TIGP, MCB & 3-program course

EAMCB and Core approaches for current molecular biology research

Mouse model and Nuclease-mediated genome editing (Dr. Ching-Yen Tsai)

Name: _____

1. Please briefly describe a transgene design for generating a transgenic mouse over-expressing a gene of interest. Please also specify (1) if the plasmid backbone should be included or excluded and why? (20%) (2) in regular cases, the relation between transgene copy number and transgene expression level (10%).
2. Please briefly describe how the nuclease-mediated genome editing works to generate a GM mouse (30%) and what kinds of modification it can do (10%).

3. Please choose the correct answer(s). (Three points for each correct answer.
   Please note: points will be deduced when choosing incorrect answer(s))

Purpose	**Embryo stage for manipulation**	**Material(s) injected into embryos**
Transgenic mouse	☐ one-cell stage ☐ blastocyst stage	☐ Transgene DNA fragment ☐ Transgene DNA in plasmid ☐ Embryonic stem cell (ES cells) ☐ sgRNAs ☐ Cas9 mRNA/Cas9 protein ☐ donor DNA
Chimeric mice	☐ one-cell stage ☐ blastocyst stage	☐ Transgene DNA fragment ☐ Transgene DNA in plasmid ☐ Embryonic stem cell (ES cells) ☐ sgRNAs ☐ Cas9 mRNA/Cas9 protein ☐ donor DNA
CRISPR/Cas9 mediated "Indel" mutation	☐ one-cell stage ☐ blastocyst stage	☐ Transgene DNA fragment ☐ Transgene DNA in plasmid ☐ Embryonic stem cell (ES cells) ☐ sgRNAs ☐ Cas9 mRNA/Cas9 protein ☐ donor DNA
CRISPR/Cas9 mediated HDR	☐ one-cell stage ☐ blastocyst stage	☐ Transgene DNA fragment ☐ Transgene DNA in plasmid ☐ Embryonic stem cell (ES cells) ☐ sgRNAs ☐ Cas9 mRNA/Cas9 protein ☐ donor DNA in any format
CRISPR/Cas9-mediated "PITCh"	☐ one-cell stage ☐ blastocyst stage	☐ Transgene DNA fragment ☐ Transgene DNA in plasmid ☐ Embryonic stem cell (ES cells) ☐ sgRNAs ☐ Cas9 mRNA/Cas9 protein ☐ donor DNA in plasmid format

Bonus question!

Please choose ONE of the following approaches shown in lecture slide #84 (i.e. PITCh, MMEJ, HMEJ, 2C-HR CRISPR etc.) for GM mouse production through CRISPR/Cas9, and describe the design of it. Please cite the reference if any