DICE, an efficient system for iterative genomic editing in human pluripotent stem cells.

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Public Summary:
Human stem cells have great potential, but in order to take advantage of them to the fullest, we need methods for adding genes to stem cells in a safe and effective manner. This publication describes a new method to add genes to human stem cells, such as embryonic stem cells and induced pluripotent stem cells. The method is precise and efficient, and it leads to excellent expression of the added genes. It is a two-step method. In the first step, a "landing pad" is added to the human genome that will be used as the location where the desired genes are placed. In order to best fulfill this role, we searched for a location in the human genome that would be a "safe harbor" for added genes. This means that genes added to this location would not activate any genes involved in cancer and would not disrupt any coding sequences, so gene addition at this location would have a minimal effect on existing genes. In addition, we wanted a location that would provide strong expression for the added genes. We used computer analysis of DNA sequences to find candidate locations and chose one, that we called H11, found on human chromosome 22. We had already shown that a similar location worked well in mice, and H11 also seems to work very well for the purpose in human cells. We used a new method, called TALEN-assisted homologous recombination, to add the landing pad to the H11 site. This method worked very well to provide precise and efficient addition of the landing pad to the desired H11 location. In the second step, we added genes into the landing pad by using two enzymes derived from bacterial viruses (phage) that have the ability to recombine specific DNA sequences that are present on the landing pad and flanking the genes we want to add. In this reaction, the genes we want to add are exhanged for the genes present on the landing pad. Hence, the reaction is called a "cassette exchange". Since two integrases are involved, we named the method Dual Integrase Cassette Exchange, or DICE. We demonstrated the DICE reaction by adding genes involved in making the neurons that are defective in Parkinson's Disease to stem cells. The DICE reaction worked very well, and we were able rapidly to create many cell lines carrying these genes, which will be used to study Parkinson's Disease. This new method should be valuable for many investigators who need to modify stem cells.

Scientific Abstract:
To reveal the full potential of human pluripotent stem cells, new methods for rapid, site-specific genomic engineering are needed. Here, we describe a system for precise genetic modification of human embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). We identified a novel human locus, H11, located in a safe, intergenic, transcriptionally active region of chromosome 22, as the recipient site, to provide robust, ubiquitous expression of inserted genes. Recipient cell lines were established by site-specific placement of a 'landing pad' cassette carrying attP sites for phiC31 and Bxb1 integrases at the H11 locus by spontaneous or TALEN-assisted homologous recombination. Dual integrase cassette exchange (DICE) mediated by phiC31 and Bxb1 integrases was used to insert genes of interest flanked by phiC31 and Bxb1 attB sites at the H11 locus, replacing the landing pad. This system provided complete control over content, direction and copy number of inserted genes, with a specificity of 100%. A series of genes, including mCherry and various combinations of the neural transcription factors LMX1a, FOXA2 and OTX2, were inserted in recipient cell lines derived from H9 ESC, as well as iPSC lines derived from a Parkinson's disease patient and a normal sibling control. The DICE system offers rapid, efficient and precise gene insertion in ESC and iPSC and is particularly well suited for repeated modifications of the same locus.

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