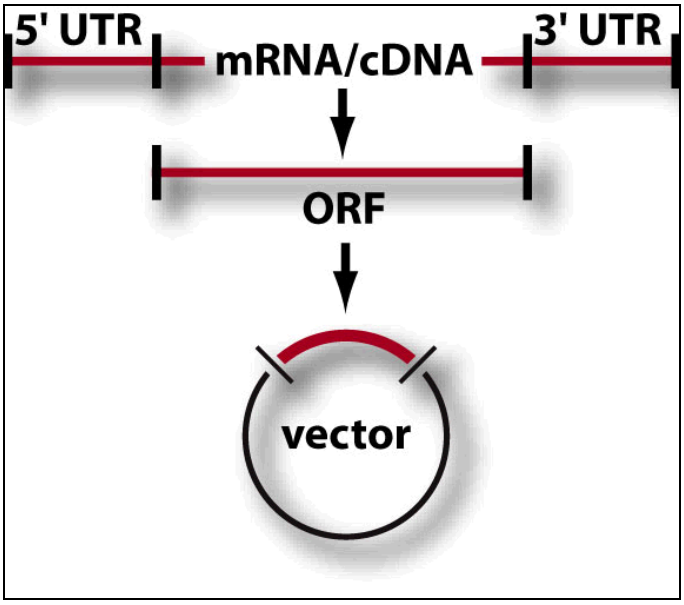
Product: Human ORFeome Collection
Catalog #: OHS1771

Product description

The Human ORFeome Library from Open Biosystems contains cloned open reading frames (ORFs) derived from fully sequenced Mammalian Gene Collection (MGC) full-length cDNA clones inserted into recombinational entry vectors. The ORF clones contain the coding sequences located exactly between the initiation and termination codons, excluding the 5' and 3' mRNA untranslated regions (UTRs)[1]. The termination codon has been engineered out of these clones to allow the researcher to utilize the Gateway system to add a 3' tag to their ORF of interest if desired. In the human ORFeome v1.1, each clone represents a mini-pool of PCR amplified inserts cloned into the pDONR223 vector, not a single unique insert. Each pool is expected to contain the source ORF without the termination codons, but it is formally possible that various by-products might have contaminated the pool during the various cloning steps. For example, although PCR conditions were optimized (high proof reading DNA polymerase and limited number of cycles) mutations will still occur at low frequency during the PCR amplification. Out of ~70,000 insert nucleotides sequenced, the misincorporation rate was 1 mutation every 35,000 nucleotides.



Individual Human ORF clones are shipped as a bacterial culture of *E. coli* in LB broth with an inert growth indicator, 8% glycerol, and spectinomycin (50 µg/ml).

Clone storage

The Human ORF collection should be stored at -80°C.

Selectable Marker

Spectinomycin is utilized for selection of bacterial cultures.

Sequencing Primer - 5'

TTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGT

Replication of plates

1. Prepare target plates by dispensing ~160µl of LB media, 8% glycerol, and spectinomycin at a concentration of 50µg/ml into each well.
2. Remove the lids of the first source plate and target plate, allowing the source plate to thaw before you begin replication.
3. Gently place the Q-Rep into the source plate and lightly move the Q-rep around inside the well to stir the culture. Make sure to scrape the bottom of the well.
4. Pull the Q-rep out of the source plate and gently place into target plate and mix gently in the same manner.
5. Dispose of Q-rep into a biohazard container. Autoclave used Q-reps when finished.
6. Replace the lids of the source and target plates.
7. Repeat steps 1-6 until all plates have been replicated.
8. Return the source plates to the freezer.
9. Place the inoculated target plates inside a 10"X12" Ziploc bag (maximum of 10 plates per bag). Place the bagged plates in a 37°C incubator for 24 hours.
10. Check the target plates for growth on the following day.
11. Place the target plates showing growth into the freezer. (You may have to retry growing individual clones that do not initially grow.)
12. After plates are frozen, seal all of the source and target plates by placing an aluminum plate seal over the frozen plate and securing the seal with a rolling device.

Note: If you do not have a Q-rep replicator, you can use a multichannel pipettor to transfer ~10 µl of culture from each well of the source plate to the target plate.

Background

Version 1.1 of the human ORF collection utilizes the Mammalian Gene Collection (http://www.openbiosystems.com/full_length_human_cdnas.php) as a starting point. Future additions will be added as the MGC collection is expanded. Bioinformatic analysis revealed that the MGC collection corresponds to over 10,000 distinct full-length ORFs. Eliminated were i) clones for which the corresponding ORFs were reported as "partial coding sequence" at NCBI (no 5' ATG detected); ii) multiple copies of identical cDNA clones ; and iii) clones that only differ in their 5' or 3' UTRs or both but not in the coding sequence. Also discarded were clones for which the ORF length is smaller than 100 nucleotides, following a convention similar to that initiated during the annotation of the yeast genome[2]. The remaining MGC clones were arrayed by increasing length and this resource constituted the starting point for cloning. ORF cloning was counted as successful if the ORF Sequence Tag (OST) obtained matched the sequence of the expected ORF insert. The ORFs that were successfully cloned and sequenced were then arrayed by increasing length, thereby generating version 1.1 of the human ORFeome (hORFeome v1.1). In total, human ORFeome v1.1 constitutes a resource large enough to constitute a platform for reverse proteomics (protein expression, localization, interactions).

Vector Information

pDONR223

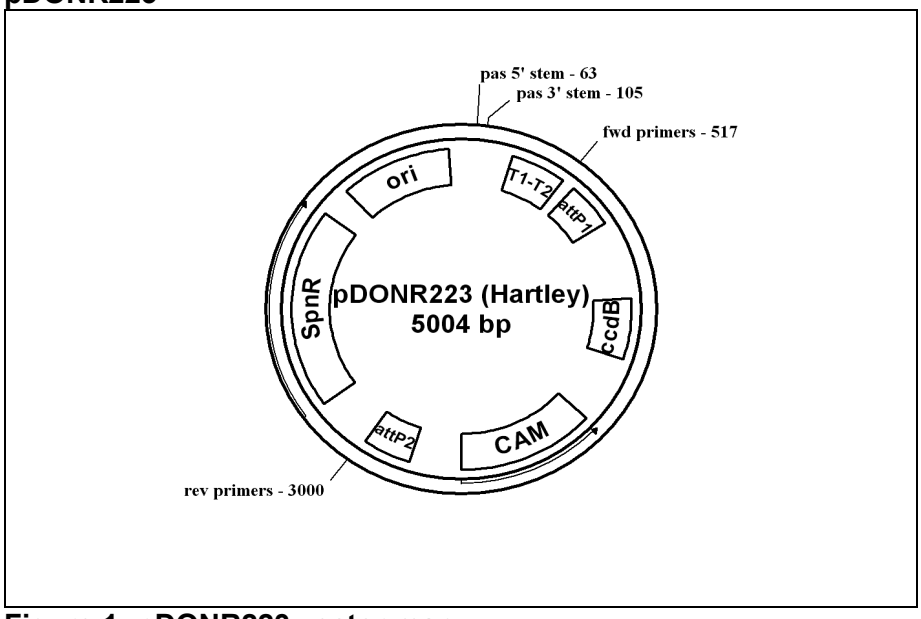
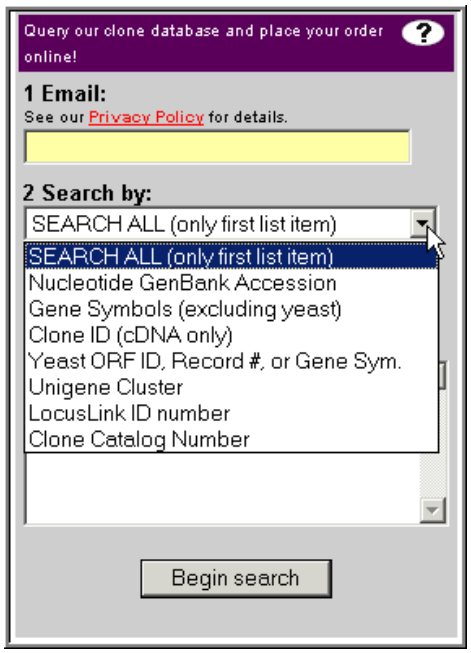


Figure 1: pDONR223 vector map

Finding further information on clones

The Open Biosystems Clone Query provides a rapid means of locating relevant clone information. In step 2 of the query, choose the appropriate search criteria from the drop-down menu (Figure 2).



Example of search criteria:

Genbank accession number: CV030778
Clone ID number: 9994

Figure 2: Open Biosystems Clone Query

In the box for step 3, enter your search list. Click "Begin Search". Example search criteria and detailed instructions are available, if necessary, by clicking the question mark icon in the upper right corner of the query (Figure 3).

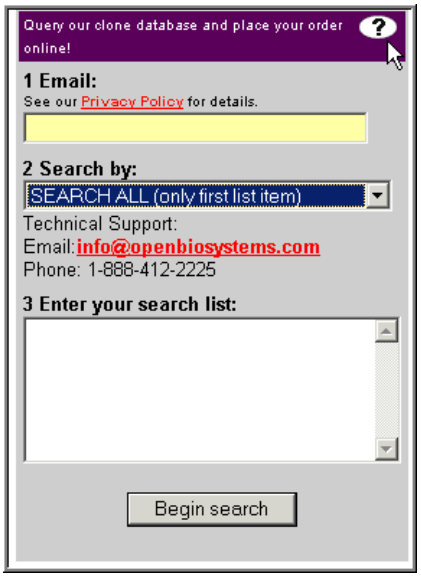


Figure 3: Query Assistance

Clicking the “View” link on the query result page will display the clone information page containing vector and sequence information (Figure 4).

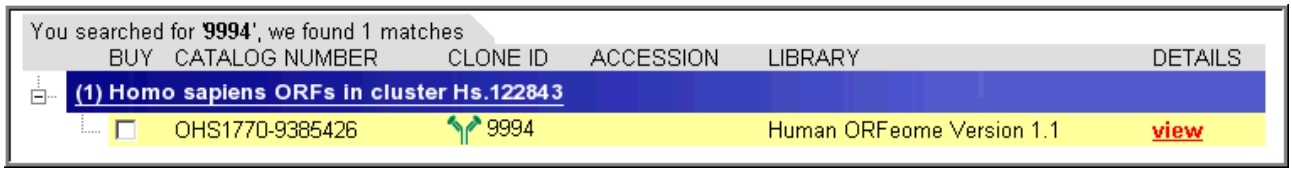


Figure 4: Open Biosystems Clone Query Results

Useful websites

- The Human ORFeome Lab http://horfdb.dfci.harvard.edu/
- The Mammalian Gene Collection http://mgc.nci.nih.gov/
- Restriction Mapper http://www.restrictionmapper.org/

References

1 Jean-Francois Rual, Tomoko Hirozane-Kishikawa, Tong Hao, Nicolas Bertin,Siming Li, Amélie Dricot, Ning Li, Jennifer Rosenberg, Philippe Lamesch,Pierre-Olivier Vidalain, Tracey R. Clingingsmith, James L. Hartley, Dominic Esposito, David Cheo, Troy Moore, Blake Simmons, Reynaldo Sequerra, Stephanie Bosak, Lynn Doucette-Stamm, Christian Le Peuch, Jean Vandenhaute,Michael E. Cusick, Joanna S. Albala, David E. Hill, and Marc Vidal. Human ORFeome Version 1.1: A Platform for Reverse Proteomics. Genome Research 2004 14:2128–2135

2 Walhout, A.J., G.F. Temple, M.A. Brasch, J.L. Hartley, M.A. Lorson, S. van den Heuvel, and M. Vidal. 2000b. GATEWAY recombinational cloning: application to the cloning of large numbers of open reading frames or ORFeomes. Methods Enzymol 328: 575-592

3 Goffeau et al. Science. 1996 Oct 25;274(5287):546, 563-7.

4 Hartley, J.L., G.F. Temple, and M.A. Brasch. 2000. DNA cloning using in vitro site-specific recombination. Genome Res 10: 1788-1795.