**MGC premier Expression-Ready cDNA clones**

TCH1103, TCM1104, TCR1105, TCB1106, TCH1203, TCM1204, TCR1205, TCB1206, TCH1303, TCM1304, TCR1305

The MGC premier Expression-Ready collection has the high quality, proven gene content from the Mammalian Genome Collection (MGC) in a choice of two expression-ready constructs. Genome scale content for human, mouse, rat and bovine is available in the following vectors:

1. pTCN - cDNA expressed from CMV promoter with a neomycin selectable marker
2. pTCP - cDNA expressed from CMV promoter with a puromycin selectable marker
3. Empty vector controls are available for both pTCN and pTCP Expression-ready cDNA clones (Human, Mouse, Rat and Bovine)

Expression of the cDNA is from a robust CMV (Human Cytomegalovirus) promoter; an RNA polymerase II promoter. The expression vector confers either puromycin resistance or neomycin resistance to transfected cells to enable selection of a population of exclusively transected cells.

![Figure 1: A schematic map highlighting vector elements common to the pTCN and pTCP vectors. Both high copy plasmids express the cDNA from the CMV promoter. Plasmids differ by selection marker (pTCN confers neomycin resistance; pTCP confers puromycin resistance).](image)

<table>
<thead>
<tr>
<th>Table 1: Vector elements present in the expression-ready vectors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pTCN and pTCP vector elements</strong></td>
</tr>
<tr>
<td>CMV promoter</td>
</tr>
<tr>
<td>Poly A</td>
</tr>
<tr>
<td>SV40 promoter</td>
</tr>
<tr>
<td>Selection marker</td>
</tr>
<tr>
<td>pUC ori</td>
</tr>
<tr>
<td>Ampicillin</td>
</tr>
</tbody>
</table>

**Expression vectors include either neomycin or puromycin resistance.**

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Figure 2: A schematic map highlighting vector elements of pCMV Sport6. The vector name is abbreviated pCS6 in gene search results.

Table 2: Vector elements present in the expression-ready vectors

<table>
<thead>
<tr>
<th>pCMV SPORT6 elements</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMV promoter</td>
<td>Drives robust cDNA expression</td>
</tr>
<tr>
<td>SV40 pA</td>
<td>Transcriptional stop</td>
</tr>
<tr>
<td>MCS</td>
<td>Multiple cloning site</td>
</tr>
<tr>
<td>pUC ori</td>
<td>Propagation of the plasmid in E. coli</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>Bacterial selection marker</td>
</tr>
</tbody>
</table>

Controls
Empty vector controls detect changes related to cellular toxicity or changes in gene expression due to transfection. Transfection of a vector of similar size with no functional insert would control for these changes. Empty vectors pTCN and pTCP available from transOMIC technologies, can be used as negative controls. Placed alongside the cDNA expression vectors during transfection, selection and expression reactions; they are used to establish the baseline against which the results of gene overexpression are measured.

An empty vector control for pCMV SPORT6 is not available. However, an Aval restriction digest will excise the insert and the backbone can be religated to create an empty vector.

MGC premier Expression-Ready cDNA human gene families and pathway focused sets
MGC premier Expression-Ready cDNA gene families and pathways arrayed from the MGC collection cloned into the pCMV SPORT6 expression-ready vector. The gene lists were created using resources from the National Institute of Health’s Cancer Genome Anatomy Project (CGAP) and Gene Ontology (GO). Annotation is based on electronic and manual literature-based curation as well as sequence analysis. (See Annotation Standard Operating Procedures from the Gene Ontology Consortium for more information.)

**Focused Sets are limited by the availability of MGC clones in pCMV SPORT6**
**There is no mammalian selection marker in pCMV SPORT 6. If transfection efficiency is high enough so that many target cells contain a plasmid, then the need for selection is obviated.**
Replication, storage and archiving
Recommended vendors and catalog numbers are provided in the protocol when available.

*E. coli* carrying MGC *premier* Expression-Ready cDNAs are best propagated in LB broth or LB broth with 8% glycerol, the latter for archiving and freezing.

<table>
<thead>
<tr>
<th>LB+Glycerol (1 liter)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>20g LB Broth-Lennox (low salt)</td>
<td>VWR EM1.00547.0500</td>
</tr>
<tr>
<td>80ml Glycerol</td>
<td>VWR EM-4760</td>
</tr>
<tr>
<td>920ml distilled H2O</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>LB media (1 liter)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>20g LB Broth-Lennox</td>
<td>VWR EM1.00547.0500</td>
</tr>
<tr>
<td>1000ml distilled H2O</td>
<td></td>
</tr>
</tbody>
</table>

Autoclave to sterilize. Cool to 60°C before adding antibiotic. Add ampicillin or carbenicillin to 100 µg/ml.

**Individual cDNA clones**
For propagation
1. Grow the MGC *premier* Expression-Ready clone in LB broth with the ampicillin or carbenicillin (100 µg/ml).
2. 2-10 ml starter cultures for plasmid purification can be inoculated using 2 to 10 µl.
   Alternatively
3. Pick a single starter colony from a freshly streaked LB agar plate containing the antibiotic and inoculate into the desired volume of LB broth for plasmid purification.
4. Grow overnight at 37° with vigorous shaking (~300 rpm).

**Growth for storage**
Place 1ml of culture grown in LB+Glycerol into a polypropylene tube. Mix well and store at –80°C.
Or
Place 920 µl of culture grown in LB into a polypropylene tube and add 80 µl sterile glycerol (8% glycerol). Mix well and store at –80°C.

**Plate replication**
96-well plates of glycerol stocks are shipped frozen with aluminum foil seals to prevent cross contamination between wells.
Seals should only be removed while the culture is frozen to prevent contamination from condensation and aspiration between wells.
Minimize the time the plates are thawed condition of plates where possible.
Always store plates at -80°C. It is recommended that an archival copy is made as soon as possible. Glycerol stocks kept at -80°C are stable indefinitely as long as freeze/thaw cycles are kept to a minimum.

1. Create destination plates  
   a. Dispense sterile LB broth 8% glycerol into 96-well microtiter plates filling each well halfway.

2. Prepare plates to be copied  
   a. Remove the foil seals (VWR 73520-056) from the frozen source plates.  
      NOTE: Removing the seals while the source plates are frozen will minimize cross-contamination.

3. Transfer culture to destination plates  
   a. Place a sterile/disposable replicator (Genetix X5054) into the thawed plate and gently rotate replicator in the wells to mix the culture. Make sure to scrape the bottom of the plate. Place the replicator into the target plate and rotate again to transfer the cells.

4. Reseal the source plates and return to the -80°C freezer.

5. Place the inoculated target plates in a 37°C incubator and incubate for 12–24 hours.

Note, if wanting to carry put a plasmid extraction in a 96-well format, please refer to the product manual of the kit from the vendor.

**Puromycin or neomycin (G418) selection - establishing stable cell lines post transfection**

MGC premier Expression-Ready cDNAs are available in two vectors. The vectors have either a puromycin or a neomycin resistance marker for selection in mammalian cells. Cell lines have varying sensitivity to these antibiotics. A kill curve should be used to determine the optimal antibiotic concentration. Below is the protocol for titrating the antibiotic of choice using as an example a 24 well tissue culture dish.

Note:

   a. Neomycin phosphotransferase (neomycin resistance), confers resistance to G418, as well as neomycin.

   b. MGC premier Expression-Ready cDNA gene families and pathway focused sets have no mammalian selection marker. If transfection efficiency is high enough so that many of target cells contain a plasmid, then the need for selection is obviated.

1. Prepare appropriate cell culture media and desired antibiotic dilutions for antibiotic titration.
   - With a stock solution of puromycin (1.25µg/µl stock recommended) make dilutions in the media of the antibiotic ranging from 0 to 10 µg/ml (final concentration) in 0.5 µg/ml increments.
   - For G418, 200 – 500 µg /ml for most mammalian cells is expected. Make a stock solution of the G418 (12.5 µg/µl) and make dilutions in the media ranging from 50 µg/ml to 1mg/ml.
Table 3: Dilutions and volumes required for establishing optimal puromycin concentration

<table>
<thead>
<tr>
<th>Volume of Antibiotic Stock Solution Added (µl)</th>
<th>Total Volume of Media plus Antibiotic per 24 Well</th>
<th>Final Concentration (µg/ml) Puromycin</th>
<th>Final Concentration (µg/ml) G418</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>500 µl</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.2</td>
<td>500 µl</td>
<td>0.5</td>
<td>50</td>
</tr>
<tr>
<td>0.4</td>
<td>500 µl</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>0.6</td>
<td>500 µl</td>
<td>1.5</td>
<td>150</td>
</tr>
<tr>
<td>0.8</td>
<td>500 µl</td>
<td>2</td>
<td>200</td>
</tr>
<tr>
<td>1</td>
<td>500 µl</td>
<td>2.5</td>
<td>250</td>
</tr>
<tr>
<td>1.2</td>
<td>500 µl</td>
<td>3</td>
<td>300</td>
</tr>
<tr>
<td>1.6</td>
<td>500 µl</td>
<td>4</td>
<td>400</td>
</tr>
<tr>
<td>2</td>
<td>500 µl</td>
<td>5</td>
<td>500</td>
</tr>
<tr>
<td>3</td>
<td>500 µl</td>
<td>7.5</td>
<td>750</td>
</tr>
<tr>
<td>4</td>
<td>500 µl</td>
<td>10</td>
<td>1000</td>
</tr>
</tbody>
</table>

2. Split/dilute cells from a confluent well of a 24 well plate (~ 5 x 10⁴ cells per 500 µl media).
3. Plate ~ 5 x 10⁴ cells per well in media without antibiotic and allow enough time for cells to attach (less than 24 hours for most cell lines) before adding antibiotic.
4. Begin antibiotic selection the following day by replacing with media containing the appropriate concentrations of antibiotic.
5. Incubate cells at 37°C, or conditions normal for the cells.
6. Observe the cells for approximately 7 days.
7. Replace the media every 3 days with media containing the correct antibiotic concentration for each well.
8. To minimize duration to achieve fully selected, stable, cell populations one should choose the lowest concentration of drug that begins to give massive cell death in 3 days and kills 100% the cells within 5 days.

Transfection

OMNIfect reagent for plasmid DNA transfection
Use the following procedure to transfect plasmid DNA into mammalian cells in a 24-well format. For other plate formats, scale up or down the amounts of DNA and OMNIfect reagent proportionally to the total transfection volume (Table4).

A. Adherent cells: One day before transfection, plate 80,000 cells/well in 500 µl of growth medium without antibiotics so that cells will be 70–95% confluent at the time of transfection.
B. Suspension cells: On the same day of transfection just prior to preparing transfection complex plate 160,000/well cells in 500 µl of growth medium without antibiotics.
Transfection complex preparation (Figure 3):

Volumes and amounts are for each well to be transfected.

1. Plasmid DNA preparation: Dilute 0.5 µg of plasmid DNA in a microfuge tube containing Opti-MEM® I Reduced Serum Media*** up to a total volume of 25 µl.
2. OMNIfect reagent preparation: In a separate microfuge tube, add 1 µL of OMNIfect into 24 µl Opti-MEM® I Reduced Serum Media*** for a total volume of 25 µl.
3. Final transfection complex: Transfer the diluted DNA solution to the diluted OMNIfect reagent (total volume = 50 µl). Mix gently and incubate at room temperature for 10 minutes.

Adding Transfection complex to wells:

1. Add the 50 µl of transfection complex to each well containing cells and medium.
2. Incubate cells at 37°C in a CO₂ incubator for 24-48 hours.
3. After 24-48 hours of incubation, assay cells for gene activity.

*** serum-free DMEM medium can also be used.

Figure 3: Transfection protocol for 24 well plates (volumes indicated are per well). To transfected the entire plate multiply all volumes and DNA amount by 24
Table 4: Suggested amounts of DNA, medium and OMNIfect for transfection of plasmid DNA into adherent and suspension cells.

<table>
<thead>
<tr>
<th>Tissue Culture Plates</th>
<th>Surface Area per Well (cm²)</th>
<th>µl Plating Medium per Well</th>
<th>µg Plasmid DNA per Well</th>
<th>µl OMNIfect per Well</th>
<th>µl Transfection Complex per Well†</th>
</tr>
</thead>
<tbody>
<tr>
<td>6- well</td>
<td>9</td>
<td>2000</td>
<td>2 (in 100 µl Opti-MEM® I)</td>
<td>4 (in 100 µl Opti-MEM® I)</td>
<td>200</td>
</tr>
<tr>
<td>12-well</td>
<td>4</td>
<td>1000</td>
<td>1 (in 50 µl Opti-MEM® I)</td>
<td>2 (in 50 µl Opti-MEM® I)</td>
<td>100</td>
</tr>
<tr>
<td>24-well</td>
<td>2</td>
<td>500</td>
<td>0.5 (in 25 µl Opti-MEM® I)</td>
<td>1 (in 25 µl Opti-MEM® I)</td>
<td>50</td>
</tr>
<tr>
<td>96-well</td>
<td>0.3</td>
<td>200</td>
<td>0.1 (in 10 µl Opti-MEM® I)</td>
<td>0.2 (in 10 µl Opti-MEM® I)</td>
<td>10-20</td>
</tr>
</tbody>
</table>

† Total volume of the transfection complex is made up of equal parts of DNA solution and OMNIfect solution.

**Optimizing Your Transfection:**
- It is important to optimize transfection conditions to obtain the highest transfection efficiency with lowest toxicity for various cell types.
- We recommend starting with the volumes and concentrations outlined in Table 4 for different plate formats.
- You can optimize your transfection efficiency by increasing or decreasing the volume of transfection complex that is added to each plate.
- When varying the plasmid DNA concentration, keep DNA mass to OMNIfect volume proportional (1 µg DNA : 2 µl OMNIfect).
- To further optimize your transfection efficiency and lower cytotoxicity, you can vary DNA (µg): OMNIfect reagent (µl) ratios from 1:1.5 to 1:2.5.

Note: If transfection conditions result in unacceptable cytotoxicity in a particular cell line the following modifications are recommended:
1. Decrease the volume of Transfection complex that is added to each well.
2. Higher transfection efficiencies are normally achieved if the transfection medium is not removed. However, if toxicity is a problem, aspirate the transfection complex after 6 hours of transfection and replace with fresh growth medium.
3. Increase the cell density in your transfection.
4. Assay cells for gene activity 24 hours following the addition of transfection complex to cells.
**Antibiotic selection for transfected cells**

While in overexpression experiments a percentage of untransfected cells is acceptable; obtaining results from very difficult to transfected cells is aided by the addition of antibiotic to the culture post transfection. Selection will for cells successfully transfected with the expression vector and reduce the background when assaying for gene expression. Subsequently a stable cell line can be generated and maintained for an extended period of time.

Note: MGC *premier* Expression-Ready cDNA gene families and pathway focused sets have no mammalian selection marker. If transfection efficiency is high enough so that many of target cells contain a plasmid, then the need for selection is obviated.

Based on the antibiotic concentration established in by the antibiotic kill curve above:

1. After 24-72 hours of incubation, begin the antibiotic selection by replacing the medium with complete medium supplemented with the optimal antibiotic concentration. Incubate.
2. Approximately every 2-3 days replace with freshly prepared selective media. Monitor the cells daily and observe the percentage of surviving cells. At some time point almost all of the cells surviving selection will be harboring the expression construct. Optimum effectiveness should be reached in 3-6 days with antibiotic. Observe the cells for approximately 7 days until you see single colonies surviving the selection. The negative control should have no surviving cells.
3. You can select a mixture of transfected resistant cells or a single colony depending on your preference.

**Getting Clone Information**

Relevant clone information for your expression-ready cDNA clone is available on the Fetch my Gene search results. Simply enter a clone ID number or accession number into the search box and click Go. Clicking the clone ID in the search results page will display the clone details page (see screenshots below).
Clone Details Page

Clone information appropriate for each MGC premier expression-ready vector can be obtained from the clone details page associated with your clone ID. Click on the clone ID from the gene search results page to access the clone details page and information on vector, sequencing primers, antibiotic selectable markers, E. Coli host strain and more including the published cDNA insert sequence.
References:


MGC (Mammalian Gene Collection) Program Team, 2002. Generation and Initial Analysis of more than 15,000 Full-Length Human and Mouse cDNA Sequences PNAS; 99(26):16899-903 and Supporting Table.


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