



# **Protocols for CodeLink™ Control RNA Spikes, Hybridization & Fragmentation Buffers**

*Including tips for assessing RNA  
concentration, purity & quality*

**Product booklet**

**Code: 67601000**

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## 1. Legal

### Product Use Restriction

The **CodeLink** RNA spikes, hybridization buffer and fragmentation buffer have been designed, developed, and sold for research purposes only. They are suitable for *in vitro* uses only. No claim or representation is intended for their use as a diagnostic tool.

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Applied Microarrays, Inc.  
7700 S. River Parkway  
Tempe, AZ 85284 USA  
Email: [sales@appliedmicroarrays.com](mailto:sales@appliedmicroarrays.com)  
Phone: (480) 775 6320 FAX: (800) 927 9315  
[www.appliedmicroarrays.com](http://www.appliedmicroarrays.com)

## 2. Handling

### 2.1. Safety warnings and precautions

**Warning: For research use only.**

Not recommended or intended for the diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

These procedures involve working with RNA; therefore, exercise great care to avoid RNase contamination. All solutions must be RNase-free; and pipette tips must be aerosol-resistant and changed before each step.

Use commercially prepared Nuclease-free Water (rather than water treated with diethylpyrocarbonate (DEPC)) for all nucleic acid steps.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes, wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice. For each reagent required but not provided, follow the manufacturer's safety requirements.

### 2.2. Expiry

For expiry date please refer to the product outer packaging label.

### 3. Components

#### 3.1. Product contents and storage conditions

##### Bacterial mRNA, Hybridization Reagents, and Fragmentation buffer

Component	Content	Storage Conditions
5× Fragmentation Buffer	580 µl	4°C
Hyb Buffer Component A	6.0 ml	Room temperature
Hyb Buffer Component B	11.2 ml	4°C
<b>Bacterial mRNA Control Set</b>		
<i>araB</i> RNA Control (0.1 µg/µl)	10 µl	-80°C
<i>entF</i> RNA Control (0.1 µg/µl)	10 µl	
<i>gnd</i> RNA Control (0.1 µg/µl)	10 µl	
<i>fixB</i> RNA Control (0.1 µg/µl)	10 µl	
<i>hisB</i> RNA Control (0.1 µg/µl)	10 µl	
<i>leuB</i> RNA Control (0.1 µg/µl)	10 µl	

#### 3.2. Materials to be supplied by user

##### Reagents

A list of reagents required for gel analysis of total RNA and cRNA can be found in Appendix 9.

##### Equipment and consumables

UV Spectrophotometer  
Pipettors (10-, 20-, 200-, and 1000-µl)  
Pipette tips: sterile, RNase-free, and aerosol resistant  
Vortex mixer  
Microcentrifuge  
Heat block or water bath (50 - 55°C, 70°C)  
Air incubator (42°C, 37°C)  
Refrigerated incubator (16°C)  
Agilent™ bioanalyzer  
SpeedVac™

## 4. Description

### 4.1. CodeLink system summary

CodeLink™ Expression Bioarray System comprises a set of bioarray products and tools for gene expression profiling experiments that allows monitoring of the mRNA levels of multiple genes simultaneously. The system includes:

- sets of carefully designed and validated bioarrays with integrated hybridization chambers that cover a wide range of discovery genes for several organisms
- cRNA target hybridization reagents
- optimized protocols for bioarray processing
- hybridization and post-hybridization parallel processing tools and fixtures
- bioarray quantitation analysis software

### 4.2. Protocol overview

#### 4.2.1. cRNA target preparation

The biotin-labelled cRNA target is typically prepared by a linear amplification method using Ambion's MessageAmp™ II-Biotin Enhanced Single Round aRNA Amplification Kit (20 reactions), Catalog # AM1791 or a similar commercially available kit. The poly(A)<sup>+</sup> RNA (mRNA) subpopulation within the total RNA population is primed for reverse transcription by a DNA oligonucleotide containing the T7 RNA polymerase promoter 5' to a d(T) sequence (Fig 4.1). After second-strand cDNA synthesis, the cDNA serves as the template for an *in vitro* transcription (IVT) reaction to produce the target cRNA. The IVT is performed in the presence of biotinylated nucleotides to label the target cRNA. This method typically produces approximately 1,000- to 5,000-fold linear amplification of the input mRNA. For some tissue sources, up to 10,000-fold amplification can be achieved.

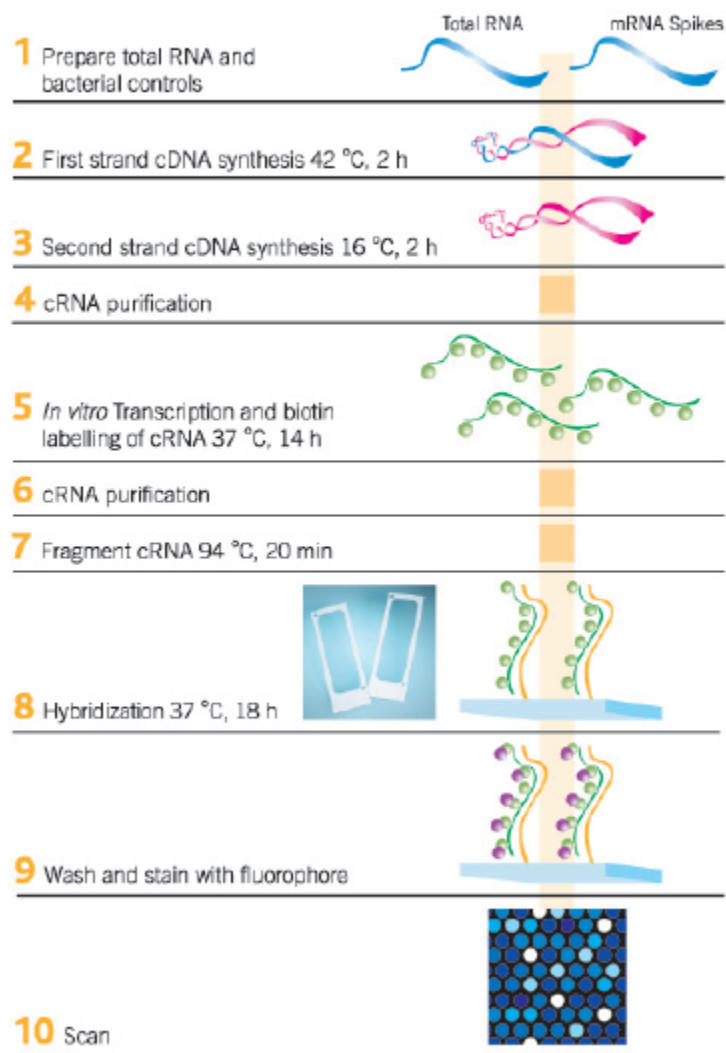
A set of bacterial mRNA controls is available through Applied Microarrays to serve as an overall platform performance control group. This set can also be used to estimate the sensitivity of RNA detection. For ordering information see Appendix 8.6 (page 21).

#### 4.2.2. Target hybridization and bioarray processing

Hybridization is performed overnight in a temperature-controlled shaking incubator using optimized hybridization buffer components. Post-hybridization processing includes a stringent wash to remove unbound and non-specifically hybridized target molecules, a secondary-labeling step using a Cy™5-Streptavidin conjugate, and several washing steps to remove unbound conjugate. Following a final rinse, the bioarrays are dried by centrifugation and scanned.

Analysis of the bioarrays with the CodeLink™ Expression Analysis software is described in the **Help** menu option that is available in the software.

## Overview of the CodeLink™ Expression Bioarray System:



**Figure 4.1.** CodeLink™ Expression Bioarray signals are produced by the hybridization of biotin-labelled complementary RNA (cRNA) target(s) to DNA oligonucleotide probes immobilized on a 3-D matrix followed by secondary labeling and signal detection.

## 5. Preparation of working solutions

### 5.1. Bacterial controls (dynamic range method)



This procedure describes a method for preparing a mixture of the supplied bacterial mRNA controls (exogenous spike-ins) that will enable the user to thoroughly evaluate the experimental performance of target preparation and/or hybridization assay on a continual basis when using the CodeLink™ Expression Bioarray platform. When this dynamic range method is employed, the bacterial controls can be used to estimate the sensitivity of RNA detection across a range of signal intensity, rather than at one set intensity based on single concentration spiking of all controls. If you are interested in exploring this method further, please refer to the Application Note entitled “External RNA controls for monitoring performance of CodeLink™ Whole Genome Bioarrays”, accessible through the website URL listed in Appendix 9.



These mRNA controls contain a poly(A)<sup>+</sup> sequence at the 3' end for priming by the T7 oligo(dT) primer. The remaining portions of the bacterial mRNA controls are amplified to produce sequences complementary to DNA probes spotted on the bioarray. The bacterial sequences have been tested across multiple organisms and tissues to ensure minimal cross-reactivity.

For a typical mammalian cell, mRNA comprises 1–5% of the total RNA content; however, this value varies depending on cell type. An estimate of the mass ratios of the bacterial mRNA controls to the target mRNA within the total RNA can be made by assuming that 5% of the total RNA is mRNA (see the Application Note in Appendix 9). In addition, we assume that the average transcript length in the mRNA is 1000 bases. The true average mRNA length can be determined for mRNA samples using an Agilent™ 2100 bioanalyzer with a 1 Kb RNA ladder. This type of length verification cannot be performed on total RNA due to the abundance of ribosomal RNAs. Once you have determined the average mRNA length for a given sample, you can adjust the level of mass ratio spiking (see the Application Note in Appendix 9).

1. Thaw the bacterial mRNA control stocks (all at 0.1 µg/µl) on ice and aliquot the following into six individual tubes, adding nuclease-free water as noted:

Tube 1:	4 µl <i>araB</i>
Tube 2:	4 µl <i>entF</i> + 6 µl H <sub>2</sub> O
Tube 3:	4 µl <i>fixB</i> + 36 µl H <sub>2</sub> O
Tube 4:	4 µl <i>gnd</i> + 156 µl H <sub>2</sub> O
Tube 5:	4 µl <i>hisB</i> + 636 µl H <sub>2</sub> O
Tube 6:	1 µl <i>leuB</i> + 636 µl H <sub>2</sub> O

2. Mix by tapping the sides of the tubes, then centrifuge for 5 seconds at ≥10 000 × g to gather the liquid. Store the tube on ice.

3. Prepare the spike-dilution mix by adding the following to Tube 1:

- 4  $\mu$ l Tube 2 *entF* (25.0 ng/ $\mu$ l)
- 4  $\mu$ l Tube 3 *fixB* (6.25 ng/ $\mu$ l)
- 4  $\mu$ l Tube 4 *gnd* (1.56 ng/ $\mu$ l)
- 4  $\mu$ l Tube 5 *hisB* (390.60 pg/ $\mu$ l)
- 4  $\mu$ l Tube 6 *leuB* (97.70 pg/ $\mu$ l)
- 36  $\mu$ l Nuclease-free water



The final volume in Tube 1 will be 60  $\mu$ l.

4. Mix well by tapping the side of the tube and centrifuge for 5 seconds at  $\geq 10\,000 \times g$  to gather the liquid. Store the tube on ice.



The resulting concentration of this spike-dilution mix will be 10 ng/ $\mu$ l. This RNA solution can be stored at  $-80^{\circ}\text{C}$  for up to one month. We recommend storing this solution in smaller aliquots to eliminate multiple freeze/thaw cycles.

5. The spike-dilution mix can now be added to total RNA samples in a 1:20,000 mass ratio, or to mRNA samples in a 1:1,000 mass ratio. Adding the spike-dilution mix at these recommended mass ratios relative to total and mRNA will yield the following individual mass-ratio levels:

- araB* 1:1,000
- entF* 1:4,000
- fixB* 1:16,000
- gnd* 1:64,000
- hisB* 1:256,000
- leuB* 1:1,024,000

#### Examples:

##### • Total RNA

For 1  $\mu$ g of total RNA, add 50 pg of spike-dilution mix prepared to 10 ng/ $\mu$ l per section 5.1 step 4). This represents a 1:20,000 mass ratio of spike to total RNA sample. To begin:

1. Make a 1:1,000 dilution of the 10 ng/ $\mu$ l spike solution in a separate tube.
2. Add 5  $\mu$ l of this 1:1,000 dilution (10 pg/ $\mu$ l) to 1  $\mu$ g of total RNA Sample.
3. Continue with target preparation using Ambion's MessageAmp™ II-Biotin Enhanced Single Round aRNA Amplification Kit (20 reactions), Catalog # AM1791 or a similar commercially available kit.

- **mRNA**

For 100 ng of mRNA, add 100 pg of spike dilution mix (prepared to 10 ng/μl per section 5.1 step 4). This represents a 1:1,000 mass ratio of spike to mRNA sample.

To begin:

1. Make a 1:100 dilution of the 10 ng/μl spike solution in a separate tube.
2. Add 1 μl of this 1:100 dilution (100 pg/μl) to 100 ng of mRNA Sample.
3. Continue with target preparation using Ambion's MessageAmp™ II-Biotin Enhanced Single Round aRNA Amplification Kit (20 reactions), Catalog # AM1791 or a similar commercially available kit.



- **Labelled-cRNA**

These exogenous controls can also be used to spike at the cRNA level after separate production of the amplified spikes. However, spiking at the cRNA level will not allow monitoring of the target preparation process. To learn more about this method, please refer to the application note entitled, "External RNA controls for monitoring performance of CodeLink™ Whole Genome Bioarrays", available at: [http://www.appliedmicroarrays.com/Reference\\_Materials.html](http://www.appliedmicroarrays.com/Reference_Materials.html)



An additional method for preparing the bacterial mRNAs controls at a single concentration can be found in Appendix 8.2. The single concentration method also enables the user to evaluate experimental performance using the CodeLink™ Expression Bioarray platform, but without dynamic range information.

## 6. Assessing concentration, purity, and quality of input total RNA

RNA samples should be of high purity, i.e., free of contaminating proteins, DNA, and other cellular material as well as phenol, ethanol, and salts associated with RNA isolation procedures. Impurities can lower the efficiency of reverse transcription and subsequently reduce the level of amplification. The size distribution of the total RNA is another important component of RNA quality. Reverse transcription of partially degraded mRNAs will typically generate relatively short cDNAs that potentially lack portions of the coding region.

### 6.1. Concentration: Measuring $A_{260}$

The total RNA input for cDNA synthesis should be quantified by UV spectrophotometry. A method for this quantification is shown below. Alternatively, use of a spectrophotometer such as the NanoDrop® will help minimize the amount of RNA sample necessary for this purpose; follow the manufacturer's instructions for this instrument.

1. Prepare a 1:50 dilution for each sample of total RNA in nuclease-free water:

2 $\mu$ l	total RNA
98 $\mu$ l	nuclease-free water
<hr/>	
100 $\mu$ l	total volume

2. Vortex the mixture and centrifuge for 5 seconds at  $\approx 10,000 \times g$  to collect all fluid at the bottom of the tube.

3. Transfer the total RNA dilution to a 100- $\mu$ l quartz cuvette (1-cm pathlength) and measure UV absorbance at 260 nm. If the  $A_{260}$  values are less than 0.15, prepare a 1:20 dilution of the total RNA and measure its UV absorbance at 260 nm.



For accurate concentration determination, the dilution must yield an  $A_{260}$  in the linear range of approximately 0.15–0.95. If the  $A_{260}$  value is less than 0.15, prepare a lower dilution of the RNA stock for more accurate UV spectrophotometric readings. If the  $A_{260}$  value is too high, prepare a higher dilution and re-measure.

4. Calculate the total RNA concentration: 1  $A_{260}$  unit = 40  $\mu$ g/ml (1-cm pathlength cuvette): concentration in  $\mu$ g/ $\mu$ l =  $A_{260} \times$  dilution factor  $\times 40 \mu$ g/ml  $\times 0.001$  ml/ $\mu$ l. For example, if the  $A_{260}$  = 0.300 for a 1:50 dilution:

$$\text{Concentration} = 0.300 \times 50 \times 40 \mu\text{g/ml} \times 0.001 \text{ ml}/\mu\text{l} = 0.6 \mu\text{g}/\mu\text{l}$$

## 6.2. Purity: Measuring $A_{260}$ : $A_{280}$ ratio

RNA purity is measured by the ratio of  $A_{260}$ : $A_{280}$ , and since this ratio is sensitive to pH, the RNA sample must be buffered to properly measure this parameter. Instructions are provided below to most accurately assess sample purity.

1. Transfer the diluted total RNA from section 6.1.3. from the cuvette to a new microcentrifuge tube and add 11.1  $\mu$ l (1/9 volume) of 0.1 M Tris-HCl, pH 7.6. Vortex the mixture.

2. Transfer the Tris-buffered total RNA ( $\sim 100 \mu$ l) to a quartz cuvette and measure UV absorbance at 260 nm and 280 nm.



**The  $A_{260}$ : $A_{280}$  ratio is a measure of sample purity and should be between 1.8 and 2.1. If the ratio is less than 1.7, do not proceed with this protocol. Instead, attempt to remove potential contaminants from the total RNA and retest the  $A_{260}$ : $A_{280}$  ratio.**

## 6.3. Quality: Measuring size distribution

To assess the integrity of the total RNA starting material prior to use in first-strand cDNA synthesis, a denaturing gel can be run (Appendix 8.1). As an alternative to a denaturing gel,

another analytical method such as the Agilent 2100 bioanalyzer can also be used. Using either method, good quality total RNA should exhibit well-defined large and small ribosomal RNA (rRNA) bands, and the ratio of the band density of 28S:18S for mammalian total RNA should approach 2. Additional bands or a smear between the two rRNA bands, or below the small rRNA band, are indicative of degradation in the total RNA sample. Degraded or partially degraded total RNA will adversely affect expression microarray results. Though a denaturing agarose gel can be used for this quality assessment, it requires the use of a large amount of sample (~1ug). Less total RNA is required to do a quality check using the Agilent 2100 bioanalyzer. With this instrument a RIN (RNA Integrity Number) can be calculated to further evaluate RNA integrity. A comprehensive metric developed by Agilent, the RIN integrates information from both the rRNA bands, as well as information contained outside the rRNA peaks (potential degradation products) to provide a fuller picture of the RNA integrity. A RIN value greater than ~7 is indicative of good quality RNA.



Search for “RIN” at the following web address for more information:  
<http://www.chem.agilent.com>



If the total RNA is not of good quality, do not proceed.

## 7. Assessment of cRNA concentration, purity, and quality

High quality cRNA is critical to an accurate and reproducible microarray experiment. The cRNA concentration, purity and quality can be assessed by several methods. Here we describe the use of UV spectrophotometry for assessment of quantity and purity. A cRNA integrity assessment, i.e., size distribution, can be made by denaturing-gel electrophoresis (Appendix 8.1.) or by an analytical method such as the Agilent 2100 bioanalyzer.



**Quality assessment of the cRNA before fragmentation and hybridization is highly recommended.**

### 7. 1. Concentration: Measuring $A_{260}$

Prepare an aliquot of the cRNA diluted in Nuclease-free Water and measure the UV absorption at 260 nm as described in section 6.1 for total RNA. If the initial reading with 1:50 dilution is not in the linear range, prepare another sample with the appropriate dilution change. Concentrate the cRNA in a SpeedVac concentrator before fragmentation if the cRNA concentration <0.5  $\mu\text{g}/\mu\text{l}$ .

### 7. 2. Purity: Measuring the $A_{260}:A_{280}$ ratio

The  $A_{260}:A_{280}$  ratio is a measure of sample purity, and can be obtained by following the instructions as for total RNA described in section 6.2. If the cRNA has an  $A_{260}:A_{280}$  ratio of 1.8–2.1 in Tris-HCl (pH 7.6), then proceed to fragmentation and hybridization; if not, do not proceed. Instead, remove potential contaminants from the cRNA by ethanol precipitation and retest the  $A_{260}:A_{280}$  ratio; if the problem persists, contact Applied Microarrays, Inc. technical support.

### 7. 3. Quality: Measuring size distribution

To assess the integrity of the cRNA prior to use in hybridization, a denaturing gel can be run (Appendix 8.1.). As an alternative to a denaturing gel, another analytical method such as the Agilent 2100 bioanalyzer can also be used. Denaturing agarose gel analysis - Amplified cRNA should appear as a smear from 250 to 5,000 nt. The average size of biotin-labelled cRNA should be approximately 1,400 nt. Agilent bioanalyzer analysis – Follow the manufacturer’s protocol for bioanalyzer analysis. The expected cRNA profile is a distribution of sizes from 250–5,500 nt, with most of the cRNA between 1,000-1,500 nt.



To compare bioanalyzer profiles of different cRNA samples, be sure to load equal mass amounts to get an accurate comparison.



**The cRNA should appear on the gel/profile as a smear centered around 1,200 – 1,400 nt. If there is no smear, or if the smear is predominantly below 500 nt, do not continue with the sample.**

## 8. Appendices

### 8.1. Assessing total RNA or cRNA quality by denaturing gel electrophoresis

To assess RNA quality, analyze 5–10 µg of the total RNA or 2 µg of cRNA by denaturing gel electrophoresis (described below).

#### Reagents to be supplied by the user:

- nuclease-free water
- ethidium bromide, 10 µg/µl
- 1 kb DNA ladder
- 1× TAE running buffer
- agarose
- 14.3 M β-mercaptoethanol (β-ME)
- 37% formaldehyde
- 3-[N-Morpholino] propanesulfonic acid (MOPS) free acid
- bromophenol blue
- formamide
- RNA 6000 molecular weight ladder (Ambion, Cat no. 7152)
- 5 M ammonium acetate

#### Materials to be supplied by the user:

- 1.7-ml microcentrifuge tubes: sterile, RNase-free
- 15- and 50-ml conical tubes: sterile, nuclease-free
- automatic serological pipettor and disposable serological pipettes

- horizontal minigel electrophoresis apparatus
- pre-cast TAE agarose gels
- power supply for electrophoresis
- gel imaging equipment

### Preparation of 10× MOPS solution

1. Dissolve the following in 470 ml of nuclease-free water:

21 g MOPS  
2.05 g sodium acetate  
10 ml 0.5 M EDTA

2. Adjust pH to 7.0 with 1 N NaOH.

3. Adjust the volume to 500 ml with nuclease-free water. Wrap the MOPS bottle in aluminum foil (MOPS is light-sensitive) and store at room temperature. The solution is stable for up to one month.

### Preparation of Denaturing Agarose Gel

1. Boil 1.0 g agarose in 72 ml of nuclease-free water.

2. Let the solution cool to approximately 60°C then add 10 ml of 10× MOPS solution (Section 8.1.) and 18 ml of 37% formaldehyde.

3. Insert the comb into the gel tray and pour the molten agarose into the gel tray. Remove any bubbles that may have formed in the gel with a pipette tip. Allow the gel to solidify and cool to room temperature.

### Preparation of samples and RNA ladder



**Due to its toxicity, all work involving formaldehyde should be performed in a chemical fume hood. Treat formaldehyde-containing gels and buffers as toxic waste.**



**Ethidium bromide is a powerful mutagen and possible carcinogen. Use proper laboratory safety procedures when working with ethidium bromide; decontaminate solutions containing ethidium bromide with commercially available kits.**

1. Mix the following reagents in a tube:
  - 20  $\mu$ l formamide (deionized)
  - 7  $\mu$ l formaldehyde
  - 4  $\mu$ l 10 $\times$  MOPS buffer
  - 2  $\mu$ l 1% bromophenol blue solution
  - 1  $\mu$ l ethidium bromide (5  $\mu$ g/ $\mu$ l)
  - 5  $\mu$ g total RNA or 2  $\mu$ g of cRNA
  - X  $\mu$ l nuclease-free water

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40  $\mu$ l Final volume

2. To prepare an RNA ladder, make a mixture of the above reagents, but replace the cRNA with 1  $\mu$ l of RNA ladder solution and 5  $\mu$ l of nuclease-free water.

### Electrophoresis

1. Fill the electrophoresis chamber with 1 $\times$  MOPS solution to submerge the gel.
2. Heat the RNA samples at 65°C for 5 minutes; then chill on ice for 5 minutes. Load the samples into the wells of the gel.
3. Electrophorese at 5 V/cm under constant voltage until the dye front is 1/3 from the bottom of the gel. Remove the gel from the electrophoresis apparatus and visualize the nucleic acids on standard gel imaging equipment.

## 8.2. Alternative bacterial mRNA controls dilution method for use in target synthesis: single concentration

Addition of control mRNAs at a single concentration at the start of cDNA synthesis allows for an accurate assessment of target preparation efficacy and assay sensitivity.



**Discard the final Working Solution after a single use because highly diluted RNA samples are not very stable, even under appropriate storage conditions.**

## Dilution Methods

1. Thaw the tubes containing the bacterial mRNA controls (all at 0.1 µg/µl) on ice and prepare the 16.7 ng/µl combined spike stock solution as follows:

5 µl	<i>araB</i>
5 µl	<i>entF</i>
5 µl	<i>fixB</i>
5 µl	<i>gnd</i>
5 µl	<i>hisB</i>
5 µl	<i>leuB</i>
<hr/>	
30 µl	Final volume

2. Mix well by tapping the side of the tube, then centrifuge for 5 seconds at  $\geq 10,000 \times g$  to gather the liquid. Store the tube on ice.

3. Aliquot the combined spike stock solution to 10 separate tubes, each containing 3 µl. Store these tubes at -80°C and use within 1 month.

4. Prepare a dilution of the bacterial mRNA control combined spike stock solution for a final concentration of 50.2 pg/µl *araB*, *entF*, *fixB*, *gnd*, *hisB*, and *leuB*:

3 µl	Combined spike stock (each spike at 16.7 ng/µl)
997 µl	Nuclease-free Water
<hr/>	
1,000 µl	Final volume

5. Mix well by tapping the side of the tube, then centrifuge for 5 seconds at  $\geq 10,000 \times g$  to gather the liquid. Store the tube on ice.

6. Users may aliquot this final dilution of the bacterial mRNA controls to separate tubes if desired, and store at -80°C. Aliquots should be used within 1 month.

7. Prepare a Working Solution of bacterial mRNA controls:

2 µl	Final dilution of the bacterial mRNA controls (each spike at 50.2 pg/µl)
998 µl	Nuclease-free Water
<hr/>	
1,000 µl	Final volume

8. Add 1.0 µl of the Working Solution of bacterial mRNA controls (0.1 pg/µl *araB*, *entF*, *fixB*, *gnd*, *hisB*, and *leuB*) for every 1.0 µg of total RNA.



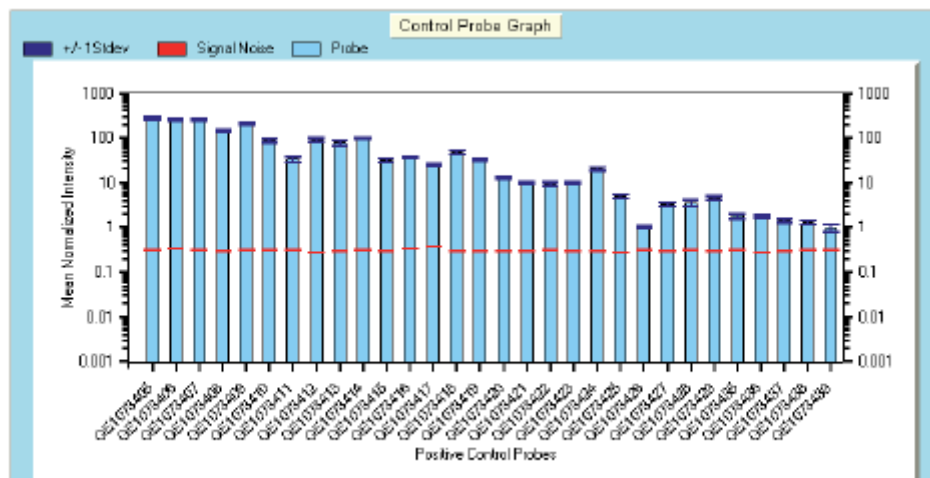
If spiking into pure mRNA, divide your input mRNA amount by 300,000 to obtain X pg. Then sufficiently dilute the 50.2 pg/ $\mu$ l final dilution of the bacterial mRNA controls, to yield X pg in 1–2  $\mu$ l.

### 8.3. Extracting positive control data

#### Expression Analysis Software

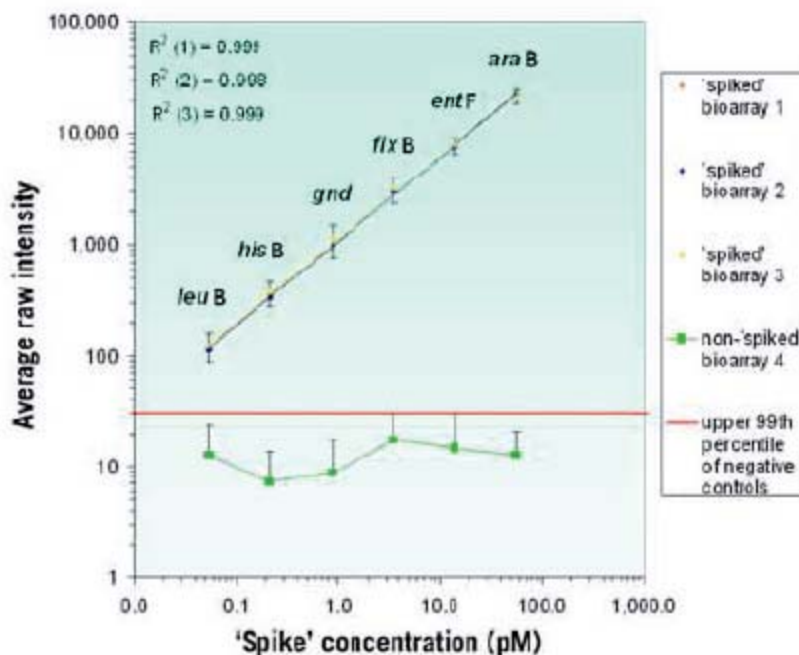
Under the Expression Analysis menu button in the CodeLink™ Expression Analysis software, select the Control Probes Report. Select to use the Positive Controls with spots of G, L, and S quality flags and Update the graph. The graph can be easily copy-pasted elsewhere for record-keeping purposes.

#### Interpretation Dynamic range spike method



**Figure A3.1.** Typical sensitivity plot showing that normalized signal from each bacterial control probe of a dynamic range set is above noise.

In the example experiment, the spikes' concentrations range from an estimated mass ratio of 1:1,000 to 1:1,024,000. On CodeLink™ bioarrays there are 5 unique probes per control gene, and the intensity of each unique probe is shown separately; therefore, each of the six bacterial controls corresponds to 5 consecutive signal intensity bars in the above figure. Note that due to sequence differences, there are some signal differences between probes targeting the same gene.



**Figure A3.2.** Using a software tool such as Microsoft Excel™, the “within-slide dynamic range” can be plotted using the values derived from the positive control report of the CodeLink™ Expression Analysis software. Here, “within-slide dynamic range” is shown for three different bioarray technical replicates; a fourth bioarray was processed without spiking and is illustrated by the horizontal green data points.

Error bars represent one standard deviation of the average raw intensity for each bacterial transcript signal. The horizontal red line represents the upper 99th percentile of the negative controls’ signals. For additional information on this type of data interpretation, please refer to the Application Note entitled “External RNA controls for monitoring performance of CodeLink™ Whole Genome Bioarrays” available at:

[http://www.appliedmicroarrays.com/Reference\\_Materials.html](http://www.appliedmicroarrays.com/Reference_Materials.html)

### Single concentration spike method



**Figure A3.3.** Typical sensitivity plot showing that normalized signal from each bacterial control probe is above noise for the set of control mRNAs spiked at an estimated mass ratio of 1:300,000. The noise level (the average mean background) for each probe is indicated by the red line.

The final mass ratios of the bacterial control mRNAs to the total RNA for the dilutions given in Appendix 8.2. are: 1:10,000,000 for *araB*, *entF*, *fixB*, *gnd*, *hisB* and *leuB*. For a typical mammalian cell, mRNA comprises 1–5% of the total RNA content. If the proportion of mRNA content within total RNA for a particular tissue is unknown, then an estimate of the mass ratios of the bacterial mRNA controls to the target mRNA within the total RNA can be made by assuming a midpoint of this range, or 3% mRNA content.

The above total RNA mass ratio then corresponds to an estimated RNA mass ratio of 1:300,000 for all the bacterial transcripts. The normalized signals from these controls are graphed within the CodeLink™ Expression Analysis software, generating sensitivity plots like that shown in Figure A3.3 above. Since the positive control probe signals are above the noise, the system is shown to be capable of detecting signal above noise at an estimated sensitivity of one copy per cell with use of a 1:300,000 mass ratio spike.

#### 8.4. Use of the total RNA control (HeLa)

Use 1 µl (1 µg) of HeLa Control RNA (1 µg/µl) in an independent reaction through both cDNA and cRNA synthesis. The yield can be assessed as described in section 7, and should be ≥80 µg of cRNA. The average size of the cRNA should be ≥1 kb. Low cRNA yield from the control sample reaction indicates a general problem with the amplification procedure or kit.

#### 8.5. Related products

Description	Product Code
CodeLink™ Human Whole Genome Bioarray	300026-6PK
CodeLink™ Rat Whole Genome Bioarray	300031-6PK
CodeLink™ Mouse Whole Genome Bioarray	300033-6PK
CodeLink™ Human Inflammation v1 16-Assay Bioarray	300076-3PK
CodeLink™ Rat Inflammation v1 16-Assay Bioarray	300077-3PK
CodeLink™ Mouse Inflammation v1 16-Assay Bioarray	300072-3PK
CodeLink™ Human Inflammation v1 16-Assay Bioarray	300076-1PK
CodeLink™ Rat Inflammation v1 16-Assay Bioarray	300077-1PK
CodeLink™ Mouse Inflammation v1 16-Assay Bioarray	300072-1PK
CodeLink™ Human ADME v1 16-Assay Bioarray	300081-3PK
CodeLink™ Human ADME v1 16-Assay Bioarray	300081-1PK
CodeLink™ Universal Shaker Kit, 12 slides	310031
CodeLink™ Expression Parallel Processing Kit, 12 slides	310010
CodeLink™ Expression Analysis v5.0 software	310035
Cy5-Streptavidin for Microarrays	28900224

## 8.6. Ordering information

Description	Product Code
CodeLink Controls and Buffer Kit (one each of products 320002 & 320003)	320001
CodeLink Control RNA Spikes	320002
CodeLink Hybridization and Fragmentation Buffers	320003

## 9. Application Note

External RNA controls for monitoring performance of CodeLink™ Whole Genome bioarrays, Amersham Biosciences, (2004)

Located at: [http://www.appliedmicroarrays.com/application\\_notes/63005456.pdf](http://www.appliedmicroarrays.com/application_notes/63005456.pdf)



7700 S. River Parkway  
Tempe, AZ 85284 USA

Email: [sales@appliedmicroarrays.com](mailto:sales@appliedmicroarrays.com)  
Phone: (480) 775 6320 FAX: (800) 927 9315  
[www.appliedmicroarrays.com](http://www.appliedmicroarrays.com)