Buck and axel:

1. why do the use mRNA instead of genomic DNA as starting material?
2. How did they design the oligos for PCR? Based on which assumption?
3. What is the difference between figure 2a and 2B? What does it mean? Explain the results for lanes 12 and 13.
4. How many "genes" are there in lane 13?
5. If they get a band like fig2. A, lane 12: does it rule out that it is really an olfactory receptor?
6. Would they get any PCR products if they use a brain cDNA library? Before answering remember the design of the oligos.
7. In fig 3: why do they do a northern blot with 20 probes? Wouldn't they loose specificty? How do they know which probe is hybridizing to which target in the northern? What are those 2 bands?
8. Why do they use a cdna library to get full length cdna clones? Why not do it by PCR?
9. Fig3, why do they isolate polyA+ RNA? Are there rna's with no polyA+?
10. How come you can figure out the number of genes by doing a southern blot (fig 7)? Why do they genomic DNA instead of cDNA from a library? Could you do the same with a northern blot?
11. What does it mea n to hybridize a library at high stringency? Why would you want to do that?
12. Fig 7: what does it mean that they do not 'cross-hybridize"?
13. these genes have no introns: how does this factor affect the choice of source of nuclei acid for PCR? What would be the results if you use: a) mRNA after reverse transcription, b) cDNA from a library, c) genomic DNA form the olfactory epithelium, d) genomic DNA from the liver.
14. What does it mean to do "nested" PCR? Why do they do nested PCr to make sure that the clones that they pick are what they really want?
15. By doing northern blot they confirm that the genes are expressed in olfactory epithelium. Explain the logic they use to assume that it is likely that these genes are expressed in olfactory neurons.
16. Do they really know which cells express the genes? What could they do to really make sure where the genes are expressed? PCR? Northern? Other techniques?