with primers SSO15/SS029 (B), under the conditions given in Figure 4. Details of the primers are given in Table 1, footnote [a]. Lanes indicated '-' represents negative control reactions using SS020 containing solid phase without RNA in the PCR reaction. Lane M contained size markers.

Table 1. Reaction conditions used in Figure 4.

lane	oligo.[a]	T[b] (°C)	t_1[c] (min)	t_2[d] (min)	mM $MgCl_2$	Gene/target sequence	Ref.	Size (bp)
A	SRS015 SRS016	60	1	2	1.50	NILE		238
B	SS003 SS004	55	1	2	2.00	Clathrin light chain B		152

C	SRS015 SRS016	60	10	2	2.00	NILE		238 H
D	SS015 SS029	55	1	2	1.50	anchor primer ID elements		various

E	SS032 SS033	55	1	2	1.50	Clathrin light chain A		256 I
F	SS003 SS015	55	1	3	1.75	Clathrin light chain B anchor primer		> 500

[a] Oligonucleotides (5' ->3')
SRS015: GCATCCGAATTCGAGGACACTGAGGTAGATTCCGAGGCCCGG
SRS016: CTCGAGAAGCTTGCCGATGAAAGAGCCATCCTCATTGAACTG
SS003: TGCCTCGAAGGTGAACCGAAC
SS004: GGTCTCCTCCTTGGATTCTTTC
SS015: GCCTTCGAATTCAGCACC
SS021: GGGGTTGGGGATTTAGC
SS020: AL-GCCTTCGAATTCAGCACCTTTTTTTTTTTT (where AL=Aminolink)
SS029: AL-GGGGTTGGGGATTTAGC (where AL=Aminolink)
SS032: TGGTACGCAAGGCAGGATGAGC
SS033: AGATCGGAGACGTAGTGTTTCCA
[b] annealing temperature in °C

[c] annealing time in min.

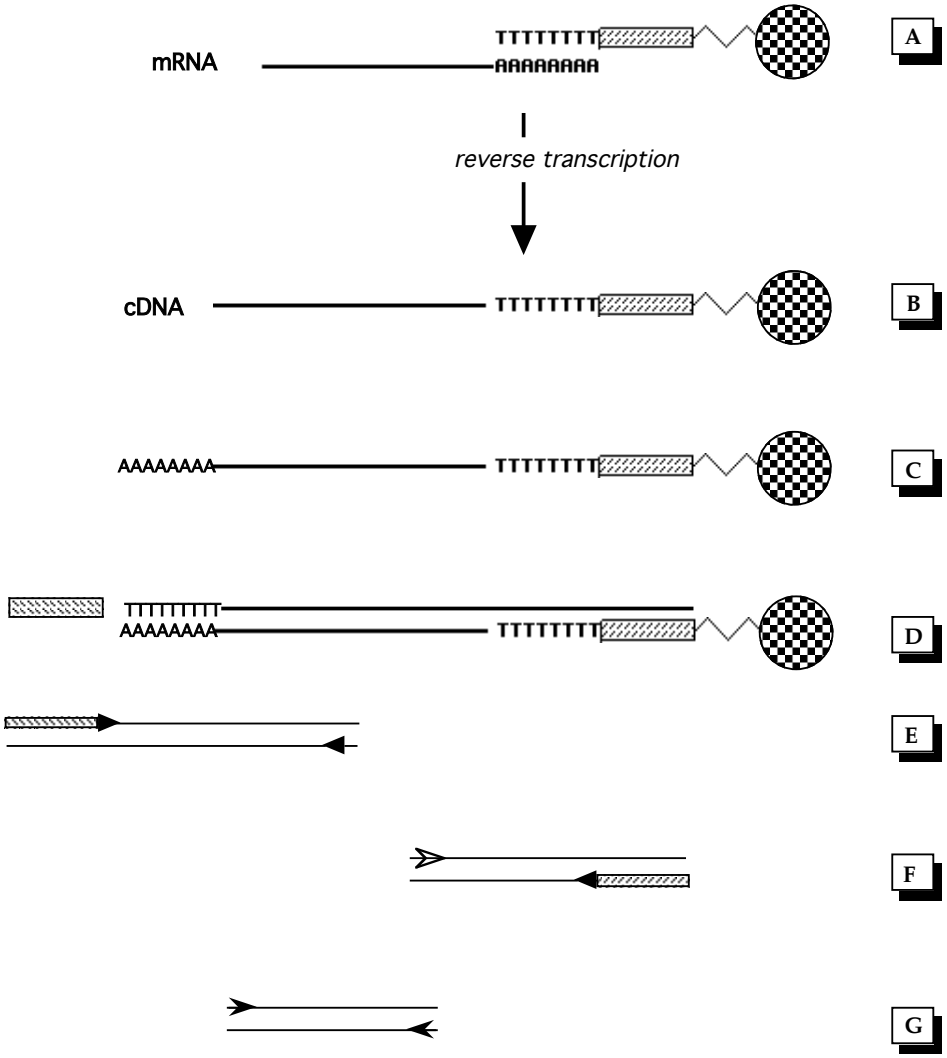
[d] extension time in min., the extension temperature was 72°C

[e] NILE: nerve growth factor-inducible large external glycoprotein

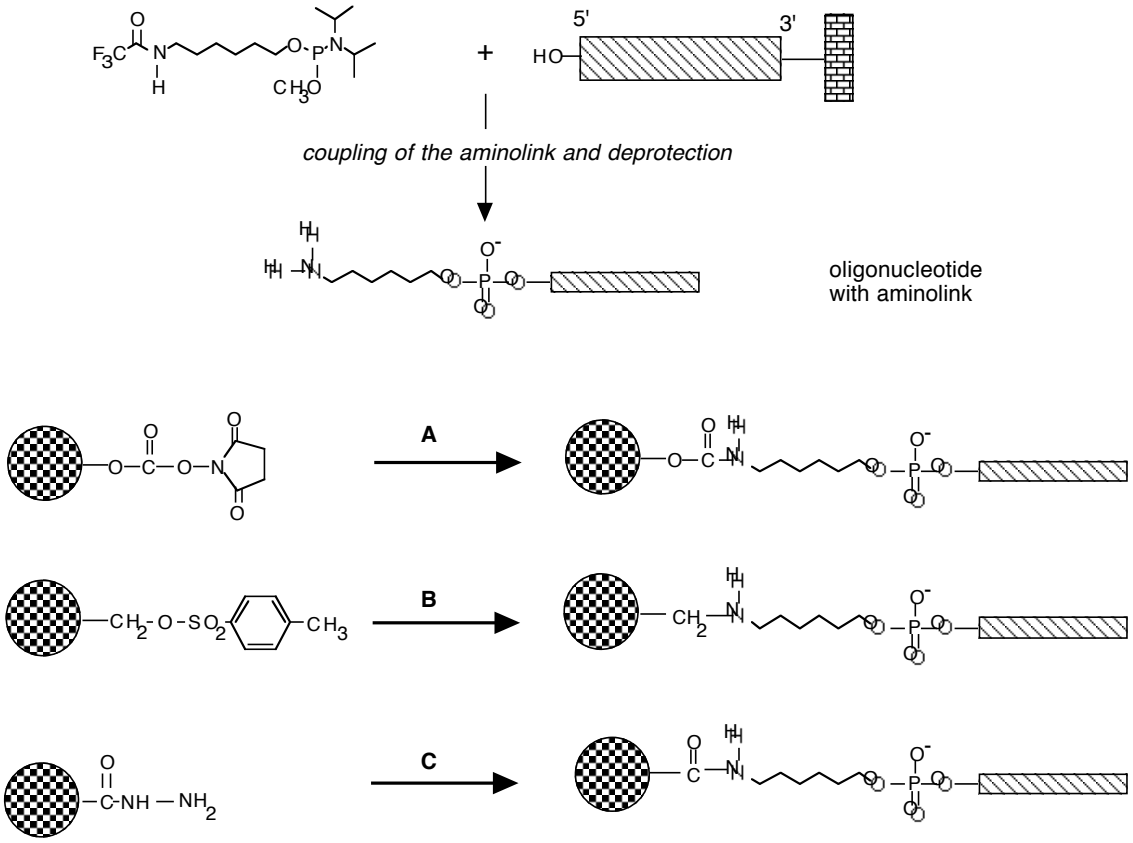
Table 2. Troubleshooting in solid phase PCR experiments

Problem	possible reason	solution
no coupling of oligonucleotide	activated matrix might be too old	check reference date on matrix, retry with new matrix;
	wrong pH of coupling buffer	use only freshly made buffers for coupling; try different activated groups;
no cDNA synthesis	oligonucleotide might not have been coupled	check oligonucleotide coupling with terminal transferase reaction (Protocol 6);
	RNA degraded or absent	When using one step RNA isolation procedures, use conventionally made RNA as control;
no PCR product	PCR reaction conditions suboptimal	titrate Mg^{++} concentration in 0.25 mM increments from 1.5 mM to 5 mM in either subsequent reamplification or parallel experiments;
	loss of solid phase	label cDNA (Protocol 4) and monitor PCR tubes for loss; use 10 min. centrifugation steps before changing the PCR products;

Stamm and Brosius/Figure 1 L



Stamm and Brosius/Figure 2



Stamm and Brosius/Figure 3