My daughter wants a pet cloned panda for her birthday. Explain how cloning of mammals is achieved, why people want to do it, and the circumstances under which it might be allowed. Discuss why Lizzie should or should not get her panda.

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Cloning is the production of genetically identical copies of biological material, from genes to entire organisms. These "clones" can occur naturally in asexually reproducing organisms such as; Planaria (e.g. *Dugesia Japonica*), Hydra (e.g. *Hydra attenuate*), starfish (e.g. *Coscinasterias tenuispina*), lizards (e.g. *Aspidoscelis neomexicanus*) through; binary fission, budding, fragmentation and parthenogenesis respectively. Pandas (giant pandas) cannot naturally produce clones, like all mammals that reproduce sexually, so producing a cloned panda would require artificial cloning. This artificial cloning of mammals has been developing since the 1970s with nuclear-transfer methods involving ovulated rabbit eggs. This developed into successfully cloning sheep/mice/cattle from blastomere nuclei, before the successful cloning using adult cells through somatic cell nuclear transfer like Dolly the sheep. Now independent companies can use this technique to clone pets (dogs, cats etc.) for huge amounts of money (Approx. 50,000-100,00 USD). So, this could also apply for pet giant pandas, which are notoriously bad for reproducing naturally. Researcher have actually been working on cloning pandas to increase the low numbers (2,000), with some experiments in 1999 and plans announced in 2004, which gained publicity again in 2015 when the same group that cloned Dolly took panda tissue samples to work towards cloning. With cloning a pet panda now established as not simply science fiction this essay will explain how to clone mammals, why people want to do it, the circumstances it might be allowed, before discussing why Lizzie (or anyone) should or should not have a cloned pet panda.

Mammal cloning through somatic cell nuclear transfer into an enucleated oocyte is achieved in 6 steps. First (1) take a somatic cell from the animal you want to be cloned that contains its complete genome, this could be anything from an embryonic stem cell to a skin cell to a mammary cell (which was the in the case for Dolly). Then (2) make an enucleated oocyte by taking the pronucleus out of an unfertilised oocyte (of the same species, although it has been done experimentally with different species). Then (3) take the nucleus out of the donor somatic cell and put it into the enucleated cell either by electrofusion or direct injection, which allows the donor DNA to act as the two meeting pronuclei in normal sexual fertilisation. The oocyte then needs to be (4) 'activated' by chemical or electrical activation, which causes the oocyte to start acting as if it has been fertilised starting the calcium transients. But activation is not quite equal to natural fertilisation an example of this is the fact that downregulation of the inositol-1,4,5-triphosphate receptor did not occur during activation while it does during normal fertilisation. The cytoplasm of the host cell also decondenses the DNA, causing a loss of silencing through things like heterochromatin, allowing it to become totipotent again (meaning it changes from the possibly specialised function to a form where it can make all the cells of the future organism again). Then just as in vitro fertilisation (IVF), some of the cells survive, divide and start to develop as normally fertilised oocytes, which like IVF are selected for and (5) implanted into the uterus of a pseudo-pregnant female (again of the same species). Where the developing clone (6) completes development as normal in utero. Dolly in 1996 was proof that this worked (even if it was 1/100), but to give a panda related example Chen took a somatic cell from a giant panda, transferred the nucleus into an enucleated rabbit oocyte and later a cat oocyte, and then implanted the cat oocyte into a cat womb. Polly in 1997 was proof of that we can clone transgenic (genetically manipulated) animals, so in the context of pandas we could use modern gene editing tools like CRISPR/Cas9 to change the clones to make them comparatively more fertile and/or libidinous, ending their reproduction problems, but

would probably make many unexpected problems including off target effects and behavioural issues.

People want to clone mammals for a number of reasons, one relating to the previously mentioned transgenic clones is that in commercial agriculture you can produce superior animals designed with specific properties that you want, e.g. cows that produce the perfect wagyu or huge amounts of milk. These could then be further genetically modified to act as bioreactors, that can produce useful macromolecules or proteins that are useful in human medicine, Polly was an example of this as she expressed the human clotting factor IX in her milk, which could then be extracted and intravenously given to people suffering from haemophilia B. This so called "pharming", although more commonly uses bacteria or yeast as bioreactors (often producing vaccines including hepatitis vaccines and human papillomavirus) there is clear progress in the large-scale use of mammalian bioreactors, with a 2017 example of 200 transgenic cloned cows producing recombinant human lactoferrin without any markers were made. Pigs have been genetically modified to reduce cross species immune barrier to allow for xenotransplantation to meet the organ transplant demand, which is so great that 18 patients in Europe die each day waiting for a transplant. Further adapting pigs to produce perfectly accepted organs or tissues for transfer then cloning them is a serious interest for cloning mammals. This would get around the extreme fears presented in the film "The Island" where human clones are produced for organ harvesting, which is clearly unethical, but also points out that some people want to clone mammals so that we can ultimately work towards cloning ourselves, just as we use animal models to study development, not necessarily because we are interested in a frogs/chickens/mice development (although people might be), but that they are good models for our own development. So mammalian clones could be useful for generating increased amounts of data whether it be with; genes (through PCR), cells (through embryonic stem cells or induced pluripotent stem (iPS) cells, through the forced expression of key transcription factors e.g. c-Myc, Oct4, Sox2 and Klf4), tissues (through tissue engineering), or organisms (through artificial cloning), this would mean that researchers could make what they want and follow its progress without having to wait for things like specific biopsies. Cloning mammals therefore gives us good models to study biology including embryonic development, cancer biology, stem cell biology, evolutionary biology allowing for faster breakthroughs in subjects like aging/immortality. Research in this area has made tailormade stem cell therapy a realistic possibility, where you can take somatic cells from an ill person, put it into a host oocyte to make a blastocyst stage clone, which you can harvest the inner cell mass to make embryonic stem (ES) cells, which you can then differentiate (using chemicals, cytokines etc.) into whatever tissue you want to then use to repair the individual that you harvested somatic cells from. This concept has been proven possible with Parkinson's as ES cells have been engineered to make functional dopamine neurons, which have been put into Parkinson's model rats improving motor coordination therefore treating Parkinson's. People may also want to clone mammals to resurrect or have multiple of their loved pet, which we know can be done, Barbra Streisand's dogs or Huang Yu's dead cat for example. So, it is not unfeasible that some people would want to clone people to make clone army (military, like in Star Wars, or army of friends, like I would like) or clone a dead leader (Mao, Lenin, or other non-communists) or great (Elvis, Newton, Darwin etc.). Since de-extinction was proven possible, if limited with its 7 minutes of life, after cloning the extinct Pyrenean ibex by taking the nucleus of a somatic cell, into a goat oocyte, and implanted into a goat.

Just because something is possible it doesn't mean it should be done, medical research is inseparable from ethics because of its goal to improve humanity, this section will discuss the circumstances cloning mammals might be allowed. Just as Nazi's conducted atrocious medical research on concentration camps inmates, and Americans conducted the Tuskegee syphilis study (looking at 400 untreated syphilis patients vs 200 controls), we too could find ourselves harming human or mammalian clones in the name of research, which is why we must not only abide by our own ethics but also The Nuremberg Code, the ethical boards of the research institutes and country (as well as it's laws). I think every researcher would/should hate to produce a piece of research that is a moral enigma such as Pernkopf's atlas (an anatomy book made by Nazi doctors), as it was produced under horrible circumstances but could be having a net good, but whether you look at it utilitarianly or through categorical imperatives it is up to you. As I am an undergraduate (medical sciences not philosophy) student in an exam I will base the circumstances on when mammalian cloning is allowed on the experiments that are published after being approved by ethics boards that I can remember and write down. Human cloning and stem cell research are huge and heated ethical debates as it is potentially destroying/creating life and since the discovery of iPS cells making all cells have the potential to create life, it makes this debate either more

complicated or has got around it, as the scientific community allows extensive iPS cell research in the pursuit of therapies I would say its got around it. Animal cloning and genetic manipulation is allowed as shown by the above examples, as these experiments (and others like it) are arguably having a net good and respect the 3Rs of animal research (Replace, Reduce, Refine) as well as the possible 4th R (Responsibility to promote animal welfare, as we are taught it as undergraduates and animal ethical approval is on every paper involving animals). I am unsure if it ethically ok to de-extinct an animal like the Pyrenean ibex only to have it die of lung failure 7 minutes later, that might be unnecessary suffering, just as resurrecting dead pets or cloning new pets might create unnecessary suffering making ethical problems, also they do not have ethical approval, and just because there are no laws shouldn't make it fine. On topic of unnecessary suffering almost all clones die sooner than their non-cloned counter parts as cloning is not without its problems these include; imprinting (like methylation inconsistencies on genes of the somatic cell interfere with expression issues), reprogramming (of the donor nucleus must be reprogrammed by the oocytes cytoplasm, optimum might be 50 percent of genes being expressed or silenced, if this process is incomplete it results in premature death), telomeres (being shorted in the "aged" somatic cells, means that they have a reduced life span), and mitochondria (as clones have mitochondrial heteroplasmy this can create issues). With further perfection of these cloning limitations it makes all forms of mammalian cloning more ethical but does not address main ethical issues of animal research and playing God, and ownership with human cloning.

Finally, why Lizzie should or should not get her pet panda is up to you as a parent (and the better scientist), but here are some arguments. Pet cloned pandas should be a thing because; working towards cloning pandas has received ethical approval for over two decades, cloning pets is actively practiced (legally), there are only 2,000 panda remaining with reproduction (naturally/assisted) being problematic so maybe introducing cloned pandas as pets can increase the global population like big cats where some species have more alive as pets as in the wild. Pet cloned pandas should not be a thing because; pandas are not pets they are all owned by the Chinese Government and used as political currency so I'd prefer to avoid that headache of ownership that could get as bad as human clones, the numbers of pandas are increasing and they are no longer endangered just vulnerable, also it does not address cause of their reduction which is the reduction of habitat, the ridiculous costs of cloning not just pandas as pets but all pets could be spent on better things like; research with an actual purpose (cloning mammalian bioreactors, stem cell therapies, anything trying to cure diseases), medical aid (vaccines), or just getting food to areas with man-made famines.

In conclusion, whether Lizzie should get her panda is up to you, it is possible to clone mammals through the above steps, and there are many reasons to clone mammals, but it is an ethical minefield with cloning humans, pharm/farm animals, and pets each having their own concerns. The technology of cloning and genetic manipulation is not perfect resulting in early death and possible unnecessary suffering but is improving to the point where pet pandas might be possible by the time I have (grand)children but if they want a pet panda they will have to pay for it and deal with it themselves after they have moved out.