Coriell CHDI Plasmid Collection Screening and Propagation

(S. Madore, March 2009)

Introduction

The CHDI Biorepository at CIMR houses an assortment of useful recombinant plasmids containing the coding sequences of proteins relevant to the research of Huntington Disease. Many of these plasmid "minigenes" have been engineered to include the necessary DNA control elements to facilitate expression various iterations of the human Htt protein in mammalian cells. In some cases the Htt cDNA sequence in the translational coding sequence in exon 1 of the Htt cDNA has been purposefully altered such that the full length proteins they encode contain varying lengths of the characteristic glutamate repeat found in the N-terminal region of the wild-type protein. Other variants contain Htt cDNAs that have been modified with premature translational stop codons. These plasmids produce truncated protein variants some of which may also contain different numbers of glutamate residues. In addition, antigenic "tags", small amino acid sequences found in the flu virus or human myc protein may be added to the mature protein to facilitate immunodetection when expressed in mammalian cells. Lastly, in addition to the myc tag, some proteins contain a C-terminal stretch of six histidines. When these proteins are expressed in bacterial cells, the resulting Htt protein can be purified by column chromatography using a matrix that selectively recognizes this histidine motif. Details of the plasmids as well as DNA sequences of the inserts can be obtained from Coriell by investigators who request these reagents.

The recombinant plasmids were constructed by collaborators of CHDI and sent to the Coriell Institute for transformation into bacteria, purification, sequence verification, and distribution.

Methodology Overview

A. Transformation and Screening

Plasmid DNA was transformed using one of two commercially available strains of chemically competent bacteria. The most commonly used was One Shot TOP-10[1] (Invitrogen Corp., Carlsbad, California, U.S.A.) and for a subset of plasmids the Chemicomp GT116[2] bacteria cell line from Invivogen Corp. (Invivogen Corp., San Diego, California, U.S.A.) was substituted. The manufacturer's standard procedure for transformation was followed. Briefly, 1- 10 ng of supercoiled plasmid DNA is added to 50 µl of TOP 10 or GT116 competent cells for 30 minutes on ice. Following a 30 second heat shock at 42° C, 0.25 mls of SOC media is added and the cells are allowed to recover for 1 hour at 37° C (30° C for GT116) in shaker at 225 RPM. Following recovery 25 to 100 µl of cell suspension is spread on LB agar plates with the appropriate antibiotic (ampicillin at 100 ug/ml or spectinomycin at 50 ug/ml) for selection. For some plasmids X-gal at 40 µg/ml was included to confirm the presence of an insert. At least 10 single colonies are selected from the lawn of bacterial transformants for further screening. To prepare overnight cultures for DNA miniprep isolations 2 mls of LB plus antibiotic was inoculated with a single colonies and grown overnight at 37° C (30° C for GT116) with shaking. Bacterial cells were recovered by centrifugation at 5000 RPM in an Eppendorf centrifuge at room temperature. The plasmid DNA was isolated using the QIAGEN QiaPrep kits according to the manufacturer's instructions. Purified DNA was subjected to restriction enzyme digestion patterns or used as template for PCR to generate amplicons using flanking primers. DNA products were visualized on 1.2 % agarose gels containing ethidium bromide. Alternatively, inserts were screened directly from transformants by using a colony PCR.

[1]The genotype of the TOP10 bacterial strain is: F- mcrA .(mrr-hsdRMS-mcrBC) F80 lacZ.M15 lacX74 recA1 araD139 (ara-leu)7697 galU galK rpsL (StrR) endA1 nupG.
[2]The genotype of the GT116 bacterial strain is: F- mcrA .(mcrr-hsdRMS-mcrBC) F80 lacZM15 .lacX74 recA1 endA1 sbcC-sbcD.

B. Propagation

For large scale propagation of plasmid DNA 0.25 mls of starting culture was used to inoculate 1 liter flasks with 250 ml of culture media with selection antibiotic and shaken overnight at 37° C (30° C for GT116). On the following day bacteria were pelleted by centrifugation and plasmid DNA purified using Qiagen (Qiagen U.S.A.) EndoFree MegaPrep Plasmid Kits following the manufacturer's procedure.

C. Assessment of Purified Plasmid DNA Quantity and Quality

The DNA concentration of the plasmid preparations was determined by spectrophotometry absorbance at 260nm. Purity was assessed by the 260/280 absorbance ratio and the 260/235 absorbance ratio. The sequence identity of smaller cDNA inserts was confirmed using sequencing primers annealing adjacent to cloning site(s). Larger inserts were analyzed using primer walking and double-stranded sequencing. The sequences obtained were compared with reference sequences provided by CHDI.