DNA Repair Deficient Cell Lines—Tools to Study Genomic Instability

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DNA Repair Pathways

There are over 150 human proteins that have been categorized as *bona fide* DNA Repair proteins (see Table 1). These fall into 14 general categories, including the classical DNA Repair pathways of Base Excision Repair (BER) (1), Direct Reversal of Damage, Mismatch Excision Repair (MMR), Nucleotide Excision Repair (NER), Homologous Recombination (HR), Non-Homologous End-Joining (NHEJ) and the Fanconi Anemia/DNA crosslink repair pathway. There are proteins that modulate nucleotide pools, DNA polymerases, and editing and processing nucleases, the Rad6 pathway; proteins that modulate chromatin structure, DNA Repair genes/proteins defective in diseases & conserved DNA Damage Response (2,3). Advances in the DNA Repair field inevitably lead to additions to each category as new proteins are found that contribute to the repair of DNA damage or to the cellular response to DNA damage, such as MRG15 involvement in HR via its interaction with PALB2 (4)or DNA2, recently identified as a structure-specific nuclease involved in nuclear and mitochondrial genome maintenance (5-7). To follow, update, and compare the DNA Repair family of genes across species, the University of Pittsburgh Cancer Institute (UPCI) has established a web-based, interactive DNA Repair database: (https://dnapittcrew.upmc.com/). It is expected to be online and available to the public by the start of this project. Most of these *bona fide* DNA Repair genes have also been summarized in a recent set of reviews and online (2,3) (http://www.cgal.icnet.uk/DNA_Repair_Genes.html).

Table 1 – Groups of DNA Repair Genes/Proteins Based on Pathway or Function

DNA Repair Pathways/Categories	# of Genes/Proteins
Base Excision Repair (BER)	20
Direct Reversal of Damage	3
Mismatch Excision Repair (MMR)	12
Nucleotide Excision Repair (NER)	30
Homologous Recombination (HR)	20
Non-Homologous End-Joining (NHEJ)	6
Fanconi Anemia/DNA Crosslink Repair Pathway	16
Proteins that Modulate Nucleotide Pools	18
DNA Polymerases	15
Editing and Processing Nucleases	6
The Rad6 Pathway	5
Proteins that Modulate Chromatin Structure	3
DNA Repair Genes/Proteins Defective in Diseases	4
Conserved DNA Damage Response genes	9
Total	167

DNA Repair Defects in Human Cancer

Since the first identification of mutations in the NER genes linked to Xeroderma pigmentosa (XP) mediated skin cancer (8), all the DNA Repair families have been linked to one or more cancers (9). For example, defects in MMR and BER are linked to elevated incidence of colon cancer (10,11) ,defects in HR elevate onset of breast and ovarian cancer (12), and defects in NER decrease survival in lung cancer (13). In all, recent estimates suggest that all cancer cells may be defective in one or more pathways of DNA repair (14) Therefore well developed and characterized isogenic cell lines each defective in one of these genes are of great value to the scientific community.

RNA interference (RNAi)

RNAi is a highly specific post-transcriptional gene-silencing mechanism that is mediated either by intracellular expression of double-stranded (ds) RNA molecules, 21 bp in length, or via the expression of dsRNA hairpins of 21- 23 bp in length (shRNA). The expressed dsRNA is cleaved by a nuclease (Dicer) into dsRNA of 21-23 bp in length to yield the siRNA. One strand of the siRNA is then incorporated into an RNA-silencing complex (RISC) to target the complementary RNA for destruction by a second nuclease, Ago2 (15-17). This method of gene regulation has become invaluable to identify gene function, validate anti-cancer gene targets and is currently being evaluated for clinical potential (18). The utility of RNA interference as a genetic tool for the study of biological pathways and stress responses in mammalian cells has made significant strides since the first demonstration of RNAi in mammalian cells in 2001 (19). This was quickly followed by the demonstration that stable-expression of short hairpin RNAs can mediate gene knockdown (20-22).

DNA Repair Capacity and Gene Expression as Biomarkers for Genotoxicant Exposure

Global gene expression analysis has proven to be an effective approach to discover cellular and/or organismal effects brought about by external stimuli such as environmental toxicants, chemotherapeutic regimens, and viral infections as well as developmental and age-related stimuli. The application of whole-genome expression analysis to toxicology, termed toxicogenomics, has provided considerable insight into possible mechanisms of toxicity and has the potential to identify a characteristic set of genes that may facilitate prediction of a compound's toxicity (23). Baseline expression analysis in all tissues in the mouse has recently been reported, demonstrating that stress response, DNA damage control and DNA repair genes are differentially expressed among tissues (24).In line with the development of these cell lines genotoxic response is suggested to be the result, in part, of variations in DNA repair capacity (24)and can further be addressed using a well characterized set of DNA repair deficient cell lines.

Product Portfolio

Presently, Trevigen offers 19 isogenic DNA repair deficient cell lines, the majority of which are included in the BER pathway. The cell lines currently available and the percent knockdown as evaluated by QPCR are shown in Table 2.

Table 2 Knockdown Cell Lines		
Target	**Catalog Number**	**Percent (%) Knockdown**
APE1	5517-001-01	90
APE2	5518-001-01	80
BRCA1	5502-001-01	84
XRCC1	5516-001-01	81
MBD4	5506-001-01	72
MPG	5511-001-01	95
MutYH	5512-001-01	87
NTHL1	5505-001-01	87
NEIL2	5507-001-01	92
NEIL3	5508-001-01	86
OGG1	5504-001-01	63
PARG	5501-001-01	84
PARP1	5500-001-01	87
PARP2	5514-001-01	83
PARP3	5515-001-01	70
SMUG1	5510-001 01	63
TDG	5519-001-01	74
UNG	5509-001-01	87
Control Cell Line	5503-001-01	N/A

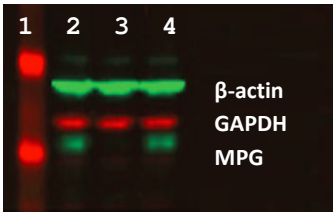
Validation

Expression is evaluated by QPCR, Western blots, and functional analysis when possible. As shown in Figure 1, MPG was assayed by incubating crude extracts of MPG knockdown lines with infrared-labeled oligonucleotide substrates. Reaction products were separated using denaturing polyacrylamide gel electrophoresis and bands quantified using LI-COR ODYSSEY. Panel A Lesion templates containing hypoxanthine (HX) were selected based on published studies.

Enzymatic Assay for MPG activity

Lane 1: Buffer alone
Lane 2: Control extract
Lane 3: MPG knockdown extract

Western Blot of MPG protein



Lane 1: Molecular weight markers
Lane 2: Scramble RNA Control
Lane 3: MPG knockdown extract
Lane 4: Control cell line

Figure 1: Enzymatic assay (panel A) and western blot analysis (panel B) for MPG activity in MPG KD cell extracts. Panel A: lane 1, buffer control; lane 2, 40 µg control extract; lane 3, 40 µg MPG KD extract. Panel B: lane 1, molecular weight markers; lane 2, scramble RNA control; lane 3, MPG KD cell extract; Lane 4, control cell line. Blots were incubated with antibodies directed against β-actin, GAPDH and MPG.

Development of Tiered Cell Bank

Figure depicts the logic by which we will develop a cell bank for cell lines developed in this proposal. The purpose of developing a cell bank is to:

- Limit the passage numbers when preparing cells for distribution. This will minimize genetic drift associated with cell lines that are maintained for long periods in tissue culture.
- Permit the relatively easy qualification of the cell lines to set both genotypic and phenotypic standards of performance to assure the cells maintain crucial characteristics during all phases of cell production.
- Provide a renewable source of qualified cells with the same phenotypic and genotypic properties. This is an essential premise for the development of a cell based screening assay dependent on a particular cell line.

Using a tiered cell bank strategy (Figure 2) three levels of cell stocks are produced: seed stock, production stock and distribution stock. Approximately 100 vials of seed stock and 100 vials of production stock are prepared. Cells grown at each level will be evaluated for the specific gene knockdown efficiency.

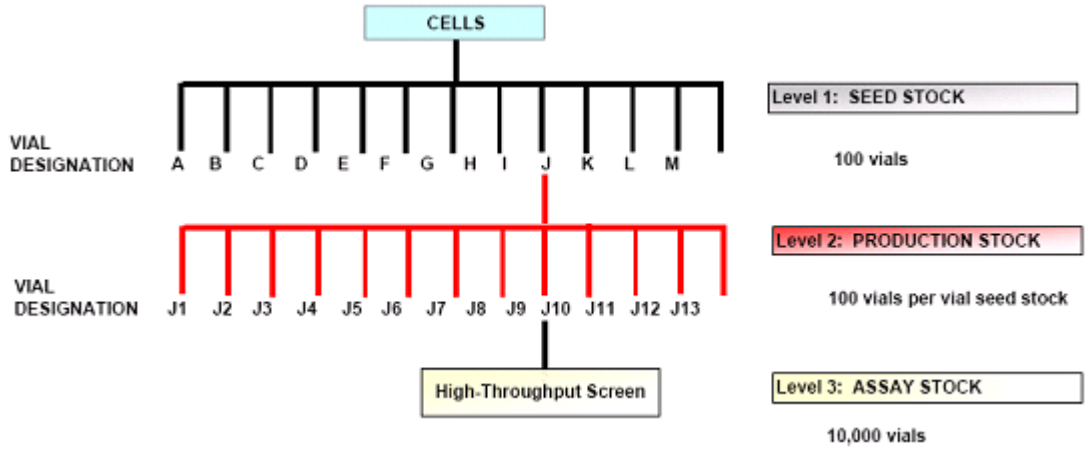


Figure 2. Cell bank structure.

Prior to cell bank construction, cells are evaluated for mycoplasma and other human viruses. The cell bank characterization will include western blot analysis and QPCR to demonstrate that during preparation knock down efficiencies have not drifted. Once the seed stock and production stock have been evaluated a vial of seed stock is grown representing assay stock (Figure 2) and evaluated as described above. These evaluations will be used to demonstrate that as a result of preparing the cell bank we did not alter specific genes or cellular properties that would hamper the performance. From every vial of seed stock we can produce 100 vials of production stock that can subsequently be used for assay stock. By using the tiered cell bank logic and inventorying of 100 vials qualified seed stock and 100 vials of qualified production stock from each vial of seed stock, we can readily produce 10,000 vials of assay stock. This strategy assures that we will have an ample supply of cells to provide the scientific community.

Impact and Significance

DNA repair pathways maintain the integrity of the genome (1), reducing the onset of cancer, disease and aging phenotypes. Conversely, the requirement for DNA repair and genome maintenance in response to radiation and genotoxic chemotherapeutics implicates DNA repair proteins as prime targets for improving response to currently available anti-cancer regimens. Further, cancer-specific DNA repair defects offer novel approaches for tumor selective therapy. As described recently by Dr. Bruce Alberts 14 "… we can expect all cancer cells to be defective in some aspect of DNA repair … There are at least 150 different proteins that catalyze DNA repair … To seed new therapies, geneticists and molecular biologists are needed to explore the detailed consequences of an alteration in each of these repair pathways…". Trevigen's DNA repair deficient cell lines provide essential tools to address this need.

***Company Background**

Trevigen, founded in 1992, develops, produces and markets products for research involving apoptosis, DNA damage, and cell behavior. Basement Membrane Extract and specialized proteins for cancer research, tissue specific *in situ* apoptosis detection kits, DNA damage assays including Comet Assay™ kits, Poly(ADP-Ribose) Polymerase (PARP), 8-oxo-dG ELISA kits, Poly (ADP-ribose) glycohydrolase (PARG) Assays, and oxidative damage detection kits are representative of the extensive product range. In addition to specific kits, Trevigen manufactures and sells numerous DNA repair enzymes and antibodies specific to DNA repair and apoptosis proteins. The company has a long and successful track record in product development and sales of DNA repair associated products to the research community.

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