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Using Taqman[™] assays to verify eQTL links arising from GWAS Studies

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ABSTRACT

Tremendous progress has been made using genome-wide association studies (GWAS) to link genetic variation with phenotypes and pathologies. In spite of these success, it has been estimated that as many as 90% of SNPs identified in GWAS studies map to non-coding regions, complicating the mechanistic interpretation of the results. It is thought that these noncoding SNPs fall into regulatory regions and influence the expression of a gene or genes. The study of expression quantitative trait loci (eQTLs) provides a method for understanding the link between genetic variants and altered gene expression, and could potentially provide new insights connecting GWAS results to molecular mechanisms. To illustrate how eQTLs can be found and verified, we generated transcriptomic information from various tumor samples using Applied Biosystems™' Clariom™ D microarrays. To find putative eQTLs, we compared the SNPs genotypes and the gene expression levels in these samples to the GTEx database. Potential eQTLs in this set of samples were verified using Applied Biosystems' Taqman[™] SNP Genotyping and Taqman Gene Expression assays. We therefore illustrate a workflow where candidate eQTLs can be confirmed and screened in larger cohorts using the more economical qPCR reagents available from Applied Biosystems.



FIGURE 1. WORKFLOW FOR IDENTIFYING eQTLS

eQTLs are emerging as important markers of gene function, and may be useful as biomarkers for certain traits or pathologies. eQTLs are typically identified in a two-step process. To do this, a collection of samples from different individuals are needed. First, as part of a GWAS, genomic SNP variants among the samples may be identified by NGS or arrays. Next, transcriptomic analysis from the same samples is performed. RNA is collected from different tissues, treatments, tumor vs normal, etc, and subjected to microarray or RNAseq analysis. Transcripts that are differentially regulated in the different groups are then identified. The SNP genotypes and gene expression changes are then correlated. Thermo Fisher Scientific provides all the reagents needed for identifying eQTLs and verifying them as biomarkers.



FIGURE 2. TRANSCRIPTOME **ANALYSIS USING CLARIOM D ARRAYS**

NA was extracted from FFPE amples from brain, kidney, lung, vary, and stomach tumor tissues nd analyzed using Clariom D licroarrays. The clustering and eat map was generated using Transcriptome Analysis Console oftware 4.0 (TAC 4.0) and represents cluster analysis of 2,742 DEGs, filtered for those where confidences of differences are highly significant (P < 0.001). DEGs from these samples are clustered by tissue type. Each row represents a single gene and each column represents a single sample. Colors represent log2 of relative expression levels (blue: low relative expression, red: high relative expression)



FIGURE 3. TISSUE SPECIFIC EXPRESSION CHANGES

Table of expression values and scatter plot showing relative expression of genes between FFPE stomach tumor and lung tumor samples. TAC 4.0 Software was used to examine pairwise relative expression of genes. The highlighted genes at the top of the table (blue rows) and in the plot (purple bubbles) were overexpressed in stomach tumor tissue relative to lung tumor tissue, consistent with previously published data. The same data can also be viewed as a volcano plot, with the same genes highlighted by purple bubbles.

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	5313	2.75988197	1.48097801	0.76130104	-5.0650539	1.02093697
mach 0.03158	8188	3.0422821	0.45259857	-1.5038033	1.94883728	2.93746567
mach 3.85096	6741	4.64403534	2.21043777	0.50680161	-1.1337051	2.07761192
mach 2.99467	7468	4.52161217	2.50423813	1.45359421	0.30448914	3.26035309
n 2.60247	7612	4.16389656	1.36025429	-1.3378773	-5.2254467	1.67616463
n 4.52767	7563	-2.7877979	0.23875999	1.45085144	4.78930664	0.19897079
n 3.19486	6237	0.46006966	-0.8761292	0.57139206	7.41348839	-1.4070377
n -0.3069	9534	-3.1536064	-1.8726311	0.46837997	4.53450394	-2.5804005
n 0.59228	8134	-1.611021	-1.9949741	-0.1994476	4.65990067	-2.8426495
n 2.29356	6003	-3.0850029	-0.0251007	-2.8416328	3.79310417	-0.470974
ry 0.582	2798	-3.6595287	-0.8409004	-3.3889256	2.34770966	-3.2522583
ry	0	-3.3572521	-0.7554302	-2.9624748	4.67296982	0.21254349
ry 4.58119	9774	2.03606033	4.34082985	1.81390381	4.79863739	5.24328995
ry -1.1364	4212	-3.3677616	-0.0032005	0	-3.2664223	-1.8469124
ry 0.05097	7389	-3.3604832	-0.7719498	0.84652328	-3.084938	-1.3164654
ry #VALU	JE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
ney 1.44779	9587	-3.3125782	-0.5813332	-0.8382988	-3.0243969	-2.5473213
ney 1.77965	5927	-2.9592018	-0.8026867	0.48974228	-1.2776356	-1.4585686
ney 1.12690	0353	-2.733345	-1.276823	0.58342743	4.70890236	-0.4497643
ney 0.82616	6806	-0.9509907	-2.1974964	-0.244833	5.38575554	-2.4411621
	11201 3.8309 mach 2.9946 n 2.6024 n 4.5276 n 3.1948 n -0.306 n 0.5922 n 2.2935 ry 0.582 ry 0.582 ry 0.582 ry 0.0509 ry 1.4364 ry 0.0509 ry 1.44779 ney 1.77963 ney 0.82611	nach 3.83096741 mach 2.99467468 n 2.60247612 n 4.52767563 n 3.19486237 n -0.3069534 n 0.59228134 n 2.29356003 ry 0.582798 ry 0 ry 0.582798 ry 0 ry 0.5097389 ry 0.05097389 ry 0.05097389 ry 1.1364212 ry 0.05097389 ry 1.1364212 ry 0.82616806	nach 2.99467468 4.04403334 nach 2.99467468 4.52161217 n 2.60247612 4.16389656 n 4.52767563 -2.7877979 n 3.19486237 0.46006966 n -0.3069534 -3.1536064 n 0.59228134 -1.611021 n 2.29356003 -3.0850029 ry 0.582798 -3.6595287 ry 0 -3.3572521 ry 0.582798 -3.6595287 ry 0 -3.3572521 ry 0.5097389 -3.36046033 ry -1.1364212 -3.3677616 ry 0.05097389 -3.3604832 ry #VALUE! #VALUE! ney 1.44779587 -3.3125782 ney 1.12690353 -2.733345 ney 0.82616806 -0.9509907	Hach3.830967414.644033342.21043777mach2.994674684.521612172.50423813n2.602476124.163896561.36025429n4.52767563-2.78779790.23875999n3.194862370.46006966-0.8761292n-0.3069534-3.1536064-1.8726311n0.59228134-1.611021-1.9949741n2.29356003-3.0850029-0.0251007ry0.582798-3.6595287-0.8409004ry0-3.3572521-0.7554302ry4.581197742.036060334.34082985ry-1.1364212-3.3677616-0.0032005ry0.05097389-3.3604832-0.7719498ry#VALUE!#VALUE!#VALUE!ney1.44779587-3.3125782-0.5813332ney0.82616806-0.9509907-2.1974964	Hach3.830967414.844033342.210437770.30680161mach2.994674684.521612172.504238131.45359421n2.602476124.163896561.36025429-1.3378773n4.52767563-2.78779790.238759991.45085144n3.194862370.46006966-0.87612920.57139206n-0.3069534-3.1536064-1.87263110.46837997n0.59228134-1.611021-1.9949741-0.1994476n2.29356003-3.0850029-0.0251007-2.8416328ry0.582798-3.6595287-0.8409004-3.3889256ry0-3.3572521-0.7554302-2.9624748ry0.5097389-3.3607616-0.00320050ry0.05097389-3.3604832-0.77194980.84652328ry#VALUE!#VALUE!#VALUE!#VALUE!ney1.44779587-3.3125782-0.5813332-0.8382988ney1.77965927-2.9592018-0.80268670.48974228ney0.82616806-0.9509907-2.1974964-0.244833	Hach5.630967414.644035342.210437770.30680161-1.1337051mach2.994674684.521612172.504238131.453594210.30448914n2.602476124.163896561.36025429-1.3378773-5.2254467n4.52767563-2.78779790.238759991.450851444.78930664n3.194862370.46006966-0.87612920.571392067.41348839n-0.3069534-3.1536064-1.87263110.468379974.53450394n0.59228134-1.611021-1.9949741-0.19944764.65990067n2.29356003-3.0850029-0.0251007-2.84163283.79310417ry0.582798-3.6595287-0.8409004-3.38892562.34770966ry0-3.3572521-0.7554302-2.96247484.67296982ry4.581197742.036060334.340829851.813903814.79863739ry-1.1364212-3.3677616-0.00320050-3.2664223ry0.05097389-3.3604832-0.77194980.84652328-3.084938ry#VALUE!#VALUE!#VALUE!#VALUE!mey1.44779587-3.3125782-0.5813332-0.8382988-3.0243969ney1.2690353-2.733345-1.2768230.583427434.70890236ney0.82616806-0.9509907-2.1974964-0.2448335.38575554



Lung Tumor Tissue



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Variant ID		Shorthand \$	rs ID (v147)		Chromoso	ome	Position	≎ MAF >= 1%	\$	Ref Allele \$	Alt Allele
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ENSG00000101181.13	MTG2	20_60768615_G_A_b37	rs6062133	dbSNP 🗹	7.0e-12	0.70	Brain - Nucleus accumbens (basal ganglia)		eQTL violin plot, IGV eQTL Browser, Multi-tissue eQTL Plot		
ENSG00000101181.13	MTG2	20_60768615_G_A_b37	rs6062133	dbSNP 🗹	1.2e-11	0.57	Colon - Sigmoid		eQTL violin plo	t, IGV eQTL Browser, Mul	ti-tissue eQTL Plot
ENSG00000101181.13	MTG2	20_60768615_G_A_b37	rs6062133	dbSNP 🗹	9.5e-10	0.28	Skin - Sun Exposed (Lower leg)		eQTL violin plo	t, IGV eQTL Browser, Mul	ti-tissue eQTL Plot
ENSG00000101181.13	MTG2	20_60768615_G_A_b37	rs6062133	dbSNP 🗹	1.4e-9	0.26	Lung		eQTL violin plo	t, IGV eQTL Browser, Mul	ti-tissue eQTL Plot
ENSG00000101181.13	MTG2	20_60768615_G_A_b37	rs6062133	dbSNP 🗹	1.5e-9	0.30	Cells - Transformed fibroblasts		eQTL violin plo	t, IGV eQTL Browser, Mul	ti-tissue eQTL Plot
ENSG00000101181.13	MTG2	20_60768615_G_A_b37	rs6062133	dbSNP 🗹	6.7e-9	0.71	Brain - Amygdala		eQTL violin plo	t, IGV eQTL Browser, Mul	ti-tissue eQTL Plot
ENSG00000101181.13	MTG2	20_60768615_G_A_b37	rs6062133	dbSNP 🗹	8.2e-9	0.61	Brain - Anterior cingulate cortex (BA24)		eQTL violin plo	t, IGV eQTL Browser, Mul	ti-tissue eQTL Plot
ENSG00000101181.13	MTG2	20_60768615_G_A_b37	rs6062133	dbSNP 🗹	2.4e-8	0.66	Brain - Hippocampus		eQTL violin plo	t, IGV eQTL Browser, Mul	ti-tissue eQTL Plot
	MTG2	20_60768615_G_A_b37	rs6062133	dbSNP 🗹	5.0e-8	0.46	Colon - Transverse		eQTL violin plo	t, IGV eQTL Browser, Mul	ti-tissue eQTL Plot
ENSG00000101181.13			********	dbSNP 🔽	7.7e-8	0.65	Brain - Spinal cord (cervical c-	1)	eQTL violin plo	t, IGV eQTL Browser, Mul	ti-tissue eQTL Plot
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The GTEx database collects and displays information about eQTLs. The database can be queried by gene, tissue, splice isoform, and SNP ID. In the example shown above, information about the SNP rs6062133 in MTG2 is displayed.

TAQMAN VERIFICATION OF GENE FIGURE 5. **EXPRESSION DIFFERENCES**

Five genes with previously-defined eQTLs were chosen for further analysis. DNA and RNA were isolated from a second cohort of tumor samples using the Recoverall[™] FFPE kit. RNA from these samples was analyzed using predesigned Taqman probes (best coverage) for the genes SLC12A2, CAMK2N1, MTG2, PLIN2, PTPRZ1 and DAAM1. These genes were chosen because the GTEx database showed there were known eQTLs that have been apped these genes. None of these genes are hypothesized to be related to the tumor phenotype; they were chosen for eQTL illustration. The table shows the $\Delta\Delta$ Ct of the gene relative to the OAZ1 reference transcript. Colored values highlight genes and tissues that have GTEx data for tissue-specific eQTLs. Note that one of the ovarian samples failed to produce any data. The accompanying graphs highlight the sample variation in $\Delta\Delta$ Ct values for each tissue.

CONCLUSIONS

FIGURE 6. GENOTYPING USING TAQMAN SNP ASSAYS

DNA the same set of samples (Figure 5) was analyzed using predesigned Taqman SNP Genotyping assays. The database of Taqman assays was queried for SNPs that covered previously-mapped eQTLs proximal to the transcription start site (TSS) of the genes above. Genotypes were analyzed using the Thermo Fisher Connect[™] (cloud-based) Genotyper (GT) application. Each sample is a different dot on each rsID plot; blue and red are called homozygous by the software, green dots are heterozygous, and yellow dots indicate no amplification in that sample.

Thermo Fisher SCIENTIFIC



FIGURE 7. CONFIRMATION OF eQTL IN MTG2

rouping expression levels of MTG2 lative to rs6062133 genotype reveals a slight increase in expression n GA heterozygotes relative to GG nomozygotes (bar graph). These esults are very similar to those in the GTEx database (violin plot). Note that none of the other SNPs (Figure 6) had diverse genotypes to confirm they were eQTLs.

MTG2





are significant in GWAS studies (circled) that fal within the SERPINH1 gene. There is potential overlap with three eQTLs in mammary tissue. (B) Entering SERPINH1 in the "Gene-eQTL Visualizer" confirms that three eQTLs have been mapped to mammary tissue, but not to whole blood. (C) Clicking on the bubbles in (B) brings up box plots showing the relationship between genotype and expression levels. (D) TaqMan Assays can be used to confirm and extend these results. TaqMan Gene Expression Assays can measure the expression levels of SERPINH1, and can be used to identify SNP genotypes of individuals at the putative eQTL.



• eQTLs can contribute to understanding link between genotype and phenotype

- Influencing expression levels of a gene can influence a trait or pathology
- May facilitate understanding how GWAS loci that fall outside coding regions act
- Software tools exist that link GWAS results with eQTL studies

TaqMan assays can be used to advance eQTL links to GWAS studies

- TaqMan Gene Expression assays can analyze transcript levels
- TaqMan Genotyping assays can analyze genotypes at specific SNPs • Other Thermo Fisher Scientific tools can be used for discovery-based eQTL research

ACKNOWLEDGEMENTS

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