Quantification of Fusion Transcripts Using TaqMan® Gene Expression Assays

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Abstract

Chromosomal aberrations such as translocations are frequently found in human cancer cells. Chromosomal translocations may result in a chimeric gene expressing a fusion transcript which is then translated into a fusion protein that affects normal regulatory pathways and stimulates cancer cell growth. A well known example is the BCR/ABL chimeric mRNA which is the result of a translocation of ABL on chromosome 9 to the BCR breakpoint cluster on chromosome 22. The resulting fusion transcript is the cause for 90% of chronic myelogenous leukemia. Current methods for identifying translocations include FISH, and karyotyping, none of which can be used to quantify the expression level of the fused gene . We have designed TaqMan® Gene Expression Assays for a set of known fusion transcripts for quantitative analysis. We first collected 214 fusion transcript GenBank mRNA Accessions representing 165 unique translocation events from two data sources (Chimer D:http://genomce.ewha.ac.kr/ChimerDB/ and **Hahn et al, *PNAS* 2004;101;13257-13261**). The transcripts were annotated and fusion breakpoint locations were identified or verified. Assays were designed such that the primer and probe spanned the breakpoint region and did not map directly to the breakpoint. Breakpoint region, SNPs and any repetitive sequences were masked before the assay was designed using the Applied Biosystems assay design pipeline. As proof of principle, several assay designs were tested against plasmids containing the translocation variant and patient samples with the translocation event. Only those samples containing the fusion transcript were amplified indicating the specificity of the assay. From a large number of assay designs, we selected 165 TaqMan Gene Expression Assays, targeting each of the 165 translocation variants. These 165 assays for quantization fusion transcripts are currently published on the Applied Biosystems Website (http://www.appliedbiosystems.com).

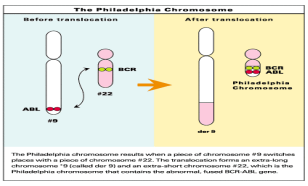


Figure 1. Philadelphia Chromosome

Introduction

One of the known causes of cancer are translocations that create fusion transcripts that result in altered protein expression. For example, the Philadelphia Chromosome, a translocation between Chromosomes 9 and 22, is found in almost all cases of chronic myeloid leukemia (CML) (Figure 1). FISH and classical cytogenetic methods detect the chromosomal translocation events and RT-PCR (SYBR® Green, gels) has been successfully used to detect the presence or absence of a fusion transcript. However, no systematic method to quantify fusion transcripts has been available so far. For this purpose, we developed TaqMan® Fusion Transcript Assays to quantitate fusion transcripts resulting from genomic translocations.

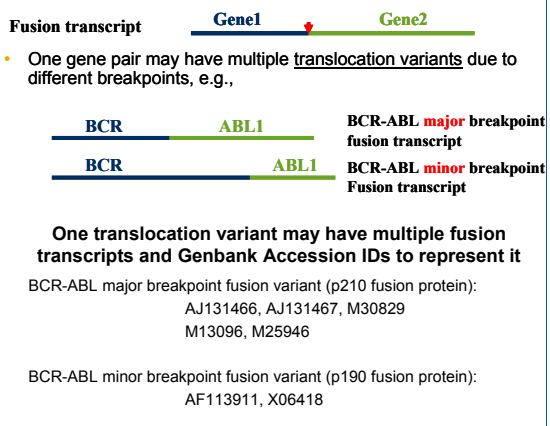
- TaqMan Gene Expression Assays consist of a target specific forward and reverse primer and FAM™-MGB labeled probe. The TaqMan® Assay is based on the 5' nuclease activity of the Taq polymerase. TaqMan Assays quantify gene expression levels with high sensitivity and specifity.

- Fusion transcripts are often recorded as ESTs or deposited as GenBank Accessions by NCBI or other databases. Using two additional database sources, Chimer DB and the database provided in Hahn et al, we identified a set of fusion transcript GenBank RNA accessions (NCBI) to use for development of TaqMan Gene Expression Assays to detect fusion transcripts (Figure 2). After annotation and verification of their fusion breakpoint on the transcript and masking sequences for snps and ambiguities, we used our standard assay design pipeline and verification processes to design the assays (Figure 3).

- As proof of principle, we designed assays for several translocation targets and tested them against samples known to contain the translocation event (Figure 4, 5, 6).

Results

Fusion Transcripts Result from Translocations



- One gene pair may have multiple translocation variants due to different breakpoints, e.g.,

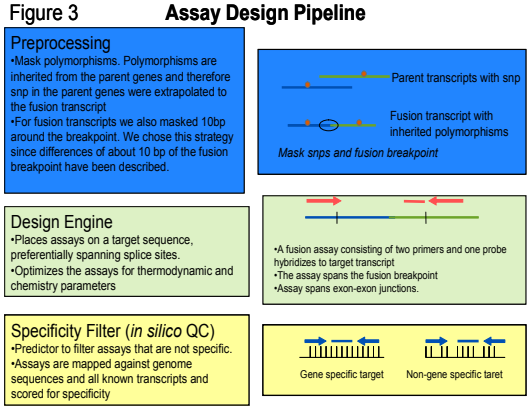
One translocation variant may have multiple fusion transcripts and Genbank Accession IDs to represent it

BCR-ABL major breakpoint fusion variant (p210 fusion protein):
AJ131466, AJ131467, M30829
M13096, M25946

BCR-ABL minor breakpoint fusion variant (p190 fusion protein):
AF113911, X06418

Figure 2. An Example of Fusion Transcript Variants for BCR-ABL.

Figure 3 **Assay Design Pipeline**



Conclusions:

- We developed a set of TaqMan Fusion Transcript Assays to measure the expression level of fusion transcripts resulting from a translocation event.
- Assays were designed to detect fusion transcript variants for the same translocation. Thus a single assay may detect all the known transcript variants for that translocation event.
- Using proof of principle assay designs, we show that TaqMan Fusion Transcript Assays are highly specific and quantifiably detect the fusion transcript over a broad dynamic range and limit of detection to <10 copies.
- We currently have 165 TaqMan® Fusion Assays which map to 133 gene fusions (go to **www.appliedbiosystems.com**).

Proof of Principle

Fusion Transcript Assay for PML/RARA

This assay was designed to detect PML-RARA BCR1 fusion transcript resulting from the t(15;17)(q22;q21) chromosomal translocation. Primers were designed on either side of the translocation breakpoint to ensure specificity for the fusion transcript. The forward primer is designed on the PML exon 6 (accession number M73778); the reverse primer on RARA exon 3 (accession numberX06538). The TaqMan® MGB probe is located downstream the breakpoint.

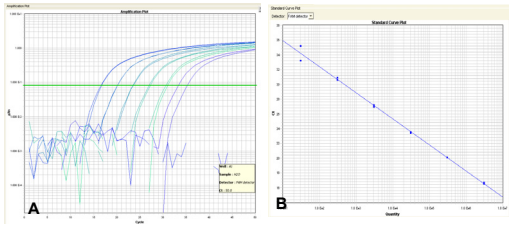


Figure 4. Standard Curve of PML/RARA fusion transcript.
A standard curve was generated using plasmid containing the fusion transcript (Ipsogen). R^ = 0.995; Amplification efficiency = 93% (slope = -3.5); detection limit <30 copies. Samples were run in duplicate. A) Amplification Plot; B) Standard Curve.

Fusion Transcript Assay for TCF3-PBX1

This assay was designed to detect TCF3-PBX1 fusion transcript (M31522.1, AY311345) resulting from the t(1;19)(q23;p13) chromosomal translocation. Primers were designed on either side of the translocation breakpoint to ensure specificity for the fusion transcript. The forward primer and probe is designed on the TCF3 exon 15 ; the reverse primer on PBX1 exon 3. The TaqMan® MGB probe is located upstream of the breakpoint.

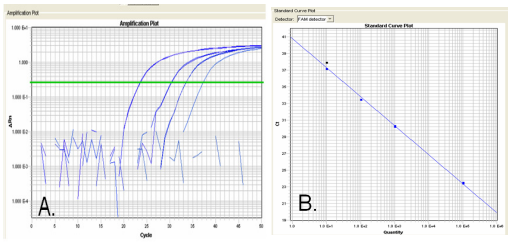


Figure 5. Standard Curve for TCF3/PBX1 fusion transcript.
A standard curve was generated using plasmid containing the fusion transcript (Ipsogen). R^ = 0.996; Amplification efficiency = 94% (slope = -3.48); detection limit <10 copies. Samples were run in duplicate. A) Amplification Plot; B) Standard Curve.

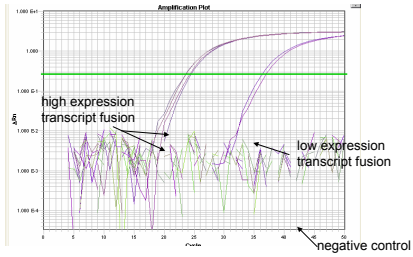


Figure 6. Fusion transcript assay for TCF3/PBX1.
Fusion assays run with total RNA from 3 clinical research blood samples. Samples were run in duplicate.

Method:

Sample: Total RNA was extracted from whole blood using Trizol methods; plasmids were obtained from Ipsogen.
cDNA: cDNA was prepared from total RNA using Applied Biosystems cDNA Archive Kit.
Real-Time PCR: TaqMan Assays were run in duplicate with 1X Universal Master Mix in 20ul final volume on Applied Biosystems 7900HT Fast Real-Time PCR System under universal cycling conditions.

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