

# Construction and Preliminary Characterization of a Series of Mouse and Rat Testis cDNA Libraries

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**ABSTRACT:** We have constructed a series of 23 cDNA libraries from mouse and rat testicular cells. These include libraries made from whole, intact adult testes; seminiferous tubule cells from adult testes; combined populations of primary spermatocytes from 18-day-old mouse testes; and isolated populations of primitive type A spermatogonia, type A spermatogonia, type B spermatogonia, preleptotene spermatocytes, leptotene plus zygotene spermatocytes, juvenile pachytene spermatocytes, adult pachytene spermatocytes, round spermatids, Sertoli cells from 6-, 8-, 17-, and 18-20-day-old mice, and peritubular cells from 18-20 day old mice, all recovered from outbred white Swiss (CD-1) mice. We also constructed libraries from whole adult testes from five other lines of mice: C57 Bl6/J, C3 HEB, BDF-1, Balb/c, and 129 Sv. Finally, there are two libraries made from populations of Sertoli cells and

peritubular cells isolated from testes of 20-day-old Sprague-Dawley rats. Enzymatic dissociation, followed by gradient separation or plating/lysing techniques, was used to prepare populations of specific cell types in purities of 85-98%. cDNAs were synthesized from poly A+ mRNA primed with oligo dT and unidirectionally cloned into the lambda Uni-Zap XR expression vector from Stratagene. Primary titers ranged from  $2.1 \times 10^5$  to  $2.9 \times 10^8$  plaque-forming units, and insert sizes averaged 1.0-1.2 kb. These libraries have been amplified once and submitted to the American Type Culture Collection (ATCC) for distribution to interested investigators. ATCC accession numbers are provided.

**Key words:** Spermatogenesis, Sertoli cells, peritubular cells, gene expression.

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The mammalian testis is a complex organ comprised of many different cell types. These can be subdivided into germ cells, which participate in the process of spermatogenesis (McCarrey, 1993); somatic cells, including Leydig cells, which produce androgens (Ewing and Keehey, 1993); Sertoli cells, which foster the process of spermatogenesis (Jégou, 1993; Griswold, 1995); and peritubular cells, which surround the seminiferous epithelium and facilitate interactions among other cell types (Skinner, 1991). The spermatogenic cell lineage is well characterized and descends from primordial germ cells in the embryo and from prospermatogonia in the fetus and neonate. These stem cells give rise to premeiotic spermatogonia, meiotic spermatocytes, and postmeiotic spermatids, which undergo the final differentiative process of spermiogenesis, leading to formation of the mature male gametes

(spermatozoa) (reviewed in McCarrey, 1993). Each testicular cell type is distinguished by unique morphological and functional characteristics that develop as a result of differential gene expression. Complimentary DNA (cDNA) libraries are constructed from messenger RNAs (mRNAs) isolated from a specific tissue or cell type and thus represent the unique collection of genes that are expressed in that particular tissue or cell type (McCarrey and Williams, 1994). We have constructed a series of cDNA libraries that have the potential to provide a useful resource for studies of the genes and/or their products that are expressed in various testicular tissues or cell types.

## Methods

### Source of Tissues and Purified Cell Types

Except where otherwise designated, outbred CD-1 white Swiss mice (Charles River) or rats (Sprague-Dawley) were used as the source of testicular tissues and cell types. All experiments were performed according to guidelines specified by the National Research Council (Institute of Laboratory Animal Resources, 1996) and approved by local institutional animal care and use committees. Following euthanasia, testes were dissected from male mice at the designated age and were decapsulated to remove the tunica albuginea. The remaining tissue was either used as such

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Table 1. Summary of testis cDNA libraries

Source tissue or cell type*	Purity of cell type	Primary titer	Percent recombinant†	Amplified titer	ATOC accession
Adult whole testis	—	$4.50 \times 10^7$	>98	$8.00 \times 10^{11}$	63413
Adult seminiferous tubule cells	—	$1.77 \times 10^8$	>98	$2.21 \times 10^{11}$	63414
6-Day primitive type A spermatogonia	≥85%	$2.50 \times 10^6$	89.5	$9.28 \times 10^{11}$	63415
8-Day type A spermatogonia	≥85%	$3.85 \times 10^6$	96.5	$2.94 \times 10^{11}$	63416
8-Day type B spermatogonia	≥85%	$2.90 \times 10^6$	96.0	$2.95 \times 10^{11}$	63417
18-Day preleptotene spermatocytes	≥85%	$2.17 \times 10^6$	96.0	$1.72 \times 10^{11}$	63418
18-Day leptotene + zygotene spermatocytes	≥85%	$4.80 \times 10^6$	98.5	$1.81 \times 10^{11}$	63419
18-Day pachytene spermatocytes	≥85%	$2.53 \times 10^5$	96.0	$1.60 \times 10^{11}$	63420
18-Day mixed primary spermatocytes‡	≥95%	$1.52 \times 10^6$	90.5	$1.10 \times 10^{11}$	63421
Adult pachytene spermatocytes	≥95%	$3.73 \times 10^7$	98.0	$3.65 \times 10^{11}$	63422
Adult round spermatids	≥95%	$8.26 \times 10^7$	98.5	$3.65 \times 10^{11}$	63423
6-Day Sertoli cells	≥85%	$3.00 \times 10^6$	95.5	$4.08 \times 10^{11}$	63424
8-Day Sertoli cells	≥90%	$6.60 \times 10^6$	99.0	$3.05 \times 10^{11}$	63425
17-day Sertoli cells	≥90%	$8.40 \times 10^6$	96.0	$1.26 \times 10^{11}$	63426
18-20-Day Sertoli cells	≥98%	$2.70 \times 10^6$	98.5	$7.20 \times 10^{11}$	63427
18-20-Day peritubular cells	≥98%	$2.90 \times 10^6$	>98	$9.60 \times 10^{11}$	63428
Adult testis C57 Bl6/J	—	$6.10 \times 10^6$	>95	$1.75 \times 10^{12}$	63429
Adult testis C3.HEB	—	$1.35 \times 10^6$	>95	$7.44 \times 10^{11}$	63430
Adult testis BDF-1	—	$5.95 \times 10^6$	>95	$1.36 \times 10^{12}$	63431
Adult testis Balb/c	—	$1.10 \times 10^7$	>95	$1.20 \times 10^{12}$	63432
Adult testis 129 Sv	—	$1.40 \times 10^7$	>95	$2.08 \times 10^{12}$	63433
20-Day rat Sertoli cells	≥98%	$3.32 \times 10^5$	89.5	$5.60 \times 10^{11}$	63434
20-Day rat peritubular cells	≥98%	$2.80 \times 10^6$	99.0	$7.20 \times 10^{10}$	63435

\* Source tissue was obtained from outbred CD-1 white Swiss mice unless otherwise noted.

† Values for libraries pooled from multiple packaging reactions are expressed as greater than or equal to a minimum percentage.

‡ Produced by mixing equal plaque-forming unit (PFU)-containing portions of primary libraries made from purified preleptotene spermatocytes, leptotene + zygotene spermatocytes, and pachytene spermatocytes isolated from 18-day-old mice.

(whole testis) or was further processed to yield the desired testicular cell types, as described below.

### Preparation of Germ Cell Populations

Enzymatic dissociation of testicular tissues was accomplished as described (Bellvé, 1993) in enriched Krebs-Ringer bicarbonate buffer (EKRB) [120.1 mM NaCl, 4.8 mM KCl, 25.2 mM NaHCO<sub>3</sub>, 1.2 mM KH<sub>2</sub>PO<sub>4</sub>, 1.2 mM MgSO<sub>4</sub>·7H<sub>2</sub>O, 1.3 mM CaCl<sub>2</sub>, supplemented with 11.1 mM glucose, 1 mM glutamine, 10 ml/L essential amino acids, 10 ml/L nonessential amino acids, 100 µg/ml streptomycin, and 100 U/ml penicillin (K<sup>+</sup> salt) (Gibco BRL, Gaithersburg, Maryland; Sigma, St. Louis, Missouri)]. An initial 15-minute treatment of decapsulated adult testes with collagenase (0.5 mg/ml) yielded seminiferous tubule sections from which interstitial cells in suspension were decanted away. These seminiferous tubules were further washed with EKRB buffer and were either used as such (seminiferous tubule cells) or were subjected to further dissociation with trypsin (see below). A similar collagenase treatment yielded seminiferous tubules from puberal (17–18 day) and prepuberal (6–8 day) testes. A subsequent 15-minute treatment with trypsin (0.5 mg/ml) followed by filtration through nylon mesh (80 µm for adult testes; 40–80 µm for prepuberal and puberal testes) yielded a fully

dissociated cell suspension from which specific cell types were purified as described below.

Spermatogenic cell types (spermatogonia, spermatocytes, spermatids, and residual cytoplasmic bodies) and 6-day Sertoli cells were purified by the Sta Put gradient/unit gravity sedimentation method as described by other researchers (Romrell et al, 1976; Bellvé et al, 1977; McCarrey et al, 1992; Bellvé, 1993). Fractions containing the desired cell types were identified on the basis of cellular morphology under phase-contrast optics. The specific cell types recovered, ages of mice from which they were recovered, and purities of each recovered cell population are summarized in Table 1.

### Preparation of Somatic Cell Populations

Sertoli cells from 17-day-old mice were isolated from seminiferous tubule cell suspensions by differential plating as described previously (O'Brien et al, 1989). Following enzymatic dissociation with collagenase and trypsin as described above, cell suspensions containing both Sertoli and spermatogenic cells were cultured in Primaria dishes (Falcon, Franklin Lakes, New Jersey) at 32°C in Eagle's Minimum Essential Medium (MEM) supplemented with 15 mM HEPES, 100 U/ml penicillin and 100 µg/ml streptomycin, 5 µg/ml insulin, and 200 ng/ml testosterone.

### *Amplification and Storage of cDNA Libraries*

At least 200,000 PFU from each primary library were used to produce amplified libraries. These PFU were mixed with XL1 Blue MRF' *E. coli* and plated as described above on four 150-mm Petri dishes at a density of 50,000 PFU per dish. Plates were incubated at 37°C for 6.5 hours to propagate phages. Phage particles were recovered by overlaying each plate with 8 ml SM buffer and gently rocking the plates at 4°C overnight to elute the phage. This phage-containing solution was then recovered, and each plate was washed with an additional 2 ml SM buffer. The combined 40 ml of solution containing eluted phage represented the amplified version of each library. After titrating, each amplified library was prepared for storage by adding DMSO to a final concentration of 7% and freezing aliquots at -80°C.

### **Results**

A total of 23 testis-derived libraries were constructed. The source tissue or cell type, purity of the source cell type (where applicable), primary library titer, percent recombinant phage in each library, amplified library titer, and the ATCC accession number for each are listed in Table 1. On the basis of the preliminary characterization performed, each library showed an average insert size of approximately 1.0–1.2 kb (data not shown).

### **Discussion**

The cDNA libraries described here represent potentially useful research resources for investigators interested in studying genes and/or their encoded products expressed in the mammalian testis. As constructed, these libraries can be screened by hybridization with nucleic acid probes or by affinity with an antibody probe or any other means based on detection of either the specific DNA sequence of the cloned cDNA or the mRNA or protein products encoded by that cDNA.

Because the cDNAs were ligated unidirectionally into the lambda vector in the same 5'–3' orientation as the endogenous lambda  $\beta$ -gal gene into which they were inserted, the normal coding DNA strand can be transcribed from all of the cDNAs as part of a chimeric mRNA produced by transcription initiated at the  $\beta$ -gal promoter following infection of host bacterial cells. However, only one out of three of the clones carrying any particular cDNA can be expected to produce a fusion protein with the same amino acid sequence as that normally encoded by the mRNA from which the cDNA was derived. The other two out of three clones will, on average, produce chimeric mRNAs in an incorrect reading frame to encode the correct amino acid sequence.

The primary titers of all of these libraries are sufficiently large to be expected to include representation of

even relatively rare transcripts expressed in the source tissue or cell type, since libraries with primary titers of  $>10^6$  should include even those mRNAs that are present as rarely as one molecule per cell (Sambrook et al, 1989). Amplification of 200,000 PFU from each primary library can be expected to include representation in the amplified library of the vast majority of the cDNAs present in the primary library.

Because each of these libraries is derived from template mRNA isolated from a different testicular tissue, cell type, combination of cell types, line of mice, or species (mouse or rat), the representation of cDNAs and, hence, expressed genes, will vary in each case. This variation will include both qualitative differences (genes that are expressed in one testicular tissue or cell type but not at all in another) and quantitative differences (genes that are expressed at relatively higher or lower levels in different testicular tissues or cell types). Quantitative differences resulting from different proportions of particular transcripts in the total mRNA pool recovered from each source tissue or cell type will be reflected by variation in abundance of each corresponding cDNA in each library. Amplification of each primary library one time is not expected to have significantly skewed this relative representation of specific cDNAs. Thus, the detection of any particular cDNA in a particular library will be indicative of expression of the corresponding gene in that source tissue or cell type, and the relative abundance of positive clones of a particular cDNA in different libraries will be indicative of the relative expression level of the corresponding gene in each source tissue or cell type.

To facilitate access by interested investigators, these libraries have been submitted to the ATCC, and the corresponding accession numbers are provided in Table 1. That accessibility, along with the background information provided in this report, should facilitate the use of these libraries in a large variety of studies.

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### **References**

- Bellvé AR. Purification, culture, and fractionation of spermatogenic cells. In: Wassarman PM, DePamphilis ML, eds. *Guide to Techniques in Mouse Development*. San Diego: Academic Press; 1993:84–113. *Methods in Enzymology*; vol. 225.
- Bellvé AR, Millette CF, Bhatnagar YM, O'Brien DA. Dissociation of the mouse testis and characterization of isolated spermatogenic cells. *J Histochem Cytochem* 1977;25:480–494.
- Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* 1979;18:5294–5299.

- Dorrington JH, Roller NF, Fritz IB. Effects of follicle-stimulating hormone on cultures of Sertoli cell preparations. *Mol Cell Endocrinol* 1975;3:57-70.
- Ewing LL, Keeney DS. Leydig cells: structure and function. In: Desjardins C, Ewing LL, eds. *Cell and Molecular Biology of the Testis*. New York: Oxford University Press; 1993:137-188.
- Jégo B. The Sertoli-germ cell communication network in mammals. *Int Rev Cytol* 1993;147:25-96.
- Griswold MD. Interactions between germ cells and Sertoli cells in the testis. *Biol Reprod* 1995;52:211-216.
- Institute of Laboratory Animal Resources (U.S.), Commission on Life Sciences, National Research Council. *Guide for the Care and Use of Laboratory Animals*. Washington, District of Columbia: National Academy Press; 1996.
- McCarrey JR. Development of the germ cell. In: Desjardins C, Ewing LL, eds. *Cell and Molecular Biology of the Testis*. New York: Oxford University Press; 1993:58-89.
- McCarrey JR, Berg WM, Paragioudakis SJ, Zhang PL, Dilworth DD, Arnold BL, Rossi JJ. Differential transcription of *Pgk* genes during spermatogenesis in the mouse. *Dev Biol* 1992;154:160-168.
- McCarrey JR, Williams SA. Construction of cDNA libraries from limiting amounts of material. *Curr Opin Biotechnol* 1994;5:34-39.
- O'Brien DA, Gabel CA, Eddy EM. Mouse Sertoli cells secrete mannose 6-phosphate containing glycoproteins that are endocytosed by spermatogenic cells. *Biol Reprod* 1993;49:1055-1065.
- O'Brien DA, Gabel CA, Rockett DL, Eddy EM. Receptor-mediated endocytosis and differential synthesis of mannose 6-phosphate receptors in isolated spermatogenic and Sertoli cells. *Endocrinology* 1989;125:2973-2984.
- Romrell LJ, Bellvé AR, Fawcett DW. Separation of mouse spermatogenic cells by sedimentation velocity. A morphological characterization. *Dev Biol* 1976;49:119-131.
- Sambrook J, Fritsch EF, Maniatis T. *Molecular Cloning: A Laboratory Manual*. 2nd ed. Cold Spring Harbor: Cold Spring Harbor Laboratory Press; 1989.
- Skinner MK. Cell-cell interactions in the testis. *Endocrinol Rev* 1991;12:45-77.
- Skinner MK, Fetterolf PM, Anthony CT. Purification of the paracrine factor, P-Mod-S, produced by testicular peritubular cells that modulates Sertoli cell function. *J Biol Chem* 1988;263:2884-2890.
- Tung PS, Skinner MK, Fritz IB. Fibronectin synthesis is a marker for peritubular cell contaminants in Sertoli cell-enriched cultures. *Biol Reprod* 1984;30:199-211.