

# Round A/B/C Random Amplification of DNA Protocol

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**This protocol was adapted from Bohlander et al. *Genomics* 13 (1992).**

The goal of this procedure is to randomly amplify any given sample of DNA which as much representation as possible. It is not a "linear" method, but is useful to compare relative enrichment between two samples. This protocol has been used successfully to amplify genomic representations of less than 1ng of DNA.

The protocol consists of three sets of enzymatic reactions. In Round A, Sequenase is used to extend randomly annealed primers (Primer A) to generate templates for subsequent PCR. During Round B, the specific primer B is used to amplify the templates previously generated. Finally, Round C consists of additional PCR cycles to incorporate either amino allyl dUTP or Cy-dye-coupled nucleotide.

**Caution:** Wear gloves and be careful of contamination as ANY DNA can be amplified by this protocol. Use filter tips!!! Always run a control sample with water only (no DNA template) to make sure the reagents are not contaminated with DNA.

## Materials

### Round A

Sequenase(13 units/ $\mu$ l) US Biochemical cat# 70775  
5X Sequenase Buffer  
Sequenase Dilution Buffer  
3 mM dNTP mix  
500ug/ml BSA  
0.1 M DTT  
40 pmol/ $\mu$ l Primer A: GTT TCC CAG TCA CGA TCN NNN NNN NN

### Round B

10X PCR Buffer (500 mM KCl, 100 mM Tris pH 8.3)  
25 mM MgCl<sub>2</sub>  
100X dNTPs (20 mM each nucleotide)  
5 unit/ $\mu$ l Taq polymerase  
100 pmol/ $\mu$ l Primer B: GTT TCC CAG TCA CGA TC

### Round C

Same as Round B except use modified dNTP mix  
100X modified dNTP mix  
25 mM dATP  
25 mM dCTP  
25 mM dGTP  
10 mM dTTP  
15 mM aminoallyl-dUTP or Cy-dUTP  
(The ratio of aa-dUTP to dTTP can be altered/optimized)

## Protocol

### 1. Round A Reactions

Denature template DNA/primer annealing

As little as 10 nanograms of DNA can be effectively amplified by this protocol.

7  $\mu$ L DNA  
2  $\mu$ L 5X Sequenase Buffer  
1  $\mu$ L Primer A (40 pmol/ $\mu$ l)  
Total Volume = 10  $\mu$ L

Heat 2 min at 94 °C

Rapid cool to 10 °C and hold 5 min at 10 °C

Add Reaction Mixture to sample:

1  $\mu$ L 5X Sequenase Buffer  
1.5  $\mu$ L 3 mM dNTP  
0.75  $\mu$ L 0.1 M DTT  
1.5  $\mu$ L 500 ug/ $\mu$ l BSA  
0.3  $\mu$ L Sequenase (13U/ $\mu$ l)  
Total Volume = 5.05  $\mu$ l

Ramp from 10 °C to 37 °C over 8 min.

Hold at 37 °C for 8 min; rapid ramp to 94 °C and hold for 2 min.

Rapid ramp to 10°C and hold for 5 min at 10 °C while adding 1.2  $\mu$ l of diluted Sequenase (1:4 dilution)

Ramp from 10 °C to 37 °C over 8 min.

Hold at 37°C for 8 min

Dilute samples with water to final Volume = 60  $\mu$ l.

### 2. Round B PCR

Round A Template	15
MgCl <sub>2</sub>	8
10X PCR Buffer	10
100 X dNTP	1
Primer B(100pmol/ $\mu$ l)	1
Taq	1
Water	63

Round B Cycles:

30 sec 94 °C

30 sec 40 °C

30 sec 50 °C

2 min 72 °C

Run 15-35 cycles, depending on the amount of starting material.

Run 5  $\mu$ L on 1% agarose gel. A “smear” of DNA should be present between 500bp –1kb.

It may be necessary to remove aliquots every 2 cycles to check the amplification in order to optimize the number of cycles. It is best to use the minimal number of cycles that generates a visible smear. Make sure there is no DNA in the negative control lane!

### 3. Round C

Use 10-15  $\mu$ L of Round B to seed the Round C reaction:

Round B Template	10-15
MgCl <sub>2</sub>	8
10X PCR Buffer	10
100X aa-dNTP/cy-dNTP	1
Primer B(100pmol/ $\mu$ l)	1
Taq	1
Water	63-68

30 sec 94 °C

30 sec 40 °C

30 sec 50°C

2 min 72 °C (even longer extension times may improve yield if directly coupling Cy dyes)

10-25 cycles can be run

If aa-dNTPs were used in Round C, the sample must be desalted (to remove Tris buffer which interferes with the coupling) prior to dye coupling. Add 400  $\mu$ L water to the sample in a Microcon 30, and spin (about 8 min, 12K). Repeat 1X with 500  $\mu$ l water.

Proceed to Cy-dye coupling as described in the Reverse Transcription protocol.