

THE PROCESS

Building the Chip:

MASSIVE PCR:
Full yeast genome =
6500 reactions

PCR PURIFICATION and PREPARATION:
IPA precipitation + EtOH washes
+ 384-well format

PREPARING SLIDES:
Polylysine coating
for adhering PCR
products to glass
slides.

PRINTING:
The arrayer: high precision
spotting device capable of
printing 10,000 products
in 14 hours, with a plate
change every 25 min.

Preparing RNA:

CELL CULTURE AND HARVEST:
Designing experiments to profile
conditions/perturbations/
mutations and carefully controlling
growth conditions.

RNA ISOLATION:
RNA yield and purity are determined
by system. PolyA isolation is
preferable but total RNA is
useable. Two RNA samples are
hybridized/chip.

Hybing the Chip:

POST PROCESSING:
Chemically converting the
positive polylysine surface to
prevent non-specific hybridization.

ARRAY HYBRIDIZATION:
Cy3 and Cy5 RNA samples are
simultaneously hybridized to
chip. Hybs are performed for 5
12 hours and then chips are
washed.

cDNA PRODUCTION:
Single strand synthesis or
amplification of RNA can be
performed. cDNA production
includes incorporation of
Aminallyl-dUTP.

PROBE LABELING:
Two RNA samples are labeled
with Cy3 or Cy5 monofunctional
dyes via a chemical coupling to
the AA-dUTP. Samples are
purified using a PCR clean-up kit.

DATA ANALYSIS:
Ratio measurements are
determined via quantification
of 532nm and 635nm emission
values. Data is uploaded to the
appropriate database (AMAD) and
high-level analysis can be
performed using software like
Cluster and Tree-View.