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A genome-wide, end-sequenced 129Sv BAC library resource for targeting vector construction

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Abstract

The majority of gene-targeting experiments in mice are performed in 129Sv-derived embryonic stem (ES) cell lines, which are generally considered to be more reliable at colonizing the germ line than ES cells derived from other strains. Gene targeting is reliant on homologous recombination of a targeting vector with the host ES cell genome. The efficiency of recombination is affected by many factors, including the isogenicity (H. te Riele et al., 1992, Proc. Natl. Acad. Sci. USA 89, 5128–5132) and the length of homologous sequence of the targeting vector and the location of the target locus. Here we describe the double-end sequencing and mapping of 84,507 bacterial artificial chromosomes (BACs) generated from AB2.2 ES cell DNA (129S7/SvEvBrd-*Hprt*$^{b\text{-}m2}$). We have aligned these BACs against the mouse genome and displayed them on the Ensembl genome browser, DAS: 129S7/AB2.2. This library has an average insert size of 110.68 kb and average depth of genome coverage of 3.63- and 1.24-fold across the autosomes and sex chromosomes, respectively. Over 97% of the mouse genome and 99.1% of Ensembl genes are covered by clones from this library. This publicly available BAC resource can be used for the rapid construction of targeting vectors via recombineering. Furthermore, we show that targeting vectors containing DNA recombineered from this BAC library can be used to target genes efficiently in several 129-derived ES cell lines.

Keywords: BAC library; Gene targeting; 129Sv; Mouse genome

The development of embryonic stem (ES) cell technology has revolutionized biology by providing the means to assess mammalian gene function in vivo and in vitro. ES cells can be manipulated in culture by homologous recombination to generate an almost limitless repertoire of modifications [1]. The first ES cells isolated, and the majority of subsequent isolates, were derived from 129 substrains or from F1 embryos derived from a cross between a 129 substrain and another inbred strain ([2–8], reviewed in [9]).

The efficiency of gene targeting is dependent on both the length and the extent of homology between the targeting vector and the target locus. Identical (isogenic) sequence ensures maximum targeting efficiencies. Targeting vectors should be constructed with genomic DNA that is derived from the same strain if efficient targeting is desired. We recently indexed a small insert targeting vector library that covers approximately 10% of the genes in the genome [10]. To generate a public resource for the entire genome that is appropriately configured for recombineering, we generated a 129Sv bacterial artificial chromosome (BAC) library from AB2.2 ES cell DNA [11]. This BAC library constitutes the first fully end-sequenced and arrayed large insert clone resource designed specifically for gene-targeting experiments.

Results

The goal of this project was to construct an indexed BAC library to facilitate the generation of gene targeting vectors. The fundamental characteristics of this library were

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choice of the appropriate mouse strain, maximal coverage of the genome, indexing, and suitability for recombineering [12–14].

Average insert size, end sequencing, and clone mapping

We generated this BAC library in two segments from separate cloning experiments. Each segment consists of 219 × 384-well plates. Segment I is composed of plates bMQ27–bMQ245, while segment II is composed of plates bMQ246–bMQ464. We double end sequenced all clones, generating 244,222 reads that passed postsequence quality processing. There were 109,217 clones for which we were able to generate quality clipped reads on both ends of the clone, which were then used to align the clones to the genome using SSAHA2 [15]. In total 91% of all reads mapped to the genome, with 67% of reads mapping to a definitive location on the NCBIm33 assembly.

In addition we generated 26,619 reads that were probable best matches to the genome. Using these data we successfully mapped 84,507 clones to the NCBIm33 assembly. The majority of these clones were localized using read pairs that mapped uniquely on both ends of the clone but we were also able to localize some clones using one read that mapped uniquely and one read, on the other end of the clone, that was the probable best genome match to the genome and mapped within 3 SD of the mean insert length of clones from the library and on the strand opposite to the uniquely mapped end read. By calculating the distance between end reads we estimate that the average insert size of clones in this library is 110.68 kb (Fig. 1A), which correlated with an experimentally derived figure of 110 kb (Fig. 1B).

We estimate the genome coverage of this library across the autosomes to be 97% (Table 1). Importantly we determined that 25,177 Ensembl-predicted mouse genes are covered by a BAC clone (99.1%; Supplementary Data 1). The average depth of coverage was 3.64-fold across the autosomes and 1.24-fold across the sex chromosomes. The lower coverage of the sex chromosomes reflects the fact that 1) AB2.2 ES cells are male (XY), and hence each of these chromosomes was not represented as highly in the DNA pool used to construct the library, compared to the diploid autosomes, 2) the assembly status of these chromosomes X and Y in NCBIm33 assembly. We used read mapping data to generate a tile path of clones (Supplementary Data 2) for use in the development of arrays for comparative genomic hybridization [16,17] and for sequence contig construction.

We have generated a DAS source to display these clones on the Ensembl genome browser (www.ensembl.org/Mus_musculus/: DAS: 129S7/AB2.2) (Fig. 2). Clones are displayed as green and pink depending on the orientation of the insert in the vector. End reads appear as gray bars. A DAS link for each clone has been established, which links to a clone order page (www.sanger.ac.uk/cgi-bin/teams/team38/CloneRequest/CloneRequest) and also to the Ensembl trace repository (http://trace.ensembl.org), where the end-read sequences for all 244,222 quality clipped reads have been deposited. FASTA files of these quality clipped reads have also been generated and deposited on the Ensembl ftp site (ftp://ftp.ensembl.org/pub/traces/mus_musculus; file name sanger-mouse-129S7-AB2.2-cloneEnds).

SNP analysis

We used SSAHA-SNP2 [18] to call 222,568 SNPs from end reads by comparing them to the NCBIm33 C57BL/6J assembly (Supplementary Data 3). We validated these SNP calls by

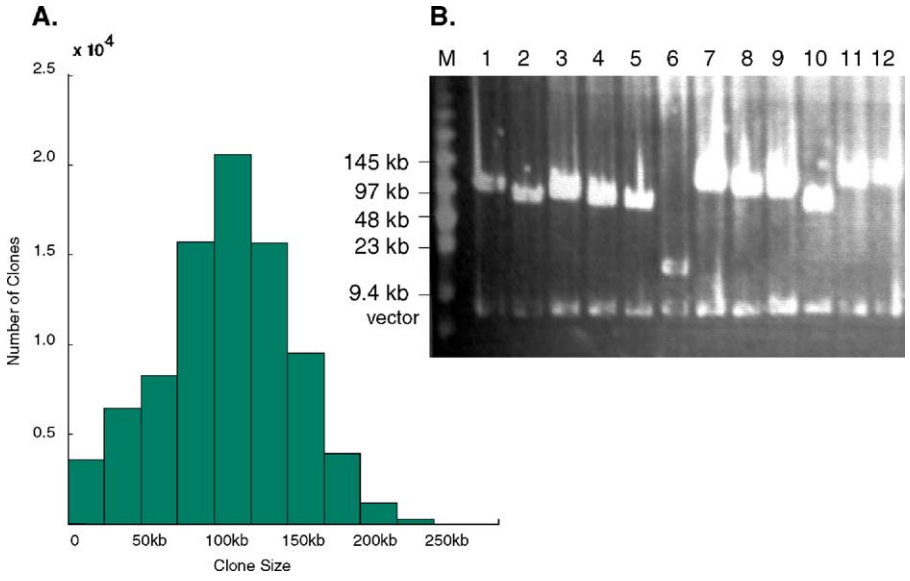

Fig. 1. 129S7/AB2.2 BAC clones have an average insert size of 110.68 kb. (A) Frequency distribution of the insert size of the 84,507 clones mapped to the genome. BAC clones were aligned to the NCBIm33 assembly and the distance between uniquely mapped end-reads was calculated. (B) CHEF gel of *Not*I-digested clones from the library indicating large insert size with an average of approximately 110 kb.

Table 1
The 129S7/AB2.2 library covers over 97% of the mouse genome at an average depth of 3.63-fold

(A) Whole library: plates bMQ27–bMQ464	
All attempted reads	313,089
All reads sequenced successfully	244,222
All templates (clones) present	129,826
All templates sequenced on both strands	109,217
All reads with an alignment to the genome	222,155 (91%)
All aligned reads with a unique match	164,073 (67%)
All aligned reads with probable best match	26,619
All aligned reads with multiple matches	31,463
All templates positioned uniquely using read pairs	84,507 (clones localized using uniquely mapped and best match read pairs)

(B) Chr	Length	Clone length	Depth	% Total coverage
1	195,203,927	195,095,131	3.280	99.944
2	181,686,250	178,477,033	3.918	98.234
3	160,575,607	157,455,046	3.354	98.057
4	154,141,344	151,041,304	3.731	97.989
5	149,219,885	146,042,576	3.838	97.871
6	149,721,531	146,568,255	3.542	97.894
7	133,051,633	129,935,902	3.655	97.658
8	128,688,707	125,532,719	3.776	97.548
9	124,177,049	121,090,951	3.693	97.515
10	130,633,972	127,348,620	3.387	97.485
11	121,648,857	118,303,758	4.340	97.250
12	115,071,072	111,884,675	3.591	97.231
13	116,458,020	113,301,926	3.422	97.290
14	117,079,080	113,906,909	3.448	97.291
15	104,138,553	100,960,960	3.720	96.949
16	98,801,893	95,659,721	3.223	96.820
17	93,559,791	90,443,258	3.668	96.669
18	91,083,707	87,743,163	3.381	96.332
19	60,688,862	57,582,339	3.989	94.881
X	160,634,946	156,967,150	1.556	97.717
Y	47,900,188	44,475,769	0.920	92.851

All data are derived from the NCBIm33 mouse assembly. Length refers to the length of chromosome in bp. Clone length refers to the cumulative length of all clones covering the chromosome. Depth = clone length/length. Coverage is the cumulative length of chromosome (bp) covered by 129S7/AB2.2 BAC clones. % Total coverage is the length of chromosome covered by a 129S7/AB2.2 clone.

designing PCR primers flanking 300 SNPs, amplifying products from 129Sv tail DNA, and resequencing the PCR products. This analysis revealed a computational SNP calling accuracy of 90%.

Targeting vector construction using recombineering

Using recombineering strategies [13] we generated three targeting vectors against three different randomly selected genomic loci: *D11Mit1, Notch1,* and *IgH*. These vectors were used to target these loci in AB2.2 and CJ-7 ES cell lines (Table 2). At the *Notch1* and *IgH* loci high efficiencies of targeting were observed in AB2.2 ES cells. Similarly, highly efficient targeting was observed at the *D11Mit1* locus. Targeting at this locus was comparable in both AB2.2 and CJ-7 cells, despite the genetic differences between these ES cell lines [19]. Although limited to a small number of loci these data confirm the utility of this resource for targeting in AB2.2 ES cells and suggest that this resource will also be useful for targeting vectors for an array of 129-derived ES cell lines.

Discussion

High-quality BAC libraries exist for several mouse strains, including C57BL/6J, MSM/Ms [20,21], C3H/HeJ, NOD, BALB/c, A/J, SPRET/Ei, and AKR/J (www.bacpac.chori.org). These libraries have been shown to be an invaluable resource for assembling genomes and in vivo functional studies, such as BAC rescue [22–24]. Here we describe the generation of an indexed 129Sv BAC library for targeting vector construction, which covers over 97% of the mouse genome and 99.1% of genes (Table 1, Supplementary Data 1). Although other 129Sv BAC libraries have been described [20], this is the first to be fully end sequenced. This resource was aligned against the C57BL/6J mouse genome and is displayed on the Ensembl genome browser, eliminating the need to perform filter hybridizations to isolate clones of interest. By mapping this library against the C57BL/6J genome we were able to call over 222,000 SNPs from the end-read sequences (Supplementary Data 3), which will be invaluable for mapping QTLs observed on a mixed 129Sv × C57BL/6J genetic background. This study brings the total SNP calls we have submitted to dbSNP for 129Sv to over 450,000 [25]. We also show that this resource is useful for the rapid generation of targeting vectors. These vectors were confirmed to target very efficiently in several 129-derived cell lines. Finally, the high density and end-sequence quality of this BAC library make it a useful tool for examining large-scale structural differences between 129Sv and other mouse strain genomes and will greatly facilitate high-throughput targeted manipulation of the mouse genome.

Materials and methods

Construction of the 129Sv BAC library

This BAC library resource was generated from male ES cell DNA taken from AB2.2 (129S7/SvEvBrd-*Hprt*$^{b\text{-}m2}$) ES cells grown as described previously [11]. Seventy-two hours prior to collecting cells to make the library they were passaged onto gelatinized plates without feeders. The cells were passaged again 24 h before collection to remove any residual feeder contamination. This ES cell line was karyotyped and found to be diploid for all chromosomes and to contain both X and Y sex chromosomes (data not shown). We have used this same passage of AB2.2 ES cells to generate several knockout lines obtaining good rates of germ-line transmission. DNA in PFGE agarose blocks prepared from 5×10^7 cells/ml was partially digested with *Sau*3AI and cloned into *Bam*HI-linearized pBACe3.6 [26].

The ligation was electroporated into DH10B cells and plated on LB agar containing 5% sucrose and 11.5 μg/ml chloramphenicol. Recombinant clones

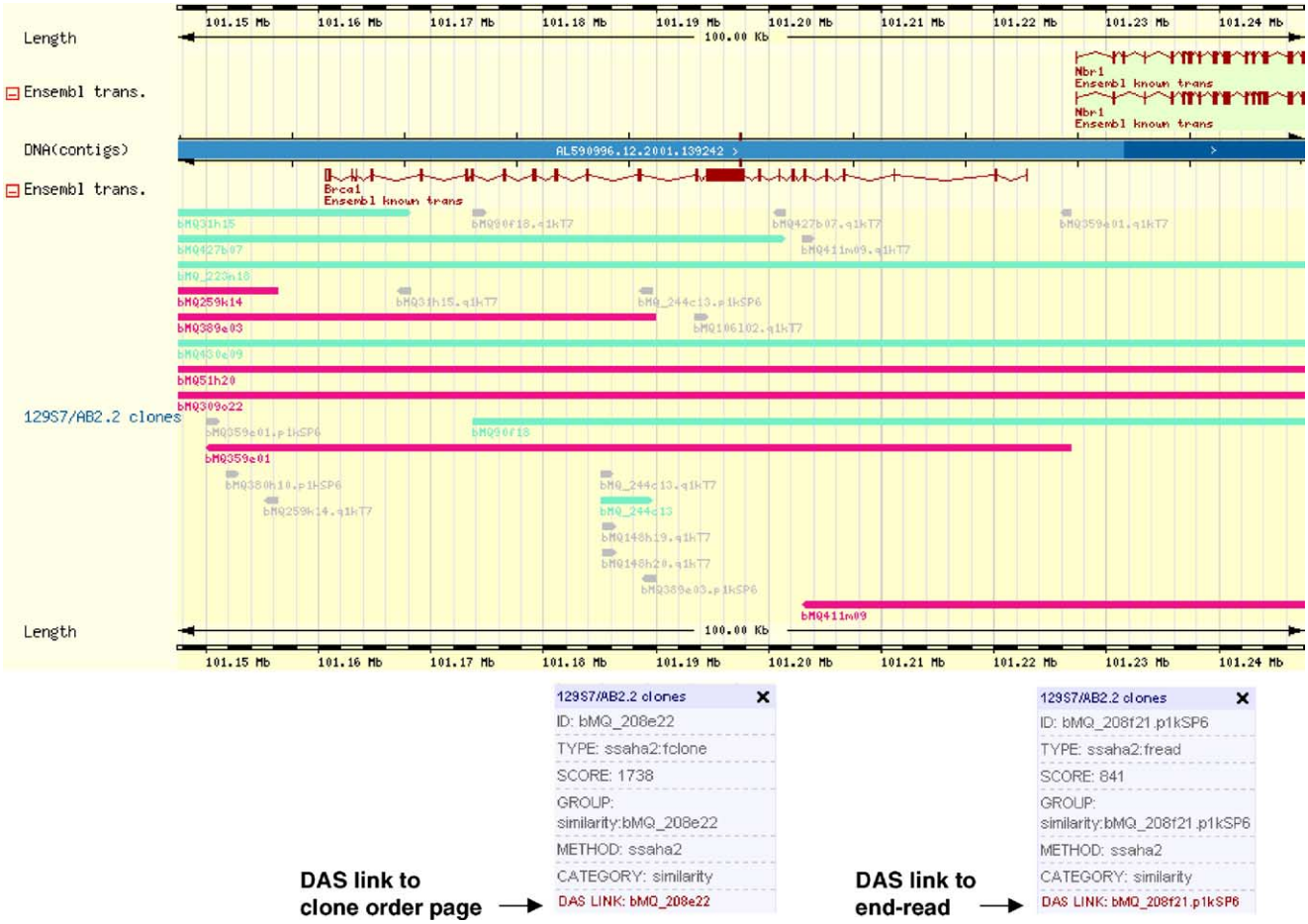

Fig. 2. 129S7/AB2.2 BAC clones are displayed on the Ensembl genome browser under the DAS source, 129S7/AB2.2. The clones are displayed as green and pink, which indicates the orientation of the DNA insert in the vector. End reads are shown as gray bars. Clones from this library have been given the designation bMQ. DAS links to a clone order page and to the end read sequences, in the Ensembl trace repository, can be found by clicking on the clone of interest. These DAS links are shown as gray boxes below the contig view.

were picked robotically into 384-well plates containing 7.5% glycerol and grown for 20 h at 37°C, after which time replicates were produced and the library stamped onto tester agar to confirm that it was free of phage and *Pseudomonas* contamination. In total 168,192 clones were picked. The plates from this library were given the designation bMQ.

End-sequence profiling of the BAC resource

In total 313,089 reads were attempted using the T7 and SP6 primers on the vector. Sequence reads were subjected to postsequence processing using ASP, which clips vector sequences and quality clips the sequence read. The number of insertless clones was determined to be 9%. Average read lengths were determined to be 739 bp.

End-sequence mapping of BAC clones

End-read data were mapped using SSAHA2 with the mapping criteria that >100 bp should map with >95% identity to the NCBIm33 assembly.

Recombineering to generate targeting vectors

Targeting vectors were generated using technology described in Liu et al. [13]. Briefly, the DH10B clones carrying the relevant 129S7/AB2.2 BAC clones were electroporated with a mini-lambda plasmid [27] carrying the λRed recombination genes and selected in tetracycline (12.5 μg/ml) and chloramphenicol (12.5 μg/ml). Bacterial cells carrying both the BAC clone and the mini-lambda were then expanded and electroporated with a pBS-based capture vector to rescue a fragment of DNA from the BAC. This product was confirmed by restriction digest and further recombination reactions were performed to introduce the selection markers. Full recombineering protocols can be obtained via the Web link http://recombineering.ncifcrf.gov/ and a detailed description of the targeting vectors is available on request.

Gene targeting in ES cells

AB2.2 and CJ-7 ES cells were cultured as described previously [11] on irradiated STO feeder cells. Linearized targeting vectors were electroporated into ES cells and selected for 10 days with 180 μg/ml G418. Colonies were picked into 96-well plates, expanded, and then analyzed by Southern blot using external DNA probes.

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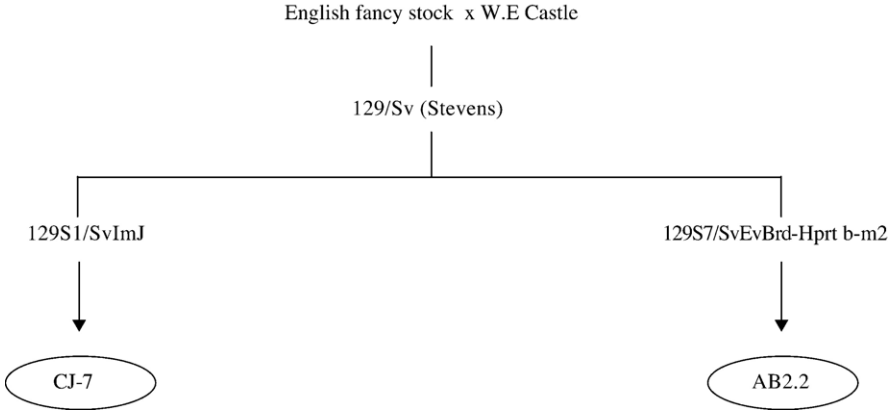
Table 2
Targeting vectors generated using 129S7/AB2.2 BACs by recombineering have a high targeting frequency in the 129-derived AB2.2 and CJ-7 ES cells

Locus	Homology (kb)			Sel	Targeting frequency	
	Total	RH	LH		AB2.2	CJ-7
D11Mit1 (Chr 11)	8	3	5	Neo	35%	38%
IgH (Chr 12)	7.5	5	2.5	Neo	15%	NT
Notch (Chr 2)	10.8	5.4	5.4	Neo	32.5%	NT

RH, homology on the right side of the selection marker; LH, homology on the left side of the selection marker; NT, not tested; Sel, selection marker.



picking, and Sarah Hunt for assistance in submitting SNP data to dbSNP.

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ygeno.2005.08.003.

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