# cDNA Array CGH

1. **Purify genomic DNA** (Trizol and phenol/chloroform method)

2. **Sonication**
   Using probe sonicator: 10 sec x 6
   (20 ug DNA in 300 μl $H_2O$)
   Check on gel--- smear 200-800bp

   **Purification with Qiagen PCR spin column protocol**.

   a. Add 900 μl PB buffer to reaction and transfer to QIAquick column.
   b. Spin at maximum speed for 1 minute.
   c. Wash with 700 μl **PE** buffer
   d. Spin at maximum speed for 1 minute.
   e. Repeat wash steps c and d.
   f. Empty collection tube and spin at maximum speed for 1 minute.
   g. Transfer column to a fresh tube, Elute with 30 μl **H2O** with 1 min incubation followed by spin at maximum speed for 1 minute.
   h. Repeat elution step g

**3. Genomic DNA labeling**

For one labeling use 3µg of the digested control DNA and test DNA.

Add

| | |
|---|---|
| DNA 3µg | 19µl |
| Random Primer (hexamers) Gibco (3 ug/ul) | 2µl |

Incubate for 10 min at 95° C, cool on ice

| | |
|---|---|
| aadNTPs 50x* | 0.5µl |
| Klenow Reaction Buffer 10x | 2.5µl |
| Klenow Fragment NEB **(50U/µl)** ** | 1µl |
| total | 25µl |

*

| |
|---|
| dATP 25mM |
| dCTP 25mM |
| dGTP 25mM |
| dTTP 5mM |
| aa-dUTP 20mM |

Note that the ratio between dTTP and aa-dUTP is 1:4

Incubate 37 ° C overnight, then stop reaction by adding 5µl 0.5 M EDTA pH 8.0.

**Probe purification with Qiagen PCR spin column protocol**.

i. Add 300 µl PB buffer to reaction and transfer to QIAquick column.
j. Spin at maximum speed for 1 minute.
k. Wash with 700 µl **phosphate wash buffer** (5 mM KPO4, pH 8.0 + 80% EtOH – use this home-made buffer to avoid Tris).
l. Spin at maximum speed for 1 minute.
m. Repeat wash steps c and d.
n. Empty collection tube and spin at maximum speed for 1 minute.
o. Transfer column to a fresh tube, Elute with 30 µl **phosphate elution buffer** (4 mM KPO4, pH 8.5) with 1 minute incubation followed by spin at maximum speed for 1 minute.
**p.** Repeat elution step g.
q. Speedvac dry (~40 minutes)

4. **Cy Dye coupling**
    a. Resuspend the cDNA in 4.5 μl 0.1 M carbonate buffer, pH 9.0 (freshly made, <1 month).
    b. Add 4.5μl NHS-Cy dyes (resuspended in DMSO. AVOID ANY MOISTURE!!)
    c. Incubate the reactions in dark for 1 hour in room temperature.

**Probe purification with Qiagen PCR spin column protocol**.

a. Add 35 μl **100 mM** NaAc pH5.2 to the reaction.
b. Add 300 μl PB buffer to reaction and transfer to QIAquick column.
c. Spin at maximum speed for 1 minute.
d. Wash with 700 μl **phosphate wash buffer** (5 mM KPO4, pH 8.0 + 80% EtOH – use this home-made buffer to avoid Tris).
e. Spin at maximum speed for 1 minute.
f. Repeat wash steps c and d.
g. Empty collection tube and spin at maximum speed for 1 minute.
h. Transfer column to a fresh tube, Elute with 30 μl H2O with 1 minute incubation followed by spin at maximum speed for 1 minute.
**i.** Repeat elution step g.

At this step, 1 μl of sample can be taken for dye incorporation analysis. Diluted sample at 1:100. Measure absorbance at 260 nm, 550 nm (for Cy3) and 650 nm (for Cy5).
For Cy3, incorporation: nucleotide/dye=17.1*OD260/OD550.
For Cy5, incorporation: nucleotide/dye=28.5*OD260/OD650.

**<u>Prehybridization:</u>**

a. Prepare prehybrization buffer (**5xSSC, 0.1% SDS and 1% bovine serum albumin, BSA Sigma Cat# A-9418**)
b. Place slides to be analysed into a staining dish, fill with prehybridization buffer, and incubate at 42 $^{o}$C for 45 minutes
c. Wash the slides in a Wheaton slide rack at room temperature with DIH2O with agitation.
d. Dip the slides in room temperature isopropanol and dry by centrifugation at 2000 rpm for 2 min. Inspect slides to make sure there is no protein marks on slides. If there is, repeat step 3 and 4.

Slides should be used immediately following prehybridization, at least within one hour.

## Hybridization:

1. Combine the Cy3 and Cy5 labeled probes (about 120 μl)

add 50 μg of Cot-1 DNA (10mg/ml) 5 μl
100 μg of yeast tRNA (4 mg/ml) 25 μl

speedvac dry it.

2. Redisolve the combined probes in 36 μl 1X hybridization solution

**Dextran hybridization solution**

| | |
|---|---|
| 50% Formamide | 500 μl of Formamide / 1ml |
| 4xSSC | 200 μl of 20xSSC / 1ml |
| 2% SDS | 100 μl of 20%SDS / 1ml |
| 10% Dextran Sulfate | 200 μl of 50% Dex Sul /1ml |

| | |
|---|---|
| Cy5/Cy3 labeled probes | 37 μl |

3. Denature at 75 $^{o}$C for 10 minutes
4. Incuabate at 37 $^{o}$C for 1 h.
5. Apply 3x7μl H2O into each modified hybridization chamber to prevent drying.
6. Apply probes to the prehybridized microarray slide, hybridize in a humidified chamber at 37 $^{o}$C for 16-24 hours.

## Wash:

Place slide into a staining dish with Wash #1 until coverslip falls off.

| | |
|---|---|
| Wash #1: | at R/T in 0.2% SDS + 1 X SSC until coverslip falls off |
| Wash #2: | 4 minutes at 42 $^{o}$C in 0.2% SDS + 1 X SSC |
| Wash #3: | 4 minutes at R/T in 0.2% SDS + 0.1 X SSC |
| Wash #4: | 4 minutes at R/T in 0.06 X SSC |

Spin immediately (1000 rpm for 2 minutes at R/T)

## Appendix:

Solutions:

**100 mM aa-dUTP** (Sigma, cat # A0410)

Disolve 10 mg in 191 ul 0.1 M KPO4 buffer, pH 7.5. Quantitate this stock solution by diluting an aliquot 1:5000 in the same buffer and measuring A289. Stock concentration in mM = $OD_{289nm}$ x 704.

## Make 10 ml 0.1 M $KPO_4$ buffer, pH7.5

1. Prepare 2 solutions: 1 M KH2PO4 and 1M K2HPO4.
2. Mix:
   - 0.802 ml 1M K2HPO4
   - 0.198 ml 1 M KH2PO4
3. Bring to 10 ml with $H_2O$. The pH of this KPO4 buffer should be at 7.5.

## 50X aa-dNTP mix

Nucleotide/dye ratio with the ratio of aa-dUTP to dTTP. We use a 2:3 ratio. Prep are a 50X stock:

| Start Concentration | Volume | Final Concentration |
|---|---|---|
| 100 mM dATP | 25 µl | 25 mM dATP |
| 100 mM dCTP | 25 µl | 25 mM dCTP |
| 100 mM dGTP | 25 µl | 25 mM dGTP |
| 100 mM dTTP | 5 µl | 5 mM dTTP |
| 100 mM dUTP | 20 µl | 20 mM aa-dUTP |

## Phosphate wash buffer

1. Prepare 2 solutions: 1 M KH2PO4 and 1M K2HPO4.
2. Make 1 M KPO4 (the pH should be 8.5-8.7) mix:
   - 9.5 ml 1M K2HPO4
   - 0.5 ml 1 M KH2PO4
3. Make 100 ml phosphate washing buffer, Mix:
   - 0.5 ml 1 M KPO4, pH 8.5
   - 15.25 ml H2O
   - 84.25 ml 95% EtOH

   (This solution will be slightlt cloudy)

## Phosphate elution buffer

Dilute 1 M KPO4 pH 8.5 to 4 mM for Phosphate elution buffer:

| | |
|---|---|
| 1 M KPO4 pH 8.5 | 0.4 ml |
| H2O | 99.6ml |
| Total | 100ml |

## Carbonate buffer 0.1 M Na2CO3, pH 9.0:

| | |
|---|---|
| Na2CO3 | 0.27g |
| H2O | 20ml |
| Adjust pH to 9.0 with | 6 N HCl |
| Total with H2O | 25 ml |

## NHS-Cy dyes

NHS-Cy3: AmershamPharmacia Cat# PA23001
NHS-Cy5: AmershamPharmacia Cat#PA25001

1. Resuspend one tube in 73 ul DMSO.
2. Use immediately, or aliquot 4.5 ul into 0.5 ml tubes and store at -80°C. Avoid moistures!!!