Mouse/Rat Pluripotent Stem Cell Assessment Primer Pair Panel

Catalog Number SC015

Reagents for the identification of mouse and rat stem cell differentiation states.

This package insert must be read in its entirety before using this product.

FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
THE SAFETY AND EFFICACY OF THIS PRODUCT IN DIAGNOSTIC
OR OTHER CLINICAL USES HAS NOT BEEN ESTABLISHED.

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INTRODUCTION

Embryonic stem (ES) cells, derived from the inner cell mass of pre-implantation embryos, have been recognized as the most pluripotent stem cell population (1, 2). ES cells can be maintained and propagated for extended periods in media containing fibroblast growth factor basic (FGF basic) (3). Differentiation of ES cells to a lineage-committed fate is typically monitored by characterization of its phenotypic properties. More recently, molecular markers for ES cell differentiation have been identified by a variety of techniques including comparison with databases, reverse transcription-polymerase chain reaction (RT-PCR*), focused cDNA microarrays, and immunocytochemistry. The identification of a common set of expressed genes contributing to the core properties of ES cells, or their "stemness" phenotype has been established (3 - 11).

MATERIALS PROVIDED

The Mouse/Rat Pluripotent Stem Cell Assessment Primer Pair Panel profiles the mRNA transcripts of fourteen genes that are frequently used as markers for molecular characterization of undifferentiated and lineage-committed mouse and rat ES cells (Table 1). A primer pair for mouse/rat GAPDH is included and can be used as a control for successful cDNA synthesis. A positive control is also included. See Table 2 in the appendix.

Table 1: *Expected gene expression patterns detected by this panel.*

Undifferentiated ES Cells	Ectodermal Lineage	Endodermal Lineage	Mesodermal Lineage	Germ Cells
DPPA5/ESG1	Nestin	AFP	Brachyury	Stella/DPPA3
Nanog	Otx2	GATA-4	—	—
Oct-3/4	TP63/TP73L	PDX-1/IPF1	—	—
SOX2	SOX2	SOX17	—	—
—	—	HNF-3β/FoxA2	—	—

Primer Pairs - Lyophilized. Each vial contains 375 pmoles of each primer. See Table 2 in the appendix for details about the primer pairs provided in this kit.

Positive Control 61 - One vial containing 1500 ng of lyophilized synthetic double-stranded DNA. The Positive Control is not intended for quantitative purposes.

*PCR is covered by US Patent Nos. 4683195 and 4683202 assigned to Hoffmann-La Roche.

ADDITIONAL SUPPLIES REQUIRED

Reagents

- RNase-free DNase (DNase I from Ambion or equivalent)
- Random Primers (R&D Systems, Catalog # RDPC2) or Oligo $(dT)_{12-18}$ primers (R&D Systems, Catalog # RDPC1)
- RNase H^- Reverse Transcriptase (Superscript II from Invitrogen or equivalent)
- Taq DNA Polymerase (Taq 2000 from Stratagene or equivalent)
- Agarose (Seakem ME agarose from Cambrex or equivalent)
- Reverse Transcription Buffer (Invitrogen or equivalent)
- 10X Taq buffer (Stratagene or equivalent)
- 2 mM dNTPs (Amersham or equivalent)
- 10 mM dNTPs (Invitrogen or equivalent)
- 0.1 M DTT (Invitrogen or equivalent)
- Autoclaved deionized water (dH_2O)
- Nuclease-free dH_2O
- Gel loading dye
- DNA markers
- Mineral oil
- 0.1X TE
- 1X TAE

Equipment

- Thermal cycler (Stratagene Robocycler or equivalent)
- Hot block or water bath at 70° C
- Hot block or water bath at 42° C
- Power supply

Disposables

- Pipettes and aerosol barrier pipette tips
- Gloves
- Thin walled PCR tubes
- 1.5 mL sterile tubes

STORAGE

The reconstituted primer pairs are stable for up to one year at ≤ -20° C* in a manual defrost freezer. Aliquot in single use portions. Avoid repeated freeze-thaw cycles.

*Provided this is within the expiration date on the kit label.

LIMITATIONS OF THE PROCEDURE

- **FOR LABORATORY RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
- The safety and efficacy of this product in diagnostic or other clinical uses has not been established.
- The kit should not be used beyond the expiration date on the kit label.
- The quality of the cells and any variations in the procedure can cause variation in results.

SPECIFICITY

All mouse/rat embryonic stem cell primer pairs are specific for mouse and rat cDNA. All primer pairs were also tested with human cDNA. The GATA-4 primer pair amplified human cDNA. Mouse/rat GATA-4 primer pair is not optimized for human. All embryonic stem cell primer pairs were tested with mouse embryonic fibroblast (MEF) cDNA and thirteen out of fourteen did not amplify a product. Mouse/rat Nestin did amplify a product from the MEF cDNA.

RECOMMENDED PROTOCOLS

I. DNase Treatment

Treatment of total RNA with RNase-free DNase is required to remove contaminating genomic DNA. Follow the procedure according to the manufacturer's instructions. If DNase treatment of RNA is not done, false positives may result.

II. Reverse Transcription Reaction

1. Thaw all reagents completely on ice. All reactions should be assembled on ice.
2. Pipette the following into a nuclease-free tube:
   - 1 - 5 μg of DNase treated total RNA (up to 11 μL)
   - 1 μL of random primers (300 ng/μL) or Oligo $(dT)_{12-18}$ primers (0.5 μg/μL)
   - X μL of nuclease-free dH_2O for a final volume of 12 μL
3. Mix and incubate at 70° C for 10 minutes. Place tube on ice immediately.
4. Briefly centrifuge the tube, and add the following to each tube:
   - 4 μL of 5X Reverse Transcription Buffer
   - 2 μL of 0.1 M DTT
   - 1 μL of 10 mM dNTPs
5. Mix and incubate at room temperature for 10 minutes.
6. Incubate at 42° C for 2 minutes.
7. Add 1 μL RNase H^- Reverse Transcriptase (200 units/μL). Mix by pipetting.
8. Incubate at 42° C for 50 minutes.
9. Incubate at 70° C for 15 minutes.
10. Dilute reactions 5-fold by adding 80 μL of nuclease-free dH_2O.
11. Assay immediately or store the cDNA samples at ≤ -20° C in a manual defrost freezer.

III. PCR Reaction

1. Resuspend each Primer Pair in 50 μL of autoclaved deionized water or 0.1X TE Buffer (1 mM Tris HCl, 0.1 mM EDTA, pH 8.0 at 25° C) for a final concentration of 7.5 pmoles/μL each primer (do not resuspend the Positive Control at this time).
2. Determine the number of PCR reactions (see Technical Hints for setting up a PCR master mix). Multiply the volumes listed below for each reagent by the number of reactions. Prepare a separate master mix for each primer pair.

   36.5 μL autoclaved deionized water

   5 μL 10X Taq buffer (with 15 mM $MgCl_2$)

   5 μL 10X dNTP mix (10X = 2 mM each dNTP)

   2 μL Primers (7.5 pmoles/μL each primer)

   0.5 μL Taq DNA Polymerase (5 units/μL)
3. Prepare the Negative Control reaction tube:
   a. Pipette 1 μL of autoclaved deionized water into a pre-labeled negative control tube.
   b. Add 49 μL of the master mix prepared above.
   c. Briefly spin tube and add 30 μL of mineral oil to prevent evaporation.
   d. Close the reaction tube and place on ice.
4. Prepare the cDNA sample reaction tube(s):
   a. Pipette 1 μL of cDNA sample into a pre-labeled PCR reaction tube.
   b. Add 49 μL of the master mix prepared above.
   c. Briefly spin tube and add 30 μL of mineral oil to prevent evaporation.
   d. Close the reaction tube and place on ice.
5. Resuspend Positive Control 61 in 150 μL of autoclaved deionized water or 0.1X TE Buffer. Centrifuge the Positive Control tube briefly. This should be done in a separate location from where PCR reactions are set up. Use different pipettes than those used for PCR set up.
   a. Pipette 1 μL of Positive Control into the pre-labeled Positive Control reaction tube.
   b. Add 49 μL of the master mix prepared above.
   c. Briefly spin tube and add 30 μL of mineral oil to prevent evaporation.
   d. Close the reaction tube and place on ice.
6. Place all tubes in a thermal cycler and perform the following program:

   94° C for 4 minutes

   94° C for 45 seconds
   55° C for 45 seconds } 30 - 35 cycles
   72° C for 45 seconds

   72° C for 10 minutes

TECHNICAL HINTS

- Thaw all reagents completely on ice before use.
- Use either Random Primers or Oligo (dT) for Reverse Transcription.
- The recommended annealing temperature is 55° C.
- To minimize the risk of amplicon contamination of the Primer Pairs and other PCR reagents, the following precautions are recommended:
  - PCR reactions should be set up in an area separate from where PCR products are analyzed.
  - Pipettes and tube racks should be specifically designated for PCR.
  - Use aerosol barrier pipette tips.
- Follow the steps below to determine the number of PCR reactions and to calculate the amount of PCR master mix necessary.
  1. Determine the number of cDNA samples that will be analyzed. Include a positive and negative control. Add 2 to the number of cDNA samples to account for the two controls.
  2. Multiply the number of samples by a factor of 1.1 to account for pipeting volume. This is the number of reactions for which the master mix should be made.

     For example, the master mix for 3 cDNA samples should be:
     - 3 cDNA samples will be analyzed.
     - Plus 2 for negative and positive controls = 5
     - 5 x 1.1 for pipeting volume = 5.5
- Primer Pairs are not validated for use in kinetic RT-PCR.

ANALYSIS OF RESULTS

The PCR products can be analyzed by 1.5 - 2% agarose gel electrophoresis. For predicted sizes of PCR products, refer to Table 2.

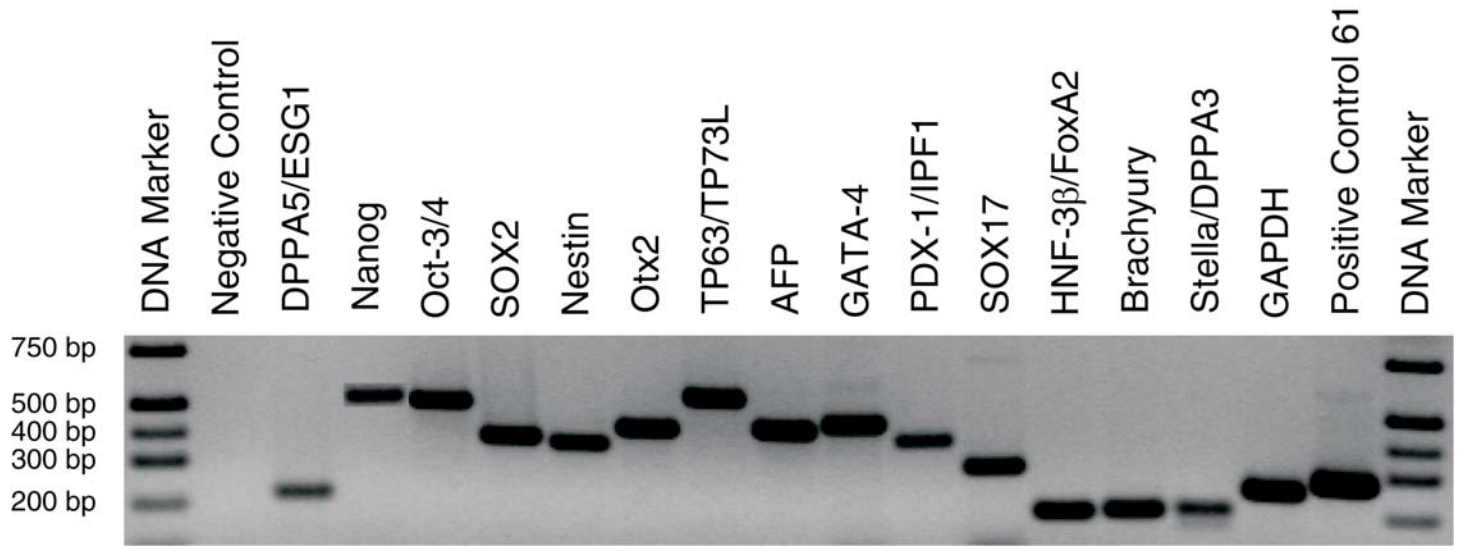

Figure 1: *PCR products derived from various tissue samples were separated by agarose gel electrophoresis.*

APPENDIX OF PROVIDED PCR PRODUCTS

Table 2: *Predicted sizes of PCR products provided in the Mouse/Rat Pluripotent Stem Cell Assessment Primer Pair Panel.*

Gene	Primer Pair Catalog #	GenBank Accession #	cDNA Product Size (bp)	Genomic Product Size (bp)	Comments
AFP	RDP-337	NM_007423 (m) NM_012493 (r)	451 (m & r)	3435 (m) N/A (r)	———
Brachyury	RDP-343	NM_009309 (m) XM_217890 (r)	216 (m & r)	1769 (m & r)	———
DPPA5/ESG1	RDP-333	NM_025274 (m) XM_236761 (r)	224 (m) 221 (r)	305 (m) N/A (r)	224 bp and 221 bp products also seen from pseudogenes.*
GAPDH	RDP-106	M32599 (m) M17701 (r)	265 (m & r)	650 (m & r)	265 bp product also seen from pseudogene.*
GATA-4	RDP-345	NM_008092 (m) NM_144730 (r)	479 (m) 476 (r)	3343 (m) N/A (r)	———
HNF-3β/FoxA2	RDP-340	NM_010446 (m) NM_012743 (r)	215 (m & r)	1217 (m) N/A (r)	———
Nanog	RDP-334	NM_028016 (m) XM_575662 (r)	547 (m) 562 (r)	1801 (m) N/A (r)	547 bp product also seen from pseudogene in mouse.*
Nestin	RDP-153	NM_016701 (m) NM_012987 (r)	388 (m & r)	3620 (m) N/A (r)	———
Oct-3/4	RDP-335	NM_013633 (m) NM_001009178 (r)	536 (m) 537 (r)	2678 (m) 1191 (r)	536 bp and 537 bp products also seen from pseudogenes.*
Otx2	RDP-342	NM_144841 (m) XM_224009 (r)	449 (m & r)	2422 (m & r)	———
PDX-1/IPF1	RDP-338	NM_008814 (m) NM_022852 (r)	421 (m) 418 (r)	4153 (m) N/A (r)	———
SOX2	RDP-336	NM_011443 (m) XM_574919 (r)	406 (m) 423 (r)	406 (m) 423 (r)	———
SOX17	RDP-339	NM_011441 (m) XM_232640 (r)	337 (m & r)	767 (m & r)	———
Stella/DPPA3	RDP-344	NM_139218 (m) XM_216263 (r)	218 (m) 224 (r)	218 (m) 224 (r)	———
TP63/TP73L	RDP-341	NM_011641 (m) NM_019221 (r)	587 (m & r)	8672 (m) N/A (r)	Amplifies all variants to give the same size product.
Positive Control 61	———	———	290	———	Synthetic double-stranded DNA.

bp = base pairs
N/A = not applicable
*Pseudogenes will not affect performance in properly DNase treated samples.

APPENDIX OF RELATED PRODUCTS

Table 3: *Related products available from R&D Systems.*

Molecule	Catalog #	Product Description
AFP	MAB1368	Human/Mouse Monoclonal Antibody (Clone 189502)
Nanog	AF2729	Mouse Affinity Purified Polyclonal Antibody
Nestin	AF2736 IC2736P MAB2736	Rat Affinity Purified Polyclonal Antibody Rat Labeled Monoclonal Antibody (Clone 307501) Rat Monoclonal Antibody (Clone 307501)
Oct-3/4	IC1759F IC1759P MAB1759	Human/Mouse Labeled Monoclonal Antibody (Clone 240408) Human/Mouse Labeled Monoclonal Antibody (Clone 240408) Human/Mouse Monoclonal Antibody (Clone 240408)
PDX-1/IPF1	IC2419A IC2419P MAB2419	Human/Mouse Labeled Monoclonal Antibody (Clone 267712) Human/Mouse Labeled Monoclonal Antibody (Clone 267712) Human/Mouse Monoclonal Antibody (Clone 267712)
SOX2	MAB2018	Human/Mouse Monoclonal Antibody (Clone 245610)
Stella/DPPA3	AF2566 BAF2566 MAB2566	Mouse Affinity Purified Polyclonal Antibody Mouse Biotinylated Affinity Purified Polyclonal Antibody Human/Mouse Monoclonal Antibody (Clone 283910)
Brachyury	**Please refer to www.RnDSystems.com for a current list of available products.**	
DPPA5/ESG1		
GATA-4		
HNF-3β/FoxA2		
Otx2		
SOX17		
TP63/TP73L		
Human Kit	SC012	Human Pluripotent Stem Cell Assessment Primer Pair Panel

TROUBLESHOOTING GUIDE

Problem	Suggestions
No PCR products obtained.	Operator error may have occurred during reaction assembly. Run positive control reaction.
	Unsuccessful cDNA synthesis. Use the GAPDH primer pair as a control to show that the RT reaction was successful.
	RNA may be degraded. Check the integrity of the RNA by gel electrophoresis. A good quality RNA preparation should show the 28S and 18S ribosomal RNAs in an approximate 2:1 ratio. If RNA appears degraded, repeat the RNA isolation.
	RNA secondary structure may be inhibiting cDNA synthesis. This can often be overcome by using random primers.
	The target RNA concentration may be too low. Increase the amount of cDNA template used in the PCR reaction or the number of cycles used to amplify the cDNA.
Smearing or bands of unpredicted size.	Non-specific priming may have occurred. Use less cDNA or reduce the number of cycles.

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NOTES