

Table of Contents

Acknowledgement	i
Abstract	ii
Introduction	1
Materials and Methods	
Materials	4
Methods	
1. Construction of expression vector for human wild type TTR and its variants	5
2. Construction of human TTR variants	5
3. <i>Pichia</i> transformation	
3.1 <i>by an electroporation</i>	6
3.2 <i>by PEG1000 transformation method</i>	6
4. Recombinant TTRs synthesis	
4.1 <i>Screening for Mut⁺ transformants</i>	7
4.2 <i>Induction for gene expression of recombinant Pichia clones</i>	7
5. Time course of TTR gene expression in yeast	7
6. Media selection	8
7. Purification of recombinant human TTRs from yeast culture	
7.1 <i>by affinity chromatography</i>	8
7.2 <i>by preparative gel electrophoresis</i>	8
8. Determination of the mass of the TTR tetramer by gel filtration	8
9. Measurement of the thyroid hormone binding affinities of recombinant human TTR and analysis of binding data	9
10. Western analysis	9
11. Expression scale up	9
12. SDS-PAGE	10
13. Preparation of L[¹²⁵ I]-thyroxine	10
14. Amyloid formation	10
15. Congo red binding	10
16. Preparation of competent cells and DNA transformation	10

Results and Discussion

12

Conclusion

20

References

21