UCD Expression Analysis Core - Illumina Sentrix Gene Expression BeadChip Arrays

This document explains the step-by-step procedure for full-service processing at our Core facility. For information on costs, click the 'Prices' tab on our web page (http://dnatech.genomecenter.ucdavis.edu/prices). Any questions about beadchips, samples, pricing, or if you have circumstances that deviate from a typical order (such as very low concentration samples), please contact Siranoosh Ashtari (sashtari@ucdavis.edu) or Vanessa Rashbrook (vkrashbrook@ucdavis.edu). Once you have read this document please fill out the 'Order Request and Sample Submission Form' (available from Siranoosh), which will initiate the beadchip ordering process.

STEP 1: CREATE AN ACCOUNT

If your PI/lab does not have an account with the UCD Genome Center, follow the instructions for creating one on the DNA Technologies and Expression Analysis Core website at http://dnatech.genomecenter.ucdavis.edu (select 'Genome Center Account Login' under the menu tab: 'Accounts/Calendars'), or go directly to http://cores.genomecenter.ucdavis.edu/index.php.

STEP 2: CHOOSE THE NUMBER OF EXPRESSION BEADCHIP ARRAYS

Each beadchip has 12 arrays on a single glass slide; the beadchips cannot be divided up. The beadchips also need to be purchased in pairs:

HumanHT-12 v.4.0 (12 arrays x 2 chips = 24 samples)

Details on beadchip specifications and gene lists can be accessed from our Illumina Gene Expression web page at: http://expression.genomecenter.ucdavis.edu/illumina_expre.html. Following sample submission, beadchips will be purchased from Illumina by Core personnel (please do not order your own beadchips). Expect a 3-4 week turnaround time on orders.

STEP 3: CHARACTERIZE SAMPLE INPUT RNA

Input material for the amplification/labeling protocol we use is total RNA. RNA can be quantified by UV spectrophotometer or Nanodrop. The input material should ideally be 100-500 ng/ul – please submit between 11 and 15 ul of each sample. If the concentration is less than 100 ng/ul, please supply 24-30 ul. Amplification will work best at concentrations of 25 ng/ul and above (the higher the better).

It is very important to have high quality RNA (i.e., low amounts of degraded RNA) to ensure meaningful gene expression results. RNA integrity is assessed with the Agilent Bioanalyzer; up to 12 samples can be run on one Bioanalyzer chip. Please provide at least 5 ul of sample in clearly labeled 0.5 ml tubes, in a concentration range of 15-500 ng/ul (medium to upper concentration is best). We will inform the customer of the results before proceeding with the labeling – the Expression Analysis Core assumes no responsibility for poor labeling/amplification if the quality & quantity of total RNA does not meet basic requirements.

STEP 4: RNA AMPLIFICATION

Total RNA samples are amplified and labeled with biotinylated nucleotides using a kit from Ambion (TotalPrep -96 RNA Amplification Kit, Cat# 4393543). The labeling protocol is a variant of the Eberwine protocol. We recommend using the same amount of input for all samples in a project, which can vary from 300 or 500 ng in a 11 ul reaction volume

HumanHT-12

Each array/sample requires 200-700 ng/ul concentration amplified/labeled cRNA, with at least three times the mass amount needed (as a backup). Each array allows up to 5 ul of sample volume that will be hybridized with a total mass of 750 ng.

STEP 5: AMPLIFIED AND LABELED cRNA QC

Occasionally RNA that looks good prior to labeling (see Step 3), that yields the expected number of micrograms following amplification with ~50 fold amplification (see Step 4), does not always produce the expected 1.2 kb average labeled cRNA. cRNA smaller than the expected 1.2 kb average will negatively affect microarray results. We therefore perform Bioanalyzer analysis to verify cRNA is at the expected 1.2 kb average size before applying to an array. If problems are encountered we will contact the customer with recommendations on whether to proceed.

STEP 6: SCANNING CONDITIONS

We scan the beadchips with the Illumina iScan using standard conditions. Raw data and image files with feature location files will be uploaded to our server for customer download through our website. For local customers we can provide the Illumina GenomeStudio software and basic software training for no additional fee.

Please deliver or send all RNA samples on dry ice.

Shipping address:
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