# A Fluorescent Quantitative PCR Approach to Map Gene Deletions in the Drosophila Genome

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ABSTRACT

We report the application of *TaqMan* quantitative PCR (QPCR) to map Drosophila chromosome deficiencies by discrimination of twofold copy number differences. For a model system, we used this technology to confirm the X chromosomal mapping of *Dspt6* given the autosomal mapping of *Dspt4*. We then used this technique on both preexisting deletion mutant flies and flies that we generated with deletions to demonstrate the presence or absence of *Dspt6*, *Dspt4*, and *swa* in various deletion mutant flies. In contrast with *in situ* hybridization studies, QPCR both vitiates the need to do these more intricate studies, and it is more accurate as the site of deletion can be known down to the $10^2$-bp level. We then successfully applied the technique to the analysis of transcription, demonstrating that the amount of *Dspt6* or *Dspt4* transcriptional product depended directly on the dosage of the *Dspt6* or *Dspt4* gene, respectively. The rapidity and precision of this method demonstrates its applicability in Drosophila genetics, the rapid and accurate mapping of Drosophila deletion mutants.

ONE of the major strengths of Drosophila genetics is the availability of flies with chromosomal deficiencies. Such stocks have been collected over the past several decades. Most of these chromosomal deficiencies have been roughly mapped physically and / or genetically. Since these deficiencies were mapped using only a limited number of markers, the region of interest is only approximate. Furthermore, mastery of the cytogenetic and *in situ* hybridization techniques to accomplish this mapping remains laborious. Therefore, the current mapping strategy cannot accomplish precise molecular mapping. Thus, there are compelling reasons to elaborate a rapid and inexpensive technology to accomplish more precise characterization of the location and extent of chromosomal deficiencies in Drosophila.

We aim to study the biological functions of the chromatin proteins *SPT4* and *SPT6*. Genetic data obtained in yeast suggest that *SPT4* and *SPT6* are involved in transcriptional regulation and chromatin structure (Winston 1992). In addition, genetic studies suggest that *SPT4* and *SPT6* are required for normal recombination (Malagon and Aguilera 1996), chromosome segregation (Basrai *et al.* 1996), and transcription elongation (Hartzog *et al.* 1998). *Dspt6* and *Dspt4* are the Drosophila homologues of the yeast chromatin proteins *SPT6* and *SPT4*, respectively.

Here, we describe the use of a rapid, facile *TaqMan* quantitative PCR (QPCR) strategy to map *Dspt4* and *Dspt6* in deficiency flies. The success of our mapping strategy provides a convenient tool for the mapping of genes of interest in deficiency flies.

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## MATERIALS and METHODS

***TaqMan* QPCR:** The *TaqMan* assay has been described previously (Higuchi *et al.* 1992; Livak *et al.* 1995; Heid *et al.* 1996). The *TaqMan* assay requires a forward primer, a reverse primer, and a probe that hybridizes between the forward and reverse primers. The *TaqMan* PCR technology exploits the 5′-3′ nuclease activity of Taq DNA polymerase, allowing direct detection of the PCR product by the uncoupling of a reporter dye from its quencher dye during PCR. The probe consists of an oligonucleotide with a 5′ reporter dye, 6-carboxyfluorescein (6-FAM), and a 3′-quencher dye, 6-carboxytetramethylrhodamine (TAMRA). When the probe is intact, the fluorescence from the reporter dye is suppressed by the proximity of the quencher dye. During PCR, if the target is present, the probe specifically anneals between the forward and reverse primer sites. Under this condition, the 5′-3′ exonuclease activity of Taq DNA polymerase cleaves the oligonucleotide probe that links the reporter and quencher. The extent of digestion, which depends directly on the amount of PCR that occurs, can be quantified accurately by measuring the increment in fluorescence that results from decreased energy transfer between the reporter and quencher fluors.

The ABI PRISM 7700 can both perform and measure the PCR reaction in real time. This sensitive measurement allows detection of the PCR reaction in the exponential phase that is required for determination of initial genomic sequence copy number. To measure the relative genomic copy numbers of two sequences in a given DNA sample, the quantity of PCR product from the first sequence with the given DNA (whose copy number is to be determined) is divided by the quantity of PCR product from the second sequence (whose copy number is known). This obviates the requirement to know the

exact concentration of DNA used for the PCR reaction. The *TaqMan* QPCR primers and probes were designed using the Primer Express software (Perkin Elmer, Norwalk, CT) and synthesized by Perkin Elmer. The *Dspt6* forward primer was 5′-GCCGCACTCCATGAATCTG-3′, and the reverse primer was 5′-CATTCACTTCGCCGCTCCTA-3′. The *TaqMan* probe was 5′-6-FAM-ATGCCACGCCCCTCTACGACGAG-TAMRA-3′. The *Dspt4* forward primer was 5′-CTCGTGGTATCTATGC CATTTCTG-3′, and the reverse primer was 5′-TCCACGATTC TTCATGTCACGTA-3′. The *TaqMan* probe was 5′-6-FAM-TCCGGAACACTGCCGCAGTCTACC-TAMRA. The *swa* forward primer was 5′-CGCTGCCGTAATGGTGATC-3′, and the reverse primer was 5′-GGCATCGCAGGCAAACC-3′. The *TaqMan* probe was 5′-6-FAM-TTGAACCGCAGCAATGTCACATA CAAGG-TAMRA-3′. The *Dspt6* forward primer for reverse transcription (RT)-QPCR was 5′-GGAGAATCTGGGCGTCAA AGT-3′, and the reverse primer was 5′-CGCTTTCGTTGT CGTGGAT-3′. The *TaqMan* probe was 5′-6-FAM-AGACGCTT GAACCGCCGTCTCTCG-TAMRA-3′. The *Dspt4* forward primer for RT-QPCR was 5′-TTGACGCGATACCCAAGGAT-3′, and the reverse primer was 5′-CTAGTGTGATCATAGACAT TGTCCTTGTT-3′. The *TaqMan* probe was 5′-6-FAM-CTCA A A T T G A T C A A A A C T C T T C A C T A G G G A G C A A A-TAMRA-3′. The *TaqMan* RT-QPCR and Core Reagent PCR kits (with MultiScribe reverse transcriptase and *AmpliTaq* Gold enzyme) were used for the analysis. The *TaqMan* RT-QPCR and QPCR reactions were performed on the ABI PRISM 7700 sequence detection system, using conditions suggested by Perkin Elmer. DNA samples for the PCR analysis were isolated by proteinase K/SDS overnight treatment followed by phenol/chloroform extraction. Ten nanograms of DNA was used for the highest concentration of each dilution. RNA samples for the PCR analyses were isolated from adult female flies using the TRIzol method (GIBCO-BRL, Gaithersburg, MD). Total RNA (1 ng) was used for the highest concentration of each dilution in the RT-PCR reaction.

**Fly strains:** *Df(1)JF5/FM7*, *Df(1)G4e$^{L}$H24i$^{R}$/FM7a*, *Df(1)N73/FM6*, and *Df(1)5D/FM6* were obtained from the Bloomington Stock Center. *y$^{1}$ w$^{1}$; P{w$^{+mC}$ = EP}EP2604/CyO* was obtained from the Berkeley Drosophila Genome Project stock center. Flies were handled according to standard procedures. Flies with *Dspt4* deficiencies were generated by the imprecise excision of a revertible *P*-element insertion in *y$^{1}$ w$^{1}$; P{w$^{+mC}$ = EP}EP2604/CyO*. *y$^{1}$ w$^{1}$; P{w$^{+mC}$ = EP}EP2604/CyO* was mobilized to generate imprecise excisions as follows: *y$^{1}$ w$^{1}$; P{w$^{+mC}$ = EP}EP2604/CyO* males were crossed to *y$^{1}$ w$^{1}$; Ki$^{1}$ P{ry$^{+t7.2}$ = Delta2-3}99B/TM3, Sb$^{1}$* females. *y$^{1}$ w$^{1}$; P{w$^{+mC}$ = EP}EP2604/+; Ki$^{1}$ P{ry$^{+t7.2}$ = Delta2-3}99B/+* males were individually crossed to *y$^{1}$ w$^{1}$; T(2;3)ap$^{Xa}$/CyO* females. Individual white-eyed *y$^{1}$ w$^{1}$; Df/CyO* males were crossed back to *y$^{1}$ w$^{1}$; T(2;3)ap$^{Xa}$/CyO* females to establish stocks. Stocks lacking non-Curly flies were kept for further analysis.

**TABLE 1**

***TaqMan* quantitative PCR mapping of the *swa*, *Dspt6*, and *Dspt4* copy number in different Drosophila**

| A. Fly | *Dspt6*/*Dspt4* | Conclusion |
|---|---|---|
| Wild-type female | 1.1 | Normal |
| 1:1 dilution | 1.0 | |
| 1:2 dilution | 1.1 | |
| 1:4 dilution | 1.2 | |
| Wild-type male | 0.5 | Normal |
| 1:1 dilution | 0.5 | |
| 1:2 dilution | 0.5 | |
| 1:4 dilution | 0.6 | |
| **B. Fly** | ***Dspt4*/*swa*** | **Conclusion** |
| *Df(1)JF5* female | 2.0 | Deletion of *swa* |
| 1:1 dilution | 2.1 | |
| *FM7* male | 2.1 | Normal |
| 1:1 dilution | 2.1 | |
| **C. Fly** | ***Dspt6*/*Dspt4*** | **Conclusion** |
| *Df(1)JF5* female | 1.0 | Normal |
| 1:1 dilution | 0.9 | |
| 1:2 dilution | 1.1 | |
| 1:4 dilution | 0.9 | |
| *Df(1)G4e$^{L}$H24I$^{R}$* female | 0.8 | Normal |
| 1:1 dilution | 0.9 | |
| *Df(1)N73* female | 1.2 | Normal |
| 1:1 dilution | 1.2 | |
| *Df(1)5D* female | 0.6 | Deletion of *Dspt6* |
| 1:1 dilution | 0.6 | |
| *FM6* male | 0.5 | Normal |
| 1:1 dilution | 0.6 | |
| *FM6/FM6* female | 1.0 | Normal |
| 1:1 dilution | 1.0 | |
| **D. Fly** | ***Dspt6*/*Dspt4*** | **Conclusion** |
| *Df(2)Dspt4-11* female | 2.0 | Deletion of *Dspt4* |
| 1:1 dilution | 2.0 | |
| *Df(2)Dspt4-11* male | 1.1 | Deletion of *Dspt4* |
| 1:1 dilution | 1.1 | |
| *Df(2)Dspt4-14* female | 2.0 | Deletion of *Dspt4* |
| 1:1 dilution | 2.0 | |
| *Df(2)Dspt4-14* male | 0.9 | Deletion of *Dspt4* |
| 1:1 dilution | 0.8 | |

Four or two twofold dilutions of genomic DNA were analyzed in the experiments.

## RESULTS

**Testing the *TaqMan* QPCR mapping strategy:** We had previously developed a fluorescent QPCR-based method to determine gene copy number (Chiang *et al.* 1996, 1999; Cairns *et al.* 1998). Given the need for rapid and accurate mapping of Drosophila deficiency chromosomes, we tested the *TaqMan* QPCR methodology (Higuchi *et al.* 1992; Livak *et al.* 1995; Heid *et al.* 1996) for this purpose. We first tested the *TaqMan* QPCR strategy on the dosage of the sex chromosomes, requiring differentiation between one (male) and two (female) copies per genome. *TaqMan* QPCR was performed on the samples using two different sets of primers, one set for the *X*-linked gene, *Dspt6*, and one set for the autosomal gene, *Dspt4*. The ratio of QPCR for *Dspt6*/*Dspt4* reflects the copy number ratio of the *X*-linked gene, *Dspt6*, to the autosomal gene, *Dspt4*. This ratio should be 1 for wild-type female flies and 0.5 for wild-type male flies. This is the case, as demonstrated by the control experiments in Table 1A. Each control consisted of a set of four serial dilutions assayed for both *Dspt6* and *Dspt4*. *TaqMan* QPCR successfully detected a twofold difference between the dilutions. Furthermore, as ex-

pected, the *Dspt6*/*Dspt4* ratio for the wild-type female fly was 1, and the ratio for the wild-type male fly was 0.5 (Table 1A). We then tested a known deletion of the *swallow* (*swa*) gene on the X chromosome. *swa* was previously shown to be deleted in *Df(1)JF5* (Stephenson and Mahowald 1987). As shown by our *TaqMan* QPCR analysis, the *Dspt4*/*swa* ratio for the *Df(1)JF5* female was 2, and the *Dspt4*/*swa* ratio for the *FM7* male was also 2 (Table 1B). These control experiments showed that *TaqMan* QPCR is suitable for determining copy number in Drosophila.

***TaqMan* QPCR mapping of *Dspt6*-deficient flies:** *Dspt6* mapped between 5E1 and 5E8 on the X chromosome (P.-W. Chiang, unpublished result). According to FlyBase (http://flybase.bio.indiana.edu:82/maps/fbgrmap.html), the 5E6 to 5E8 region is deleted in the deficiency fly *Df(1)JF5*, 5E3 to 5E8 to 6B is deleted in the deficiency fly *Df(1)G4e$^{L}$H24i$^{R}$*, 5C2–5D6 is deleted in the deficiency fly *Df(1)N73*, and 5D1 to 5E is deleted in the deficiency fly *Df(1)5D*. We applied *TaqMan* QPCR to female *Df(1)JF5*, *Df(1)G4e$^{L}$H24i$^{R}$*, *Df(1)N73*, and *Df(1)5D* flies. We also tested the nonfertile *FM6*/*FM6* female and the *FM6* male. The *Dspt6*/*Dspt4* ratio was close to 1 for female *Df(1)JF5*, *Df(1)G4e$^{L}$H24i$^{R}$*, *Df(1)N73*, and *FM6*/*FM6* flies, and the *Dspt6*/*Dspt4* ratio was close to 0.5 for male *FM6* flies. However, the *Dspt6*/*Dspt4* ratio was close to 0.5 for female *Df(1)5D* flies, showing a deletion of *Dspt6* in *Df(1)5D* (Table 1C). Thus, *TaqMan* QPCR mapping placed *Dspt6* in the 5E1 to 5E6 region covered by the deletion in *Df(1)5D*.

***TaqMan* QPCR mapping of *Dspt4*-deficient flies:** According to the BDGP database, a *P* element is inserted into an intron of *Dspt4* in *EP(2)2604* (P.-W. Chiang, unpublished result). *EP(2)2604* was mapped between 49B7 and 49B11. The homozygous *P*-element insertion flies are semilethal. Two Dspt4 deficiency flies, *Df(2)Dspt4-11* and *Df(2)Dspt4-14*, were generated through imprecise *P*-element excision from the *EP(2)2604* fly. Both *Df(2)Dspt4-11* and *Df(2)Dspt4-14* could not complement *EP(2)2604*. We then applied *TaqMan* QPCR to female and male *Df(2)Dspt4-11* and *Df(2)Dspt4-14* flies. In both cases, the *Dspt6*/*Dspt4* ratio for the female fly was close to 2, and this ratio for the male fly was close to 1 (Table 1D). Therefore, *TaqMan* QPCR confirmed that one copy of *Dspt4* is deleted in both heterozygous deficiency flies.

**Analysis of transcriptional dosage by *TaqMan* RT-QPCR:** *TaqMan* RT-QPCR was performed to determine whether the genomic deletion of *Dspt4* or *Dspt6* in the deficiency flies resulted in transcriptional dosage. In this experiment, we measured the relative amount of *Dspt4* mRNA against the amount of *Dspt6* mRNA. To avoid contamination from any genomic DNA present in the RNA preparation, primer sets specific for *Dspt4* and *Dspt6* mRNA were used in the analysis. These sets used at least one primer bridging two adjacent exons of the gene, eliminating amplification by genomic sequences containing the intervening intron. As expected, no amplification was detected using a DNA template for either primer set (data not shown). As shown in Table 2, the mRNA-derived ratio of *Dspt6*/*Dspt4* was close to 1 for the wild-type fly. The *Dspt6*/*Dspt4* ratio was close to 2 for *Df(2)Dspt4-11* and *Df(2)Dspt4-14* flies. This result was independent of the sex of the fly that was utilized, reflecting dosage compensation for the *Dspt6* gene on the X chromosome. Furthermore, the *Dspt6*/*Dspt4* ratio was close to 0.5 for the *Df(1)5D* fly. This RT-QPCR analysis confirmed that the deletion of a single genomic copy of *Dspt4* or *Dspt6* in either of these deficiency flies resulted in a concomitant decrease of *Dspt4* or *Dspt6* mRNA transcript.

**TABLE 2**

***TaqMan* quantitative RT-PCR of the *Dspt6*/*Dspt4* copy number in Drosophila**

| Fly | *Dspt6*/*Dspt4* | Conclusion |
|---|---|---|
| Wild-type female | 1.0 | Normal |
| 1:1 dilution | 1.1 | |
| Wild-type male | 1.2 | Normal |
| 1:1 dilution | 1.2 | |
| *Df(2)Dspt4-11* female | 1.8 | Deletion of *Dspt4* |
| 1:1 dilution | 1.9 | |
| *Df(2)Dspt4-11* male | 2.0 | Deletion of *Dspt4* |
| 1:1 dilution | 2.4 | |
| *Df(2)Dspt4-14* female | 2.3 | Deletion of *Dspt4* |
| 1:1 dilution | 2.2 | |
| *Df(2)Dspt4-14* male | 2.4 | Deletion of *Dspt4* |
| 1:1 dilution | 2.2 | |
| *Df(1)5D* female | 0.5 | Deletion of *Dspt6* |
| 1:1 dilution | 0.5 | |

## DISCUSSION

Because this is the first application of *TaqMan* QPCR for Drosophila, we tested several factors essential for the success of this mapping strategy. Dosage for the *X*-linked gene, *Dspt6*, with a known copy number difference between males and females, was repeated four times at different dilutions, and the data were highly reproducible (Table 1A). In this analysis, twofold dilutions of each sample were tested, and the technique readily detected a twofold difference. As expected, the relative ratio of *Dspt6*/*Dspt4* for a wild-type female is 1, and the relative ratio of *Dspt6*/*Dspt4* for a wild-type male is 0.5. We also tested a known deletion of the *swa* gene on the X chromosome (Stephenson and Mahowald 1987), and *TaqMan* QPCR successfully detected the deletion (Table 1B). Thus, *TaqMan* QPCR is highly reproducible with the ability to resolve twofold differences in Drosophila.

We then used *TaqMan* QPCR to determine the copy number of *Dspt6* in specific flies. The transmission rate

of *Dp(1;f)J21A* through females to progeny is 28%, and *Df(1)JF5* weakly increases this transmission rate (Cook *et al.* 1997). Furthermore, yeast *SPT6* was shown to be involved in faithful chromosome segregation (Basrai *et al.* 1996). This suggested that *Dspt6* might be deleted in the deficiency line *Df(1)JF5*. However, our *TaqMan* QPCR mapping data demonstrated that *Dspt6* is not deleted in *Df(1)JF5*. We also showed that there is no *Dspt6* deletion in *Df(1)G4e$^{L}$H24i$^{R}$*, *Df(1)N73*, and *FM6*/*FM6*. In contrast, *Dspt6* is deleted in *Df(1)5D*. This placed *Dspt6* between 5E1 and 5E6, illustrating the utility of developing the QPCR method as a fast and accurate way to map quantitative alterations in the Drosophila genome.

To further illustrate the applicability of *TaqMan* QPCR for mapping, we analyzed the relative ratio of *Dspt6*/*Dspt4* for two deficiency flies that we constructed. *Df(2)Dspt4-11* and *Df(2)Dspt4-14* were generated through imprecise *P*-element excision from *EP(2)2604*. The homozygous deficiency is lethal for both the *Df(2)Dspt4-11* and *Df(2)Dspt4-14* flies, and both deficiency flies could not complement *EP(2)2604*. Our *TaqMan* QPCR mapping demonstrates the deletion of *Dspt4* in these two flies, consistent with the complementation data.

The amount of transcript generated from any specific gene generally falls within the limits of a direct correlation with its chromosomal dose. With a direct correlation, a chromosomal reduction would result in 50% of normal expression. From our *TaqMan* RT-QPCR analysis, the regulation of *Dspt4* or *Dspt6* expression correlated directly with their gene dosage. A reduction of 50% from the normal expression level of *Dspt4* occurred in both *Df(2)Dspt4-11* and *Df(2)Dspt4-14*, and a reduction of 50% from the normal expression level of *Dspt6* occurred in *Df(1)5D* (Table 2).

This *TaqMan* QPCR-based approach will be useful to establish sequence copy number in the Drosophila genome. Although this task can also be accomplished by *in situ* hybridization, the QPCR mapping approach is easier, faster, cheaper, and detects deletions at the $10^2$-nucleotide level rather than at the $10^3$-nucleotide level required for *in situ* hybridization. Thus, the molecular order of gene deletions could be determined more precisely in any deficiency fly using the *TaqMan* QPCR approach. This approach will become even more useful as the genomic sequences are elaborated by the Drosophila Genome Project. The need for a simple and efficient method to map deficiency flies more precisely down to the level of specific genes will become especially useful. Using *Dspt4*, *Dspt6*, and *swa*, we demonstrated that the *TaqMan* QPCR approach represents a valuable tool for merging this initiative with the mapping of gene deletions in deficiency flies. Furthermore, *TaqMan* RT-QPCR represents a facile tool for monitoring the expression of genes associated with perturbations resulting in changes of gene copy number.

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