

Candidate tumor suppressor gene (n = 65) screening



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graph TD; A[Candidate tumor suppressor gene (n = 65) screening] --> B[Confirmation with DNA methylation datasets from TCGA/GEO  
Criteria 1: Mean Methylation percent (β value) > 0.25 in ESCC samples  
Criteria 2: Mean Methylation percent (β value) < 0.25 in adjacent control samples  
Criteria 3: Mean Methylation percent (β value) < 0.25 in PBMC and PBL of normals  
Criteria 4: At least 2 CpG sites on candidate gene fulfills criteria 1,2 and 3]; B --> C[Candidate tumor suppressor genes (n = 6) for further validation]; C --> D[Four candidate biomarkers for multiplex PCR design:  
ADHFE1, EOMES, SALL1, TFPI2]; D --> E[94 pairs of ESCC tumors and adjacent control tissues for validation with targeted bisulfite sequencing]; E --> F[Model Evaluation: Accuracy, Sensitivity, Specificity, AUC];
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