Buck and axel:

1. why do the use mRNA instead of genomic DNA as starting material?

One criteria of olfactory receptor is that it is expressed only in nasal epithelium but not in other region. If one starts from genomic DNA, there is no guarantee that whatever amplified by PCR belongs to olfactory receptor gene family. Also, the genomic DNA contains both exon and intron. There is no guarantee that there is no introns between the two regions selected to design PCR primers. If there is intron(s) between the two regions, first PCR may not work. Also, the size of PCR product cannot be a selection criterion of candidate gene.

2. How did they design the oligos for PCR? Based on which assumption?

Olfactory receptors belong to 7-transmembrane G-protein coupled receptor. At that point, there was a dozen of such receptor family already known (rhodopsin, beta-adrenergic receptor etc). By comparison between these genes, one can find homologous region with in this family of protein. They assumed that the olfactory receptor protein also share the same sequence feature.

3. What is the difference between figure 2a and 2B? What does it mean? Explain the results for lanes 12 and 13.

2A is before digestion with HinfI and 2B is after. 2A is to confirm that the size of PCR products are within expected range as G-protein coupled receptor. 2B test if the bands consist of multiple different DNA or not. In 2B lane 13 consists multiple bands which sum up much longer than undigested DNA. This is indicative of presence of multiple species of fragments in undigested DNA. In contrast, the

length of digested DNA will add up to the length of undigested DNA in lane 12 (compare with marker).

4. How many “genes” are there in lane 13?

The authors estimated that the length sum to 5- to 10-fold of the undigested DNA. This means there are at least 5-10 different genes.

5. If they get a band like fig2. A, lane 12: does it rule out that it is really an olfactory receptor?

It is still possible that this particular primer pair only amplified single species of olfactory receptor.

6. Would they get any PCR products if they use a brain cDNA library? Before answering remember the design of the oligos.

The oligos are designed to detect G-protein family receptor. It is possible that other G-protein coupled receptor (but not olfactory receptor) is cloned.

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?

There is ~1000 olfactory receptor genes and if one use only one probe and each of may be expressed only in a small fraction of olfactory receptor. Therefore, the amount of expression may not be sufficient to detect if they use only one probe. The two bands may correspond to different subtypes of olfactory receptor.

8. Why do they use a cdna library to get full length cdna clones? Why not do it by PCR?

One can try 5' RACE (rapid amplification of cDNA end) and 3' RACE to get full-length clone. For RACE, one use primer that anneals to a know part of cDNA and another primer in the vector (phage vector or plasmid vector, which is already known) sequence. This will allow one to get unidentified portion of cDNA. However, if you have a sufficiently long cDNA fragment which you can use as probe, screening cDNA library using plaque hybridization is more standard. One reason is that PCR tends to incorporate mutations. Second reason is that it does not involve further work to connect original PCR fragment with new ones.

9. Fig3, why do they isolate polyA+ RNA? Are there rna's with no polyA+?

Transfer RNA and ribosomal RNA do not have polyA. Also, there are a number of minor RNAs such as shRNA, do not have polyA either. There are also messenger RNAs lacking polyA. Some of mRNAs coding nuclear protein belong to such family. Histon is a typical example.

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?

Restriction enzyme digests various olfactory receptor DNA into fragment at different length. In addition, if you cut with 6 cutters such as EcoRI or HindIII (appears 1 out of 4kb), the average fragment should contain only 1 receptor. By doing blotting at low stringency and counting the number of positive bands, one

should be able to estimate the number of family protein. In contrast, mRNA has less diverse in their length. It seems to be around 2 kb in Northern blotting even they used a mixture of 20 different cDNA as probe. So this makes it difficult to estimate the number of genes. From the same reason, it is difficult to estimate the number of independent gene using southern blotting against cDNA.

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11. What does it mea n to hybridize a library at high stringency? Why would you want to do that?

DNA double helix is stabilized at low temperature and high salt concentration. If you use higher temperature and low salt concentration, only a perfectly matched DNA anneals together. By using such condition, one can identify DNA fragment exactly matches the probe.

12. Fig 7: what does it mean that they do not 'cross-hybridize"?

Under low temperature and high salt concentration, even the mismatched DNA can anneal together. It is called low stringency. If you want to detect other members of the same family, you can use low stringency.

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.

Olfactory cDNA made by RT reaction (a) or olfactory cDNA library (b) should amplify olfactory receptor. Genomic DNA from olfactory epithelium and liver (c and d) may be also used but G-protein coupled genes other than olfactory receptor may

be also amplified and requires extra effort to characterize it. (c) and (d) should give identical results. The length of product, if any, will be approximately the same because there is no intron in-between.

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?

In nested PCR, one first amplifies DNA with a pair of primers and then recovers the PCR product. Using it as a template, the second round PCR will be carried out using primers located with in the product region. Because two sets of primers determine the specificity, it will be more accurate than only one set is used. Erroneous products amplified with only first set of primer will not be amplified in the second PCR.

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.

It has been postulated that the cilia of olfactory neuron is the site of odor detection (p175, right column). Selective removal of cilia abolishes the olfactory response. The specific binding of amino acid (in fish, it is odorant), is found in cilia. Also, by application of odorant, adenylate cyclase is activated GTP dependently and open cyclic nucleotide gated channels in cilia.

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?

In situ hybridization will be the best technique to identify the cell type expressing a certain gene. One can counter-stain tissue to identify cell types.