9-30-1998

The mouse deafness locus (dn) is associated with an inversion on chromosome 19

Ana M. Viñas
University Medical Center New Orleans

Stacy S. Drury
Louisiana Stt. Univ. Med. C.

Margaret M. Deangelis
Louisiana Stt. Univ. Med. C.

Zhining Den
Louisiana Stt. Univ. Med. C.

Jer Min Huang
University Medical Center New Orleans

See next page for additional authors

Recommended Citation

Viñas, A., Drury, S., Deangelis, M., Den, Z., Huang, J., Berlin, C., Hunt, J., Batzer, M., Deininger, P., & Keats, B. (1998). The mouse deafness locus (dn) is associated with an inversion on chromosome 19. *Biochimica et Biophysica Acta - Molecular Basis of Disease, 1407* (3), 257-262. https://doi.org/10.1016/S0925-4439(98)00050-7

ELSEVIER

Biochimica et Biophysica Acta 1407 (1998) 257–262

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Rapid report

The mouse *deafness* locus (*dn*) is associated with an inversion on chromosome 19

Ana M. Viñas [a], Stacy S. Drury [b], Margaret M. DeAngelis [b, d, f], Zhining Den [b], Jer-Min Huang [c, h], Charles I. Berlin [c, e, h], Jay D. Hunt [a, e, g], Mark A. Batzer [a, b, d, e, f, g], Prescott L. Deininger [a, e, f, g], Bronya J.B. Keats [b, c, e, f, g, h, *]

[a] *Department of Biochemistry and Molecular Biology, Louisiana State University Medical Center, New Orleans, LA 70112, USA*
[b] *Department of Biometry and Genetics, Louisiana State University Medical Center, 1901 Perdido Street, New Orleans, LA 70112, USA*
[c] *Department of Otorhinolaryngology, Louisiana State University Medical Center, New Orleans, LA 70112, USA*
[d] *Department of Pathology, Louisiana State University Medical Center, New Orleans, LA 70112, USA*
[e] *Center for Molecular and Human Genetics, Louisiana State University Medical Center, New Orleans, LA 70112, USA*
[f] *Neuroscience Center of Excellence, Louisiana State University Medical Center, New Orleans, LA 70112, USA*
[g] *Stanley S. Scott Cancer Center, Louisiana State University Medical Center, New Orleans, LA 70112, USA*
[h] *Kresge Hearing Research Laboratory, Louisiana State University Medical Center, New Orleans, LA 70112, USA*

Received 21 May 1998; accepted 22 June 1998

Abstract

Recombination data for the mouse *deafness* locus (*dn*) on chromosome 19 are consistent with the presence of an inversion for which one of the breakpoints is between *D19Mit14* and *D19Mit96*, a distance of less than 226 kb. Fluorescence in situ hybridization studies using a bacterial artificial chromosome on interphase (G_1) nuclei provide additional support for the presence of an inversion. The *dn* gene is probably the orthologue of the human DFNB7/DFNB11 gene on chromosome 9.

Keywords: Non-syndromic hearing impairment; *Deafness*; Mouse; Chromosome 19; Inversion

The deafness (*dn*/*dn*) mouse has profound sensorineural hearing impairment with early degeneration of the organ of Corti, stria vascularis, and occasionally the saccular macula. Deol and Kocher [1] described it as an autosomal recessive mutant of the curly-tail stock, and they found no other associated phenotypic anomalies. Hair cells of the organ of Corti show abnormal development and degeneration by 15 days after birth [2,3], and Webster [4] demonstrated that by 45 days after birth all cell types in the organ of Corti have degenerated completely. Interestingly, he found evidence of regeneration of support cells but not hair cells in the apical turn between 45 and 90 days after birth. Physiological studies show that deafness (*dn*/*dn*) mice do not produce an auditory brainstem response (ABR) [5] and they have no spontaneous activity of cochlear origin [6]. Keats et al. [7] mapped the *dn* locus to mouse chromosome 19 by analysis of 230 intersubspecific backcross progeny from the mating (*Mus musculus molossinus* × *dn*/*dn*)F_1 × *dn*/*dn* (designated as the *MOLF* cross). Each offspring was genotyped for 66 microsatellite

* Corresponding author, at address b. Fax: (504) 5688500; E-mail: biombjk@lsumc.edu

0925-4439/98/$ – see front matter
PII: S0925-4439(98)00050-7

markers spanning the mouse genome and no recombinants were found between the *dn* locus and the marker *D19Mit14*. This region of mouse chromosome 19 is homologous to human chromosome 9q13-q21, and two loci for autosomal recessive non-syndromic hearing loss, DFNB7 and DFNB11, have been mapped to this region [8,9]. Thus, the *dn* gene is likely to be the mouse orthologue of one of these human genes. In this paper, we demonstrate that the *dn* locus is associated with an inversion on mouse chromosome 19 based on (1) additional linkage data and (2) fluorescence in situ hybridization (*FISH*) of interphase nuclei using a bacterial artificial chromosome (BAC) spanning one of the breakpoints.

The 230 backcross offspring produced from the *MOLF* cross [7] were genotyped for 11 DNA markers (*D19Mit128*, *D19Mit129*, *D19Mit41*, *D19Mit110*, *D19Mit16*, *D19Mit23*, *D19Mit60*, *D19Mit14*, *D19Mit96*, *D19Mit111*, *D19Mit45*) that are closely linked to *D19Mit14*. Primers for these dinucleotide repeat polymorphisms were obtained from Research Genetics, and the protocol for genotyping was described previously [7]. A second set of 124 progeny was obtained from matings between heterozygous (+/*dn*) and homozygous (*dn*/*dn*) curly-tail mice (designated as the *CT* cross) because different allele sizes were found in curly-tail +/+ and *dn*/*dn* mice for seven of the markers in the vicinity of *D19Mit14*. (The allele sizes were the same for *D19Mit60*, *D19Mit23*, *D19Mit128*, and *D19Mit129*, as well as for 66 markers spanning the genome.) Each of these progeny was classified as hearing or deaf by performing an ABR test following the protocol described by Huang et al. [10]. Briefly, the mouse was anesthetized intraperitoneally and ABRs were evoked by 50 μs alternating polarity clicks at 90 dB peak sound pressure generated from a Nicolet Pathfinder II. The acoustic stimuli were presented at a rate of 27.7/s and delivered to a Realistic Minimus 7 Speaker. Then a latency-intensity function was approximated by dropping the intensity of the clicks until the responses fell below a 0.05 μV peak-to-peak value. After the ABRs were completed, the mice were sacrificed, and DNA was extracted from one of the kidneys and genotyped for the seven informative markers.

No recombinants were found between the *dn* locus and the markers *D19Mit41*, *D19Mit110*, *D19Mit16*, *D19Mit23*, *D19Mit60*, *D19Mit14* for the sets of offspring from either the *MOLF* or the *CT* crosses (*D19Mit23* and *D19Mit60* were not informative for the *CT* cross). Among the 230 *MOLF* backcross progeny, three recombination events were observed between *dn* and *D19Mit128*, *D19Mit129*, and two between *dn* and *D19Mit96*, *D19Mit111*. (Two recombination events between *dn* and *D19Mit41* previously reported [7] were found to be genotyping errors when all samples were reanalyzed.) Similarly, for the progeny from the *CT* cross, one recombinant out of 124 progeny was found between *dn* and *D19Mit96*, *D19Mit111*. (*D19Mit128* and *D19Mit129* were not informative for the *CT* cross.) In contrast, the map generated from 92 meioses (46 intercross offspring) using *Mus musculus castaneus* (designated as the *CAST* cross) [11] shows no recombination between *D19Mit14* and *D19Mit96* but has a distance of approx. 3 cM between *D19Mit41* and *D19Mit14* (Fig. 1). The difference in the two maps for this interval is highly significant ($P < 0.01$). For the flanking regions on chromosome 19 and also for other chromosomes, the maps for the *MOLF* cross tend to be longer than those constructed from other crosses, a result that is in agreement with Reeves et al. [12]. Another map that includes this region of chromosome 19 [13] shows less than 4% recombination between markers that are at least 8 cM apart in the maps constructed from the *MOLF* and the *CAST* crosses. The lack of recombination across this interval in the progeny

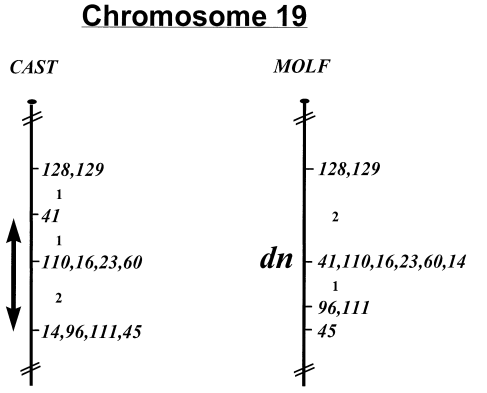

Fig. 1. Linkage maps constructed from the progeny of the *MOLF* and *CAST* crosses. The map from the *CT* cross is identical to that from the *MOLF* cross for those markers that are informative. The arrow delineates the region that is inverted on the *dn* chromosome.

from the crosses involving the *dn*/*dn* mouse is consistent with the presence of an inversion, or similar rearrangement that suppresses recombination, on the *dn* chromosome relative to the normal (±) curly-tail chromosome as well as to the *M. musculus molossinus* chromosome. An inversion would also explain the different allele sizes between the *dn*/*dn* and +/+ curly-tail mice for markers in this interval, but not elsewhere in the genome.

Screening of the Whitehead mouse YAC library with markers from across the region identified a 460 kb YAC (91C10) that was positive for both *D19Mit14* and *D19Mit96*. This result demonstrates that *D19Mit14* and *D19Mit96* are probably within an interval of less than 460 kb and is consistent with the lack of recombination between these markers in the map from the *CAST* cross [11]. Combining this result with the linkage data from the crosses involving *dn*/*dn* suggested that one of the breakpoints of the inversion may be between *D19Mit14* and *D19Mit96*, and a minimal tiling path of BACs was constructed in this region.

Clones were isolated using polymerase chain reaction (PCR) analysis from a total mouse genomic BAC library commercially available from Research Genetics using the methods described by DeAngelis et al. [14]. The libraries were screened with the three markers *D19Mit14*, *D19Mit96*, and *D19Mit111* using the PCR conditions described in Keats et al. [7]. To identify possible overlaps, all BACs were amplified using inter B1 and B2 repetitive element PCR primers [15]. PCR products from combinations of B1 and B2 as well as only B1 and B2 amplifications were compared to identify similar fragments as an indicator of overlap. *Eco*RI restriction digest patterns were also analyzed to help identify overlaps and they were confirmed by Southern blot analysis [16]. The BACs were digested with *Eco*RI, electrophoresed, and transferred by capillary action onto HyBond N^+ positively charged nylon membrane (Amersham). Hybridization was carried out in 0.5 M sodium phosphate, 7% SDS, 0.001 M EDTA with 1 μg/ml of salmon sperm DNA and 0.25 μg/ml of mouse Cot-1 DNA (BRL) at 65°C overnight. The inter B1 and B2 combined repetitive element PCR products were radiolabeled with ^{32}P by random priming using the rediprime DNA labeling system from Amersham and used to probe the blots. Washes were 20 min in 2×SSC+0.1% SDS at room temperature, 20 min in 2×SSC+0.1% SDS at 65°C, 20 min in 1×SSC+0.1% SDS at 65°C, and 10 min in 0.5×SSC+0.1% SDS at 65°C. Individual BAC clones were sized by digestion with the restriction enzyme *Not*I and fractionation on 1% agarose gels using a clamped homogeneous electric field (CHEF) mapper (BioRad) as previously described [14,17]. After confirmation of overlap by Southern blot analysis, a minimal BAC contig across the presumed breakpoint was established (Fig. 2). BACs 183G22 (160.5 kb) and 124J3 (93.6 kb) were both positive for *D19Mit14* and they were shown to overlap by Southern blot analysis. Similarly, BAC 124J3 was confirmed to overlap with BAC 222M20 (132.0 kb) which is positive for *D19Mit96*. Thus, the maximum distance between *D19Mit14* and *D19Mit96* is less than 226 kb. Finally, BAC 222B7 overlapped with both BAC 124J3 and BAC 236G18 which is positive for *D19Mit111*. Thus, a minimal contig was constructed spanning the markers thought to flank one of the inversion breakpoints, and utilized for *FISH* analysis.

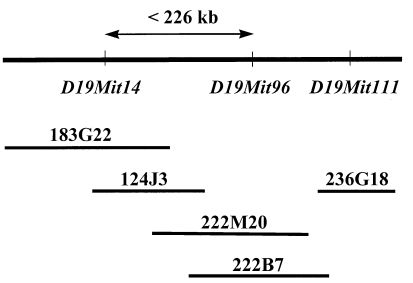

Fig. 2. BAC tiling path across the region encompassing *D19Mit14*, *D19Mit96*, and *D19Mit111* which contains one of the breakpoints of the inversion.

Four of these BACs (183G22, 124J3, 222B7, 222M20) were labeled by nick translation with biotin-11-dUTP or digoxigenin-11-dUTP (Boehringer) using an Oncor nick translation kit. Labeled DNA was combined with mouse Cot-1 DNA in a 1:200 ratio and the probe/blocking DNA mixture was completely dried down and resuspended in 50% formamide (in 2×SSC). Chromosome spreads were prepared by standard procedures [18], and slides were aged at room temperature for 1–2 weeks before use. Following denaturation in 70% formamide in 2×SSC (pH 7.0) at 70°C, the slides were dehydrated in a graded ethanol series 70%, 80%, and 95% at

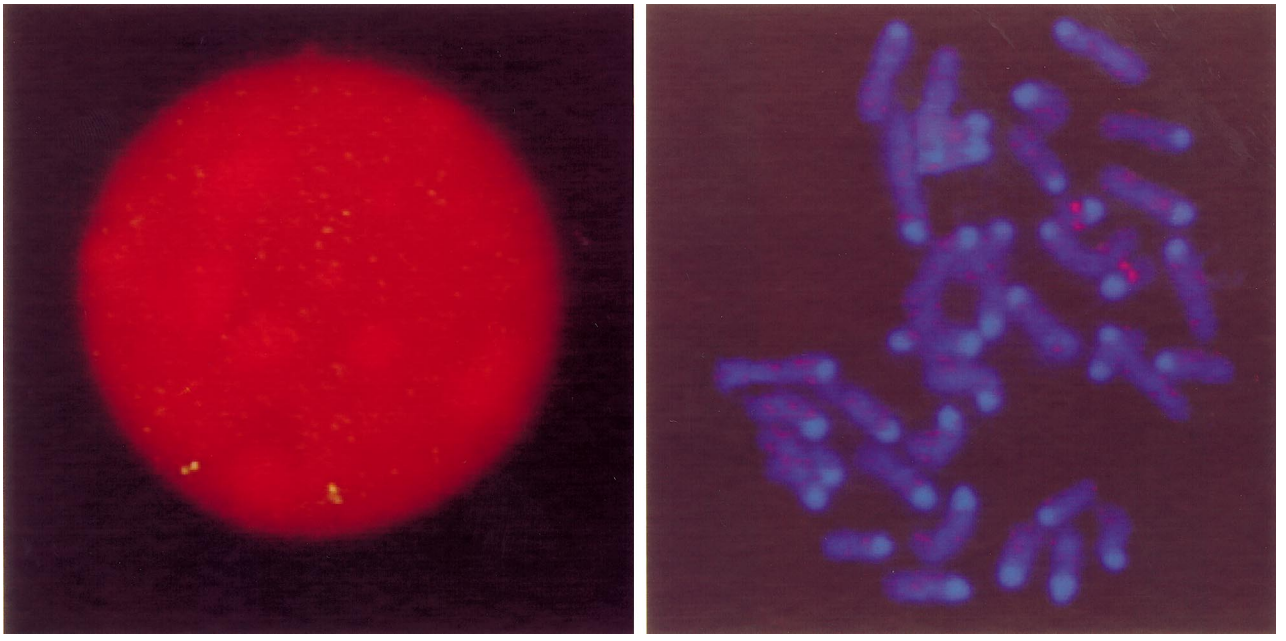

Fig. 3. Interphase (left panel) and metaphase (right panel) *FISH* using BAC 124J3 as the probe on cells from *dn*/*dn* mice. BAC 124J3 was labeled with biotin (left panel) or digoxigenin (right panel) and secondarily labeled with FITC or rhodamine, respectively. The interphase *FISH* was background stained with propidium iodide and the metaphase spreads were counter-stained with DAPI. The interphase image shows two closely spaced pairs of dots, consistent with separation of regions of homology with the probe on the mouse chromosomes. The metaphase shows single hybridizations per chromosome consistent with the lower resolution on condensed chromosomes. Images were collected with a 100× Plan-Achromat lens and a Cohu CCD video camera.

−20°C for 2 min each and dried before hybridization. The hybridization mixture containing 300 ng of labeled probe in 30 μl was denatured at 70°C for 5 min and applied to the slides. The hybridization was performed overnight in a moist chamber at 37°C. The slides were washed in 2×SSC (pH 7.0) at 70°C for 5 min. The signal detection and amplification were done using fluorescein or rhodamine detection kits (Oncor). Biotin-labeled probes were detected with FITC and digoxigenin-labeled probes with rhodamine. Slides were counterstained with propidium iodide or DAPI and photographed with a Zeiss Axioskop.

Metaphase in situ hybridization with the four BACs showed a strong signal on chromosome 19 in both normal (+/+) and deaf (*dn*/*dn*) curly-tail mice. (BAC 124J3 is shown in Fig. 3.) Using metaphase chromosomes, individual probe signals can generally be resolved if they are at least a megabase apart. Because no signal differences were detected between metaphase spreads for +/+ and *dn*/*dn* mice, hybridization patterns were visualized on interphase nuclei, which are less condensed and have a resolution of 50–100 kb. Samples of interphase nuclei from +/+ and *dn*/*dn* curly-tail mice were examined and the numbers of cells with one, two, three, and four spots for BACs 124J3 and 222B7 are shown in Table 1. (Fig. 3 shows a cell with four spots for BAC 124J3.) Although the results are complicated because not every potential locus is detected in every cell and

Table 1
Number of cells from *dn*/*dn* and +/+ curly-tail mice with one, two, three, and four spots after interphase *FISH* using biotin-labeled or digoxigenin-labeled BAC 124J3 and BAC 222B7 and detected with FITC or rhodamine, respectively

	Number of spots per cell			
	1	2	3	4
BAC 124J3				
dn/*dn*	15	52	21	12
+/+	48	40	10	2
BAC 222B7				
dn/*dn*	23	42	5	0
+/+	12	25	3	0

also because a few S or G_2 phase cells may have replicated, these results suggest that BAC 124J3 contains one of the inversion breakpoints. In +/+ mice, most of the nuclei had one or two spots, with only a small percentage having three or four spots. In contrast, a much greater percentage of the cells from *dn*/*dn* mice had three or four spots. A χ^2 test for this 2×4 contingency table gave a value of 29.9 with 3 degrees of freedom which has a probability of less than 0.001. Thus, the differences in the number of cells with one, two, three, and four spots in the two types of mice are highly significant. On the other hand, for BAC 222B7 (and also 183G22 and 222M20) the majority of cells have one or two spots for both +/+ and *dn*/*dn* mice, and the differences are not significant. These results provide additional support for the presence of a breakpoint in BAC 124J3.

The combination of linkage analyses and *FISH* studies provides convincing evidence for the presence of an inversion that is associated with a mutation at the *dn* locus. The marker allele differences between the curly-tail + and *dn* chromosomes in the vicinity of this inversion provided the necessary variation to test the hypothesis that the *dn* chromosome is inverted relative to the + chromosome. The linkage results are consistent with this hypothesis because no recombination was observed over a 3–4 cM interval. The *FISH* results add support for the presence of an inversion, although we cannot rule out a rearrangement that is more complex than a simple inversion.

While the linkage and *FISH* results demonstrate the presence of an inversion junction within the BAC 124J3 region, they do not pinpoint the location of the *dn* gene. An attractive hypothesis is that one of the breakpoints of the inversion caused the mutation; however, the *dn* gene could be located anywhere within the inversion. The different marker alleles on the curly-tail + chromosome versus the *dn* chromosome suggest that the *dn* mutation arose before the curly-tail inbred stock was established. With many generations of inbreeding and forced selection to maintain both + and *dn* alleles in the colony, the different alleles at markers within the inversion, and just outside it, would be maintained because of the lack of recombinant offspring. This unexpected variation allowed us to demonstrate that the inversion is not a characteristic of chromosome 19 in the curly-tail mouse relative to *M. musculus molossinus*.

The interval containing the *dn* gene is homologous to the region of human chromosome 9 to which two non-syndromic hearing impairment loci (DFNB7 and DFNB11) have been mapped. Greinwald et al. [19] published a contig spanning this region and excluded two candidate genes. The possibility that one of these hearing impairment genes may be the human homologue of the mouse *dn* gene will facilitate the refinement of the location and identification of the *dn* gene on mouse chromosome 19.

This work was supported by US Public Health Service grant DC00379, the Foundation Fighting Blindness, and the Marriott Foundation.

References

[1] M.S. Deol, W. Kocher, A new gene for deafness in the mouse, Heredity 12 (1958) 463–466.
[2] G.R. Bock, K.P. Steel, Inner ear pathology in the *deafness* mutant mouse, Acta Otolaryngol. 96 (1983) 39–47.
[3] R. Pujol, A. Shnerson, M. Lenoir, M.S. Deol, Early degeneration of sensory and ganglion cells in the inner ear of mice with uncomplicated genetic deafness (dn): preliminary observations, Hear. Res. 12 (1983) 57–63.
[4] D.B. Webster, Degeneration followed by partial regeneration of the organ of Corti in deafness (*dn*/*dn*) mice, Exp. Neurol. 115 (1992) 27–31.
[5] K.P. Steel, G.R. Bock, The nature of inherited deafness in *deafness* mice, Nature 288 (1980) 159–161.
[6] D. Durham, E. Rubel, K. Steel, Cochlear ablation in *deafness* mutant mice: 2-deoxyglucose analysis suggests no spontaneous activity of cochlear origin, Hear. Res. 43 (1989) 39–46.
[7] B.J.B. Keats, N. Nouri, J.M. Huang, M. Money, D.B. Webster, C.I. Berlin, The *deafness* locus (*dn*) maps to mouse chromosome 19, Mamm. Genome 6 (1995) 8–10.
[8] P.K. Jain, K. Fukushima, D. Deshmukh, R. Arabandi, E. Thomas, S. Kumar, A.K. Lalwani, B. Ploplis, H. Skarka, C.R.S. Srisailapathy, S. Wayne, R.I.S. Zbar, I.C. Verma, R.J.H. Smith, E.R. Wilcox, A human recessive neurosensory nonsyndromic hearing impairment locus is a potential homologue of the murine deafness (*dn*) locus, Hum. Mol. Genet. 4 (1995) 391–394.
[9] D.A. Scott, R. Carmi, K. Elbedour, S. Yosefsberg, E.M. Stone, V.C. Sheffield, An autosomal recessive nonsyndromic hearing loss locus identified by DNA pooling using two inbred Bedouin kindreds, Am. J. Hum. Genet. 59 (1996) 385–391.
[10] J.M. Huang, M.K. Money, C.I. Berlin, B.J.B. Keats, Audi-

tory phenotyping of heterozygous sound-responsive (+/*dn*) and *deafness* (*dn*/*dn*) mice, Hear. Res. 88 (1995) 61–64.

[11] W.F. Dietrich, J. Miller, R. Steen, M.A. Merchant, D. Damron-Boles, Z. Husain, R. Dredge, M.J. Daly, K.A. Ingalis, T.J. O'Connor, C.A. Evans, M.M. DeAngelis, D.M. Levinson, L. Kruglyak, N. Goodman, N.G. Copeland, N.A. Jenkins, T.L. Hawkins, L. Stein, D.C. Page, E.S. Lander, A comprehensive genetic map of the mouse genome, Nature 380 (1996) 149–152.

[12] R.H. Reeves, M.R. Crowley, B.F. O'Hara, J.D. Gearhart, Sex, strain, and species differences affect recombination across an evolutionarily conserved segment of mouse chromosome 16, Genomics 8 (1990) 141–148.

[13] M.E. Rusiniak, E.P. O'Brien, E.K. Novak, S.M. Barone, M.P. McGarry, M. Reddington, R.T. Swank, Molecular markers near the mouse brachymorphic (bm) gene, which affects connective tissues and bleeding time, Mamm. Genome 7 (1996) 98–102.

[14] M.M. DeAngelis, J.P. Doucet, S.S. Drury, S.T. Sherry, M.B. Robichaux, Z. Den, M.Z. Pelias, G.M. Ditta, B.J.B. Keats, P.L. Deininger, M.A. Batzer, Assembly of a high-resolution map of the Acadian Usher syndrome region and localization of the nuclear EF-hand acidic gene, Biochim. Biophys. Acta (1998) in press.

[15] D.H. Kass, M.A. Batzer, Inter-Alu polymerase chain reaction: advancements and applications, Anal. Biochem. 228 (1995) 185–193.

[16] L.K. Ashworth, M. Alegria-Hartman, M. Burgin, L. Devlin, A.V. Carrano, M.A. Batzer, Assembly of high-resolution bacterial artificial chromosome, P1-derived artificial chromosome, and cosmid contigs, Anal. Biochem. 224 (1995) 564–571.

[17] R.S. Hubert, S. Mitchell, X.N. Chen, K. Ekmekji, C. Gadomski, Z. Sun, D. Noya, U.J. Kim, C. Chen, H. Shizuya, M. Simon, P.J. de Jong, J.R. Korenberg, BAC and PAC contigs covering 3.5 Mb of the Down syndrome congenital heart disease region between D21S55 and MX1 on chromosome 21, Genomics 41 (1997) 218–226.

[18] W. Wray, E. Stubblefield, A new method for the rapid isolation of chromosomes, mitotic apparatus, or nuclei from mammalian fibroblasts at near neutral pH, Exp. Cell Res. 59 (1970) 469–475.

[19] J.H. Greinwald, D.A. Scott, J.R. Marietta, R. Carmi, J. Manaligod, A. Bamesh, R.I.S. Zbar, M.L. Kraft, K. Elbedour, Y. Yairi, M. Musy, A.B. Skvorak, G. Van Camp, C.R.S. Srisalapathy, M. Lovett, C.C. Morton, V.C. Sheffield, R.J.H. Smith, Construction of P1-derived artificial chromosome and yeast artificial chromosome contigs encompassing the DFNB7 and DFNB11 region of chromosome 9q13-21, Genome Res. 7 (1997) 879–886.