

## **AceGene® Protocol** **(aRNA labeling)**

Version 2.0 (2005/05/09)

### **Materials**

- Amino Allyl MessageAmp™ aRNA kit (#1752 : Ambion) OR amino-allyl RNA amplification Kit (Sigma)!
- 3M Sodium acetate (S7899: Sigma-aldrich) or 5M Ammonium acetate (09691 : Sigma-aldrich)
- 100% Ethanol, 70% Ethanol
- 0.2M Sodium Bicarbonate buffer (NaHCO<sub>3</sub>-Na<sub>2</sub>CO<sub>3</sub> (pH9.0)) (AceGene®)
- Dimethylsulfoxide(DMSO) (7898-1 : CLONTECH)  
(or comes with Amino Allyl MessageAmp™ aRNA kit)
- Cy3 Mono-Reactive Dye (PA23001 : Amersham Bioscience)\*1
- Cy5 Mono-Reactive Dye (PA25001 : Amersham Bioscience)\*1
- Micro Bio-Spin Column P30 (732-6223 : BioRad)!
- Microcon YM-30 (42409 : Millipore)!
- Microcon YM-10 (42406 : Millipore)!
- 5 × Fragmentation buffer (AceGene®)
- 20 × SSC
- 10% SDS
- GAP cover glass (CG00044: Matsunami)
- 50 × Denhardt's solution \*2
- Salmon sperm DNA (15632-011: Invitrogen)
- Formamide deionized (SIGMA :F-9037) \*2
- Hybridization Solution (AceGene®)

### **Optional**

- Glycogen (for molecular biology) (901 393 : Roche)
- HAIBURI-KUN® (K-W026-001: Hitachi Software Engineering)
- Human Cot-1 DNA (15279-011 : Invitrogen)  
or 5 × Competitor I (Human) (TX808 : TaKaRa)

\*1 Preparation of CyDye

Dissolve a pack of Cy-Mono-Reactive Dye in 45µl of Dimethylsulfoxide (DMSO), and store at -20 in the shade.

\*2 Store these solutions in aliquots at -20

! For detailed usage of kits, please follow instructions recommended by the manufacturers.

### Coupling and Purification

- Ethanol-precipitate aRNA(5µg/sample) amplified using Amino Allyl MessageAmp™ aRNA kit (Ambion)
- Rinse pellet using 70% ethanol
- Dissolve aRNA sample in 5µl of 0.2M Sodium bicarbonate buffer(pH9.0)
- Add 5µl of CyDye (dissolved in 45µl of DMSO/vial ;Amersham Bioscience ) to sample
- Mix well by vortex
- Incubate for 1 hour at 40 keep in DARK
- Add 40µl DW to sample and remove unincorporated Cy-dyes using Micro Bio-Spin Column P30 (Bio-Rad)
- Concentrate sample using microcon YM-30. (wash twice with 100µl of DW and bring sample volume to 32µl; Cy3- and Cy5-labeled samples can be combined in this step)
- Add 8µl of 5 × fragmentation buffer
- Incubate at 94 for 15 minutes and immediately cool on crushed ice
- Remove ca. 1/20 volume of labeled sample to a new tube and add formamide to a final concentration of 40% for electrophoresis in a 3% agarose gel (See figure below: Cy3-labeled samples before(left lane) and after(right lane) fragmentation)
- Purify the fragmentated sample using microcon YM-10 and bring sample volume to 15.5µl

### Hybridization

Add the following reagents to sample

|                          |        | (final concentration) |
|--------------------------|--------|-----------------------|
| Cy-dye labeled samples   | 15.5µl |                       |
| 20 × SSC                 | 12.5µl | 5 × SSC               |
| 10 % SDS                 | 2.5µl  | 0.5%SDS               |
| 50 × Denhardt's solution | 4µl    | 4 × Denhardt's        |
| hybridization solution   | 10µl   | 20%Hyb.Solution       |
| Total                    | 44.5µL |                       |

- Incubate at 95 for 2minutes, immediately cool with crushed ice.
- Add the following reagents to the above solution.

|                            |        | (final concentration) |
|----------------------------|--------|-----------------------|
| target solution            | 44.5µL |                       |
| salmon sperm DNA (10mg/mL) | 0.5µL  | 100 µ g/ml            |
| formamide                  | 5µL    | !10%                  |
| Total                      | 50µL   |                       |

**! You may need to adjust the concentration of formamide for optimal result.**

- Incubate at 50 °C for 5 minutes to clear precipitation of SDS in high ionic solution when cooled on ice
- Place softly a GAP coverslip onto AceGene® Oligo Chip and inject the target solution slowly in between
- Do not move the coverslip if small bubbles formed as they will disappear (hopefully)
- Incubate at 50 °C for 16-20 hours with HAIBURI-KUN® or in a humid chamber (prepared by soaking KIM towel with 4 × SSC)

**! Do not let the AceGene® oligo chip come to direct contact with KIM-towel.**

### **Wash**

Preheat the following solutions at 40 °C for at least 20 minutes.

- Remove coverslip by dipping in 4 × SSC-0.1%SDS solution
- Incubate in 4 × SSC-0.1%SDS solution (40 °C) for 5 minutes intervened by gentle plunging
- Incubate in 2 × SSC-0.1%SDS solution (40 °C) for 5 minutes intervened by gentle plunging
- Incubate in 2 × SSC solution (40 °C) for 5 minutes intervened by gentle plunging
- Rinse in 1 × SSC solution (RT).
- Dry AceGene® by centrifugation (1000rpm, 2 minutes, RT) or using an air dust spray
- Scan promptly and keep from light

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### **Update History**

|          |                                      |
|----------|--------------------------------------|
| 2004July | AceGene protocol version 1           |
| 2004May  | AceGene protocol version 2 (Current) |