

## THE READIT™ SNP GENOTYPING SYSTEM: SINGLE NUCLEOTIDE POLYMORPHISM DETECTION IN NEUROSCIENCE

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### Introduction

Humans are 99.9% genetically identical (1). The most common type of genetic variability found in humans occurs in the form of single nucleotide polymorphisms (SNPs). SNPs (pronounced “snips”) are points where there are two or more possible nucleotides at a specific, mapped location in the genome and occur in the human genome with an estimated frequency of one for every 1,200–1,500bp (1). As sources of genetic variability, SNPs hold great promise in the understanding, treatment and prevention of disease. SNPs may also reveal the genetic influences of many aspects of human behavior and personality.

Upon publication of the rough draft of the human genome, scientists had identified over a million SNPs (1,2). To date, over 1.5 million SNPs have now been identified and placed in a database, as a result of collaborative efforts between the Human Genome Project and groups such as the SNP Consortium, a nonprofit foundation supported by the Wellcome Trust and eleven pharmaceutical and technology companies (3).

Not all SNPs cause disease; they can also help determine how a person responds to therapeutic treatments or act as markers for populations at risk for developing a disease. Examples of the variety of consequences of SNPs include the missense mutation (A→U) that causes Sickle Cell Anemia, the APOE ε4 allele implicated in susceptibility to late-onset Alzheimer’s disease (4), the Factor V 1691G→A allele (FV Leiden) involved in hereditary deep-vein thrombosis (5) and several forms of the cytochrome p450 (CYP) gene that affect drug metabolism (6,7).

### Identifying SNPs Associated with Neurological Disease

Since close to half of all human genes are expressed in the brain, the potential for SNPs to help explain the diseases of the brain is tremendous. The human SNP database will be a valuable tool to help researchers pinpoint genetic variations associated with many diseases and mental disorders (8). For example, microsatellite allele frequency in the D2 dopamine receptor may be linked to the genetic susceptibility to schizophrenia (9), and SNPs in the variable number tandem repeat (VNTR) in the 3’ untranslated region (3’UTR) of the human dopamine transporter (DAT) may play a role in attention deficit hyperactivity and other dopamine-related disorders (10).

For most mental disorders, finding the genetic cause will be more complicated than simply identifying one gene or a few SNPs, because these disorders most likely result from the interaction of several genetic and environmental factors (11). Different alleles of multiple genes may act in combination to create a predisposition to illness, while the onset of illness may be triggered by developmental events or exposure to unidentified environmental risk factors (12).

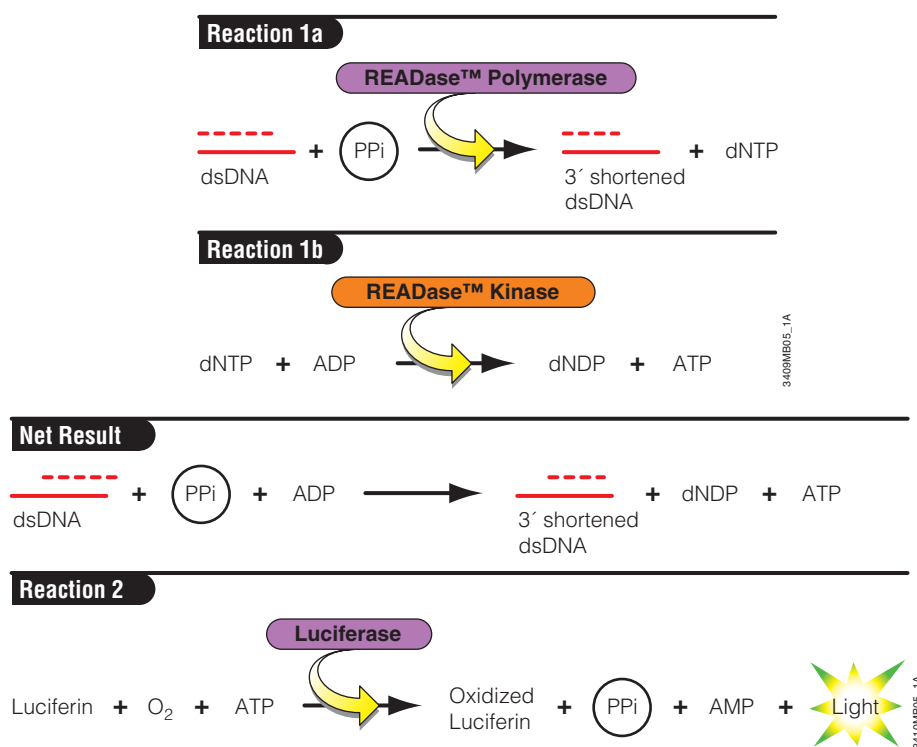
### Additional Benefits of SNP Genome Mapping

In addition to producing discernable phenotypes, SNPs can also be used as landmarks on a genetic road map created to point scientists to an important gene. Because a SNP located close to the sequence that codes for a gene will most likely be inherited along with that gene, researchers can compare an individual’s “map” of known SNPs to a map of the same SNPs from a control group. If the pattern from the affected person varies, those differences could point to a genetic cause. Estimates of the number of SNPs required to create a useful map have ranged from 100,000 (one SNP per every 30kb of DNA) to 1 million (one SNP per every 3kb or less). In general, the more SNPs on the map the better, and the ideal number is probably between 600,000 and 1 million. However, the number of SNPs on the map must be balanced against the cost of identifying them (13). Costs should drop with the completion of the Human Genome Sequence, and useful maps can still be made using a smaller number of markers. For example, maps with SNPs spaced 30kb apart have been successfully used to identify genes involved in diseases such as psoriasis, migraine, Alzheimer’s and diabetes (13).

With the potential number of SNPs stretching into the millions, researchers face the daunting task of finding the ones that are informative. One way to do this is to screen a piece of DNA for sequence changes. Screening technologies identify sequence changes by comparing the results from an experimental sample to those of a sample with a known sequence. Screening samples for sequence changes is more time-efficient than sequencing and helps to narrow the field to those samples with the most promise of revealing an interesting SNP.

### Introducing the READIT™ SNP Genotyping System

Once a useful SNP has been identified, whether it is a marker or a coding sequence change, the next hurdle is identifying samples that contain the SNP. The relatively new world of SNP genotyping is becoming increasingly more competitive. A useful detection method must meet several important criteria: i) accuracy—new methods must be validated against existing “gold standard” methods, and an



**Figure 1. READIT™ Assay coupled reaction.** READase™ Polymerase catalyzes pyrophosphorylysis to produce free dNTPs (Reaction 1a); READase™ Kinase uses the dNTPs to generate ATP (Reaction 1b). The luciferase/luciferin reaction (Reaction 2) generates a light signal proportional to the amount of ATP produced in the first reaction.

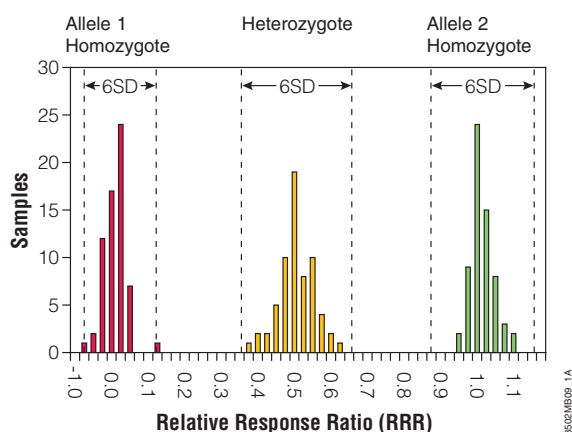
accuracy of >99% is essential; ii) reliability—repeating tests because of assay failure increases costs, delays results and wastes precious samples; iii) convenience—minimizing complexity and hands-on time reduces costs and decreases the possibility of operator errors; and iv) affordability—reagent costs, technician time, equipment requirements and royalty payments all contribute to the overall cost of a method (14). The READIT™ SNP Genotyping System<sup>(a)</sup> now available from Promega is a versatile and accurate assay that offers a new approach to genotyping and SNP scoring (14,15).

The READIT™ Assay is a three-step system that interrogates PCR amplified samples for a specific sequence and produces a light signal to identify which target sequence is present. The assay uses hybridization specificity and a coupled reaction using two thermostable enzymes, READase™ Polymerase and READase™ Kinase, to generate high-energy adenosine triphosphate (ATP). When luciferase/luciferin reagent is added, the ATP is used to produce light detectable with a luminometer. The procedure is compatible with several automated systems and is simple to perform. The companion READIT™ Calculator software (Cat.# MD1240) processes the results

downloaded from the luminometer and assigns a genotype to each sample. The software can perform statistical analysis on a defined group of samples and contains error-checking algorithms to identify problematic samples.

### The READIT™ Reaction

To detect a SNP, the assay requires two unmodified DNA probes (interrogation probes) that differ at the 3' end, each forming perfect hybrids to one of the two possible sequences. If the probe matches the target sequence, the READase™ Polymerase catalyzes depolymerization, or pyrophosphorylysis, of the DNA target, shortening the 3' end of the target and releasing a high-energy deoxynucleotide triphosphate (dNTP). In a coupled reaction, READase™ Kinase transfers the terminal phosphate from the liberated dNTPs to adenosine diphosphate, producing ATP. ATP is an essential cofactor in a reaction with luciferase and luciferin that produces light, and the light is used to monitor ATP production (Figure 1). The light signal produced is proportional to the ATP present in the reaction. Aside from SNPs, the READIT™ Assay can also analyze samples for sequence variations such as deletions, insertions and chromosomal



**Figure 2. READIT™ Assay results.** Thirty-two representative allele 1, heterozygous and allele 2 samples were amplified for a total of 96 samples. Duplicate purifications were performed on each sample, and the READIT™ Assay was performed on all purified samples. The READIT™ Calculator plotted the frequency of samples with increasing relative response ratios. Dashed lines represent the  $\pm 3$  standard deviation values from the response ratio mean of each genotype population and are overlaid on the graph.

translocations. For more detailed information about the READIT™ Assay, refer to the *READIT™ SNP Genotyping System Technical Manual* (#TM053, 16).

In a study analyzing over 500 DNA samples, 100% concordance was observed between the READIT™ Assay and the genotyping assignments made in independent testing laboratories (15). A larger study of over 2,000 samples confirmed the accuracy of the READIT™ System (unpublished data). Figure 2 shows sample results from the READIT™ Assay. Existing standard PCR parameters can be used to generate the template DNA, and interrogation probes can be designed for any sequence. This allows researchers to develop their own assays. Additional information about designing a READIT™ Assay can be found in the *READIT™ SNP Genotyping System Technical Manual* (#TM053) as well as online at [www.promega.com/readit/](http://www.promega.com/readit/).

The READIT™ SNP Genotyping System is an accurate and cost-effective solution for the SNP scoring and genotyping market. In addition to SNP detection, the flexibility of the READIT™ System allows it be used in many other applications including detecting insertions, deletions and translocations, detecting pathogens, and determining zygosity in amplified samples.

### Conclusions

As the scientific and medical communities begin to decipher the secrets within the human genetic code, the way we approach the diagnosis, treatment and prevention

of disease will change dramatically. With only a fraction of the genome decoded, scientists have already created large databases filled with thousands of single nucleotide sequence changes. Some of these SNPs could define the genetic basis of health. The development of detection technologies such as the READIT™ Assay will benefit researchers by allowing them to develop their own assays while maintaining a high level of accuracy and thus help realize the full potential of the Human Genome Project and the upcoming SNP revolution.

### References

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- The READIT™ SNP Genotyping System Technical Manual* #TM053, Promega Corporation.

### Protocol

#### **The READIT™ SNP Genotyping System Technical Manual #TM053**

[www.promega.com/tbs/tm053/tm053.html](http://www.promega.com/tbs/tm053/tm053.html)

### Ordering Information

Product	Size	Cat.#
READIT™ Interrogation Module	100 samples (3 reactions/sample)	MD1200
READIT™ Purification Module	100 samples (3 reactions/sample)	MD1210
READIT™ System Verification Module	10 samples	MD1220
READIT™ Calculator	1 each	MD1240

For Research Use Only. Not for use in diagnostic procedures. The READIT™ Assay requires both the READIT™ Interrogation Module (Cat.# MD1200) and the READIT™ Purification Module (Cat.# MD1210). The READIT™ Calculator software is not included in the system and needs to be ordered separately at no additional cost. ©U.S. Pat. No. 6,159,693 has been issued to Promega Corporation for nucleic acid detection using depolymerization. Other patents are pending. READase and READIT are trademarks of Promega Corporation.