Lectures 28 and 29 – applications of recombinant technology

I. Manipulate gene of interest
   A. site-directed mutagenesis

B. in vitro mutagenesis by PCR
   1. anneal primer 1
   2. first cycle of PCR
   3. after many rounds of PCR

   why?

C. reporter transgenes
   - fuse regulatory sequences to reporter
   - use lacZ (encodes β-galactosidase), gfp (jelly fish green fluorescent protein) or luciferase (firefly enzyme)

   - eg: ceh-23::gfp

   - why?

II. Transgenic organisms - introduce gene from one species into genome of second species
   A. transgenic bacteria – generally introduce gene on plasmid

   - why?
B. transgenic yeast
   1. how?

   2. uses of yeast transformation

C. transgenic plants
   1. can be transformed by particle gun or by Agrobacterium Ti plasmid

   2. Ti plasmid:

   3. engineering plants

   4. types of engineered plants
      a. luciferase (from firefly)
      b. β-galactosidase (from E. coli)
      c. glyphosate resistant (from Salmonella)
      d. Bt toxin (from Bacillus thuringensis)

Genetically modified plants

<table>
<thead>
<tr>
<th>Crop</th>
<th>Modification</th>
<th>US</th>
<th>World</th>
</tr>
</thead>
<tbody>
<tr>
<td>soybean</td>
<td>herbicide resistant</td>
<td>93%</td>
<td>77%</td>
</tr>
<tr>
<td>field corn</td>
<td>herbicide resistant, Bt</td>
<td>86%</td>
<td>26%</td>
</tr>
<tr>
<td>cotton</td>
<td>Bt</td>
<td>93%</td>
<td>49%</td>
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5. GM (= genetically modified) plants
   a. many plants engineered to improve yield, etc.
b. potential benefits
- improved yield
- improved crops
- reduced herbicide and pesticide use
c. concerns

d. transgenic mice – why? reporters, models for human genetic disorders, etc.
1. DNA into embryonic stem cells
2. vector usually modified retrovirus

E. “transgenic” humans – gene therapy
1. can recombinant DNA technology be used to correct genetic disorders?
2. two general types
   a. germ line therapy – not done, problems:
   b. somatic – try to provide wild-type gene in some somatic cells
      - provide wild-type gene to some cells, restores production of protein in specific cells
3. approaches to somatic therapy – use different vectors
   a. retrovirus – inserts into chromosome at random
   b. adenovirus – persists extrachromosomally
   c. adeno-related virus – advantages of adenovirus
   d. lentivirus – less prone to insertional mutagenesis
F. an aside - cloning whole organisms

1. still technically challenging
   - most embryos fail/die early
   - many of those that develop have defects
2. can you clone yourself?

G. another aside - stem cells

1. what are they?
   a. share two characteristics
      i. unspecialized cells, renew through long periods through division
      ii. can be induced to differentiate

2. three types
   a. embryonic
      i. 
      ii. 
   b. adult
      i. 
      ii. produce type of tissue in which they reside
   c. induced pluripotent stem cells (iPS)
      i. introducing 4 genes into differentiated cells can cause them to revert to a stem-like state
      ii. may cause elevated risk of producing tumors; still being investigated

3. potential benefits
   a. possibly used to treat number of different diseases
      egs: Parkinsons, diabetes, heart disease, spinal cord injury, duchenne muscular dystrophy, Huntington’s, amyotrophic lateral sclerosis, multiple sclerosis, etc.

embryonic          adult          iPS

4. restrictions on research
   a. privately funded, no restrictions
   b. publicly funded, can use but not derive embryonic stem cells
   for more info, see: http://www.nih.gov/news/stemcell/primer.htm
IV. Reverse genetics
   A. what is it?
      1. forward genetics
      2. reverse genetics
   B. how do you get the mutation?
      - use knock-out techniques
   C. yeast knock-out mutations
      1. 
      2. 
      3. 
   D. C. elegans (also flies, mammals, plants, etc.)
      1. RNA mediated interference (RNAi)
      2. prepare double-stranded RNA from gene of interest
      3. inject germ cells or early embryo
      4. what it does:
   E. mouse knock-out
      1. similar to procedure for generating transgenic mice
   F. CRISPR (= Clustered Regularly Interspaced Short Palindromic Repeats): A different way to perform gene therapy or knock out gene
      1. Bacterial defense mechanism, produces a nuclease that clips DNA complementary to a guide RNA
      2. Possible uses:
3. How CRISPR/Cas9 works

A bacterial nuclease (Cas9) can be guided to any DNA sequence using a complementary RNA sequence (guide RNA)

The nuclease creates a double-strand break (DSB) that can be repaired by:

- Non-homology end joining to disrupt a gene or
- Homologous recombination (HR) to insert a new/corrected gene