

ReadMe

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by UCB Neurospora Microarray Team

10,918 oligomers are on the chip. 10,526 oligos are based on MIPS or Broad Institute predicted ORFs. 8 oligos are Abmion ArrayControl Sense Oligo Spots (#1781). The remaining 384 oligos (ChIP_Chip) were mainly designed in the intergenic regions.

40uM oligos in 3x SSC were printed onto Corning UltraGAPS slides. Slides are not post-processed. Rehydration is optional. Oligos need to be cross-linked with 600mJ UV.

Slides should be stable for a year at room temperature in desiccator.

Slides printed in October 2005 or before have sticker labels on. Some batches of the labels seem to increase background level. **Please peel them off** and wash carefully with 95% ethanol prior to UV-crosslinking.

Slides may display unacceptable levels of green background and only way we know to remove is using the Pronto!™ Background Reduction Kit (Item #40029) from Corning. **This step is absolutely essential!** It is not 100% effective so we recommend prescanning after prehybridization and identifying spots that are 3x standard deviation above background for possible elimination.

A standard protocol for reverse transcription and hybridization can be found here but we recommend to use Corning/Promega kit, now sold separately:

Promega Z4000 ChipShot™ Indirect Labeling and Clean-Up System,
Corning 40026 Pronto!™ Hybridization Kit, 25x and
RPN5661 Amersham Post-labeling Reactive Dye Pack,

which are expensive but always give brilliant results.

GAL files for spot gridding are batch dependant. Please choose the appropriate one.

ID corresponds to gene names from which oligos were designed. A typical oligo name looks like:

ID	Name
3nc442_550_1875	NCU00340.1

For the majority of oligos, MIPS gene models were used. The adjunct “_1875” above indicates distance between oligomer’s 3’-end and the last base of ORF. Corresponding NCU genes are given as “Name”. Since we created oligomers,

MIPS has changed 52 of corresponding NCU counterparts. Also NCU models have been recently assigned to 454 MIPS models. Each of the total of 329 NCU models correspond to more than one MIPS models. When it happened, postfix “a, b, or c” were added to NCU models as:

ID	Name
xnc105_070_1566	NCU09113.1a
xnc105_080_1191	NCU09113.1b

We have updated information for NCU counterparts and created new gal files with “_Mar06” postfix. In addition, for the gal files NC6_05.gal, NC8_05.gal, “ID” is used for NCU counterparts and “Name” is used for MIPS genes, which should have been the other way around. This inconsistency has also been fixed. Changed oligo names can be found in “GeneTemplateMar_06”. Note that 3/9/07-made slides have 384 spots duplicated (printed twice).

Slides	gal files	updated gal files
NC 6/2/05	NC6_05.gal	NC6_05_Mar06.gal
NC_8/23/05 UCB	NC8_05.gal	NC8_05_Mar06.gal
NC_10/26/05 CNR	NC9_05_CNR.gal	NC9_05_CNR_Mar06.gal
NC_10/04/05 UCB	NC10_05.gal	NC10_05_Mar06.gal
NC_11/22/05 UCB	NC10_05.gal	NC10_05_Mar06.gal
NC_6/7/06 UCB		NC10_05_Mar06.gal
NC_3/7/07 UCB		NC3_07A.gal
NC_3/9/07 UCB		NC3_07B.gal
NC_2/20/08 UCB		NC10_05_Mar06.gal
NC_2/22/08 UCB		NC10_05_Mar06.gal

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