1a.

32^n>1031

n=3

>> 3 parallel homing barcodes would be needed to map the lineage of all cells in a male *C. elegans* nematode worm.

$32^n = 8\times10^{10}$

n=8

>> 8 homing barcodes would be need for human brain.

1b. (*I based my answer on the assumption that the barcode mutation occurs naturally, and doesn't occur in a controlled manner by Dox induction as in the article)*

My answer would base on presuming the mutation is not time controllable , that is in a natural way, which Cas9 is not controlled by Dox induction as article mentioned.

When the cells are fast dividing, faster than the mutation rate, then there would be lot of cell with the same barcode, it's hard to trace the lineage, it's possible mother (1^{st}), daughter (2^{nd}) and granddaughter(3^{rd}) possess the same barcode.

When the cell is dividing slowly, that would cause more than one barcode (*mutation)* in the same generation. That would waste barcodes, and barcodes would run out before all the cells are done dividing.

In my opinion, although it would be more complex to analyze and barcode might run out before cells are done dividing in slow dividing cell, it could still distinguish between generations before the barcode runs out; however, for a fast dividing cell, that's more challenging to separate them.

1c.

The insertion or deletion (mutation) of the original guide sequence would lead it to lose its ability to recognize the invader's (virus's DNA or RNA) sequence, and lost the adaptive immunity.

2a.

Due to the transcription initiation requirement of a 'G' base for human U6 promoter, I add a G in front of 20 bases.

gRNA_primer_forward (5'-3'): GTGGAAAGGACGAAACACCGTTTACCCAGATCTAT GCAACGGGTTAGAGCTAGAAATAGC

gRNA_primer_reverse (5'-3'): AAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTG ATAACGGACTAG

2b.

synthetic_grna: GCCAUUAACUCUUAGAGACG GGGUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGU GCUUUUUU

target: GCCATTAACTCTTAGAG|ACGGGGCCGCCCTCAGTAGA

homo_primer_forward: GCCATTAACTCTTAGAGGAGGGCCTATTTCCCATGAT

homo_primer_reverse: TCTACTGAGGGCGGCCCCGTAAAAAAGCACCGACTCGGTG

2c.

i. Rate of insertion and deletion, the length of nucleotide.

ii. The longer the initial barcode length, that can get more barcodes with 17 bases nucleotide, so the linage depth would be bigger.

iii.

$$Cas9 + gRNA \xrightarrow{k1} Cas9\_gRNA$$

$$Cas9\_gRNA \xrightarrow{k2} Cas9 + gRNA$$

$$Cas9\_gRNA + DNA \xrightarrow{k3} DNA_{DSB} + Cas9\_gRNA$$

$$DNA_{DSB} + nProtein_X + mNTP \xrightarrow{k4} DNA_{insersion} + nProtin_X + mPPi$$

$$DNA_{DSB} + iProtein_Y \xrightarrow{k5} DNA_{deletion} + iProtin_Y + DNA_{cut\ out}$$

$Omit\ "k"\ of\ each\ reactant\ and\ product\ above\ to\ \emptyset.$

3a.

$$\begin{bmatrix} 1 & 0 & -1 \\ -1 & 1 & -1 \\ 0 & -1 & 2 \end{bmatrix}$$

3b.

$$\begin{bmatrix} k1CxCb \\ k2CyCb \\ k3CxCy \end{bmatrix}$$

3c.

$$\begin{bmatrix} \frac{dCx}{dt} \\ \frac{dCb}{dt} \\ \frac{dCy}{dt} \end{bmatrix} = \begin{bmatrix} k1CxCb - k3CxCy \\ -k1CxCb + k2CyCb - k3CxCy \\ -k2CyCb + 2k3CxCy \end{bmatrix}$$

4a.

$$\begin{bmatrix} 1 & -1 & 0 \\ -1 & 0 & 1 \\ 0 & 1 & -1 \end{bmatrix}$$

4b.

$$\begin{bmatrix} k1CaCb \\ k2CcCa \\ k3CbCc \end{bmatrix}$$

4c.

$$\begin{bmatrix} \frac{dCa}{dt} \\ \frac{dCb}{dt} \\ \frac{dCc}{dt} \end{bmatrix} = \begin{bmatrix} k1CaCb - k2CcCa \\ -k1CaCb + k3CbCc \\ -k2CcCa - k3CbCc \end{bmatrix}$$